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An expert system for honeybee species identification and information retrieval

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Abstract

Detecting honeybee species is important for ecological and agricultural research, as it helps researchers understand their behavior, population, movement pattern, and pollination habits. This paper proposed a honeybee Identification system categorizing five honeybees namely: *Apis cerena indica, Apis mellifera, Apis florea, Apis dorsata,* and *Trigona.* Input images of honeybees were preprocessed to improve quality and eliminate any noise. Data augmentation methods were used to increase the dataset size, ensuring effective model training. The architecture of VGG16 which is popular for the image classification tasks identified the morphological characteristics present in the data set that were more relevant for species categorization rather than behavior analysis. Additionally, Rectified Linear Unit (ReLU) and Softmax layers were added, increasing the model's efficiency. The support Vector Machine model was trained to classify 5 classes of honeybees. After training, the model made accurate predictions of different honeybee species with high levels of precision and recall. This system performed exceptionally well in species classification, providing advancements in ecological and agricultural studies, through the implementation of VGG16 and SVM.

Keywords: ecosystem conservation, Honeybees, Computer vision, Deep learning, VGG16, SVM

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Bal Arısı türlerinin tespiti ve bilgi erişimi için uzman sistem

Özet

Bal arısı türlerinin tespit edilmesi, araştırmacıların davranışlarını, popülasyonlarını, hareket şekillerini ve tozlaşma alışkanlıklarını anlamalarına yardımcı olduğundan ekolojik ve tarımsal araştırmalar için önemlidir. Bu makale, beş bal arısını kategorize eden bir bal arısı Tanımlama sistemi önermiştir: *Apis cerena indica, Apis mellifera, Apis florea, Apis dorsata* ve *Trigona*. Bal arılarının girdi görüntüleri, kaliteyi artırmak ve gürültüyü ortadan kaldırmak için önceden işlendi. Veri kümesi boyutunu artırmak ve etkili model eğitimi sağlamak için veri artırma yöntemleri kullanıldı. Görüntü sınıflandırma görevleri için popüler olan VGG16 mimarisi, veri setinde bulunan ve davranış analizinden ziyade tür kategorizasyonuyla daha alakalı olan morfolojik özellikleri tanımladı. Ayrıca Rektifiye Linear Unit (ReLU) ve Softmax katmanları eklenerek modelin verimliliği artırıldı. Destek Vektör Makinesi modeli, 5 sınıf bal arısını sınıflandırmak için eğitildi. Eğitimin ardından model, farklı bal arısı türlerine ilişkin yüksek düzeyde hassasiyet ve hatırlamayla doğru tahminler yaptı. Bu sistem, VGG16 ve SVM'nin uygulanması yoluyla ekolojik ve tarımsal çalışmalarda ilerlemeler sağlayarak tür sınıflandırmasında olağanüstü iyi performans gösterdi.

Anahtar kelimeler: ekosistemin korunması, Bal arıları, Bilgisayarla görme, Derin öğrenme, VGG16, SVM

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1. Introduction

In recent years, there has been a growing interest in leveraging computer vision techniques for honeybee identification, driven by the need to monitor bee populations efficiently and effectively. The advantages of pollination for people and the environment are in danger as bee numbers worldwide decrease due to which there is a pressing need for advanced methods to monitor bees species efficiently.

Honeybees are a diverse group of insects belonging to the class genus *Apis*. Their role is crucial for honey production and pollination. *Apis mellifera* is the most well-known species, usually called Western honeybee, spread all over and domesticated for its keeping of honey and agricultural pollination. Others are the Asian honeybee (*Apis cerena*), giant honeybee (*Apis dorsata*), and dwarf honeybee (*Apis florea*). Each has unique behaviors as well as adaptations for instance; *Apis dorsata* constructs large, exposed nests on tree branches while *Apis cerena* has a high resistance to certain diseases and parasites. Although bee pollination's role in advancing sustainable development goals by fostering biodiversity and food security is well known, there are still many undiscovered advantages that bees offer. It's crucial to remember that although image analysis can identify morphological characteristics that are particular to a species, it cannot immediately disclose behaviors that are specific to that species, such as *Apis dorsata's* aggression or *Apis florea's* restricted flight range. While identifying species is a first step in comprehending ecological responsibilities, this research introduces a computer vision-based method that does not directly evaluate behavioral patterns. Bees have the potential to support at least 30 SDG targets and 15 of the 17 Sustainable Development Goals (SDGs) [1]. Ecological research, apiary management, and conservation initiatives all heavily rely on information and species identification systems for honeybees. Knowledge of the distribution, behavior, and ecological roles of honeybee species within ecosystems depends on accurate species identification. Manual procedures, which are labor-intensive, time-consuming, and need specialized taxonomic skills, were the norm for species identification in the past. Recently, though, technological advancements have created new opportunities for automating this process, especially in the areas of deep learning, computer vision, and remote sensing. Through the use of image processing techniques, these systems can distinguish between distinct species and subspecies of honeybees by analyzing complex morphological traits such as wing venation patterns. This technique provides a productive and non-invasive way to identify species, eliminating the requirement for labor-intensive human labor and a high level of taxonomic knowledge. DeepABIS deep learning method is used for identifying honeybee subspecies using wing photos of eight different species of honeybees e.g. Carnica, Siciliana, etc. The models that are used to train these datasets are CNN models such as MobileNetV2, B-CNN, and Inception-ResNet. By removing intricate features, DeepABIS gained an accuracy of 93.95% for (top-1) and 99.61% for (top-5). A smartphone application and website named CloudABIS is used to input the image and for the graphical user interface [2]. Xilotheques in the 21st century have a unique opportunity to advance with AI and deep learning for enhanced forest species identification. Challenges include limited image datasets, lack of automated identification focus, and uneven taxonomic distribution. Solutions involve digital sample photography, standardized data sharing, and prioritizing sampling methods for comprehensive image collections, positioning xilotheques as crucial hubs for forest species research [3].

To monitor honeybee colony conditions such as brood rearing and swarming, the study presented a system that uses temperature data from one sensor per hive. It successfully recognizes these states by using artificial neural networks for pattern identification. Limitations include possible algorithm complexity and reliance just on temperature data, despite the promising nature of the approach. Still, the strategy looks promising for better colony management of honeybees [4]. An imaging device to track the activity of honeybees at hive entrances is shown in this work. It tracks individual bees using infrared technology and character-encoding tags, obtaining great accuracy in bee identification (86%) and character recognition (98%) respectively. The method shows its potential for effectively investigating honeybee behavior by offering insights into everyday foraging behavior [5]. The Hungarian algorithm and Kalman Filter are used in this study's real-time imaging system to track honeybee activity at hive entrances, providing precise bee detection and background reduction. A 4G LTE router is used to send data to a distant server for analysis, allowing for long-term observation of colony health and behavior, including the impacts of pesticides. This economical system provides important information on colony's health and honeybees' activity [6]. The findings of research describes an audio recording technique near hive entrances for early swarming mood detection in bee colonies. Mel Frequency Cepstral Coefficients (MFCCs) and their derivatives are analyzed to identify significant coefficients for worker bee and drone bee categorization. The study surpasses the typical MFCC coefficients' accuracy of up to 90% by improving detection accuracy to slightly above 95% with the chosen set of signal attributes through the use of an autoencoder neural network [7]. This study used computer vision and deep learning to automate the identification of bumble bee species. Using 89,000 photos from 36 different species, it assesses four models; InceptionV3 achieves 91.6% accuracy in 3.34 milliseconds. Bee Machine, a user-friendly identification tool that, and highlights the technology's revolutionary potential is presented [8]. The methodology used geometric morphometrics to analyze forewing vein patterns to produce a quick and simple approach for identifying populations, ecotypes, subspecies, or hybrids of honeybees. Reference samples comprising 187 honeybee colonies from 25 subspecies spanning four evolutionary lineages were taken from the Morphometric Bee Data Bank. While evolutionary lineage identification turned shown to be very consistent, subspecies identification accuracy varied, with African honeybees demonstrating less consistency. For

analysis, the acquired data were exported to the Identify programmer. All things considered; the study helps create effective instruments for identifying different subspecies of honeybees [9]. The study introduced a hierarchical multi-task structural learning algorithm designed to enhance plant species identification on a large scale. A visual tree is utilized to organize numerous plant species in a coarse-to-fine manner, facilitating the identification of interrelated learning tasks and guiding hierarchical classifier training. By effectively controlling error propagation between levels, the developed tree classifiers demonstrate superior discrimination power for large-scale plant species identification. Experimental results showcase competitive performance in terms of identification accuracy and computational efficiency compared to existing approaches. Additionally, the proposed algorithms have potential applications in large-scale image classification tasks [10]. A developed Neural Network was applied to detect two honeybee colony states. It detected 11 possible swooning cases [11]. The research presents an alternative solution for the automated identification of tree species using macroscopic woodcut images. They propose a convolutional network trained from scratch achieving 93.6% top-1 accuracy and further improve results with a pre-trained ResNet50 model reaching 98.03%. Despite successful application, the immediate challenge lies in building larger datasets, with ongoing efforts focused on a database of Costa Rican tree species. The importance of xylotheques in various fields underscores the significance of this research [12]. Findings of the research demonstrates the effectiveness of image processing in identifying fish species with a high degree of accuracy. However, limitations exist in the scope of fish species covered and the static nature of the image acquisition process. Future research should focus on expanding the range of freshwater fish species studied and developing dynamic image processing methods to enable real-time and online identification, thereby improving practical applicability in production settings [13]. To classify datasets of different bee species, this paper uses the DeepABIS deep learning system that uses MobileNetV2, B-CNN, and Inception-ResNet. By using these models, they got accuracies of 85%, 87%, and 92%, respectively. They have also made a website using Flask and PHP for the backend for inputting the image. The novelty of this paper is anomaly detection for species identification tasks where the number of different existing species is often unknown so that's why detecting outliers is important [14]. The main part of paper that makes it novel is the region of interest locator network can be trained with a minimum number of hand-annotated images; it can handle crowdsourced data with a wide range of image quality; it doesn't require manual image cropping before genus/species identification; and it can accurately classify multiple bees in a single image. A dataset from the Bee Spotter website is used for this paper, comprising 15,347 crowd-sourced images of bees annotated for species by an expert. The method used for both bee identification and classification tasks is Faster R-CNN with a Resnet 101+FPN backbone. The model's accuracy on average is 91% [15]. The dataset which contains 5000 images of different bee subspecies like Italian bee, Russian bee, Western Honey bee, etc is used in this paper with health conditions using a two-layer CNN. The identification of the bees is done in this paper. The CNN model has 86.5% accuracy for subspecies and 84.9% accuracy for health classification. Data Preprocessing is done by SMOTE Data Balancing. To enhance generalization, data augmentation techniques are applied to the images. Relu and SoftMax are used as activation functions [16]. The work proposes a method for the recognition of pollen-bearing honeybees in hive entrance videos to monitor foraging behavior. Tests were conducted on a newly annotated dataset with several models, including baseline classifiers and Convolutional Neural Networks. Although the simple CNN performed the best, its predictions were somewhat degraded due to misalignments and the visual noise of manual annotations. Networks shallower in depth did not lead to better performance due to limited data and computational demands. However, in the future, large, high-quality datasets should be developed by automatic image collection and validation to enhance model performance and applicability in real practice [17]. Deep learning-based models for monitoring honeybee hives to detect healthy bees, pollen-bearing bees, and anomalies such as Varroa parasites, ants, hive robberies, and small hive beetles are reflected in the research. Transfer learning with pre-trained deep neural networks (DNNs) and support vector machines (SVMs) under a variety of feature sets are tested on three datasets consisting of 19,393 images. The models reach as high as 99.07% accuracy, proving to be useful in smart beekeeping and real-time hive monitoring. Although no DNN was best across all datasets, the accuracy of these models and the short classification time make them promising tools for improving the safety of bees and enhancing the efficiency of beekeeping. Future work will adapt these models to other hive conditions and evaluate the performance of DNNs in further detail [18]. A bee hive health monitoring system using image processing with Convolutional Neural Networks has been proposed in the work. It includes two models: one for detecting bees and another for health classification. In health classification, it exceeded existing methods with an accuracy of 95%, while the detection of bees was lower, at 82%. While health classification is quite fast, at more than 500 FPS, bee detection is slower at 2-3 FPS, which makes the system unsuitable for real-time usage. Future work will then focus on improving speed and accuracy with the potential use of Faster R-CNN. This system promises to reduce stress in monitoring bees and support global food production through better bee health [19]. Findings describes a process of classifying images of pollen-bearing bees using Convolutional Neural Networks, with the goal of future implementation on low-cost FPGA hardware. A new dataset of images of bees was taken at hive entrances. This paper has investigated multiple configurations of CNNs, up to 3 hidden layers with up to 15 filters per layer, and with filter sizes ranging from 15x15 to 3x3. The most appropriate configuration found is a three-hidden-layer CNN with a 7-7, 5-5, 3-3 configuration, which reaches 94% of accuracy in classification, balancing the computational requirements. This trained CNN will be implemented on FPGA for the real-time video detection of pollen-bearing bees. To identify the regions of interest, a histogram of oriented gradients and background subtraction

techniques will be applied [20]. The color of the bee depends on the form of the bee, whether the bee is a drone, worker bee, or queen bee. In these 3 forms of bees, the color and pattern variation is observed. The color variation ranges from yellow to black. Yellow workers are the result of the cross of the yellow queen with a black drone. Black drones are produced by yellow-laying workers. The body color is an expression of the gender of the bees [21]. The genetics based on the origin of the brood influences the coloration of abdominal tergites. The pattern on the abdomen varies based on temperature and the surrounding ecosystem [22]. A morphometric study of honeybee species *Apis cerena* is carried out in [23]. This study relies heavily on color patterns and detailed visual features of honeybees. AI-based technique using ResNet for classification of bumble bees from non-bumble bees is mentioned in [24]. 91.6 % accuracy of classification was achieved in this work. Geometric Morphometric Analysis was used to classify honeybees based on shape morphology. Deviations in the intersections of the wing vein angles was used as the distinguishing parameter [25].

Honeybees are the most important pollinators. They are a vital part of the forest ecosystem and agriculture. Identification and classification of honeybees are necessary to maintain a record of the honeybees and for track the movement of their colony. Honeybees can be identified based on morphology, foraging behavior, and defensive mechanisms. However, in the forest surrounding it is difficult and time-consuming to observe in detail the color shades and visual patterns on the body of a honeybee. Only an expert and well-trained individual will be able to identify such cases. This work uses computer vision, machine learning, and deep learning to identify the honeybee based on image input. Based on the identification results, the system fetches details about the honeybee species from the dataset. The system generates the details which include scientific name, distribution, and habitat. In this work, a more inclusive environment is developed with an expert system for honeybee species identification from the captured image. The computer vision-based model is trained by well-identified input images. The honeybee identification must be carried out with their habitat in the background. Hence honeybee images with a background of natural surroundings like flowers and leaves are used for testing the model in the proposed work. Table 1. Displays the work that has already been done for classification utilizing various algorithms

Table 1. Comparison of prior art

Reference No.	Database/Dataset used in literature	Algorithm
[13] (2020)	Datasets for Fish Species Datase 1 : 9942 images, 166 genera, and 881 species. Dataset 2: 300 images with exactly 60 images per population (30 males, and 30 females).	MobileNetV2, Tensorflow, Keras, B-CNN, Inception-ResNet
[1] (2022)	Total of 9887 images which includes 8 classes for Honeybees.	ResNet 50, MobileNet V2, Inception Net V3, and Inception ResNet V2
[2] (2018)	9000 photographs of some 1800 species of hard commercial timber in the world	MnasNet, ResNet, Wide ResNet

The research outcomes of prior work are consisely presented in Table 1 that shows a variety of methods for classifying species using deep learning. As an instance, the Study [13] used MobileNetV2, B-CNN, and Inception-ResNet to classify a large, heterogeneous dataset of fish species, emphasizing image analysis for an aquatic species group that is distinct to a population and cross-gender. Although Study [1] examined honeybee classification it used larger classes of honeybees with numerous model architectures, such as ResNet50 and InceptionNet V3, with the goal of recognizing general species rather than differentiating between subspecies. Similarly, to identify timber species, Study [2] used MnasNet, ResNet, and Wide ResNet, which necessitated algorithms tailored to plant morphology. In contrast, this research optimizes model accuracy to capture subtle morphological distinctions essential for ecological and agricultural research by combining VGG16 with SVM in a unique way to specifically identify five honeybee classes. This customized strategy sets this methodology apart from previous studies' more generalized classification objectives and promotes it as a focused option for honeybee biodiversity monitoring.

2. Materials and methods

The paper presents a thorough approach to computer vision-based honeybee identification that includes image capture, preprocessing, feature extraction, and classification steps. To improve feature extraction, image data is preprocessed using techniques like resizing, normalization, and Gaussian blur. However, as the input photos and extracted characteristics only offer morphological information, excluding behavioral analysis.

The dataset includes 5 types of honeybees and honeybees are classified into 5 classes as given below:

1. *Apis cerena indica* (Indian Honey bee)
2. *Apis dorsata*
3. *Apis florea*
4. *Apis mellifera*
5. *Trigona*

2.1 Details of the Information System

Honeybees are colonial insects having a complex social hierarchy with one queen, many worker bees, and drones. The fact that they can repurpose information about food sources through waggle dance is amazing for them. Their pollinating activities make them crucial parts of ecosystems due to their contribution towards the reproduction of many flowering plants hence maintaining biodiversity including agricultural productivity. A description of the class of honeybees and the difference between them in terms of features and location is shown in Table 2. Honey bees are climate indicators. The information system is designed to fetch information about the identified honeybee. This expert system records their presence, location, day, date, and Time of occurance. A scientific study of this pattern of occurance helps in monitoring and assessing the health of the environment, and predict future changes. This will aid in taking measures to take positive measures towards biodiversity conservation.

Table. 2. Desciption of honey bees classified in this work

Sr	**Names**	**Category**	**Features / characteristics**	**Location**
1	*Apis cerena lica*	Subspecies	Slightly Larger, relatively non-gressive, and rarely exhibiting arming behavior. Has 4 stripes on the domen	India, Pakistan, Nepal, Myanmar, ngladesh. Srilanka, Thailand, ainland Asia
2	*Apis mellifera*	Species	Western Honey bee, Lifespan 30-60 ys. Has 3 abdominal stripes	Africa, Europe and the Middle st.
3	*Apis florea*	Species	Dwarf honeybees, small in size	South-east Asia
4	*Apis dorsata*	Species	very large (17-20 mm), golden in lor. Abdominal stipes yellow to black.	South-east Asia
5	*Trigona*	Genus	Stingless bees, Black spiny feet	Mexico, Columbia, South nerica, and Venezuela

2.1.1 Honeybee identification architecture

The honeybee category detection project using computer vision starts by gathering honeybee images and then goes through preprocessing tasks like resizing and normalization. To increase the variety in the dataset, techniques like rotation and flipping are applied for data augmentation. The VGG16 architecture, which is a pre-trained convolutional neural network (CNN), is used to extract features from the images. Non-linearity for classification is introduced with Relu activation functions, and a SoftMax layer generates probability distributions for different species. Ultimately, the model that has been trained predicts the honeybee species by analyzing input images using the features and probabilities it has learned. Figure1. shows the block architecture of the honeybees species identification system.

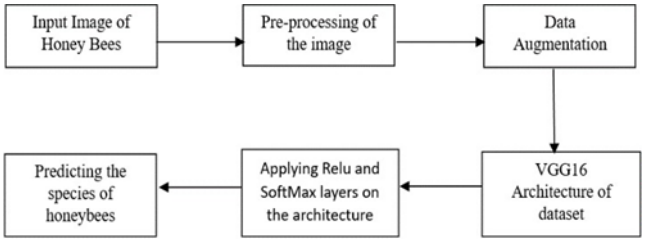

Figure 1. Honeybee Identification system

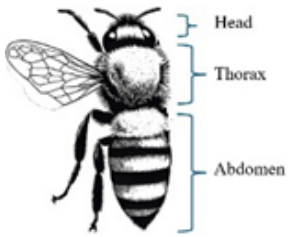

Figure 2. Major external organs of a honeybee

The anatomy of a honeybee has 3 major parts namely the head, thorax, and abdomen as shown in Figure 2. Abdomen is the most visible part of the honeybee image. The color, size, and shape of the stripes on the abdomen and

the size and shape of the abdomen are important for species identification. The image processing and feature extraction techniques are used to capture the underlying distinguishing pattern for a particular species.

2.1.2 Dataset/Preprocessing details

The dataset is collected from the iNaturalist website. iNaturalist is a well-known website and community-based database where people can share their sightings of different species of plants and animals, including honeybees. The dataset contains images of 5 types of honeybees namely *Apis cerena indica*, *Apis mellifera*, *Apis florea*, *Apis dorsata*, and *Trigona*. Each species contains approximately 200 images, therefore, overall, 1070 images are used in the dataset as shown in Figure 3.

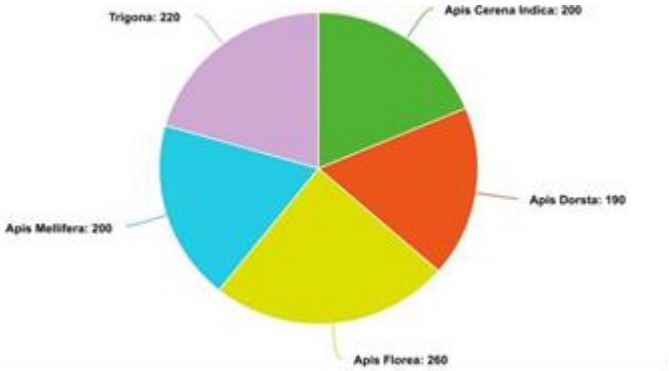

Figure 3. Dataset taken for each subspecies

Preprocessing: During dataset preprocessing, the first step is to label and resize images to ensure consistency in representation. Gaussian blur is applied to remove noise and to enhance feature extraction for SVM after resizing, while VGG16 only requires resizing. Normalization adjusts pixel values and data augmentation helps diversify the dataset, improving model robustness. These steps improve dataset preparation and ultimately boost model performance in machine learning or deep learning tasks.

Figure 4. Honeybees images with and without natural surrounding

Figure 4 depicts the images of honeybees belonging to catoried mentioned in this paper. The images include honeybees perched on flowers or leaves as it is their natural surroundings. These images are then used for dataset preprocessing. Figure 6 depicts the preprocessed images of *Apis mellifera* where Gaussian blurring has been performed.

Figure 5. Labeled images for 5 classes

Figure 6. Gaussian Blur filtered images of *Apis mellifera type*

2.1.3. Classification of honeybees

This work implements two techniques for classification. The machine learning technique uses SIFT feature classification using a Support Vector Machine (SVM). The deep learning technique uses the VGG-16 model for class identification.

SIFT features and SVM-based classification: SIFT is a feature extraction algorithm used to detect the key points and descriptors of the given image. It is renowned for its robustness to variations in illumination, scale, and rotation which makes it suitable for extracting key points and descriptors from grayscale images. Edges, corners, and texture patterns, these types of local features are extracted from the image. SIFT first identifies key point locations in the image based on gradient information and then computes descriptors around these key points to capture their local image information. Equation 1 describes the process of extracting key points from an image using the SIFT method.

$$Z = -\frac{\partial^2 D^{-1}}{\partial x^2}\frac{\partial D}{\partial x} \qquad (1)$$

(a) (b) (c)

Figure 7. SIFT feature detection of morphological parameters of honeybee image

It can be seen from Figure 7 a and b that the distinct organs of the honeybee are mapped and key points are detected. The wings, thorax, and abdomen key points are identified based on pixel intensities and neighborhood. Figure 7 c shows three types of stipes patterns on the abdomen of honeybee. The key points identified in this image carry important information about the underlying pattern. SIFT features are invariant to rotation, scale, blur, Illumination, warping, and noise. Hence the system trained on SIFT features is robust and can identify the species even under the following adverse conditions. It is observed that honeybees curl their abdomens while in flight and under restraint. Their body part could get occluded by flowers or leaves. Light intensity could be low due to shadow or low ambient conditions. Honebees beat their wings 230 times per second while flying. Rhythmic thorax pulsation and wing movement make it difficult to capture sharp images of honeybees. The image of a moving honeybee could get blurred especially for low shutter speed cameras. Figure 8 shows features for *Trigona* genus. and Figure 9 exhibit the key points on wings of *Apis mellifera* class honeybee detected after applying the SIFT feature extraction method.

Figure 8. Keypoints of *Trigona* class detected by SIFT

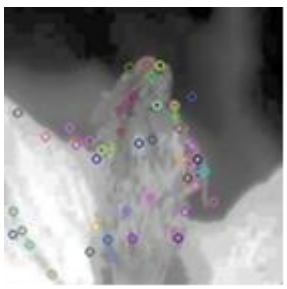

Figure 9. Keypoint of *Apis mellifera* class detected by SIFT

Images are divided into training and testing datasets. From each class, 20% of the images were reserved for testing and 80% of the images were used for training. The SIFT features are extracted from the image dataset. Extracted features are then reduced by using the k-means algorithm. The reduced feature set represents a one-row vector for each image. The feature set is generated for all classes and provided to an SVM model for classification training. The SVM model is trained to predict the class of honeybees by analyzing the keypoint descriptors extracted from the images. This combination of SIFT for feature extraction and SVM for classification increases the robustness of SIFT in handling variations and the effectiveness of SVM in classification between different species based on the extracted features.

VGG 16 Model for classification: The paper proposes a deep learning-based classification system where the VGG16 model has been implemented. Figure 10. represents the system architecture of VGG16. The VGG16 is a convolutional neural network architecture developed by the Visual Geometry Group at the University of Oxford. It is known for its deep structure, which includes 16 layers, hence the name "VGG16." The classifier model used to classify honey bee images is based on transfer learning. It allows to use a pre trained deep learning model trained on huge dataset. The model is pre trained on a diverse and rich feature representation for wide range of images. This feature representation is transferred to classify a small number of training images involving 5 classes of honeybees. This improves classification performance and training time as compared to training the model from scratch [18].

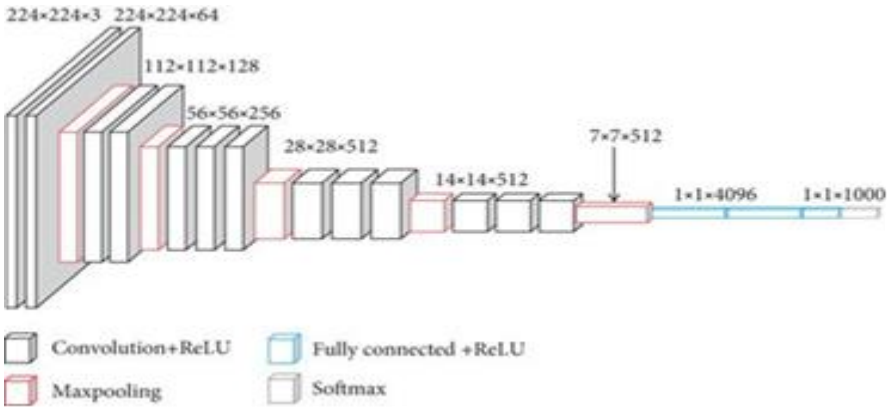

Figure 10. System Architecture of VGG16 [18] weights='imagenet': This parameter specifies that the pre-trained weights of the VGG16 model trained on the

ImageNet dataset should be used. ImageNet is a large dataset with millions of labeled images across thousands of categories, and pre-training on it can give the model good generalization capabilities. input_shape = (256, 256, 3): This argument defines the expected shape of the input images that will be fed into the model. It specifies 256 pixels in height, 256 pixels in width, and 3 color channels (RGB). include_top=False: This parameter indicates that the fully connected layers (also known as the "top" layers) of the VGG16 model should not be included. The fully connected layers are typically responsible for the final classification, and by excluding them, you can use the convolutional base of the VGG16 for feature extraction in a different task.

Setting conv_base.trainable = False has the following effects:

1. Freezing Weights: It prevents the weights of the convolutional layers within conv_base from being updated during the training process. This means that the learned feature representations from ImageNet will remain unchanged. 2. Faster Training: By freezing the majority of the model's weights, you significantly reduce the number of parameters that need to be adjusted during training. This leads to faster training times and less computational resources required. 3. Transfer Learning: This technique is often used in transfer learning, where you leverage the knowledge of a pre-trained model on a different task. By freezing the base layers, you preserve the generic feature extraction capabilities and focus on fine-tuning the new layers you add on top for your specific task. Transfer learning in VGG16 is shown in Figure 11 and Figure 12. represents the working of VGG16.

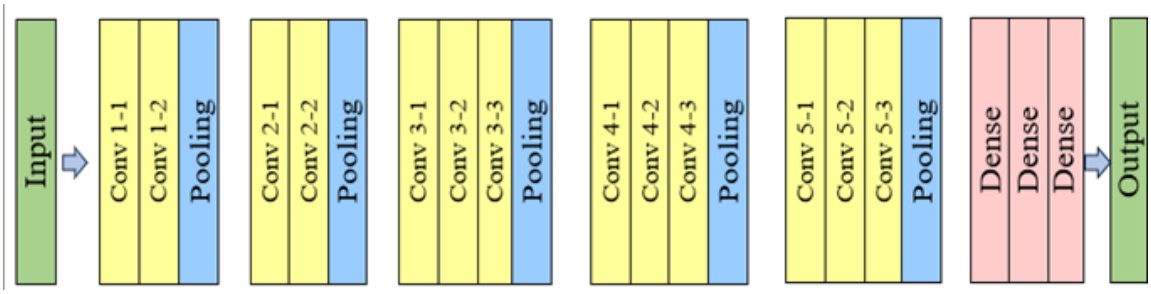

Figure 11. Transfer learning in VGG16

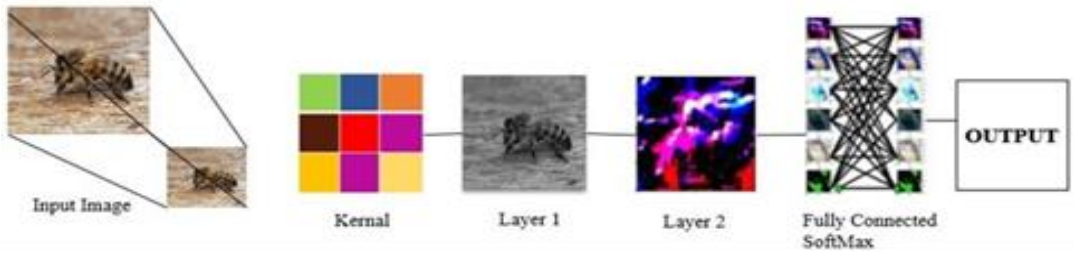

Figure 12. Working of VGG16

Algorithm: Identification Using VGG16
Input: Honey Bee image
Output: Identified *Apis cerena indica, Apis mellifera, Apis florea, Apis dorsata, and Trigona*

1. Split the dataset into train, test, and valid
2. for image in the folder
3. Normalize and resize the image
4. Apply 2D CNN with filter size 3
5. Applying f*f filters from 256 to 32
6. Activation Function using R(Z) = max(0,Z)
7. Neuron Dropout by a factor of 0.2
8. Flat 2D output matrix to 1D
9. end for

Algorithm 1 displays the Activation Function (relu) along with neuron dropout by a factor of 0.1 to avoid overfitting. Max-Pooling followed by converting the feature matrix into a dense layer is applied along with the softmax function and uses Adam optimizer while compiling the model.

2.1.4 *Implementation of a website to integrate the trained model*

Flask API was used to integrate the trained model into the self-designed website. HTML, CSS, and JavaScript are used to design the website's front end. The goal of creating a user-friendly website is to integrate the model and to provide a better experience to the user. The website is designed to record the predicted class, sighting date, Time alongwith the location. The website gives details of natural habitat and typical characteristics of the predicted class of honeybee.

3. Results

The paper focuses on identifying honeybees, encompassing five classes namely: *Apis cerena indica*, *Apis mellifera*, *Apis florea*, *Apis dorsata*, and *Trigona*. A dataset comprising 1000 images underwent preprocessing procedures including Gaussian blur and histogram equalization to enhance image quality. Histogram equalization enhances image contrast, while Gaussian blur reduces noise and simplifies details. The use of VGG16, a Convolutional Neural Network (CNN) architecture, helps in identifying and classifying species in this study. This paper introduces an innovative approach for honeybee species identification employing VGG16 and SVM algorithms. SVM shows an accuracy of 90%. Visualizations showing training and validation accuracy, as well as loss metrics, are included. The model accurately predicts different bee classes, giving a clear visual representation of the classification performance shown in Figure 13 and Figure 14. The classification model achieves high accuracy in species identification, resulting in reliable differentiation based on physical traits visible in images. However, these findings are not behavior-related. For instance, *Apis dorsata* is known for its aggressive nature and extended flight range, qualities that images cannot convey. This methodology is an effective tool for honeybee class identification, indirectly supporting ecological studies but not replacing direct behavior analysis methods.

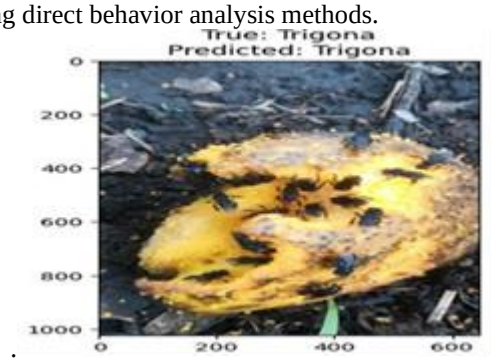

Figure13. Results predicted by the model for *Trigona* Species

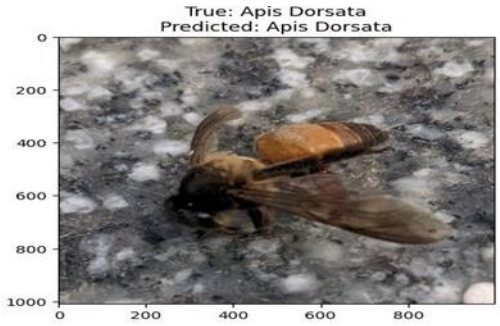

Figure14. Results predicted by the model for *Apis Dorsata* Species

The Train accuracy and validation accuracy graph for training and validation is shown in Figure 15 and Figure 16. represents Training Loss and Validation Loss for VGG16. It is seen that as the epochs increase, the model gets trained the accuracy improves and losses reduce. The x-axis shows a number of epochs.

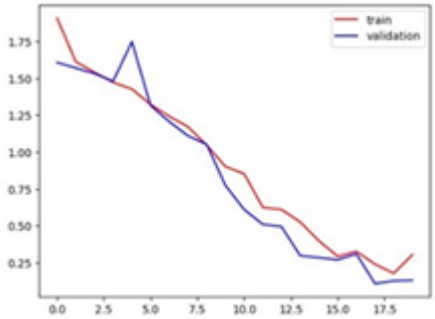

Figure15. Train accuracy and validation accuracy

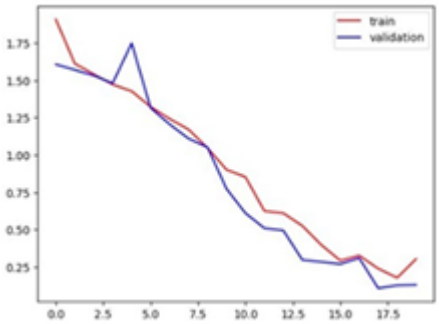

Figure 16. Train loss and validation loss

The dataset used in this research includes pictures of flowers and leaves with honeybees on them, which makes extracting features difficult and lowers accuracy. Therefore, it is important to include clearer images in the training dataset to overcome this limitation and improve the feature extraction methods' effectiveness. Table.3. represents performance for the SVM and VGG16 Models. According to the results obtained, it is seen that the model VGG16 has a better performance than SVM.

Table .3. SVM and VGG16 classification performance (in %)

Classification Performance	**SVM Classifier**	**VGG16 Classifier**
Accuracy	90.00	99.6
Precision	95.00	98.00
Recall	83.33	96.21
F1 Score	87.40	97.17
AUC Score	90.00	95.00

Figure17. depicts the information system for honeybee species in a self-designed website. The model is trained and then integrated into a website as shown. The input image of a honeybee is identified as the class *Apis cerena indica* successfully.

Figure17. Model integrated into the self-designed website

Figure18. Information system for honeybees subspecies

Figure18 represents the Information System developed in this work details the honeybee habitat and the characteristics of the identified honeybee. This system is aimed to aid the conservation of forests. The natural inclination of honeybees towards specific types of flower pollens drives the colonies to settle and grow at the location. The expert system identifies the honeybee and provides the information that the forest conservators can use for constructive measures. It acts as an information system even for a learner involved in keeping records of the forest health and ecosystem.

4. Conclusions and discussion

This research endeavors to contribute to the field of honeybee species identification by proposing a robust classification system that can distinguish between five different subspecies: *Apis cerena indica*, *Apis dorsata*, *Apis mellifera*, *Apis florea*, and *Trigona*. Through the integration of VGG16 and SVM algorithms, our approach demonstrates promising accuracy in identifying these subspecies. Furthermore, the seamless integration of the trained model into a user-friendly website interface enhances accessibility and usability for stakeholders interested in honeybee

classification. Accurately identifying different species of honeybees is crucial for beekeepers, researchers, and policymakers. It helps with biodiversity conservation, monitoring ecosystems, and promoting sustainable agriculture. Therefore, this study not only introduces a practical technological answer but also emphasizes the importance of accurate class recognition which is vital for taking measures to protect the honeybee population and maintain ecological harmony.

This research could be advanced by broadening the dataset to include more species of honeybees and a wider range of geographical areas, investigating more complex deep learning architectures, incorporating real-time data collection techniques, adding more features like genetic markers or behavioral traits, utilizing transfer learning techniques, and expanding the application to larger ecological studies like habitat monitoring and biodiversity assessments.Further techniques such as direct observation, are required for in-depth behavioral insights. Future work can integrate sensors or movement that would help track complement image-based species identification for a fuller understanding of behavior.

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Chemical profile and antioxidant activities of *Teucrium polium* subsp. *polium* and *Teucrium scordium* subsp. *scordioides*

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Abstract

The *Lamiaceae* family has a wide geographical distribution capacity and includes endemic species in some places. *Teucrium* species are members of this family and can be used in traditional methods due to their medicinal properties. The aim of this study was to determine the essential oil content, constituents, and antioxidant activities of two endemic *Teucrium* species. The components of the essential oils obtained using Clevenger apparatus were determined by GC-MS. Antioxidant determination: 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method and Folin-Ciocaltaeu method was used to determine the total phenolic content. According to the results of the analysis, the essential oil ratio of *T. polium* subsp. *polium* was determined as 0.80% and the main components of the essential oil were β-pinene (27.85%), germacrene D (18.84%), α-pinene (14.73%). *T. scordium* subs. *scordioides* species was found to contain 0.32% essential oil. The major components of the essential oil were found to be β-pinene (16.83%), caryophyllene oxide (13.06%), α-pinene (10.96%). DPPH antiradical activity of *T. polium* subsp. *polium* was 16.99±0.16% and phenolic content in terms of gallic acid was 11.60±2.45 mg/100 ml, while DPPH antiradical activity of *T. scordium* subsp. *scordioides* had DPPH antiradical activity of 17.09±1.95% and phenolic content in terms of gallic acid was 10.46±2.32 mg/100 ml.

Keywords: antioxidant activity, essential oil, GC-MS, *Lamiaceae*, *Teucrium*

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Teucrium polium L. subsp. *polium* ve *Teucrium scordium* L. subsp. *scordioides* (Schreb). Arcang taksonlarının kimyasal profili ve antioksidan aktivitesi

Özet

Lamiaceae familyası geniş bir coğrafi yayılım kapasitesine sahip olmakla beraber kimi yerde endemik olarak bulunan türleri bünyesinde barındırmaktadır. *Teucrium* türleri, bu familyanın bir üyesi olup, tıbbi özelliklerinden dolayı geleneksel yöntemlerde kullanılabilmektedirler. Bu çalışmada da endemik iki *Teucrium* türünün uçucu yağ oranı, bileşenleri ve antioksidan aktivitelerinin belirlenmesi amaçlanmıştır. Clevenger aparatı kullanılarak elde edilen uçucu yağların bileşenleri GC-MS ile belirlenmiştir. Antioksidan tayininde: 2.2-difenil-1- pikrilhidrazil (DPPH) serbest radikal süpürme metodu, toplam fenolik madde miktarının belirlenmesinde ise Folin-Ciocaltaeu yöntemi kullanılmıştır. Analiz sonuçlarına göre *T. polium* subsp. *polium'* un uçucu yağ oranı %0.80 olarak belirlenmiş, uçucu yağın ana bileşenlerinin ise β-pinene (%27.85), germacrene D (%18.84), α-pinene (%14.73) olduğu tespit edilmiştir. *T. scordium* subsp. *scordioides* türünün ise % 0.32 uçucu yağ içerdiği belirlenmiştir. Uçucu yağın major bileşenlerinin ise β-pinene (%16.83), caryophyllene oxide (%13.06), α-pinene (%10.96) olduğu tespit edilmiştir. *T. polium* subsp. *polium'* un DPPH antiradikal aktivitesi %16.99±0.16, gallik asit bakımından fenolik madde içeriği ise 11.60±2.45mg/100 ml olduğu tespit edilirken, *T. scordium* subsp. *scordioides'* in DPPH antiradikal aktivitesi %17.09±1.95, gallik asit bakımından fenolik madde içeriği ise 10.46±2.32 mg/100 ml olarak tespit edilmiştir.

Anahtar kelimeler: antioxidan activity, essential oil, GC-MS, *Lamiaceae*, *Teucrium*

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1. Giriş

Ülkemiz bulunduğu iklim kuşağı ve coğrafi konumu nedeniyle birçok bitki çeşidine ev sahipliği yapmaktadır. Bu sayede ülkemiz birçok bitkinin gen merkezi haline gelmiştir. Bu bitki türleri gıda olarak faydalanılmalarının yanı sıra tıbbi alanda, boya sanayinde, parfüm sanayinde, hayvan beslenmesinde yemlik ve hatta insanların geçim kaynağı ürünler olarak kendilerine yer edinmişlerdir. Lamiaceae familyası da bu özellikleri barındıran türler içermektedir. Yeryüzünde yaklaşık 236 cins ve 7200 türü bulunan *Lamiaceae* familyasının önemli bir gen merkezide Türkiye olarak kabul edilmektedir [1]. Bu familyaya ait en büyük cinsler ise *Salvia* (900 tür), *Scutellaria* (360 tür), *Stachys* (300 tür), *Plectranthus* (300 tür), *Hyptis* (280 tür), *Teucrium* (250 tür), *Vitex* (250 tür), *Thymus* (220 tür), *Nepeta* (200 tür) ve *Sideritis* (100 tür) cinsleridir [2]. Dünya da *Teucrium* cinsi 400'den fazla üyeye sahipken Türkiye 'de 19'u endemik olmak üzere 50 takson yayılış göstermektedir [3]. *Teucrium* türleri 2000 yılı aşkın süredir tıbbi bitki olarak kullanılırken, önemli özellikleriyle de tanınmaktadırlar [4]. Geleneksel tıpta, birçok farmakolojik aktivite *Teucrium* cinsinin antioksidan, antispazmodik, antihelmintik, antiromatoik, diüretik, antidiyabetik ve antikanser etkilerine atfedilmiştir [5].

"Acı yavşan" olarak adlandırılan *Teucrium polium* subsp. *polium* halk arasında bitki çayı olarak yaygın olarak kullanılmaktadır [6]. Ayrıca bu türün kaynatılması ve infüzyonu diyabet, böbrek, karaciğer hastalıkları, mide ve hemoroid tedavisinde kullanılır. *Teucrium polium* subsp. *polium* birçok araştırıcı tarafından çalışılmış, literatürlerde genel olarak ekstraktların biyolojik aktiviteleri değerlendirilmiştir [7]. Yine *Teucrium polium* subsp. *polium* ' un antispazmodik, antimikrobiyal, antiinflamatuar özelliklere sahip olduğu bilinmektedir [8].

Kurtluca [6] ismiyle anılan *Teucrium scordium* subsp. *scordioides* çok yıllık (10-60 cm) stolonifer otsu bir bitkidir ve orta derecede dallanmış, yükselici veya yatık ve yumuşak tüylü gövdelere sahiptir. Yapraklar tüylü ila tüysüz, yumurtamsı ila dikdörtgenimsi, kaba dişli, kısa saplı veya sapsızdır. Yayılış alanı Avrupa'nın büyük bölümünün yanı sıra Asya'nın batı kısımlarını da kapsar. Morumsu çiçekler yanal sürgünlerin tepelerinde çiçek salkımlarında gruplanır [9]. *Teucrium scordium* subsp. *scordioides* antiseptik ve antelmintik olarak kullanılmaktadır [10]. Ayrıca sentetik antioksidanların sitotoksik etkilere sahip olduğu belirlenmiştir. Bu nedenle bitkilerin biyolojik aktivite ve kimyasal bileşenlerinin araştırılması oldukça önemlidir. Fenolik asitler, flavonoidler, karotenler, tanenler ve esansiyel yağları içeren bitkilerden elde edilen doğal antioksidanlar, daha düşük sitotoksisiteleri ve kalıntıları nedeniyle sentetik antioksidanlardan daha iyidir [11].

Bu çalışma floradan toplanan *T. polium* subsp. *polium* ve *T. scordium* subsp. *scordioides* türlerinin uçucu yağ oran ve bileşenleri ile antioksidan özelliklerini belirlemek amacıyla yürütülmüştür.

2. Materyal ve yöntem

2.1. *Bitki materyali*

Çalışılan türlerden *T. polium* subsp. *polium* 13 Ağustos 2020 tarihinde Mersin İli, Anamur İlçesi, Çukurabanoz Köyü Çevresi 600-950 m yükseklikten, *T. scordium* subsp. *scordioides* ise 23 Temmuz 2020 tarihinde Karaman İli, Sarıveliler İlçesi, Dumlugöze-Daran Köyleri arasından Dr. Ömer ÇEÇEN tarafından toplanmış ve teşhisi yapılmıştır. Herbaryum örnekleri Karamanoğlu Mehmetbey Üniversitesi Biyoçeşitlilik Araştırma ve Uygulama Merkezi Herbaryumunda (*T. polium* subsp. *polium*: KMUB 6977, *T. scordium* subs. *scordioides*: KMUB 6640) muhafaza edilmektedir.

2.2. *Uçucu yağ izolasyonu*

Bitkilerin gölgede kurutulan toprak üstü kısımlarından uçucu yağ elde etmek için su distilasyon yöntemi kullanılmıştır. Distilasyon balonuna 40 gr bitki koyularak içine 200-250 ml su ilave edilmiş ve distilasyon balonu mantolu ısıtıcı ile 150 °C'yi aşmayacak sıcaklıkta ısıtılmıştır. Yaklaşık 3 saat süren ısıtma işleminin ardından taksimatlı boruda su yüzeyinde toplanan uçucu yağ, taksimatlı boru yardımıyla ml cinsinden miktarı tespit edilerek, muhafaza edilmek için viallere alınmıştır. Elde edilen yağlar analiz edilene kadar +4°C'de muhafaza edilmiştir [25]. Uçucu yağ bileşenlerinin analizi GC-MS (Gaz Kromatografisi-Kütle Spektrofotometre) kullanılarak yapılmıştır [1].

2.3. GC-MS analizleri

Her numunenin uçucu yağlarının ana bileşenlerinin analizi, TR-FAME kapiler kolon (%5 Fenil Polisilfenilen-siloksan, 60 m×) ile donatılmış Thermo Scientific ISQ Single Quadrupole Gas Chromatography- Mass Spectrometry cihazı (Milano, İtalya) kullanılarak yapılmıştır. 0.25 mm iç çap × 0.25 µm film kalınlığı). Taşıyıcı gaz olarak 1 ml/dakika akış hızında helyum (%99.9) kullanılmıştır. Kütle spektrumları 70 eV'de kaydedildi, kütle aralığı 1.2 ila 1.200 m/z idi. Veri toplama için Tarama Modu kullanıldı. MS transfer hattı, iyonizasyon kaynağı ve enjeksiyon portu sıcaklıkları sırasıyla 250, 230 ve 220 ℃ idi [12].

2.4. Antioksidan aktivite

Örneklerin antioksidan tayini için bazı modifikasyonlarla DPPH yöntemi kullanılmıştır. Buna göre numune ekstraktlarının20 µl si metil alkol ile 10 ml ye tamamlanmıştır. 0.002g/100 ml konsantrasyonda DPPH çözeltisi metil alkol ile hazırlanmıştır. Numune ekstraktının 1 ml si 1 ml DPPH çözeltisi ve metil alkol ile 3 ml'ye tamamlanmıştır. Elde edilen karışımlar 20dakika karanlıkta bekletildikten sonra 517 nm de kontrole karşı absorbans değerleri okutulmuştur. % radikal süpürme aktivite değerleri;

$\%radikal\ süpürme\ aktivitesi = \frac{A_{Kontrol} - A_{Örnek}}{A_{Kontrol}} x100$ denklemi yardımıyla hesaplanmıştır.

Deneyler üç tekrarlı yapılmış olup deney sonuçları ortalama ve standart sapma değerleriyle birlikte Tablo 1'de verilmiştir.

2.5. Toplam fenolik madde miktarı

Numunelerdeki toplam fenolik madde miktarı Folin-Ciocaltaeu yöntemine göre yapılmıştır. %99.9'luk metanolle gallik asidin farklı konsantrasyonda çözeltileri hazırlandı. 20 µl bu çözeltiden alınıp üzerine 680 µl distile su, 400 µl 0,5 N folin reaktifi(suda), 400 µl %10'luk Na_2CO_3 (suda) ilave edilip 760nm dalga boyunda absorbsiyonları okundu. Ayrıca 700 µl distile su, 400 µl 0.5 N folin reaktifi(suda), 400 µl %10'luk Na_2CO_3 (suda) karıştırılarak kör numune hazırlanıp kör numuneden gelen absorbsiyon değerleri numuneler ve standart numuneler için sıfırlanmıştır. Ekstakte edilen bitki çözeltisinden 20 µl alınıp üzerine 680 µl distile su, 400µl 0.5 N folin reaktifi (suda), 400 µl %10'luk Na_2CO_3 (suda) ilave edilerek 30 dakika sonunda 760 nm de köre karşı absorbsiyon değerleri okunmuştur. Okunan absorbsiyon değerleri elde edilen denklemde yerine koyularak gallik asit cinsinden fenolik eşdeğerleri hesaplanmıştır. Deneyler 3 tekrarlı yapılmış olup sonuçlar Tablo 1'de verilmiştir.

3. Bulgular

3.1. Uçucu yağ oranı ve kimyasal bileşimi

Clevenger aparatı ile distile edilen *Teucrium polium* subsp. *polium*'nin uçucu yağ oranı %0.80 olarak belirlenmiştir. Uçucu yağda, toplam yağın %98.24 'ünü temsil eden 41 bileşen tespit edilmiştir. Uçucu yağın ana bileşenleri ise β-pinene (%27.85), germacrene D (%18.84), α-pinene (%14.73), trans-caryophyllene (%7.20), limonene (%4.91) ve bicycloelemene (%4.84) olarak belirlenmiştir (Tablo 1).

T. scordium subsp. *scordioides* türünün ise %0.32 uçucu yağ içerdiği belirlenmiştir. Uçucu yağda, toplam yağın %99.15'ini temsil eden 39 bileşen tespit edilmiştir. Uçucu yağın major bileşenlerinin ise β-pinene (%16.83), caryophyllene oxide (%13.06), α-pinene (%10.96), spathulenol (%9.44), trans-caryophyllene (%8.66), germacrene D (%4.59) ve limonene (%4.91) olduğu tespit edilmiştir.

3.2. Antioksidan aktivite ve toplam fenolik madde miktarı

Teucrium türlerinin DPPH radikal süpürme aktivitesi deneyleri üç tekrarlı olarak yapılmış ve sonuçların ortalama ve standart sapma değerleri Tablo 1'de verilmiştir. DPPH Antiradikal aktiviteleri *Teucrium polium* subsp. *polium* L. %16.99±0.16, *T. scordium* subsp. *scordioides* %17.09±1.95 oranlarında olduğu, her iki türünde RSA (Radikal Süpürme Aktivitesi) değerlerinin birbirine yakın değerlerde olduğu sonucuna varılmıştır.

Teucrium türlerinin gallik asit cinsinden fenolik eşdeğerleri Tablo 1' de verilmiştir. *Teucrium polium* subsp. *polium* 'un gallik asit cinsinden fenolik içeriği 11.60 mg/100ml±2.45 *T. scordium* subs. *scordioides* 10.46±2.32 mg/100 ml olarak belirlenmiştir.

Tablo 1. *Teucrium polium* subsp. *polium* ve *T. scordium* subsp. *scordioides* Uçucu Yağ Oran ve Bileşenleri

RT	Bileşen Adı	*T. polium* subsp. *polium*	*T. scordium* subsp. *scordioides*
6.58	α-Pinene	14.73	10.96
8.40	β-Pinene	27.85	16.83
9.00	Myrcene	0.91	1.43
10.03	Limonene	4.91	3.24
10.98	Sabinene	0.25	-
12.05	Eucalyptol	-	0.62
12.71	p-Cymene	0.47	1.95
17.20	1-Octen-3-ol	0.28	0.28
19.01	α-Copaene	0.41	0.32
19.27	α-Muurolene	0.18	0.43
20.32	β-Bourbonene	0.67	0.84
20.68	Linalool	1.18	0.78
23.30	α-Campholene aldehyde	0.19	0.63
23.99	trans-Caryophyllene	7.20	8.66
24.65	Alloaromadendrene	-	0.38
25.19	trans-Pinocarveol	1.20	0.70
25.77	Bisabolene	0.50	-
26.22	Verbenol	1.18	0.64
26.46	Cadinene	2.61	0.99
27.04	Elemol	0.28	-
27.44	Germacrene D	18.84	4.59
27.47	Ledene	-	0.61
28.43	Bicycloelemene	4.84	2.59
28.66	Myrtenal	1.04	0.69
28.84	Nopinone	0.20	-
29.53	Myrtenol	0.55	0.65
30.68	trans-Carveol	0.43	0.85
31.66	Carvone	0.19	-
32.90	α-Calacorene	0.16	-
36.54	Longifolene	0.14	-
37.78	α-Cadinol	0.21	0.79
38.46	Cubenol	0.25	0.79
40.11	Caryophyllene oxide	0.56	13.06
40.36	Spathulenol	1.79	9.44
40.94	Aromadendrenepoxide	0.40	1.69
41.69	Veridiflorol	0.71	3.10
42.25	Torreyol	0.92	0.61
42.45	Cadalene	0.17	1.13
43.96	β-Ionone	1.19	1.76
45.50	Aromadendenepoxide	0.21	0.37
46.12	Ledene	0. 22	-
46.54	cis-Jasmone	0.45	2.06
46.92	Junipene	0.40	0.42
48.56	Cedrol	0.37	0.72
49.39	Nootkatane	-	2.19
49.55	Pentatriacontane	-	0.50
53.35	Nonacosane	-	0.86
	Bileşen Sayısı	41	39
	Toplam (%)	98.24	99.15
	Uçucu Yağ Oranı (%)	0.80	0.32
	Antioksidan Aktivite (%)	16.99±0.16	11.60±2.45
	Toplam Fenolik Madde Miktarı (mg/100 ml)	17.09±1.95	10.46±2.32

4. Sonuçlar ve tartışma

T. polium subsp. *polium* ve *T. scordium* subsp. *scordioides* türlerinin uçucu yağ bileşenlerinin benzerlik gösterdiği majör bileşenlerinin oranlarında farklılıkların olduğu Tablo 1'de görülmektedir.

Stankovic ve ark. [9] *Teucrium scordium* uçucu yağının ana bileşenlerini α-pinene (%25.77), ß-pinene (%15.59), β-caryophyllene (%9.49), caryophyllene oxide (%8.74) ve β-eudesmol (%7.29) olarak bildirmişlerdir. Piras ve ark. [10] *T. scordium* subs. *scordioides* ile yaptıkları çalışmada uçucu yağın germakren D (%25,1), δ-cadinene (%12,9) ve alloaromadandren (%11.3) açısından zengin olduğunu belirtmişlerdir. Hayta ve ark. [13] çalışmalarında *Teucrium polium* subsp. *polium* uçucu yağında α-pinene (%10.2) ve seskiterpen germacrene D (%10.1) belirlenirken, *Teucrium multicaule*' de daha yüksek bir seskiterpen caryophyllene oxide (%31.1) içeriği gözlemlemişlerdir. Monoterpenlerin ana bileşenleri (α-, β-pinene), seskiterpenler germacrene D ve caryophyllene oxide tıbbi amaç, kozmetik ve doğal verim açısından incelenen taksonu oluşturduğunu bildirmişlerdir. Sevindik ve ark. [8] yaptıkları çalışmada *T. polium* subsp. *polium* 'dan elde edilen bileşiklerden en yüksek yüzdeye sahip olanları (Z)- ß-farnesen (%15.49), ß-phellandrene (%10.77) ve α-farnesen (%10.71) olarak bildirmişlerdir. Raei ve ark. [14] yaptıkları çalışmada *T. polium* subsp. *polium* 'un uçucu yağ oranını %0.50 olarak belirlemişlerdir. Uçucu yağın %93.6'sını temsil eden 20 bileşik tanımlamışlardır. Ana bileşenleri ise β-caryophyllene (%29), farnesen (%13), β-pinene (%11), germacrene D (%6.5) ve α-pinene (%5.5) olarak bildirmişlerdir. Belmekki ve ark. [15] *Teucrium polium* subsp. *polium* uçucu yağının kimyasal analizi ve antimikrobiyal aktivitesi belirlemek amacıyla yaptıkları çalışmada uçucu yağ oranını %0.21 olarak bildirmişler, uçucu yağda toplam 27 bileşen belirlemişlerdir. Uçucu yağın ana bileşenlerinin ise germakren D (%25.81), bicyclogermacrene (%13.0), ß-pinene (%11.69) ve karvakrol (%8.93) olduğunu bildirmişlerdir. Mahmoudi ve ark. [16] *T. polium* subsp. *polium* uçucu yağının ana bileşenlerini spathulenol (%15.1), β-pinene (%11.0), β-myrcene (%10.0), germacrene B (%10.1), germacrene D (%8.1), bicyclogermacrene (%8.2) ve linalool (%4.0) olarak tespit etmişlerdir. Radulovic ve ark. [17] *Teucrium* türleriyle yaptıkları çalışmada *T. polium* subsp. *polium* ve *T. montanum* yağlarının temel olarak seskiterpenlerden (sırasıyla %64.3 ve %72.7), ana bileşenler olarak germacrene D (%4; %31.0) ve d-cadinene'den (%10; %8.1) oluştuğunu, buna karşılık, *T. scordium* ssp. yağında monoterpen mentofuran (%1.0; %11.9) daha yoğun olduğunu bildirmişlerdir. Moghtader, [18] *T. polium* subsp. *polium* yağının %99.75'ini oluşturan ve %0.75 yağ verimine sahip 28 bileşik tanımlanmıştır. Uçucu yağın ana bileşenlerini ise α-pinene (%12.52), linalool (%10.63), caryophyllene oxide (%9.69), β-pinene (%7.09) ve β-caryophyllene (%6.98) olarak bildirmiştir. Çakır ve ark. [19] yaptıkları çalışmada Türkiye'de yetişen *T. polium* subsp. *polium* 'un uçucu yağında 37 bileşen tespit etmişler, ana bileşenleri β-pinene (%18.0), caryophyllene (%17.8),α-pinene (%12.0), caryophyllene oxide (%10.0), mirsen (%6.8), germacrene-D (%5.3), limonen (%3.5) ve spathulenol (%3.3) olarak belirlemişlerdir. Ebrahimi Anaraki ve ark. [20] *T. scordium*'un uçucu yağ oranını %0.36 olarak bildirmiştir. Uçucu yağın ana bileşenlerini ise trans-α-bergamoten (%52.3), (Z)-α-trans-bergamotol (%18.1), linalool (%3.0) ve piperitenon oxide (%2.9) olarak belirlemişlerdir. *Teucrium* türleri üzerine yapılan çalışmaların çoğu α ve β-pinenlerin yanı sıra β-caryophyllene, germacrene D, caryophyllene oxide gibi bileşenleri de ana bileşenler olarak içermektedir. Yaptığımız çalışmada elde ettiğimiz sonuçlar *T. polium* subsp. *polium* uçucu yağının ana bileşenlerini α-pinen, β-pinen ve germacrene D, *T. scordium'*un uçucu yağının ana bileşenlerini ise α-pinene, β-pinene ve caryophyllene oxide olduğunu göstermektedir (Tablo 1). Yukarıda verilen çalışmalarla yaptığımız çalışmayı kıyasladığımızda elde edilen uçucu yağların ana bileşenlerinin benzer ancak oranlarında farklılıkların olduğu belirlenmiştir. Aynı bitkinin uçucu yağ bileşenleri arasındaki farklılıkları açıklayabilecek bir dizi neden vardır. Bunlar arasında çok sayıda alt türün dağılımı, coğrafi konumlar ve çevresel-fizyolojik koşullar yer almaktadır.

Savcı [21] *T. polium* subsp. *polium*'un antioksidan ve antiradikal özelliklerine baktığı çalışmasında *T. polium* subsp. *polium*'un lipit peroksit giderim yüzdesinin standart antioksidanlara çok yakın olduğunu belirlemiştir. Stankovic ve ark. [22] yaptıkları çalışmada *T. polium* subsp. *polium*'un her bir ekstraktının fenolik konsantrasyona bağlı temizleyici etkiler sergilediğini bildirmişlerdir. Panovska ve ark. [23] çalışmalarında *T. polium* subsp. *polium*'un antioksidan aktivitesinin β-carotene-linoleic acid modeli ile analizi sonucunda elde edilen ekstraktların dikkate değer aktivite gösterdiğini belirlemişlerdir.

Fenollerin, içerdikleri hidroksil grupları serbest radikalleri temizleme yeteneklerinden dolayı çok önemli bitki bileşenleridir. Bu nedenle bitkilerin fenolik içeriği antioksidan etkilerine doğrudan katkıda bulunabilir [24]. Yine yapılan çalışmalarda γ-terpinene, β-phellandrene ve terpinolene'in antioksidan aktivite gösterdiği, terpenler ve karyofilenin lipidler üzerinde antioksidan aktiviteye sahip olduğu bildirilmiştir [24]. Yapılan çalışmada *Teucrium* türlerinin uçucu yağlarının ana bileşenlerinin α-pinene, β-pinene, germacrene D ve trans-Caryophyllene olduğu Tablo 1'de görülmektedir. Söz konusu bileşenlerin Teucrium uçucu yağının antioksidan aktivitesine katkıda bulunmuş olabileceği düşünülmektedir.

Daha önce yapılan çalışmalar incelendiğinde *Teucrium* cinsine ait türlerin gerek uçucu yağ oranları gerekse antioksidan içeriği birçok araştırıcı tarafından çalışılmıştır [10]. Çalışmamızda yapılan analizler birçok çalışmada aynı seksiyona ait türlerin uçucu yağ bileşenlerinin birbirine benzer, ancak farklı oranlarda olduğu ortaya konulmuştur. Uçucu yağlar, farklı bileşenleri içeren kompleks karışımlardan oluştukları için biyolojik tesirleri açısından da farklılıklar ortaya koyarlar. İçeriklerin farklı oranlarda bulunmasının cinse ait türlerin yetiştiği coğrafyanın, iklimin, örnekleme alınma zamanlarının farklılıklarından kaynaklanabileceği düşünülmektedir. *Teucrium* cinsine ait bitkilerin

uçucu yağlarının biyolojik özellikleri koyun, keçi ve ineklerde ishal, yara, uyuz, kolik tedavisi ve gıda endüstrisinde doğal koruyucu madde olmaları gibi birçok alanda etkili olduğu bildirilmiştir. Bu çalışmada da *Teucrium polium* subsp. *polium* ve *T. scordium* subsp. *scordioides*' den elde edilen uçucu yağ kompozisyonunun ve antioksidan içeriğinin de benzer alanlarda kullanım imkânı sağlayacak sınırlarda olduğu belirlenmiştir.

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Floral biology, pollination and reproductive success of *Campanula tomentosa* Lam. in west Anatolia

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Abstract

Campanula tomentosa, an endemic species, is distributed only in the Western Anatolia Region of Türkiye. Research on the reproduction and conservation biology (pollination biology and reproductive success) of the plant was carried out for the first time. Pollen and stigma viability (floral biology), pollination biology, flower visitation, pollinator identification and reproductive success were investigated. The results of the pollen viability test in which 1% TTC (1,2,3-triphenyl tetrazolium chloride) was used indicated that 100% of the pollen grains were viable at the loading stage. In Peroxidase Test Papers and DAB stigma viability tests, the stigmas were completely curled and they were viable during the pollen presentation phase. The main pollination in the plant was realized by *Xylocopa valga* (Gerstacker, 1872) and *Evylaeus setulellus* (Strand, 1909). In *C. tomentosa*, fruit set and seed setting rates were 93.42% and 73.12%, respectively. On the other hand, it was revealed that the total area where it was distributed was 28.7 km^2 and that it was classified as endangered.

Keywords: *Campanula tomentosa*, reproductive success, pollen viability, Türkiye

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Batı Anadolu'da yayılış gösteren *Campanula tomentosa* Lam'ın çiçek biyolojisi, tozlaşması ve üreme başarısı

Özet

Endemik bir tür olan *Campanula tomentosa*, Türkiye'nin sadece Batı Anadolu Bölgesi'nde yayılış göstermektedir. Bitkinin üreme ve korunma biyolojisi (tozlaşma biyolojisi ve üreme başarısı) üzerine araştırmalar ilk kez tarafımızdan gerçekleştirilmiştir. Bu kapsamda polen ve stigma canlılığı (çiçek biyolojisi), tozlaşma biyolojisi, çiçek ziyareti, tozlayıcıların tanımlanması ve üreme başarısı araştırılmıştır. %1 TTC'nin (1,2,3-trifenil tetrazolyum klorür) kullanıldığı polen canlılık testi sonuçları, yükleme aşamasında polen tanelerinin %100'ünün canlı olduğunu göstermiştir. Peroksidaz Test Kağıtlarında ve DAB stigma canlılık testlerinde stigmalar tamamen kıvrılmış ve polen sunum aşamasında canlı kalmıştır. Bitkide esas tozlaşma Xylocopa valga (Gerstacker, 1872) ve Evylaeus setulellus (Strand, 1909) tarafından gerçekleştirilmiştir. *C. tomentosa*'da meyve tutumu ve tohum tutumu oranları sırasıyla %93,42 ve %73,12'dir. Ayrıca, yayılış gösterdiği toplam alanın 28,7 km^2 ve neslinin tükenme kategorisi sınıfında olduğu tespit edilmiştir.

Anahtar kelimeler: *Campanula tomentosa*, üreme başarısı, polen canlılığı, Türkiye

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1. Introduction

According to the results of the global screening conducted by the International Union for Conservation of Nature (IUCN) in 2020, of the 240,000 known plant species to date, about 15% are endangered. Of these endangered species, more than 90% are endemic. These plant species in the world are pollinated by approximately 300,000 animal species [1]. On the other hand, it is estimated that 25,000 to 30,000 plant species are pollinated only by bee species. Environmental variables such as elevation, ambient temperature, wind speed, relative humidity and light intensity, and demographic characteristics such as population size of a plant species are closely related to the behavior and number of pollinators [2].

The genus *Campanula L.* is represented by approximately 420 species in subtropical and temperate regions and approximately 150 species in the Mediterranean Region [3]. In many countries, studies have been carried out on the genetic diversity and polymorphism, pollination and reproductive system, population biology and conservation biology of the *Campanula* taxa. For instance, it was investigated the genetic diversity of populations of *Campanula* taxa [4]. Conservation genetics were studied in the *Campanula rotundifolia* L. population [5]. In another study, it was carried out studies aimed at identifying the flowering phenology of *Campanula bononiensis* L., and determining flowering abundance, population density and pollinators observed on the same species [6].

In Türkiye, the family Campanulaceae is represented with the following 6 genera: *Campanula, Symphayndra, Asyneuma, Michauxia, Legousia* and *Jasione*. Of these genera, Campanula has 6 sub-genera: *Campanula, Megalocalyx, Sicyodon, Roucela, Brachycodonia and Rapunculus* [7]. In these sub-genera, there are 139 taxa belonging to 127 species and the endemism rate is approximately 52% [8]. Of these sub-genera, the *Quinqueloculares* section, one of the 13 sections of *Campanula* subgenus which has the highest number of species, is represented by 9 species and 6 of these species are endemic to Anatolia. Our review of the studies conducted on this species in Türkiye demonstrated that investigated the anatomy of some *Campanula* taxa, investigated the morphology of seeds of *Campanula* species (sect of *Quinqueloculares*), and some soil parameters were revealed [9]. In other studies, endemic *Campanula tomentosa* Lam. and *C. vardariana* Bocquet species, seed germination studies were carried out [10], and conservation biology of the species *Campanula teucrioides* Boiss (Campanulaceae) was investigated [11]. Türkiye is rich in endemic species, and the ratio of the endemism to the total flora is about 34.4%. In recent years, in Türkiye, emphasis has been placed not only on autoecologic studies on the protection of endangered plants but also on studies aiming to reveal life-cycles of these plants [12].

In the present study, reproduction and conservation biology of endemic *C. tomentosa* which has a limited distribution in the Western Anatolia region of Türkiye was studied for the first time. Within this context, pollen and stigma viability (floral biology), pollination biology, flower visitation and pollinator identification and reproductive success were investigated. In addition, the number of individuals in the plant population and the areas they are distributed in were calculated, and its place in the IUCN Red List Categories was updated [13].

1.1. Study Sites and Species Description

The study was conducted on 5 populations of *C. tomentosa* distributed in Meryem Ana (Izmir); National Park-South of Doğanbey, National Park- North of Doğanbey, Çamlık-Ortaklar, and Akçakonak-Priene (Aydin) located in the Aegean region of Türkiye between May 2012 and September 2014 (37º55 '- 37º39'N and 27º27 '- 27º09' E) (Figure1). The average annual temperature in the region is 16.7°C, and the annual average rainfall is 659.4 mm. The geologic structure of the region is reported to have marble, schist and calcschist character.

C. tomentosa has a woody base and branched stem is 30-70 cm in height, and is covered with flat, dense and long tomentose hair. Stem leaves are oval-, oval-triangular- or lyrat-shaped, sessile or petiolate, 1.5-7.5 x 0.7-2.7 cm, with crenate-serrate edges. The flowers are violet-blue. The corolla is 26-53 x 18-45 mm in size, has a bell-like or wide funnel shape, and its outer part is hairy. Corolla tube is 18-42 x 12-37 mm in size, stigma and ovary each has 5 loculi, and fruits have a porocidal capsule. While the pistil is 13-37 x 2.5-3.5 mm in size, the stamen is 8-18 x 0.9-1.1 mm. The capsule has dense tomentose hairs, and the seeds are 0.4-0.5 x 0.25-0.35 mm in size, oval-shaped, and mostly light brown. Flowering season is from May to June. It grows on walls and bottom of rocks and calcareous rocks at elevations ranging between 1 and 100 m. It is endemic to Eastern Mediterranean region.

2. Materials and methods

2.1. Floral Biology

To determine its floral morphology, 30 flowers and flower buds were brought to the laboratory in FAA (acetic acid 5%, formaldehyde 5% and ethanol 90%). In the laboratory, the diameter and length of the corolla tube, height and width of the calix, flower size and pistil lengths in mature flowers were measured using a digital caliper and a digital microscope (Dino-Lite AM313). On the other hand, in 15 individuals, stigma lobes, anther and style lengths, stigma diameters, stigma-anther distance in mature flowers (with pollen presentation) and flower buds (without pollen

presentation) were calculated. Voucher specimens of *C. tomentosa* were deposited in the EGE Herbarium at the Department of Botany, Ege Universty (EGE – 41688, EGE – 41689; EGE –41690; EGE – 41691).

Nectar volumes were measured between 09:00 and 10:00 a.m., 12:00 and 1:00 p.m. and 4:00 and 5:00 p.m. using 0-5µ micropipettes in 30 flowers from the randomly selected individuals in the distribution areas of the species. Sugar concentration in these samples was determined using a hand refractometer, 0-50% Brix (Bellingham and Stanley model 45-81, Tumbrige Wells, UK). The total sugar content was calculated using the exponential regression formula [14].

Pollen viability was tested at the pollen presentation and pollen loading stages. To carry out the test, 1% TTC (1,2,3-triphenyl tetrazolium chloride) was applied on 20 flowers and flower buds. After this application, to examine the slides, a light microscope (Leica: 10x18 ocular; 4x/0.10 objective) was used. The numbers of viable (%) and nonviable (%) pollen grains were counted from ten randomly chosen fields of view at 10x. After the pollen preparations were first stained and then incubated at 30°C, they were examined under the light microscope at 160-400x magnification. To calculate the viability percentages of pollen taken from anthers of flower buds and flowers, 100 pollen grains were used. The procedure was repeated 5 times.

To determine stigma viability, Peroxidase Test Papers (Perex Tesmo KO, Macherey-Nagel) and the DAB test (Sigma FastTM 3.30-diaminobenzidine tablets; Sigma D-4168) were used [15]. These applications were tested for different floral phases.

2.2. Flower Visitation and Pollinator Identification

These observations were performed in randomly selected 15 individuals for 3 days between 09:00 a.m. and 10:00 a.m., between 12:00 p.m. and 1:00 p.m. and between 4:00 p.m. and 5:00 p.m. when the weather conditions were appropriate (sunny and slightly windy). Pollinators observed were caught and brought to the laboratory in alcohol (70%). Identification of the pollinators was performed at Hacettepe University Faculty of Science, Department of Biology, Department of Zoology. In addition, the collections belonging to these pollinators were recorded with ZDEU24 / 2013-ZDEU46 / 2013 inventory numbers in the museum, part of the Department of Zoology, Department of Biology, Ege University Faculty of Science.

2.3. Reproductive success

To demonstrate the reproduction success of the species, such parameters as (1) Fruit Set Rate (FSR), (2) Seed Setting Rate (SSR) were used.

2.4. The Effect of Cross Pollination on Seed Production

To determine the rates of fruit set, 100 flowers (two flowers per individual) were randomly selected from the *C. tomentosa* populations and labeled, and then the average number of flowers per plant (n = 50) and the average number of fruits per plant (n = 50) were calculated.

The Fruit Set Rate was calculated using the following equation:

Fruit Set Rate (%) = the average number of fruits per plant x 100 ÷ the average number of flowers per plant

To determine the Seed Setting Rate, firstly, the average number of seeds per fruit and the average number of ovules per flower were calculated. While calculating the average number of seeds per fruit, the number of the seeds from 100 mature fruits of 50 individuals (two fruits per individual) was determined. When the average number of ovules per flower was calculated, the ovaria of 200 flowers (not in the seed-bearing stage) taken from 20 individuals (10 ovaria per individual) were opened, and then the number of ovules was counted using a light microscope.
Seed Setting Rate (%) was calculated using the following equation:

Seed Setting Rate (%) = the average number of seeds per fruit x 100 ÷ the average number of ovules per flower

2.5. The Effect Self - Pollination on Seed Production

In order to determine the pollination type of the species, 100 flowers from 50 individuals were taken using pollination bags to repel possible pollinators. After about 1 month, flowers that were packed were brought to the laboratory, and fruit and seed development was observed.

2.6. Determination of the Number of Individuals and the Size of the Distribution Areas

The average number of individuals in 5 populations of *C. tomentosa* was calculated as 50 by taking sample areas of 10m x 10m (100 m^2) between May 2013 and July 2013. The Average Number of Individuals calculated was

adapted to all populations and the number of potential individuals in the distribution area was calculated. All of the aforementioned research was carried out in population in the National Park- South of Doğanbey. When calculating the size of the distribution area for each population, first the spatial data were recorded using the GIS (Geographic Information System), including the boundary distribution areas in the areas where the plant was distributed. Then the recorded spatial data were transferred to the "Google Earth Pro" program, and km. extension polygons were created. Finally, numerical maps were created by transferring the generated polygons to "ArcMap ver.10.0" program and field sizes were calculated. Thus, the total distribution area of the plant and the number of potential individuals were calculated. In the light of these data, *C. tomentosa's* place in the IUCN Red List Categories (2001) Ver. 3.1 was updated.

3. Results

3.1. Floral Biology

The measurements made on 30 flowers belonging to *C. tomentosa* demonstrated the following average values: corolla diameter: 34.52 mm, corolla length: 34.76 mm, calyx length: 20.47 mm, calyx width: 8.20 mm, and pistil length: 33.67 mm (Table 1). As a result of our floral morphology study, the phase at which the "Secondary Pollen Presentation" took place was determined. Our observations demonstrated that pollen loading (adhesion of pollens to styles by anthers) occurred when the stigma lobes were closed prior to anthesis, that stigma lobes gradually curled backward after anthesis (secondary pollen presentation), and that stigmas were completely curled in the fully opened flowers. At the end of the pollen presentation, mean floral measurements of *C. tomentosa* flowers showed that the stigma lobe length was 5.45 mm, stigma diameter was 5.61 mm, stigma-anther distance was 3.35 mm, anther length was 13.49 mm, and style length was 24.78 mm.

Table 1. Floral morphological measurements of *C. tomentosa*

Floral features		*C. tomentosa*	
		Min-Mak (mm)	Average (mm)
Corolla diameter		23,40-43,34	34,52±5,93
Corolla length		29,25-39,57	34,76±2,81
Calix	Length	15,60-28,22	20,47±3,09
	Width	5,00-10,78	8,20±1,31
Pistil		29,01-39,64	33,67±2,38
Stigma diameter		2,78-8,6	5,61±1,56

In *C. tomentosa*, the average values of these floral measurements in the flowers at the pollen loading phase were 4.12 mm; 2.85 mm, 2.36 mm; 12.66 mm and 16.52 mm, respectively (Table 2). During the day, average sugar values and sugar weight per volume for the flowers of *C. tomentosa* were measured. The highest value of sugar was measured in the evening (Table 3).

Table 2. Morphological measurements of pistil and stamen in flowers at the pollen loading and presentation stages of *C. tomentosa*.

Stage	The number of the individuals (n)	Stigma lobe length (mm)	Stigma diameter (mm)	Stigma-anther distance (mm)	Anther length (mm)	Style length (mm)
Pollen loading	15	4,12±0,69	2,85±0,90	2,36±2,14	12,66±0,77	16,52±1,65
Pollen presentation	15	5,45±1,24	5,61±1,56	3,35±1,33	13,49±1,03	24,78±3,33

Table 3. Nectar values in *C. tomentosa* flowers measured during the day.

Times	The Number of Flowers Used		Sugar Concentration (%)	Nectar in micro pipette (cm)	Net Volume V (µl)	Sucrose (mg /µL)
09:00 a.m 10:00 a.m	30	Ort.±SH	34,93±10,67	0,76±0,65	1,26±1,08	0,40±0,14
		min-mak	19,00-52,00	0,15-2,3	0,25-3,83	0,20-0,64
12:00 p.m 1:00 p.m	30	Ort.±SH	41,76±13,89	0,74±0,51	1,24±0,85	0,50±0,18
		min-mak	10,00-60,00	0,20-2,5	0,33-4,16	0,10-0,77
4:00 p.m 5:00 p.m	30	Ort.±SH	45,24±7,18	0,99±0,65	1,64±1,08	0,54±0,10
		min-mak	32,00-54,00	0,30-2,8	0,50-4,66	0,36-0,67

For pollen viability, 1%TTC (1,2,3-triphenyl tetrazolium chloride) test applied to 20 *C. tomentosa* flowers. Of the 538 pollen grains counted during the pollen presentation phase (the stage when the pollen is in the style, 382 were stained, and pollen viability was calculated as 71% on average. On the other hand, during the pollen loading stage (the stage when the pollen is not in the style), pollen grains in the anthers belonging to 20 flower buds from ten fields of view at 10× (4cm^2) were counted and pollen viability was determined as 100%. According to the results of peroxidase test papers and DAB stigma viability tests on 20 flowers and flower buds at different floral phases belonging to *C. tomentosa*, stigmas at the pollen loading phase and stigmas in the presentation phase but not curled were found to be non-viable (no staining, negative). On the other hand, it was determined that the stigmas which were completely curled in the pollen presentation phase were viable (positive staining) (Table 4).

Table 4. Pollen / stigma viability of flowers in different floral phases.

	Pollen loading stage	Pollen presentation stage
Pollen viability (%)	100	71,00±6,45
Stigma viability	No staining, Negative	Staining, Positive
Stigma lobe length (mm)	4,12±0,69	5,45±1,24
Style length (mm)	16,52±1,65	24,78±3,33

3.2. Flower Visitation and Pollinator Identification

In our observations, *C. tomentosa* was visited by only 2 pollinators between 11:00 a.m. and 3:00 p.m.: *Xylocopa valga* (Gerstacker, 1872) from the Apidae family and *Evylaeus setulellus* (Strand, 1909) from the Halictidae family. In addition, the following passive pollinators were observed: members of the Curculionidae, Hemiptera and Cantharidae (Table 5). While *Xylocopa valga* did not visit the plant between 09:00 a.m. and 10:00 a.m., between 3:00 a.m. and 4:00 p.m. and between 4:00 p.m. and 5:00 p.m., *Evylaeus setulellus* did not visit the plant between 09:00 a.m. and 10:00 a.m., between 10:00 a.m. and 11:00 a.m., between 3:00 a.m. and 4:00 p.m. and between 4:00 p.m. and 5:00

p.m. (Figure1). *X. valga* visited the plant most during the day between 10:00 a.m. and 11: 00 a.m. (145 times) and the mean duration of visits was 17.5 ± 4.68 seconds. *E. setulellus* visited the plant most during the day between 11:00 a.m. and 12:00 p.m. (12 times) and between 1:00 p.m. and 2:00 p.m. (12 times), and the mean duration of visits was 30.58 ± 8.1 seconds. The temperatures during the day varied from 19.04ºC (09:00 a.m.) to 27.02ºC (17:00 p.m.) (Figs. 2, 3).

Table 5. Flower visitors of *Campanula tomentosa*

Flower Visitors	Category	Resource Used*
Xylocopa valga (Gerstacker, 1872)	L	P/N
Evylaeus setulellus (Strand, 1909)	L	P/N
Curculionidae spp.	O	P
Hemiptera spp.	O	P
Cantharidae spp.	O	P

Abbreviations: L = legitimate pollinator; O = occasional pollinator, *Floral resources used by the visitors/pollinators: P: pollen; N: nectar.

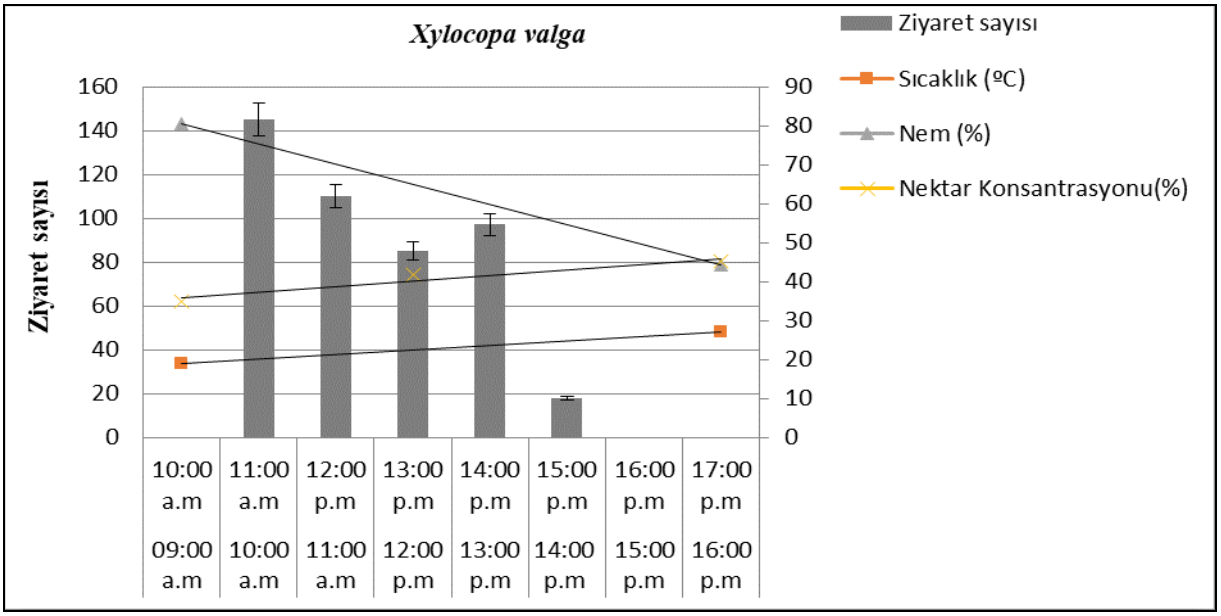

Figure 1. The number of flower visits by *Xylocopa valga* (Gerstacker, 1872), and temperature and humidity values

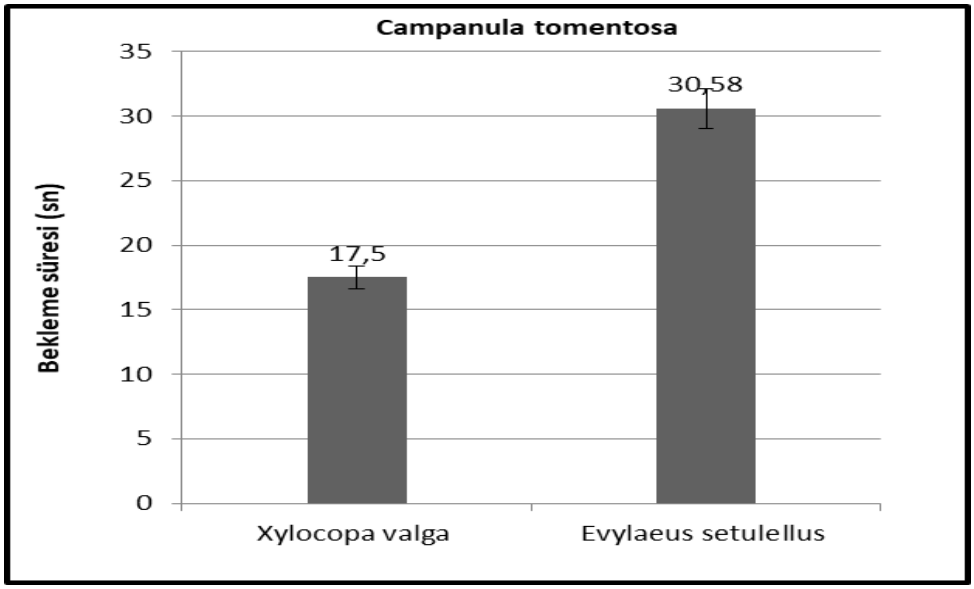

Figure 2. Mean duration of visits by *C. tomentosa* pollinators

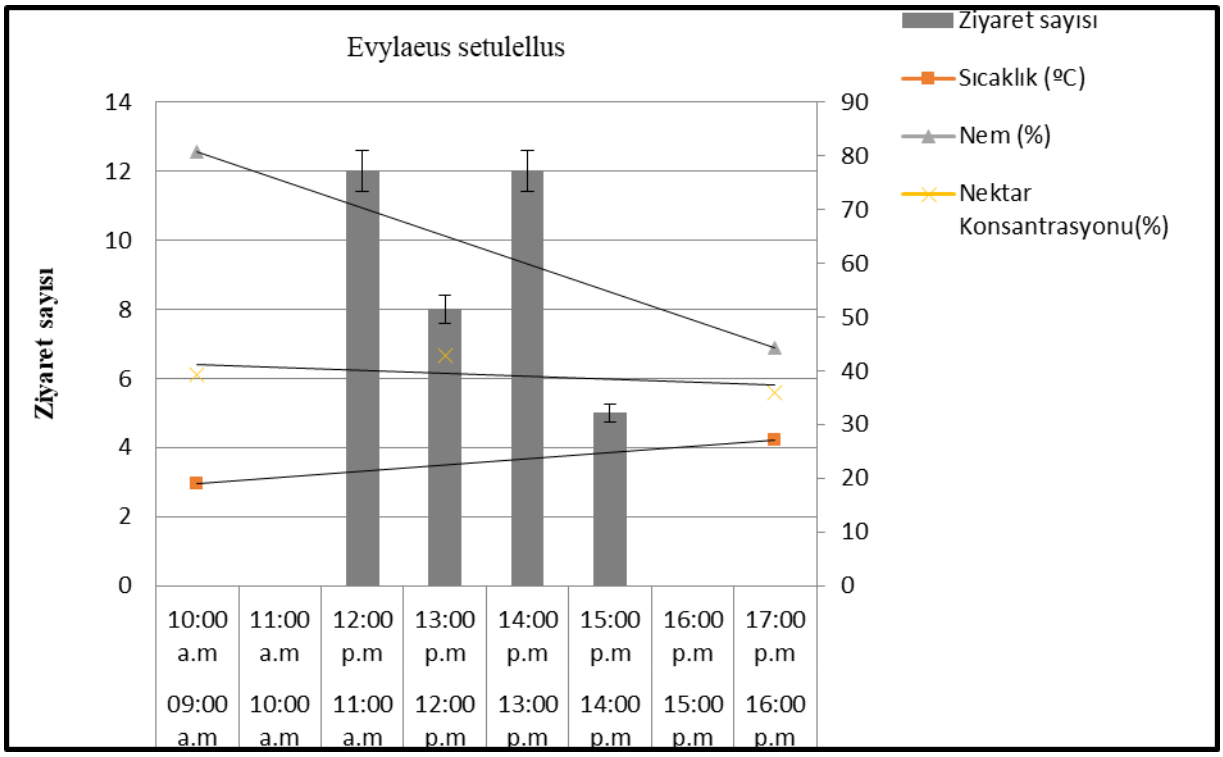

Figure 3. The number of flower visits by *Evylaeus setulellus* (Strand, 1909), and temperature and humidity values

3.3. Reproductive success

At the end of the trials on the effects of cross pollination on seed production in individuals exposed to pollinator visits under natural conditions, the average number of flowers per *C. tomentosa* plant was calculated as 110.64. The fruit counts of *C. tomentosa* demonstrated that the average number of seeds per fruit was 726.12 and the average number of ovules per flower was 992.94. At the end of the seed counts of these fruits, seed setting rate of the ovules was 73.12% (Table 6).

Table 6. Comparison of reproductive success parameters of *C. tomentosa*

Reproductive success parameters	The average number of flowers per plant	The average number of fruits per pant	Fruit set rate (%)	The average number of seeds per fruit	The average number of ovules per flower	Seed setting rate (%)
n	50	50	93,42	50	20	73,12
Min.-Mak.	22-207	11-202		510-1152	500-1198	
Ortalama	110,64±49,38	103,36±48,11		726,12±118,02	992,94±205,23	

n: *the number of the samples*

At the end of the trials on the effect of self-pollination on seed production, no fruit formation was observed in the plant.It was calculated that *C. tomentosa* was distributed in an area of 28.70 km^2 and that the average number of individuals and the number of potential individuals in 5 populations were 16.56 ± 6.41 and 4,753,713 respectively. On the other hand, it was determined that populations with the largest size and number of individuals were in Akçakonak-Priene and National Park, South of Doğanbey respectively (Table 7).

Table 7. Size and number of individuals of *C. tomentosa* populations in different of populations

Name of the population	The number of sample areas	The average number of individuals	Size of the population per km²	The number of potential individuals
Meryem Ana	7	16,71±6,57	3,99	667,397
National Park- South of Doğanbey	17	18±7,39	6,00	1081,440
National Park- North of Doğanbey	6	13,71±6,57	1,14	155,608
Çamlık - Ortaklar	7	12,85±5,63	3,08	396,936
Akçakonak -Priene	13	19,57±6,85	14,49	2,833,736
Total	**50**	**16,56±6,41**	**28,70**	**4,753,713**

4. Conclusions and discussion

Our floral morphology observations revealed that the pollen presentation occurred during the opening of the buds, that the full pollen presentation occurred in fully opened flowers, that the stigma lobes enlarged after the pollen presentation and that the stigma lobes were closed before the pollen presentation. On the other hand, our morphological measurements demonstrated that the length of the styles which was 16.52 mm during the pollen loading phase increased by 8.26 mm and reached 24.78 mm during the pollen presentation phase (Table 2). It was reported that *Campanula* flowers were protoandric, that pollen grains were loaded before the enlargement of the stigma lobes, and that although not always, pollen grains were dispersed during the budding phase or opening of the buds [19]. Researchers have reported that pollen grains are rapidly released when anthers open during the budding stage of *C. bononiensis* flowers and that all the pollen grains released are presented on the styles when the corolla is opened. They have also reported that the pollen presentation in *C. bononiensis* flowers is extended by about 2 days per flower due to the secondary pollen presentation. On the other hand, it was reported that in protandric species, the prolongation of the male phase was considered as an advantage based on phenology, and the ovules maturated rapidly during the flowering period [16].

In our studies on *C. tomentosa*, it was determined that the stigmas of flowers were not functional during the pollen loading phase, and that completely curled stigmas were functional only during the pollen presentation phase. However, the percentage of viability of the pollen grains belonging to the flowers decreased in this phase. These results were supported by pollen and stigma viability tests (Table 4), which is undoubtedly a mechanism that prevents self-pollination and encourages external pollination. While fruit formation does not occur in *C. tomentosa* buds closed during the budding phase, the presence of proterandry suggests that pollination is totally dependent on a pollinator. It has been reported that members of the genus *Xylocopa* display polylectic behaviors and that they prefer large, fancy flowers with a lot of pollen and nectar [17]. Our observations also demonstrated that *X. valga* (Gerstacker, 1872) was one of the major pollinators of *C. tomentosa*, but did not visit other *Campanula* taxa with much smaller flowers probably due to their body size. The duration of *X. valga'*s visit (Gerstacker, 1872) (17.5 ± 4.68 sec) is shorter than that of *E. setulellus* (Strand, 1909) (Figure 2). However, according to the floral morphology data of *C. tomentosa*, the average diameter of the corolla tube is 34 mm and the diameter of the stigma in the middle of this tube is 5.6 mm, and the body size of the *X. valga* (15x30 mm) is greater, which clearly indicates that their contact with the stigma is essential at every time they visit flowers (Table 1; Figure4). In addition, the fact that the body structure of *X. valga* is more hairy than that of *E. setulellus* (Strand, 1909) (according to comparison of museum samples) also leads to a more effective pollination of the plant.

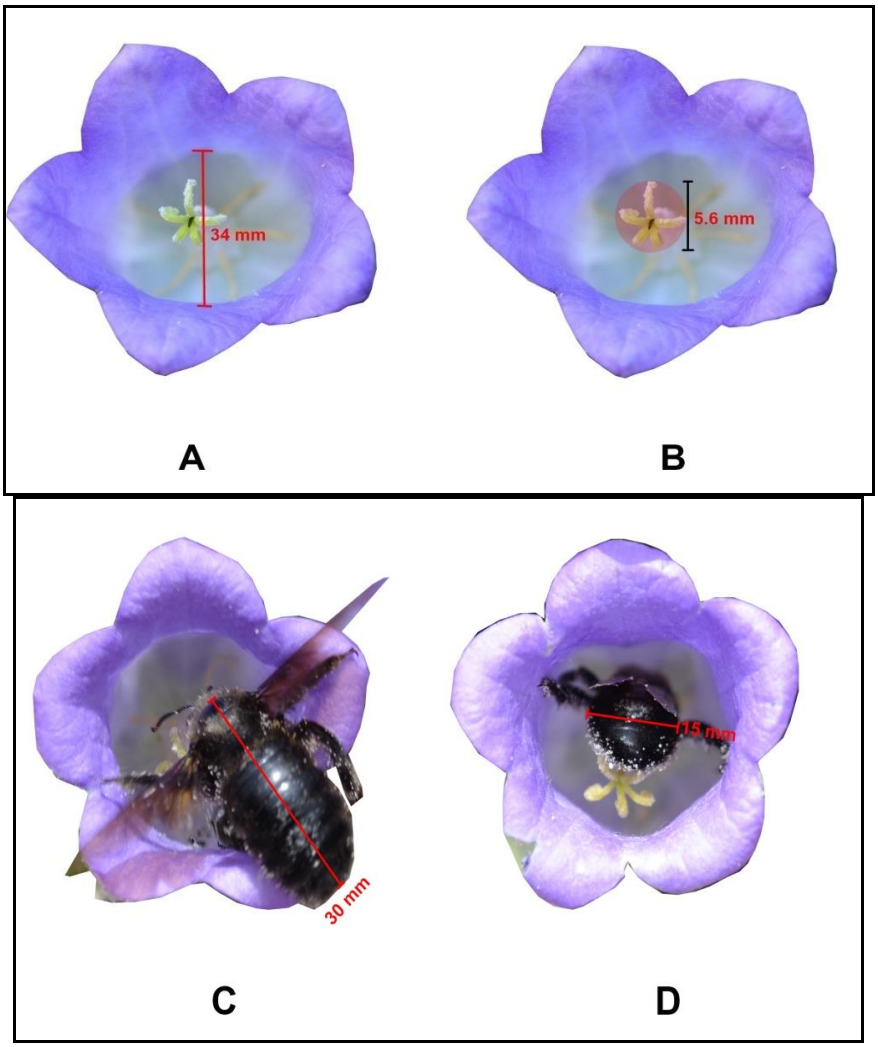

Figure 4. Morphological values in *C. tomentosa* and *X. valga*;
A: Corolla diameter; **B:** Stigma diameter; **C** and **D:** *X. valga*'s body size values.

As reported by researchers, when the main pollinator (Bombus spp.) of *Campanula punctata* enters its corolla to collect nectar, there will be an interaction between the dorsal surface of its chest and the style provided that there is a good match between the pollinator and flower [18], and size matching between the style and pollinator is expected to greatly affect the efficiency of pollination since the style of *C. punctata* has both male and female functions. In the same study, it was also reported that the duration of the visit by *Bombus* would significantly affected the accumulation of pollen on the stigma. The same researchers also reported that during their flower visits, the Bombus bees change their position around the corolla to insert their galea, which is a relatively solid organ, into the nectar spaces. It has been reported that the prolongation of this type of hunting behavior will contribute to the increase of pollen accumulation on the stigma. However, it was reported that although members of Megalchilide were locally important pollinators of *Campanula*, Halicutidae and Bombus bees were much more effective [19]. The same researchers also determined the mating and sleeping patterns of diptera species and of ants in the flowers of this species. *Campanula* flowers have a unique secondary pollen presentation. In this system; introrse anthers which dehisce inward before anthesis disperse pollen grains on the pollen-gathering feathers of the style [20]. At the beginning of the anthesis, all the pollen grains stick on the style, and the stigma lobes are yet neither opened nor functional. Another study reported that, flower visitors are oriented toward the stigma from the base of the style because the pollen tufts lean towards the style at this stage. Within a few days, as the stigma lobes are opened, the stigma becomes functional and accepts pollen grains. In the species we worked on, it was found that the hair on the style leaned towards the stigma from the base of the style [21]. When the average sugar concentration and sucrose measurements from the flowers of *C. tomentosa* and the changes in temperature-humidity and visit numbers during the day are examined, (Figure3), although the nectar concentration increased in the early evening hours, the number of visits to *X. valga* gradually decreased due to the increase in temperature (decrease in the humidity) (Figure3). Bees are ectothermic like other insect taxa and must protect their body temperature while flying. The thermal properties of their surroundings are important in their pollination activities. The high surface-to-volume ratio of small bees ensures rapid absorption of heat at high ambient temperatures and rapid cooling at low ambient temperatures. All the bees having a body mass greater than 35-50 mg

can produce endothermic heating, in other words internal heat production [22]. Examples of bee pollinators having a body weight of greater than 35 mg are Apis, Bombus, Xylocopa and Megachile. Examples of small bee pollinators are found in the Halicidae family, including the genus Lasioglossum. Many bees, in addition to endothermy, can control their temperatures before, during and after flying via physiological and behavioral ways. Endothermic abilities and thermal requirements vary between different groups of bees [23]. While *Xylocopa pubescens* generally cannot display nutritional activity at temperatures lower than 18°C, more thermophilic *X. sulcatipes* and *X. capitata* show nutritional activity when the ambient temperature reaches 22-32°C [24]. Nectar sugar concentration is mainly associated with the solar irradiance and temperature and is less associated with the bee activity. It was observed that *C. tomentosa* nutrition activity started at temperatures above 19°C, but that flower visits decreased as the temperature increased and that there were no visits after the temperatures reached 27°C (Figure3, 4). Given the reproductive parameters in the present study, it was determined that 93.42% of *C. tomentosa* flowers turned into fruit and that the Seed Setting Rate (SSR) in these fruits was 73.12%. The possible cause of this decline in seed setting rates can be said to be associated with the pollinators' behaviors. These results are considered to be an important parameter of the limited distribution.

Researchers report that *C. tomentosa* is distributed at rock formations at elevations of 100-130 meters located across from the cement plant in Söke, a district of Aydın, Türkiye [25]. However, in our field studies, *C. tomentosa* was not distributed anywhere in Söke, except for. Akçakonak-Priene, which suggests that there was a decrease in the number of distribution localities of *C. tomentosa*. In our field studies, compared to the previous year, a decrease was observed in the number of mature individuals of *C. tomentosa* in the north of the National Park, especially in the rocky areas near the entrance of the canyon. These significant habitat losses in *C. tomentosa* population is thought to be caused by the activities of Akçakonak stone crushing plant located in Söke-Akçakonak. In another study, *C. tomentosa* was classified as VU according to the IUCN Red List Categories (2001) [13]. However, when all populations of the plant were considered in the present study, approximately 4,753,713 potential individuals were identified in an area of 28.7 km². According to this, it was observed that the area occupied by all populations was less than 500 km^2 (B2). It was also determined that the habitat quality (b, iii), the number of distribution localities (b, iv) and the number of mature individuals (b, v) decreased. *C. tomentosa* was classified as EN B2b (iii, iv, v) (Endangered) according to IUCN 2001v3.1 (the Red List Categories and Criteria version 3.1, 2001).

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Analysis of landscape structure changes in Marmara Lake and its surrounding area using remote sensing data

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Abstract

This study investigates temporal changes in the landscape structure of Lake Marmara (Gölmarmara, Manisa, Türkiye), a designated bird sanctuary and registered wetland, over 10 years period using remote sensing techniques. The analysis employed the Normalized Difference Vegetation Index (NDVI) on satellite imagery from 2015 and 2024, processed through the ArcGIS/ArcMap software, to assess landscape metrics within the study area. The Normalized Difference Water Index (NDWI) was also utilized to evaluate the interactions between the water body, wetlands, and their surroundings. The findings were further supported by surface temperature analysis (STA) and comparative data. NDVI analysis revealed a 39.38% reduction in the Lake Marmara water body, an 11.09% decrease in forested areas, and an 8.28% decline in tree/shrub areas over 10 years. The dominant tree species in the forest vegetation was *Pinus brutia*, while other prevalent species included *Quercus coccifera, Cistus creticus, Pistacia terebinthus, Pistacia lentiscus, Arbutus andrachne, Arbutus unedo, Olea europaea, Erica arborea, Phillyrea latifolia*, and *Sarcopoterium spinosum*. Conversely, residential areas expanded by 21.21%, and agricultural areas increased by 4.11%. NDWI analysis indicated a 14.46% reduction in moderately dry areas and a 41.92% increase in dry areas. Furthermore, surface temperature data showed an increase over the 10 years. This study is expected to contribute significantly to the field by providing insights into sustainable land use and planning strategies. It offers a comprehensive perspective on wetland management and conservation of biological diversity by elucidating the temporal and spatial relationships between wetlands and their environment, considering both ecological and anthropogenic factors.

Keywords: ecology, remote sensing, landscape character analysis, NDVI, NDWI.

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Marmara gölü ve yakın çevresinin peyzaj karakterinde meydana gelen değişimin uzaktan algılama verileri kullanılarak araştırılması

Özet

Bu çalışmada, kuş cenneti ve tescilli bir sulak alan olan Marmara Gölü (Gölmarmara, Manisa, Türkiye) ve yakın çevresinin peyzaj karakterindeki değişimler, zamansal olarak 10 yıllık süreçte uzaktan algılama yöntemleri aracılığıyla incelenmiştir. Bu kapsamda 2015 ve 2024 yıllarına ait uydu görüntüleri üzerinde ArcGIS/Arcmap programı aracılığıyla bitki örtüsü tabanlı analizler (NDVI) yapılarak çalışma alanının peyzaj metrikleri incelenmiştir. Çalışma alanı ayrıca su kütlesi ve sulak alanların çevresiyle olan ilişkisi üzerinden normalize fark su indeksi analizi ile (NDWI) değerlendirilmiştir. Sonuçlar yüzey sıcaklık analizi (YSA) verileriyle desteklenerek karşılaştırılmıştır. NDVI analiz sonuçlarına göre, 10 yıllık süreçte Marmara Gölü su kütlesinin 39.38%, ormanlık alanlarının 11.09% ve ağaç/çalı alanlarının ise 8.28% oranında azaldığı tespit edilmiştir. *Pinus brutia* orman vejetasyonunun baskın bitkisidir. *Ouercus coccifera, Cistus creticus, Pistacia terebinthus, Pistacia lentiscus, Arbutus andrachne, Arbutus unedo, Olea europaea, Erica arborea, Phillyrea latifolia, Sarcopoterium spinosum* ise alanda sık rastlanan diğer türlerdir. Buna karşın yerleşim alanlarının 21.21%, tarımsal alanların ise 4.11% oranında arttığı belirlenmiştir. NDWI analiz sonuçlarına göre ise, orta derecede kurak alanların 14.46% azalmasına karşılık, kurak alanların 41,92% oranında arttığı görülmüştür. Ayrıca 10

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yıllık süreçte yüzey sıcaklığının da arttığı belirlenmiştir. Sulak alanların çevresiyle olan ilişkisini ekolojik ve antropojenik etmenler dahilinde zamansal ve mekansal boyutta ortaya koyması nedeniyle, bu çalışmanın sulak alan yönetiminde bütüncül bir bakış açısı ile birlikte alan kullanım/planlama ve biyolojik çeşitliliğin korunması stratejilerinde sürdürülebilir eylemlerin oluşturulması açısından alana katkı sağlayacağı düşünülmektedir.

Anahtar kelimeler: ekoloji, uzaktan algılama, peyzaj karakter analizi, NDVI, NDWI.

1. Introduction

The factors that define landscape character are identifiable and comprehensible, whether positive or negative, about the landscape structure. These factors emerge from ecological and anthropogenic influences within landscape metrics and vary according to the balance between land cover and land use [1]. Detecting land cover changes, such as spatial degradation, fragmentation, and reformation caused by ecological and anthropogenic factors, and understanding their impacts in terms of cause-effect relationships, are crucial for developing and planning new land-use strategies [2].

Changes in land cover can be detected through temporal and spatial change analyses using remote sensing methods, which is one of the key approaches for identifying deteriorations in landscape metrics [3]. Spatial analyses of green areas can be performed both temporally and spatially using two-dimensional data derived from multi-spectral satellite images, allowing the examination of the heterogeneous structure of vegetation (Normalized Difference Vegetation Index - NDVI) [4]. Numerous studies in the literature focus on detecting changes in landscape metrics through time-series analyses and multi-structural change detection approaches based on remote sensing systems [5]. Such studies also facilitate the detection and modeling of land degradation, regeneration areas, and changes in photosynthetically active vegetation [6]. Furthermore, remote sensing applications play a critical role in the development of land use planning and management policies due to their capacity to support various applications, such as spatial planning, land management, and the processing of data layers [7].

Wetlands and their interaction with the surrounding environment are critical components of the landscape due to their role in sustaining ecosystems [8]. Water plays a central role in shaping entire ecosystems, influencing life sustainability, vegetation, biodiversity, land cover classifications, and land use. Therefore, it is essential to examine the condition of wetlands and their environmental interactions [9]. Remote sensing methods, such as the Normalized Difference Water Index (NDWI), allow for the assessment of temporal and spatial changes in wetlands, providing valuable data that can serve as a reference for wetland management [10].

Marmara Lake (Gölmarmara, Manisa, Türkiye) is an alluvial barrier lake covering approximately 6,000 hectares. It is fed by groundwater sources, the Gördes Stream, and various feeding channels. The lake hosts 144 species of water birds and is listed as a Wetland of International Importance. Additionally, it is recognized as a nationally significant wetland and a registered bird sanctuary. However, due to significant water loss in recent years, the lake is at risk of extinction, having reportedly dried up completely in August 2021 [11]. Given its national and international significance, Marmara Lake and its surrounding area were chosen as the study site. The objective was to assess the temporal and spatial changes in the landscape metrics of Marmara Lake and its immediate surroundings over 10 years period using remote sensing methods.

2. Materials and methods

2.1. Research area

The study area encompasses Marmara Lake and its immediate surroundings in the Manisa province of Türkiye (Figure 1). The total area covers approximately 67,873 hectares.

2.2. Materials

Landsat 8 satellite images were utilized for this study, sourced from the official website of the United States Geological Survey (USGS). The images selected were from June 2015 and June 2024, spanning 10 years. To ensure high image quality, efforts were made to select images with minimal cloud cover and temporal proximity. Specifically,

data from June 18, 2015, with a cloud cover of 3.76%, and June 16, 2024, with a cloud cover of 0.01%, were chosen for analysis. The analyses were conducted using the relevant satellite bands from these images.

Figure 1. Satellite image of the study area

2.3. Methods

The procedures conducted within the study are outlined in the workflow plan (Figure 2). Initially, satellite data was acquired (https://earthexplorer.usgs.gov/). In the second phase, analyses were performed using the ArcGIS/ArcMap software. These analyses included NDVI, NDWI, and surface temperature analysis (STA). The analysis results were classified based on reflectance values, followed by spatial calculations of the classified data. Finally, the obtained results were compared.

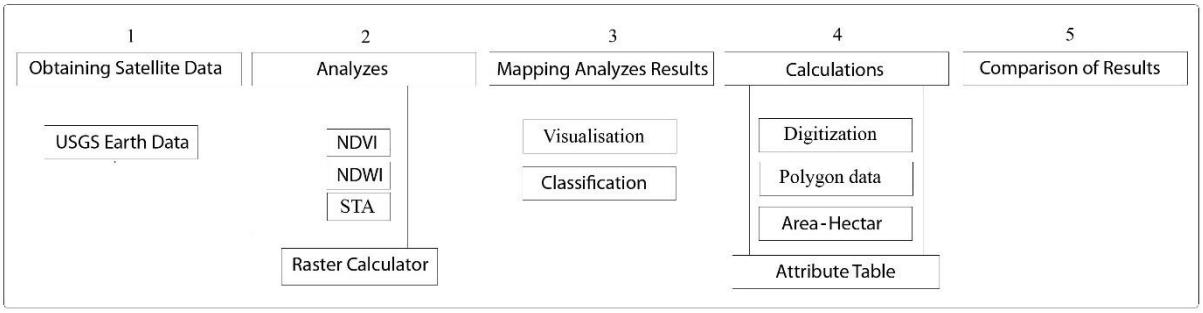

Figure 2. Workflow plan

2.3.1. NDVI (Normalized Difference Vegetation Index) Analysis

NDVI analysis was conducted on satellite data from 2015 to 2024 to assess vegetation density in the study area. The analysis was performed using the ArcGIS software, applying the formula provided below. The red and near-infrared bands from Landsat 8 satellite data were used for the study area. The resulting images were classified based on their reflectance values. This classification divided the area into five categories: forest areas, tree/shrub areas, agricultural lands, residential areas, and water bodies, which were then visualized accordingly.

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

2.3.2. NDWI (Normalized Difference Water Index) Analysis

NDWI analysis was conducted on all images to identify the spatial distribution of water within the study area. For this analysis, Band 3 and Band 5 data from Landsat 8 satellite images were utilized. The analysis was performed using the ArcGIS software, following the formula provided below. The resulting data were classified based on their reflectance values. This classification categorized the area into four groups: dry areas, moderately dry areas, humid areas, and water bodies, which were subsequently visualized.

$$NDWI = \frac{(Band\ 3 - Band\ 5)}{(Band\ 3 + Band\ 5)}$$

2.3.3. Surface temperature (ST) analysis

Surface temperature analysis was conducted on all satellite data to determine the surface temperature of the study area. The relevant bands from the satellite data were used for thermal analysis. The analysis was carried out using the ArcGIS software, following the formula provided below. The results were classified based on reflectance values and visualized by dividing the data into four categories.

$$Tb = \frac{K2}{\ln(\frac{K1}{L\lambda}+1)} - 273.15$$

$$Pv=(\frac{NDVI-NDVImin}{NDVImax-NDVImin})^2$$

$$\varepsilon TM6 = 0.986 + 0.004\, P\, v$$

$$Ts = \frac{Tb}{1+(\lambda\times\frac{Tb}{\frac{h\times c}{b}})\times ln\varepsilon\lambda}$$

(*Tb: Thermal band, Pv: Proportion of vegetation,* **ε: Emissivity, Ts: Surface temperature)**

3. Results

3.1. NDVI (Normalized Difference Vegetation Index) Analysis

The results of the NDVI analysis are shown in Figure 3 and the corresponding area calculations are shown in Table 1. In June 2015, the forest area was 6,448.89 ha, while by June 2024 it had decreased to 5,733.56 ha, representing a decrease of 11.09% over the 10 years. Areas with tree and shrub vegetation were recorded at 16,912.54 hectares in June 2015, decreasing to 15,511.56 hectares by June 2024, reflecting an 8.28% reduction over the same period. Agricultural areas increased from 19,961.04 hectares in June 2015 to 20,780.62 hectares in June 2024, showing a 4.11% increase. Residential areas expanded from 16,709.03 hectares in June 2015 to 20,252.67 hectares in June 2024, resulting in a 21.21% increase over the 10 years. Meanwhile, water bodies, which covered 5,706.3 hectares in June 2015, had decreased to 3,459.22 hectares by June 2024, marking a 39.38% decline over the 10 years.

In the research area, *Pinus brutia* is the predominant tree species within the forest vegetation. Other common species in the region include *Quercus coccifera*, *Cistus creticus*, *Pistacia terebinthus*, *Pistacia lentiscus*, *Arbutus andrachne*, *Arbutus unedo*, *Olea europaea*, *Erica arborea*, *Phillyrea latifolia*, and *Sarcopoterium spinosum*. Aquatic plants such as *Phragmites australis*, *Potamogeton natans*, *Potamogeton perfoliatus*, *Potamogeton nodosus*, *Potamogeton pectinatus*, *Myriophyllum spicatum*, and *Ranunculus* spp. are also present. The herbaceous vegetation predominantly includes species characteristic of maquis and garrigue ecosystems [3].

Landscape character analysis is a method for promoting sustainable land use by detecting, monitoring, and quantifying changes in biodiversity and landscape metrics [12]. NDVI analysis, a crucial technique in remote sensing for monitoring vegetation changes, provides insights into variations in landscape metrics, including the number, size, shape, and distribution of vegetation patches in ecosystems [13, 14]. This study examines changes in landscape metrics, such as forest areas, tree and shrub areas, agricultural lands, residential areas, and water bodies, alongside spatial, areal, and dimensional changes in landscape patches. According to area calculations for June over the past 10 years, there has been a reduction in forest and tree/shrub areas, as well as a decrease in water bodies. These reductions are primarily attributed to anthropogenic factors, and climate change, including rising temperatures, irregular precipitation patterns, and decreased precipitation levels [15]. Conversely, residential and agricultural areas have increased, largely due to the expansion of living spaces to accommodate population growth and anthropogenic influences, including the expansion of agricultural land [16]. While the increase in agricultural areas is not directly linked to heightened agricultural activities within the context of anthropogenic factors, it is hypothesized that agricultural activities may have decreased in the previous 10 years due to reduced water bodies. However, between 2015 and 2024, agricultural activities are thought to have risen due to population growth and the expansion of new agricultural areas. The 2024 data also suggests that fallow and uncultivated lands, which have seen reduced agricultural activity, may have been reclassified as agricultural areas, reflecting an overall increase in agricultural land over 10 years.

To obtain the analysis results, land cover classification was performed based on reflectance values. Examination of the analysis images reveals confusion in area classification based on these reflectance values, manifesting as displacement and spatial changes in landscape metrics. For instance, Figure 3 illustrates that the water body appears to be concentrated in areas outside of Marmara Lake. This indicates that the reflection values used for area classification may correspond to the same reflectance values as those representing the water body. Consequently, this visual evidence suggests an anticipated increase in the water mass. However, the area calculations indicate that the water body in Marmara

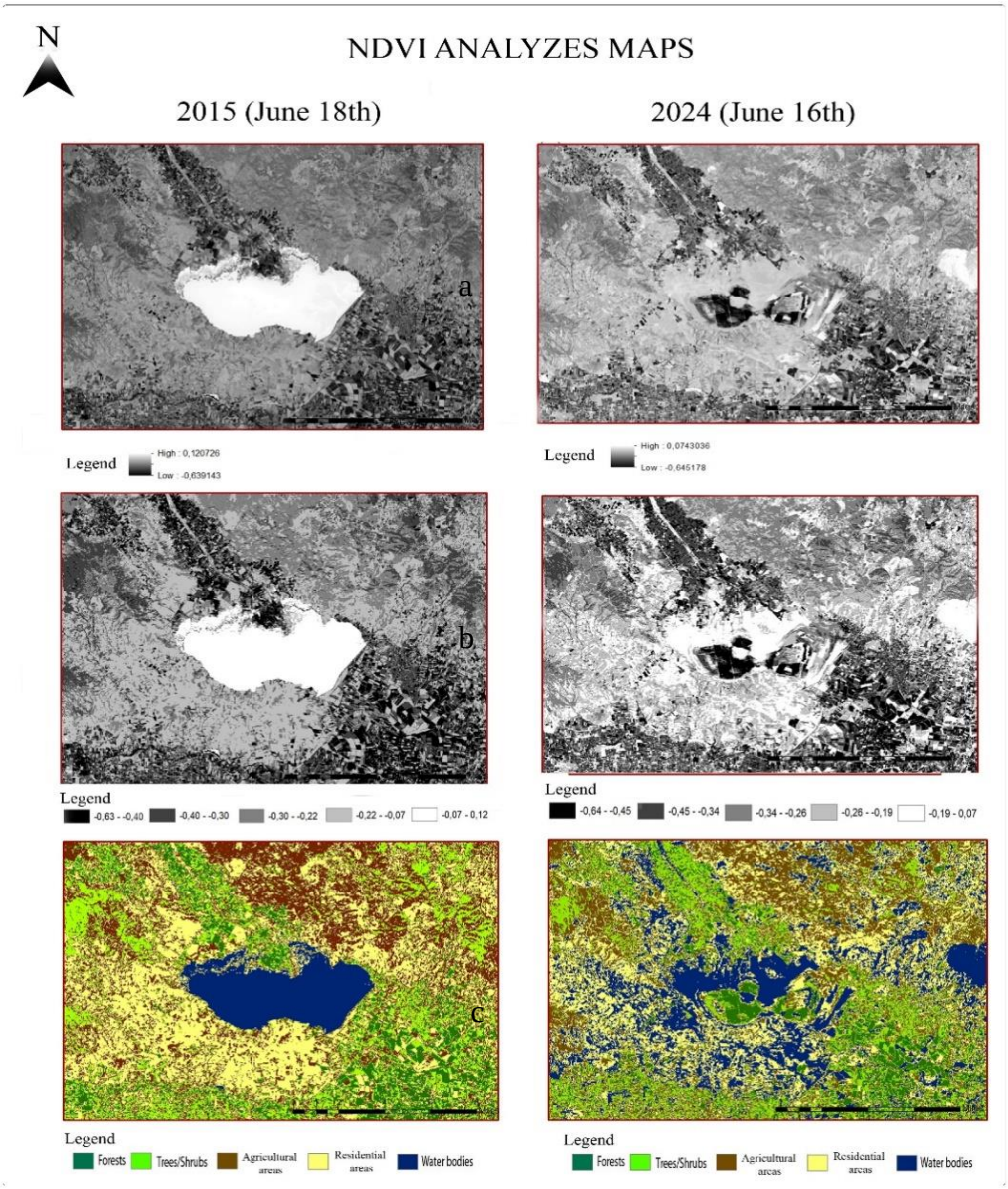

Figure 3. Results of normalized difference vegetation index (NDVI) analyses
(a. Maximum/minimum levels of reflection values, b. Classification of reflection values,
c. Classification of vegetation and water bodies)

Table 1. Numerical values of vegetation and water bodies in the research area

Categories	**2015**	**2024**	**Change**
	(June, 18th)	**(June, 16th)**	**(%)**
Forest Areas	6.448,89	5.733,56	-11.09
Tree/Shrubs	16.912,54	15.511,56	-8.28
Agricultural areas	19.961,04	20.780,62	4.11
Residential areas	16.709,03	20.252,67	21.21
Water bodies	5.706,13	3.459,22	-39,38

Lake has actually decreased in size, implying that the reflection values may lead to discrepancies in the representation of landscape metrics [20].

3.2. NDWI (Normalized Difference Water Index) Analysis

The analysis results are presented in Figure 4, with area calculations detailed in Table 2. As of June 2015, dry areas covered 10,214.16 hectares, increasing to 14,495.64 hectares by June 2024, reflecting a 41.92% increase. Moderately dry areas, which covered 20,785.78 hectares in June 2015, decreased to 17,779.55 hectares by June 2024, showing a reduction of 14.46%. Humid areas increased from 29,031.56 hectares in June 2015 to 30,003.22 hectares in June 2024, representing a 3.35% increase. Conversely, the water body, which covered 5,706.13 hectares in June 2015, decreased to 3,459.22 hectares by June 2024, indicating a 39.38% reduction in the area occupied by water bodies.

NDWI (Normalized Difference Water Index) analysis enables the examination of the natural water balance in landscape character and the temporal and spatial changes in wetlands, which are represented as water bodies in land cover classifications. Understanding the relationship between wetlands and their environment, as well as their role in the landscape structure, is crucial [8]. This study's NDWI analysis reveals a significant reduction in the water body of Lake Marmara and its surroundings over the past 10 years. Such a substantial loss of water is expected to have a negative impact on the ecosystem.

In this study, the focus was on calculating the areal extent of water bodies rather than their volumetric quantity. It is anticipated that a reduction in the areal size of water bodies will correspond with a decrease in their volumetric amount. The observed areal loss in water bodies is attributed to rising temperatures due to climate change, alterations in precipitation patterns, and reductions in precipitation levels. However, it is noted that the shoreline has shifted, with the water mass and surrounding land expanding towards the water. Changes in water surfaces manifest in two ways: complete disappearance or drying of water bodies in shallow lakes due to global warming and its effects, and coastal retreat at varying rates in deeper lakes. It is believed that coastal retreat has occurred in Marmara Lake, which reportedly dried up completely in August 2021 [11]. NDVI analysis further suggests that the reduction in water bodies may also be linked to anthropogenic activities, such as the expansion of agricultural and residential areas.

In the study area, while there is a modest increase in humid areas (3.35%), the increase in dry areas is considerably larger (41.92%). This observation is consistent with the substantial water loss recorded. Additionally, examination of the analysis images reveals that landscape patches representing various metrics have changed in spatial distribution, form, and density over the 10 years. These changes are likely attributable to the rising temperatures observed in June 2024 [18].

3.3. Surface temperature (ST) analysis

Analysis visuals are given in Figure 5 and numerical results are given in Table 3. Accordingly, in June 2015, the temperature in an area of 7,883.16 hectares was between 22.56 - 26.72 °C, in an area of 17,396.35 hectares was between 26.72 - 31.25 °C, in an area of 25,639.34 hectares was 31.25 - 34 °C and in an area of 16,954.87 hectares was between 34.29 - 41.50 °C. According to the weighted average result made by taking into account the temperature spectrum and areas, the average temperature of June 2024 was determined as 36.88 °C while it was 32.14 °C in June 2015. In this case, it is seen that the average temperature of the area increased by 14.76% in 10 years.

The analysis visuals are presented in Figure 5, and the numerical results are provided in Table 4. In June 2015, temperatures were recorded as follows: 7,883.16 hectares had temperatures ranging from 22.56 °C to 26.72 °C; 17,396.35 hectares ranged from 26.72 °C to 31.25 °C; 25,639.34 hectares ranged from 31.25 °C to 34 °C; and 16,954.87 hectares ranged from 34.29 °C to 41.50 °C. The weighted average temperature for June 2015 was 32.14°C, while for June 2024 it was 36.88 °C, calculated based on the temperature ranges and corresponding areas. This represents a 14.76% increase in the average temperature over the 10-year period.

The observed increase in average temperature is thought to have contributed to the reduction in water bodies and the expansion of dry areas in the region. It is suggested that temperature alone may not be the sole factor responsible for the decrease in water mass; anthropogenic factors also play a significant role [18]. Spatial changes, as reflected in satellite images, can lead to confusion in landscape metrics [19]. However, these changes do not affect land use classification since areas with the same reflectance value are represented collectively [20]. Consequently, landscape patches may exhibit variations in shape and size [21], which is attributed to differences in reflectance values across distinct areas [17]. The diversity in landscape structure is linked to ecological corridors [22]. Structurally, a corridor is a landscape element that differs qualitatively from adjacent areas [23]. To address deteriorations in landscape character, the creation of ecological corridors is recommended [24]. This study suggests adopting sustainable ecosystem-based approaches to address existing issues related to the degradation of Marmara Lake's landscape structure and to manage area losses in the future. Such approaches may include green infrastructure or blue-green infrastructure initiatives, rainwater management, climate adaptation, mitigation of heat island effects, sustainable energy production, and provision of clean water [25].

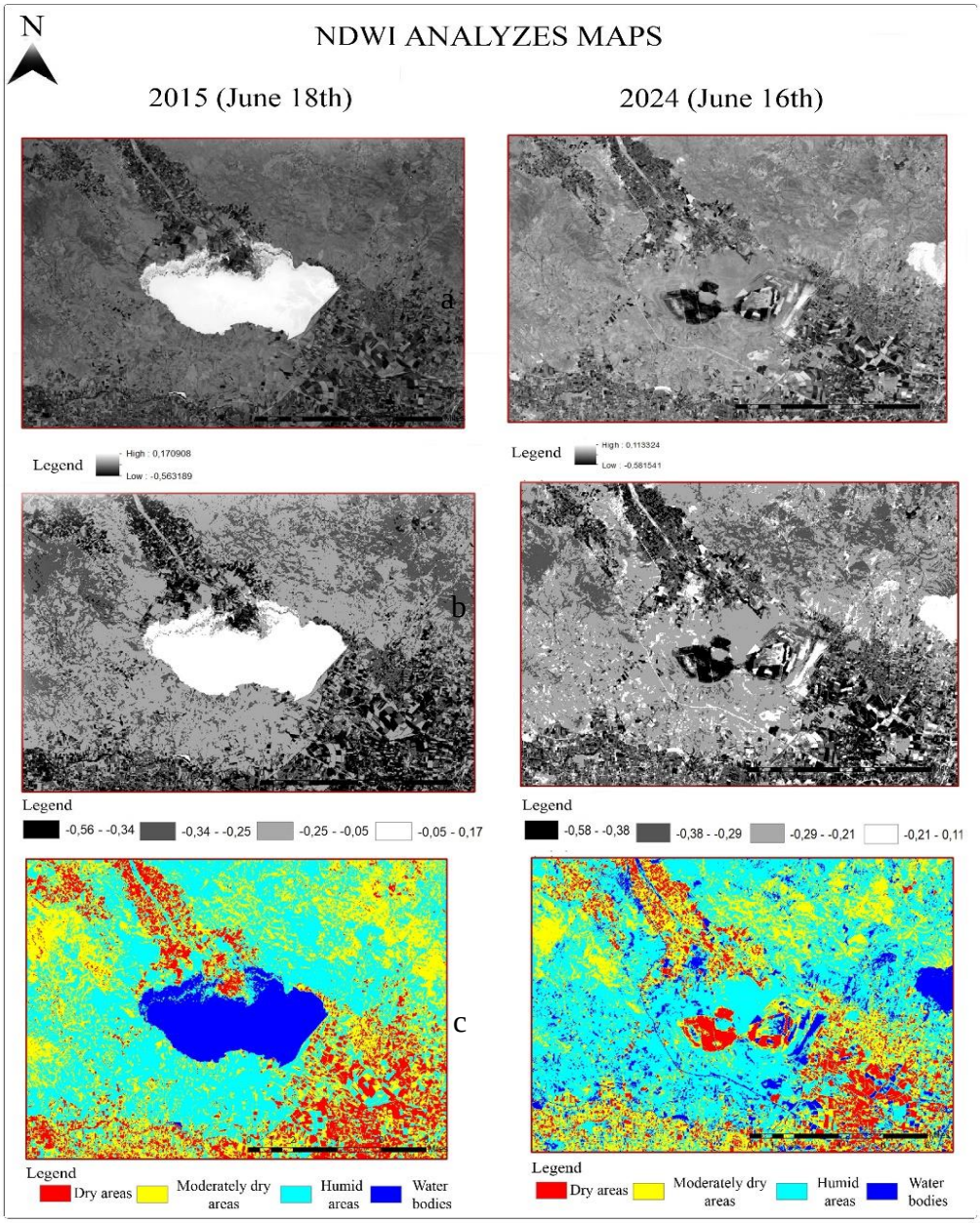

Figure 4. Results of normalized difference water index (NDWI) analysis
(a. Maximum/minimum levels of reflection values, b.Classification of reflection values, c.Classification of areas according to water content)

Table 2. Numerical values of areas according to water content

Categories	**2015**	**2024**	**Change**
	(June 18th)	**(June 16th)**	**(%)**
Dry areas	10.214,16	14.495,64	41,92
Moderately dry areas	20.785,78	17.779,55	-14,46
Humid areas	29.031,56	30.003,22	3,35
Water bodies	5.706,13	3.459,22	-39,38

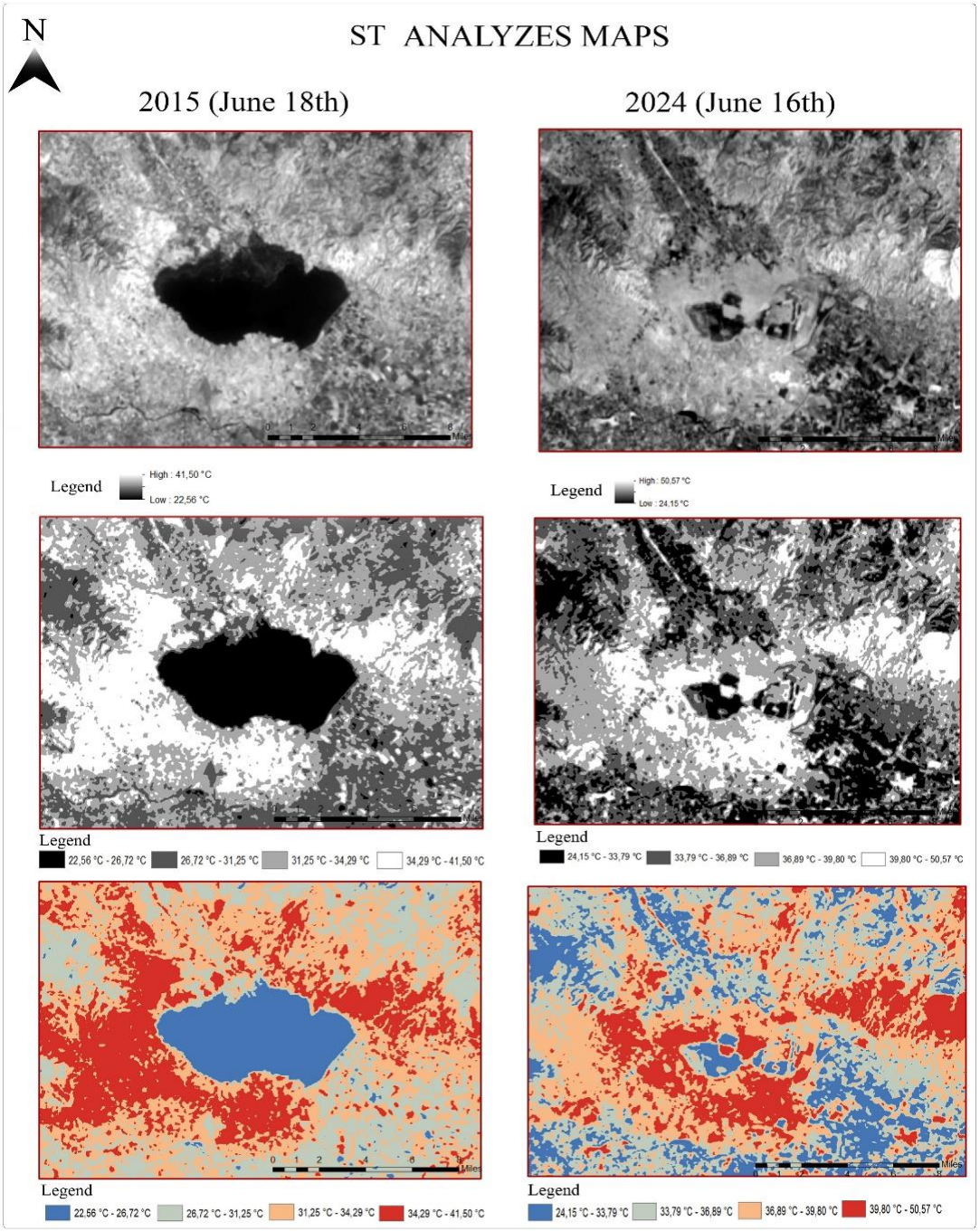

Figure 5. Results of surface temperature (ST) analysis
(a.Maximum/minimum levels of reflection values, b.Classification of reflection values, c.Classification of areas according to surface temperature)

Table 3. Numerical values of surface temperature analysis of the research area

2015 (June 18th)		**2024 (June 16th)**	
Temperature Spectrum (^{0}C)	**Area (ha)**	**Temperature Spectrum (^{0}C)**	**Area (ha)**
22,56 --- 26,72	7.883,16	24,15 --- 33,79	12.640,95
26,72--- 31,25	17.396,35	33,79--- 36,89	20.471,28
31,25---34,29	25.639,34	36,89--- 39,80	22.980,99
34,29--- 41,50	16.954,87	39,80--- 50,57	11.780,49

Over the 10-year period, changes in land cover have been influenced by anthropogenic factors, resulting in deterioration of landscape metrics, fragmentation, and changes in the extent and density as well as temperature values of the area. Analyses of the water body status, as indicated by NDWI, reveal a decrease in the area covered by water bodies in Marmara Lake, suggesting a potential for water scarcity in the near future. Consequently, it is crucial to re-evaluate and adjust wetland management policies and land use regulations to address these emerging challenges.

4. Conclusions and discussion

This study aimed to elucidate the changes in landscape character and metrics of Marmara Lake and its surrounding areas, along with their underlying causes. The investigation involved analyzing land cover changes in Marmara Lake and its vicinity through vegetation and wetland analyses (NDVI and NDWI), with the findings supplemented by climatic factors (surface temperature analysis). Notably, over the 10 years from 2015 to 2024, there has been a significant reduction in forest and tree/shrub areas, alongside an increase in agricultural and residential areas. Additionally, the substantial rise in dry areas, in response to a severe decrease in water bodies, represents a concerning trend for Marmara Lake and its surroundings. We believe that the insights gained from this study will contribute to the field, assist decision-makers, and inform the development of effective conservation policies for both biological diversity and nature.

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A scientific approach to the health and obstetrical problems of female genital mutilation of Somali women living in Türkiye

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Abstract

Mutilation is a general name given to the partial or complete injury and mutilation of the female genital organ under the name of tradition and culture. It is quite common in Africa. It is estimated that it is applied to more than 200 million women, especially in the Middle East and the Far East. Different applications continue in each country. After mutilation, anatomical tissue loss occurs in the female genital area. Therefore, lifelong physiological, psychiatric and obstetric problems arise. In this study, the mutilation status of Somali women living in Türkiye was investigated. The effects of mutilation on birth and the attitudes of women towards this practice were revealed.

For this purpose, the status of 190 women in 15 provinces where Somali citizens are most present were examined. For this purpose, a face-to-face survey was conducted with 174 participants. The results were evaluated using statistical chi-square tests and continuous quantitative variables. The data were given as parametric and non-parametric results of the tests. As a result, it was determined that almost all of the Somali women living in Türkiye were circumcised, although slightly below the world average. It was proven that economic status, education, cultural traditions and family structure are closely related to mutilation. It has been observed that female circumcision causes many obstetric and psychiatric problems. It has been determined that the attitude of Somali women living in Türkiye towards female circumcision has changed significantly with the increase in education level, household income, awareness and communication opportunities. Education, cultural change, financial status, level of health services and midwifery services have been proven to reduce female circumcision effectively.

Keywords: health care, midwifery, obstetrics, Somali women living in Türkiye, genital mutilation

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Türkiye'de yaşayan Somalili kadınların kadın sünnetinin sağlık ve doğum sorunlarına bilimsel bir yaklaşım

Özet

Mutilasyon; gelenek ve kültür adı altında kadın cinsel organın kısmen veya tümüyle yaralanmasıyla sakatlanmasına verilen genel bir isimdir. Afrika'da oldukça yaygındır. Orta doğu ve Uzak Doğu başta olmak üzere 200 milyondan fazla kadına uygulandığı tahmin edilmektedir. Her ülkede değişik uygulamaları sürmektedir. Sakatlama sonrası kadın genital bölgesindeki anatomik doku kaybı oluşur. Bu nedenle yaşam boyu süren fizyolojik, psikiyatrik ve obstetrik sorunlar ortaya çıkar. Bu çalışmada Türkiye'de yaşayan Somalili kadınların mutilasyon durumları araştırıldı. Mutilasyonun doğuma etkileri, kadınların bu uygulamaya karşı tutumları ortaya konuldu. Bunun için Somalili

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vatandaşların en çok bulundukları 15 ilde 190 kadının durumu incelendi. Bunun için 174 katılımcı ile yüz yüze bir anket yapıldı. Sonuçlar istatistiksel ki-kare testleri ve sürekli niceliksel değişkenler kullanılarak değerlendirildi. Veriler testlerin parametrik ve non-parametrik sonuçları olarak verildi. Sonuçta; Türkiye'de yaşayan Somalili kadınların dünya ortalamasının biraz altında olsa da neredeyse tamamının sünnetli olduğu tespit edildi. Ekonomik durum, eğitim, kültürel gelenekler ve aile yapısının mutilasyonla yakından ilişkili olduğu kanıtlandı. Kadın sünnetinin birçok obstetrik ve psikiyatrik soruna neden olduğu görüldü. Türkiye'de yaşayan Somalili kadınların kadın sünnetine yönelik tutumunun, eğitim düzeyi, hane geliri, farkındalık ve iletişim olanaklarının artmasıyla önemli ölçüde değiştiği tespit edildi. Eğitim, kültürel değişim, mali durum, sağlık hizmetleri düzeyi ve ebelik hizmetleri kadın sünnetini etkili bir şekilde azalttığı kanıtlandı.

Anahtar kelimeler: sağlık hizmetleri, ebelik, obstetrik, Türkiye'de yaşayan Somalili kadınlar, kadın sünneti

1. Introduction

Female circumcision (FGM) is a procedure that involves cutting, piercing, or otherwise damaging the genitals of girls and has no medical benefit. Every year, 3-4 million girls are subjected to this primitive cruelty and torture. It has decreased in the last 50 years thanks to information and communication opportunities. However, it is still practised under the guise of "tradition-culture". FGM is a clear violation of fundamental human rights [1]. This disgusting practice, which has no place in any divine religion, has been practised since the 5th millennium BC. Unfortunately, more than 200 million girls and women in more than 30 countries are subjected to this practice. It is expected to be eliminated by 2030. However, if FGM is not prevented through education, communication and health services in FGM regions, it can lead to significant disasters [2]. FGM is a substantial violation of women's rights in Sub-Saharan Africa. Somalia is a country that practices its most severe form (type-3) by more than 97%. The exact number and amount are unknown. The surveys are based on unreliable data from underdeveloped countries [3]. It has traditionally become a strong social norm. This power must be motivated by the myth that religions support it. In fact, in the geographies where FGM is practised, there are tribes and communities of different faiths, such as Muslims, Christians and Animists. Each tribe has found a religious cover for this brutal tradition. Over time, FGM myths have been fabricated and have become a cultural tradition [4].The countries where FGM is most common are Somalia (97.9%), Egypt (95.8%), Guinea (95.6%), Sierra Leone (94%), Djibouti (93.1%), Mali (91.6%) and Eritrea (88.7%). The rate of infibulation (cutting the lips of the vagina and sewing the vulva) is highest in Somalia, Ethiopia and Eritrea. FGM is also performed among immigrant communities in Europe, Canada, the United States, Australia and New Zealand [5]. In 2001, 72% of the 10,501 African women who came to Switzerland from countries where FGM is performed were over the age of 15, and approximately 6,000 had FGM [6]. United Nations reports indicate that FGM has decreased since 2012 [7]. Strengthening the information infrastructure, expanding the means of communication and universalizing people's access to information may, over time, develop a severe resistance to mutilation in new generations. On the other hand, the real danger is that immigrants will resist their attachment to their origins [8]. FGM is practised in four different ways around the world. In Ethiopia, Kenya [7], and Eritrea, the clitoris is partially or wholly removed (type 1). In West Africa, the clitoris and labia vaginalis are partially or wholly removed (type II). Benin, Sierra Leone, Gambia and Guinea. In addition to types 1 and 2, the labia minora are also cut. In Sub-Saharan African countries such as Somalia, Djibouti, Northern Sudan, and the surrounding areas, the tissues are repositioned by stitching in addition to these cuts. This type of FGM is a horror and nightmare for girls. The vaginal opening is narrowed with a gasket (type III). In Northern Nigeria, abrasive substances that penetrate the vagina are inserted, scraped and injured. It is known and practised in the region as "gishiri" (type-IV) [9]. The health risks of FGM are assessed in several categories: acute life risks, inflammatory, obstetric, chronic risks and psychiatric risks [10]. Post-traumatic stress disorder (PTSD), anxiety, depression, neuroses and psychoses are common delayed complications associated with FGM. It is complex to realistically define and monitor these conditions in countries where FGM is practised. Failure to treat these psychological traumas can lead to mental anxiety and even permanent damage later in life [11]. This study aims to evaluate the health and birth problems of Somali women living in Türkiye. In light of the information received from institutions, 15 provinces with the highest density of Somali citizens living in Türkiye were selected. Somali women over the age of 18 living in these provinces were included in the study cross-sectionally. Those living in Türkiye for education, work, civil service, and more than six months were included.

2. Materials and methods

An informed consent form was prepared. Data collection was carried out between January 1 and March 31, 2022. To scan Türkiye in general and to obtain a more precise estimate, 15 provinces where Somali individuals live were selected as the research area from official sources. Surveys were shared with 209 universities (131 State, 78 Private and Foundation Universities) through our university with official letters. Somali women were also reached through Google Forms (hhttps://forms.gle/SrUypLyy FPHFTUL18) https://mail.google.com/mail/u/1/#inbox). Women under the age of 18 were excluded. The surveys were collected in the Excel data pool [12]. Two-sample T-Test Power

Analysis was used. At least 190 women aged 18 and over were accepted as the lower limit with 99% statistical accuracy. Estimated group standard deviations of 1.9 and 1.6 to 2.2 were considered significant. A two-sample t-test was used in the study. The 0.05000 level was accepted as the reference. Statistically, 99.99% accuracy was accepted for n=174 [13]. In our research, n=190 was made. The chi-square test was used for analysis. Continuous quantitative variables were evaluated in normality tests. Data were presented with parametric or non-parametric tests by looking at the Kolmogorov-Smirnov and Shapiro-Wilk results. Permission was obtained from the Karabük University Presidency Non-Interventional Clinical Research Ethics Committee on 15.12.2021 with the number E-77192459-050.99-88241 (2021/777). This scientific research is a cross-sectional descriptive study.

Selection criteria: Qualified healthcare personnel, visually and hearing impaired, learning and mentally disabled were excluded from the study. Those with advanced orthopaedic problems, personal care, speech or self-expression and mental-psychological disabilities were excluded from the study. Severely chronic patients, people with epilepsy, diabetics and individuals with acute inflammation were not included in the study.

3. Results

The survey results were obtained regarding the participants' characteristics such as place of birth, education level, occupation, spouse's occupation, age at first marriage, age at spouse, age at first menstruation and age at conception (Table 1). It was observed that even if the place of birth was not in a rural area, it traditionally affected FGM. Education level was highly determinant to FGM. It was determined that occupation and work status could not overcome the rigid cultures of tradition.

Table 1. Individual characteristics of the participants

	n	**%**	**% of total**
Place of birth			
City Center	99	53,80	52,11
District	49	26,63	25,79
Town center	13	7,07	6,84
Village	23	12,50	12,11
Total	184	100,00	96,84
Educational status			
İlliterate	11	5,98	5,79
Primary school	25	13,59	13,16
Middle school	15	8,15	7,89
High school	55	29,89	28,95
University	58	31,52	30,53
Degree	20	10,87	10,53
Total	184	100,00	96,84
Job			
Own workplace	22	12,57	11,58
Officer-worker	22	12,50	11,58
Student	54	30,68	28,42
No Profession	26	14,77	13,68
Doesn't have a job	29	16,48	15,26
Other	22	12,50	11,58
Total	175	99,43	92,11
Spouse profession			
Not working	5	5	2,63
Own working place	11	11	5,79
Officer-worker	42	42	22,11
Student	7	7	3,68
Busy with trade	35	35	18,42
Total	100	100	52,63
First marriage age			

Table 1. Continued

Before age 18	21	19,63	11,05
18-22	34	31,78	17,89
23-27	45	42,06	23,68
28--32	7	6,54	3,68
Total	107	100,00	56,32
Spouse age			
27-35	36	37,89	18,95
36-45	30	31,58	15,79
46-55	17	17,89	8,95
56-76	12	12,63	6,32
Total	95	100	50,00
First menstrual age			
9--11	47	25,13	24,74
12--14	131	70,05	68,95
Over 15	9	4,81	4,74
Total	187	100,00	98,42
Age at conception			
<18	20	21,05	10,53
18-23	36	37,89	18,95
24-29	38	40,00	20,00
30-35	1	1,05	0,53
Total	95	100,00	50,00
First Birth			
Normal birth	72	73,47	37,89
Caesarean section	21	21,43	11,05
Total	98	100,00	51,58

Marriage, monthly income, purchasing power, social security, insurance, education, and age at first marriage of Somali women living in Türkiye were influential in the traditional continuation of FGM. Figure 1, which shows the FGM situation, is indicated.

Figure 1. The situation of Somali women living in Türkiye

The FGM status, living and health conditions and attitudes towards this issue of Somali women living in Türkiye were investigated (Table 2). The women were questioned about their knowledge of FGM and whether they had received training on the subject. It was learned whether they had had FGM themselves. It was determined which type of FGM they had and under what conditions. This situation is presented in Table 2. Almost all of them have had FGM. Since they had FGM at a young age, family pressure is at the forefront. When they had Vaginal Circumcision, they had no knowledge about FGM. Legends were fabricated under the name of culture and told to the children. Regardless of whether they were convinced or not, FGM was forced to be done by their families and relatives.

Table 2. FGM Status, conditions and attitudes of Somali women living in Türkiye

	n	**%**	**% of total**
Do you know about female circumcision?			
Yes	190	100,00	100
No	0	0,00	0,00
Total	190	100,00	100,00
Information or training on FGM			
Yes	136	73,51	71,58
No	49	26,49	25,79
Total	185	100,00	97,37
Why is FGM done?			
Tradition	102	55,74	53,68
Religion	23	12,57	12,11
Required for marriage	2	1,09	1,05
Provides genital beauty	1	0,55	0,53
It is a violation of women's rights	4	2,19	2,11
A false tradition	51	27,87	26,84
Total	183	100,00	96,32
Infringement + False Tradition	55	30,05	28,95
Tradition + religion + Marriage + Genital beauty	128	69,95	67,37
Have you become FGM?			
Yes	183	97,34	96,32
No	5	2,66	2,63
Total	188	100,00	98,95
Where was circumcision done?			
Birth house	9	4,89	4,74
At home	163	88,59	85,79
Hospital	12	6,52	6,32
Total	184	100,00	96,84
Who practiced circumcision?			
Traditional circumcision woman	143	77,72	75,26
Village midwife	24	13,04	12,63
Health employee	17	9,24	8,95
Total	184	100,00	96,84
Which type of FGM was practiced			
Pharaoh	77	41,40	40,53
Sunni	109	58,60	57,37
Total	186	100,00	97,89
Is anesthesia used in FGM?			
Yes	168	90,32	88,42
No	18	9,68	9,47
Total	186	100,00	97,89
Are your feet cross-linked after FGM?			

Table 2. Continued

Yes	112	60,87	58,95
No	72	39,13	37,89
Total	184	100,00	96,84
FGM age			
0-5 Years	16	8,74	8,42
6-10 Years	156	85,25	82,11
11-14 Years	11	6,01	5,79
Total	183	100,00	96,32
Are you aware of the laws regarding FGM?			
İllegal	104	55,91	54,74
Normal in Africa	42	22,58	22,11
Normal in some countries	14	7,53	7,37
Legal	26	13,98	13,68
Total	186	100,00	97,89
How do you agree with the WHO's views on FGM?			
Yes	132	71,35	69,47
No	34	18,38	17,89
No idea	19	10,27	10,00
Total	185	100,00	97,37
Who has a say in ending FGM?			
Dr and paramedic	112	59,89	58,95
Traditional midwives	18	9,63	9,47
Religious leaders	57	30,48	30,00
Total	187	100,00	98,42
Have you received training on stopping FGM?			
Yes	46	24,73	24,21
No	140	75,27	73,68
Total	186	100,00	97,89
Is FGM a religious obligation?			
Yes	31	16,67	16,32
No	155	83,33	81,58
Total	186	100,00	97,89
Is virginity possible with FGM?			
Yes	42	22,46	22,11
No	145	77,54	76,32
Total	187	100,00	98,42
Would you circumcise your daughter?			
Yes	55	29,73	28,95
No	130	70,27	68,42
Total	185	100,00	97,37
What is your spouse's opinion about FGM?			
Supports	16	14,81	8,42
No idea	40	37,04	21,05
Opposed	52	48,15	27,37
Total	108	100,00	56,84
Have you changed your mind about FGM after coming to Türkiye?			
Yes, it has changed	45	24,46	23,68
No, it has not changed	129	70,11	67,89
Other	10	5,43	5,26

Table 2. Continued

Total	184	100,00	96,84
Should FGM be stopped?			
Yes	141	75,40	74,21
No	46	24,60	24,21
Total	187	100,00	98,42
Would you marry a girl who was not female circumcised?			
Yes	145	79,23	76,32
No	38	20,77	20,00
Total	183	100,00	96,32
Does female circumcision have adverse effects?			
Yeah I think	118	71,52	62,11
No, I do not think	47	28,48	24,74
Total	165	100,00	86,84
What are the adverse effects?			
Maternal gynecological diseases increase	41	33,06	21,58
Mother's psychology suffers	53	42,74	27,89
A woman cannot enjoy sexual intercourse	12	9,68	6,32
A woman cannot please her husband	18	14,52	9,47
Total	124	100,00	65,26
Does FGM affect saturation quality?			
Yes	106	66,25	55,79
No	50	31,25	26,32
I do not know	4	2,50	2,11
Total	160	100,00	84,21
How does FGM affect sexual satisfaction?			
Reduces	97	52,43	51,05
Constant	27	14,59	14,21
No idea	61	32,97	32,11
Total	185	100,00	97,37
Reduces + No Idea	158	85,41	83,16

Fear, pain and anxiety during FGM were investigated. It was determined how the participants' life and health status changed after FGM. What kind of obstetric-health problems developed the problems of adolescence were revealed. It was observed that the effects of FGM on women's health, birth and postpartum care could be life-threatening. The pain of the first menstrual bleeding and the first sexual intercourse were questioned. It was determined whether infibulation was performed and whether deinfibulation was applied at birth. The results are clearly given in Table 3.

Table 3. FGM effects and attitudes of women

	n	**%**	**% of total**
Severe pain at first menstrual bleeding after FGM?			
No	82	46,33	43,16
Yes	95	53,67	50,00
Total	177	100,00	93,16
Was the first sex after FGM painful?			
Yes	95	86,36	50,00
No	15	13,64	7,89
Total	110	100,00	57,89

Table 3. Continued

Did you have a normal birth after FGM?			
Yes	77	73,33	40,53
No	28	26,67	14,74
Unanswered	85		44,74
Total	105	100,00	55,26
Has the incision been made?			
Yes	69	92,00	36,32
No	6	8,00	3,16
Unanswered	115		60,53
Total	75	100,00	39,47
Abortion (miscarriage)			
Yes	12	13,48	6,32
No	77	86,52	40,53
Total	89	100,00	46,84
How many curettage?			
1 time	10	83,33	5,26
2 times	2	16,67	1,05
Total	12	100,00	6,32
How many miscarriages did you have?			
1 time	21	58,33	11,05
2 times	12	33,33	6,32
3 times	3	8,33	1,58
Total	36	100,00	18,95
How many children do you have?			
1--2	31	34,83	16,32
3--5	29	32,58	15,26
6--8	21	23,60	11,05
9 and up	8	8,99	4,21
Total	89	100,00	46,84
Insurance			
Available	28	15,30	14,74
None	155	84,70	81,58
Total	183	100,00	96,32
Monthly income			
200--300	66	35,68	34,74
300-500	45	24,32	23,68
500-1000	59	31,89	31,05
1000-3000	14	7,57	7,37
5000 and above	1	0,54	0,53
Total	185	100,00	97,37
Residential Property			
Homeowner	24	12,83	12,63
Rent	155	82,89	81,58
Dormitory	8	4,28	4,21
Total	187	100,00	98,42

4. Conclusions and discussion

Our research revealed that FGM is common in infants and children aged 0-6. FGM was performed by first-degree relatives such as mothers, grandmothers, aunts, and paternal aunts by holding or tying their hands and feet. No medical intervention was performed for acute pain and bleeding after FGM. It was stated that the bleeding could not be

stopped, so the legs were cross-tied for hours, sometimes days (60.87%). No anaesthetic drugs or hemostatic agents were used during FGM. The clitoris, which is the most sensitive tissue, including the pudendal nerve, was removed together with the vaginal lips. Then, the wound lips were stitched, leaving a tiny opening. Severe pain was felt during FGM. Tissue swelling, inflammation and infection are common in FGM. The WHO reports that pain shock and deaths occurred in infants aged 0-2. Tissue healing took months. There are cases where the cutting of the pudendal nerve arms caused spinal cord injury and gait disturbance. Almost all women who had FGM could not experience a normal adolescence. Births were difficult. It usually requires surgical intervention. Women's first bleeding was mostly very painful (53.67%) and severe. It was determined that their first intercourse with their partners was extremely painful (86.36%) and like a nightmare. It was determined that all married women could not enjoy sexual intercourse (66.25%) [14]. On the other hand, it was determined that FGM, which is mythologized in traditional culture, has a severe place in women's minds. Adherence to FGM has become an unconditional and unquestioned belief. Despite all the violence experienced during and after FGM, a significant number of women believe that this tradition is necessary. The rate of those who stated that it reduces sexual power is 56.43%, and the rate of those who think that it should not be discussed traditionally is 32.97%. Those who did not express the opinion that it dulls sexuality and reduces power is 85.41%. They were made to believe that women do not have sexual power and should only perform the function of reproduction. In a similar study, it was reported that the probability of dyspareunia in women exposed to FGM is more than 52%. It has been shown that sexual desire decreases more than two-fold due to pain and complications, and sexual satisfaction cannot be achieved. [15].

15.46% of women lost at least one baby during childbirth. Although the reasons are not explained, FGM is one of the leading causes of obstetric complications. FGM is a risk factor due to genitourinary infections, abscess formation, septicemia and HIV. In our study, FGM complications were higher than 1/3. 3/5 of the complications were found to be chronic[16]. Similarly, the long-term complications of FGM were investigated in 28,000 women. It was shown that postpartum haemorrhage, perineal trauma tears and cesarean section tendency increased. It was reported to cause perinatal death in 1-2%. Chronic complications of women with FGM are remarkable. They are listed as prolonged labour, perineal rupture, cesarean section, episiotomy, birth requiring surgery, bleeding and difficulty in birth.[4]. Most Somali women in Türkiye (97.5%) have undergone FGM. This rate is similar to studies conducted by the WHO and other international civil or official organizations. The acute effects of FGM, which are pain, excessive menstrual bleeding, painful intercourse and lack of pleasure from sexual intercourse, are evident. Post-traumatic psychological problems, back pain, shame and hatred of parents or relatives who perform FGM are evident. Post-traumatic psychological state disorder from childhood is present. Recurrent, persistent and challenging to-treat groin infections are evident.

In this scientific study, the rate of women who got married as children and got pregnant before the age of 18 was determined as 21.05%. One in every 5 Somali women encountered in Türkiye got married as children and got pregnant. Women got pregnant more than five times. This situation is clear evidence of the population density of 7 and above. 17.7% of women had at least one abortion. Although these abortions are not the subject of our scientific research, it is clear that FGM plays an active role here. Stillbirth is 5.1%, and abortion is 6.6%. These rates are not definitive in FGM studies. Women have experienced long-term painful complications in FGM tissues and other limbs. It is definite that FGM reduces sexual performance, creates sexual reluctance and reduces sexual potency. Long-term effects of FGM, such as depression, anxiety and post-traumatic stress disorder, are not the subject of this scientific study. However, there are similar results in previous scientific studies [17]. Somali women think that FGM has adverse effects on their family members by 71.52%. 83.33% of women know that FGM is not a religious obligation. 77.54% stated that their virginity cannot be protected with FGM. They believe that FGM is a crime and violence against women (67.60%). 75.40% of women demand the abolition of circumcision. It can be said that women in Somali families in Türkiye are proportionally ahead of men in terms of education [18]. In our analysis, the prevalence of women who have undergone FGM is 96.32%, while only 2.63% have not undergone FGM. The countries with the highest practice rates are Somalia, Egypt, Sudan, Mali, Djibouti, Eritrea, Sierra Leone and Guinea. The prevalence of FGM in these countries is over 70%. [19]. The most common type of FGM in Somalia is type-3. This is the most harmful type of practice. 99.7% of women have been subjected to this practice [20]. In a scientific study conducted by Abdulcadir et al. on Somali women who migrated to Switzerland, this rate was stated as 60.7%. This difference is probably due to the comparison with those living in Somalia. This rate was different for Somali women living in Türkiye.[21]. Type 3 was found to be in 41.53% of Somali women living in Türkiye ($p<0.05$). In a study conducted in 2002 on FGM practices in maternity hospitals in Mogadishu, the capital of Somalia, mostly type-2 and type-3 were detected. This result supports our scientific research. [11]. This scientific research is very close to other research (97.89%), but a significant difference was found. Somalia has a very sharp culture of traditional blunts. Therefore, a 1% difference can be considered very important. A decrease of 0.908% was clearly detected in this scientific research.

Type-3, Pharaoh circumcision is expressed as 60.7% in studies. It is obvious that Somali society gradually reduced this practice in favour of type-1-2 due to severe physiological problems experienced during or after the Pharaoh circumcision practice. In addition, most of the families of women who come to Türkiye for education purposes are university graduates. They probably have a good awareness of FGM. Although no distinction was made between type 1 and type 3 in our research, Pharaoh circumcision, namely type-3, is proportionally 41.40%. Sunni type circumcision

(type-1-2) is 58.60%. The increase in type 1-2 circumcision is the main reason for the decrease in Pharaoh-type circumcision. Social change, communication, cultural change and integration seem to have been effective in this change. Most participants (84.24%) had this practice between the ages of 6-10. The age of the practice is usually not clearly stated in the literature. The WHO reports that girls are mostly subjected to FGM at any time from birth to age 15. In the Somali tradition, FGM is applied to girls between the ages of 4-8. Some studies state the age range as 6-10 or 4-8 [22]. In our scientific research, the FGM rate between the ages of 0-5 is 8.7%. In contrast, the FGM rate between the ages of 6-10 is 84.78%. The FGM rate is low in the 11-14 age group (5.98%) [23] Although the age range for FGM is 4-8 years, the FGM rate between the ages of 6-10 is high and statistically highly significant (p=0.01). 90% of Somali women living in Türkiye have had FGM between the ages of 4-10. During FGM, the tissues innervating the clitoris are injured or cut. The pudendal nerve, dorsal artery of the clitoris, superficial dorsal veins, clitorial fascia and venules are seriously damaged. The subcutaneous membrane layer of the labia minora, the arteriole, venule, pudendal, perineal nerve and surrounding tissues are injured. Pudendal and perineal nerve damage causes severe and shock pain. Cutting the small arteries causes severe bleeding. Venules and tissue damage causes serous leakage, prolonged bleeding, inflammation and necrosis. In this scientific study, more than half of the participants stated that their feet were crossed after FGM. It was stated that the first menstrual bleeding was severely painful and that there was pain during sexual intercourse. During FGM (cutting the head of the clitoris), the pudendal nerve and its branches come out. Regeneration occurs with the bleeding in the tissue. This regeneration forms a neuroma in the form of a solid and rough tumour. Neuroma puts severe pressure on the spinal cord. As a result, it causes chronic pain that lasts a lifetime. This pain causes gait disorders and deformities due to the pressure [3]. As a result, severe psychiatric conditions such as post-traumatic stress disorder, anxiety, panic, nightmares, insecurity, IQ deficiency and memory loss occur. [18].

When acute problems resulting from FGM are combined with chronic pain and psychiatric problems, maternal function is compromised. Since FGM is seen as a family and private matter, it will probably never be possible to obtain accurate data. Apart from acute and chronic health problems that occur after obstetric examinations, FGM continues to remain a mystery. There is no precise official data from any country regarding FGM. There are laws related to the prohibition and restriction of FGM. These legal texts are more legal texts than the health problems of FGM. Difficulties in childbirth, prolonged birth, cesarean section, episiotomy, recto-vaginal fistulas, difficulty in defecation and urination problems are common in women who have FGM. Although a connection between sexually transmitted diseases and mutilation has not been established, FGM increases the tendency to sexually transmitted diseases because there is no mention of hygiene or medical precautions in FGM practice. FGM is less common in urban areas. It is more common in rural and nomadic populations. Mortality rates resulting from FGM performed in rural areas are unknown. In this uncertainty, it can be understood that babies circumcised during infancy died from blood loss and pain shock.

In this scientific study, the rate of severe pain (86.36%) during the first menstrual period and the first sexual intercourse in those exposed to FGM is high. Acute emergencies and post-traumatic chronic disorders are covered with the introverted steel armour of social tradition and culture. In Somali women in Türkiye, almost one in every five women got married when they were children. According to traditional culture, having children as soon as marriage is desired. Therefore, this 1/5 rate of women gave birth or had an abortion at an early age. 15.46% of those exposed to FGM lost a baby during birth. The infant mortality rate in Somalia decreased from 44.9% to 36.8% between 2001 and 2020. However, FGM is one of the leading causes of infant deaths at birth. Risks such as blood loss, episiotomy, perineal tear risk, prolonged birth, cesarean section, stillbirth, need for resuscitation, and neonatal death in FGM are known anonymous facts.[24].

In conclusion, in this scientific research, 92% of the participants had an episiotomy at birth. The infant mortality rate is 15.46%. 63.68% of the participants believe that circumcision is not a correct traditional culture but cruelty-torture. Unfortunately, 28.94% think that FGM is reasonable and necessary for the future of their daughters. The main reasons for continuing circumcision are customs, culture 53.68%, religion 12.11%, marriage obligation 1.05% and tradition (26.84%). In some studies, [22] 81% of women do not want their daughters to be circumcised. 18% reported that they wish to FGM for their daughters. This scientific study has proven that the demand for FGM for their daughters is higher. Women believe that it is tough to end the practice. Instead, they agree that type 3 should be abandoned. They tend to have their daughters undergo type 1 and type 2 FGM. In this scientific study, Somali women support the continuation of type 1-2 FGM instead of abandoning the practice of FGM. They believe that their daughters' virginity will be secured and their future will be positively affected. Because a significant portion of them (58.60%) think that the tendency of Somali society towards FGM has surpassed traditional culture. İt has been determined that the FGM tradition has become a norm of belief among Somali women. Young women think differently than older adults in terms of belief in FGM. Women over the age of 45 (20.77%) believe that FGM is a feminine requirement that should be implemented. The younger generation (<45) believes this tradition infringes on personal rights in the modern world. It is widely accepted that it is possible to marry a girl who has not undergone FGM (79.23%). However, unfortunately, traditions are stabbing individual freedoms like a double-edged dagger in Somali women. The effectiveness of WHO's studies on the abandonment of FGM has been 71.36% effective. The rate of those who oppose is 18.38%. It is clear that WHO's studies on Africa are meaningful and productive. It has undoubtedly provided a sociological orientation towards integration with the Universal Culture. Protecting new generations from FGM, which is done secretly, can be possible with education and communication. The severe humiliation, shame, and parents' attention to the most valuable body

parts in FGM are remembered as a lifelong psychiatric syndrome. The crossing of the feet for a few hours to 8-10 days to stop the bleeding is the most crucial cause of psychiatric trauma (60.87%). The painful-frightening nightmare of FGM causes extremely painful menstruation after menarche (73.33%). It causes sexual intercourse in marriage to turn into a terrible trauma (86.36%). The situation is awful and more severe in <18 marriages. According to the literature, a proportional decrease is seen. However, this decreasing change is not statistically significant ($p>0.05$). The truth is that 6-7 out of every 10 Somali women we see probably have obstetric-based severe psychiatric problems. 83.33% of Somali women living in Türkiye do not see FGM as a religious requirement ($p<0.05$). The rate of those who think it is a religious requirement is insufficient. Traditional culture surrounds privacy with sharp boundaries. Therefore, it can be concluded that there is a cultural blindness within the family. They believe that female circumcision does not protect the virginity of girls (77.54%). Even more interestingly, the rate of those who define FGM as violence is 67.60%. When combined with those who silently approve this but are hesitant to express it, this rate increases to 69.74% ($p<0.05$).

Dissemination of health information through voluntary midwifery services is essential for the reduction of FGM. Perhaps if it were taught as an introductory course or curriculum in schools, it could be foreseen that this tragic trauma would end.

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Ethical approved

Karabuk University, Non-Interventional Clinical Research Ethics Committee, dated 15.12.2021, E-77192459-050.99-88241, number 2021/777.

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Second record of *Panorpa tatvana ressli* Willmann, 1975 (Mecoptera: Panorpidae) in Bolu province, West Black Sea Region, Türkiye

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Abstract

This study documents the second occurrence of *Panorpa tatvana ressli* Willmann, 1975, a subspecies in the Panorpidae family, in Bolu province within the West Black Sea Region of Türkiye. Nearly five decades after its initial sighting, this reaffirms the enduring presence of *Panorpa tatvana ressli* in the area. This information provides a valuable contribution to the knowledge of the Mecoptera fauna of Turkey, and it also highlights the importance of continuous monitoring for understanding species continuity and ecosystem changes.

Keywords: Mecoptera, *Panorpa tatvana ressli,* Panorpidae, Scorpionfly, Türkiye

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Panorpa tatvana reslii' nin Bolu'daki (Türkiye) ikinci kaydı

Özet

Bu çalışma, Türkiye'nin Batı Karadeniz Bölgesi'nde yer alan Bolu'da, Panorpidae familyasından bir alttür olan *Panorpa tatvana ressli* Willmann, 1975'nin varlığını ikinci kez ortaya koymaktadır. İlk kaydından neredeyse elli yıl sonra *Panorpa tatvana ressli*'nin bölgede kalıcı varlığı yeniden teyit edilmiştir. Çalışma, Türkiye'nin Mecoptera faunası bilgisine değerli bir katkı sağlamakta ve aynı zamanda tür devamlılığının ve ekosistem değişikliklerinin anlaşılmasında sürekli izlemenin önemini vurgulamaktadır.

Anahtar kelimeler: Mecoptera, *Panorpa tatvana ressli,* Panorpidae, Akrep sineği, Türkiye

1. Introduction

Mecoptera stands out among other insect orders due to the presence of a rostrum housing mouthparts at its apex, accompanied by fore- and hind wings characterized by comparable size and venation. The family Panorpidae holds the distinction of being the most extensive within Mecoptera, encompassing 500 species distributed among one extinct and eight extant genera. Its primary habitat is predominantly in the Northern Hemisphere [1]. Only 13 Panorpa species have been identified in Türkiye [2], yet recent studies from neighboring regions e.g., in the Caucasus [3], in the Balkans [4] highlight the dynamic nature of Panorpidae distributions and the need for ongoing targeted surveys. Wilmann reported *Panorpa tatvana ressli* for the first time in 1975, especially in Abant (Bolu). The same author reported the distribution areas of this subspecies as Kızılcahamam (Ankara), Bolkar Mountains (Çamlıyayla, Mersin), Ilgaz Mountains (Kastamonu), Bozdağlar (Izmir, along Manisa) [5].

Panorpa species prefer cool higher elevations where morning mist moistens the vegetation [6]. Adults of Panorpidae species exhibit a diverse feeding behavior consuming a range of substrates such as decaying animal and plant matter, as well as deceased insects; certain species among them are phytophagous [7]. However, some researchers have reported that Panorpidae species also feed on flowering plants [7-10].

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Research on the family Panorpidae in Türkiye is scarce, likely due to two factors: Their lack of economic importance as pests or beneficial organisms to date, and insufficient reference materials for species identification, have contributed to this research gap. Analyzing habitat distribution by examining the relationship between habitat variables and the distribution and habitat preferences of animal species is critical for understanding their ecological roles. Information on the presence, abundance, distribution, and feeding status of animals within an area can be inferred from the condition and quality of the habitat. Through habitat status, anticipated positive or negative changes in a species or population, as well as the consequent impact on the habitat itself, can be assessed, enabling timely intervention if necessary [11].

New studies on rarely recorded species like *P. tatvana ressli* are essential to update regional biodiversity knowledge and renew scientific interest in this overlooked taxon. This study presents the second documented occurrence of Panorpa tatvana ressli in the Western Black Sea region. This finding not only verifies the presence of the subspecies in the specified geographical area but also attests to the establishment of a native population within the region. The identification of an indigenous population of P. tatvana ressli in the Western Black Sea region contributes significantly to our understanding of the subspecies' distribution and ecological dynamics in this particular geographical context.

2. Material and Methods

Panorpa tatvana ressli was collected Bolu, Western Black Sea, Türkiye. The samples were collected with the help of a sweep netting from the slopes of the mountain, which are rich in trees and shrubs (1005 m, N 40°41.27', E 31°40.24'). The collection of samples took place between July and October, with field visits conducted every 15 days. In the sampled area, coniferous trees such as pine and fir dominate the higher elevations, while deciduous species like oak, beech, and hornbeam are prevalent at mid and lower altitudes. Dense shrubs, herbaceous plants, and ferns populate the forest understory, providing habitat for various small animals and insects. The confirmation of identification was undertaken by Rainer WILLMANN (Germany) through a detailed examination of the male genitalia, with a specific focus on relevant paramers.

3. Results

Scorpionflies, or as they are often referred to, Mecoptera, make up an order that currently has a very low number of species and individuals. Their small numbers and rather secretive lifestyles where the reasons why very little research had been conducted on this group for a long time. Scorpionflies were first mentioned by Aristotle, but it was only in the 17th century that they began to be studied and described. However, all these descriptions were fragmented and unsystematic. In 1921, the first monograph on this group was published by Esben-Petersen [12]. The species of the family Panorpidae in Europe and America belong to the same genus and do not exhibit particularly sharp species differences, suggesting that they settled in relatively recent geological times. The taxonomy of modern representatives of this order is primarily based on morphological differences between the abdomens of males and females. Wing characteristics serve only as auxiliary traits [13].

The second record of *Panorpa tatvana ressli*, which was described a new subspecies for science by Rainer WILLMANN in 1975, and photographs of the subspecies are given below.

***Panorpa tatvana ressli* Willmann, 1975**

Diagnosis: The Panorpidae (Insecta: Mecoptera) showcases members with an orthognathous head, featuring uniform antennae and three ocelli on its frontal region along with compound eyes, contributes to the distinctive appearance of *Panorpa* species (Figure 1, 2, 5). The elongated facial structure, a result of modified 'beak' shaped jaws formed by the clypeus and labrum, is a notable characteristic. In males, the terminalia exhibit remarkable development, adopting a spherical morphology akin to the post-abdominal segment of scorpions. This unique feature, defining the order as 'scorpion flies,' encompasses gonopods and a distinct copulatory organ, typically characterized by two cerci in the tenth row (Figure 3). The copulatory organ's hypovalves, crucial in species determination, facilitate the mating structure's grip during copulation, ensuring contact with the spermatheca duct. Females, on the other hand, possess an ovipositor terminating in two cerci (Figure 4) [7, 10, 14-17].

Material examined: Türkiye, Bolu, 1005 m, N 40°41.27', E 31°40.24', 1 ♀, 1 ♂, 26.VII.2023 (leg. F. N. Elma).

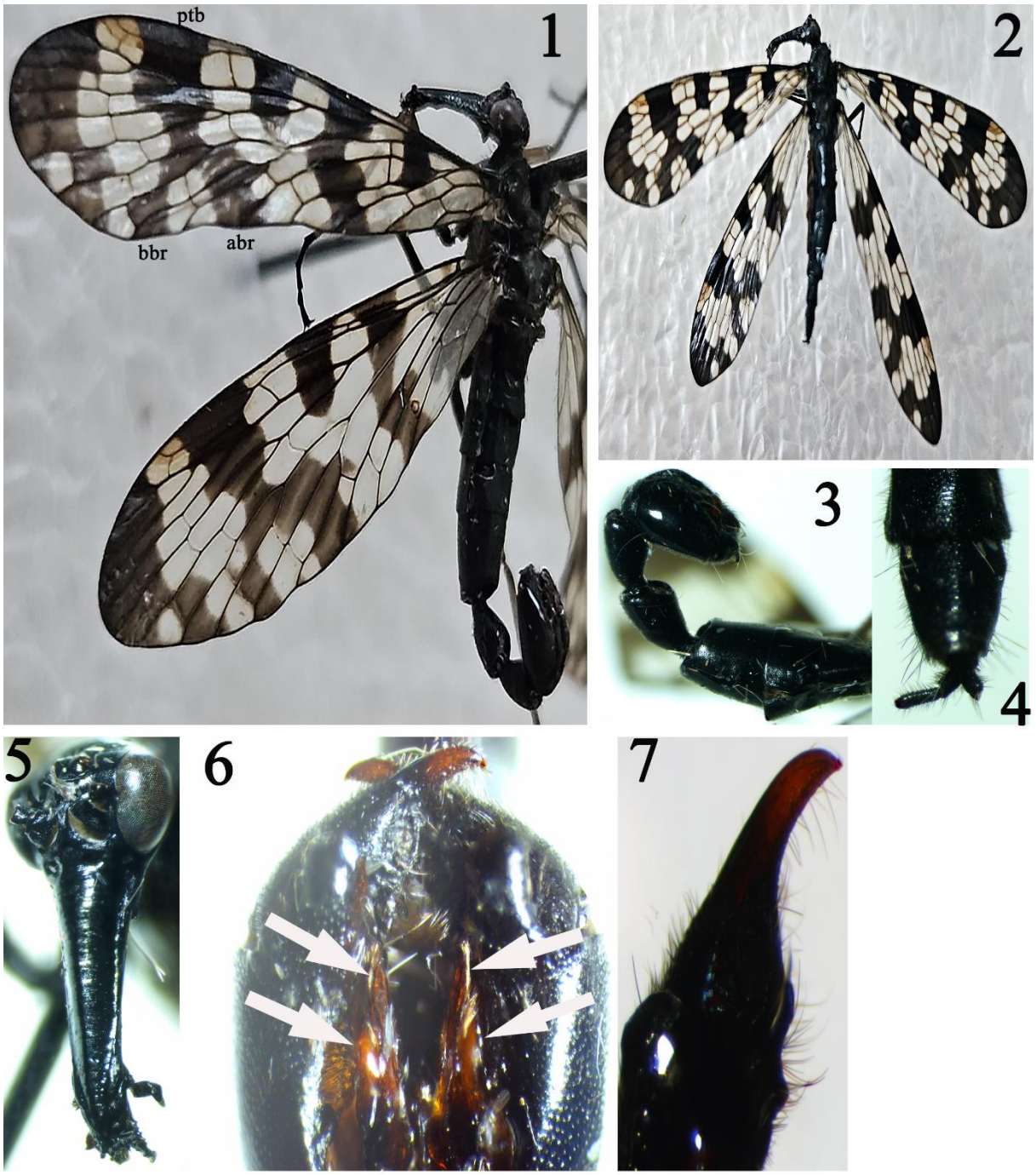

Figure 1–7. *Panorpa tatvana ressli* Willmann. (1) male left wings in dorsal view (ptb: pterostigma band, abr: apical branch, bbr: basal branch); (2) female habitus in dorsal view; (3) male terminal abdomen V–IX in lateral view; (4) female abdominal segments IX–XI in ventral view and cercus; (5) male head in frontal view; (6) male genital bulb in ventral view, white arrows indicated hypovalve; (7) male gonostylus in ventral view.

4. Conclusions and discussion

The second detection of *Panorpa tatvana ressli* Willmann, 1975 Mecoptera: Panorpidae) in the Western Black Sea Region (Bolu) not only contributes significantly to the in-depth understanding of the region's biodiversity and ecosystems but also introduces a novel perspective on the distribution of this species in Türkiye. Despite its inclusion within the *Panorpa aspoecki* species group, comprising seven known species and ten subspecies, including *P. tatvana tatvana* Willmann, 1974 found in the European part of Türkiye [18], the lack of specific identification keys poses challenges for future research. These keys, essential for distinguishing between closely related species, necessitate referencing Willmann's earlier works for accurate classification. However, future research efforts should aim to establish updated and comprehensive identification tools, focusing on both morphological traits and molecular markers to aid species delimitation.

Despite relying on the shape of the sub genital plate (sclerite 9) as a determining factor for *Panorpa* species, variations exist among European species and most Anatolian members of the *aspoecki* group. While some display a caudal recess in their sub genital plate, this feature remains species-specific, absent in others or showing weaker indentations, serving as individual distinctions. The distinct characteristics of the *aspoecki* group include a completely black body coloration, well-defined wing color patterns, and distinct male genitalia [19]. Given the current reliance on morphological features, exploring molecular phylogenetics offers promising avenues for future studies. By utilizing techniques such as mitochondrial DNA sequencing (e.g., mtCOI gene), researchers could unravel the evolutionary relationships between *Panorpa tatvana ressli* and other species within the *aspoecki* group, potentially revealing cryptic diversity or more precise species boundaries. Molecular studies could also provide insights into the species' biogeographical history and dispersal patterns across Anatolia and neighboring regions.

The differentiation and identification of the species primarily rely on their terminal morphological characteristics, particularly the localized shapes of the hypovalves (Figure 6). The entire body and wing markings display a black to black-brown hue. Notably extensive, the wing marks consist of a basal band that doesn't extend to the wing's rear edge. The apical branch (abr) is linked to the basal branch (bbr) of the pterostigma band (ptb). The male genitalia exhibit a broadly oval shape. The hypovalvae barely reach the midpoint of the gonopods, excluding their terminal scales. Adjacent to the terminal scales, each hypovalvae showcases a distinct, narrow longitudinal groove, continuing caudally beyond their base. The basal segment of the ventral parameres is broad and shield-shaped, with a slender gonostylus (Figure 7).

As of current records, *P. tatvana ressli* remains reported solely from its type locality in Türkiye, , specifically Bolu, Abant lake [19]. This paper presents the second documented instance of this subspecies in Türkiye. This information makes a significant contribution to Turkey's Mecoptera fauna and provides information on the continued continuity of the species.

In conclusion, the detection of *P. tatvana ressli* in Bolu significantly enhances our knowledge of the species' distribution and underscores the importance of continued research in the region. Future studies focusing on molecular phylogenetics, ecological interactions, and broader biogeographical comparisons will not only clarify the species' place within the *aspoecki* group but also contribute to a more profound understanding of Mecoptera diversity in Türkiye and beyond.

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Analysis of cytochrome *c* oxidase subunit I gene of *Canis lupus* in Türkiye

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Abstract

This study is based on the analysis of the cytochrome c oxidase subunit I gene of 20 grey wolf samples obtained from different regions of Türkiye between 2014-2018. The results from this study were compared with sequences from *Canis lupus* records from other localities available in GenBank. This comparison showed that *Canis lupus* in Türkiye originates from the same genetic source as the *Canis lupus* sequences in GenBank with a very high degree of similarity in sequencing (97-100%).

Keywords: Mitochondrial DNA, cytochrome *c* oxidase, gray wolf, Türkiye

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Türkiye'deki *Canis lupus*'un sitokrom oksidaz subunit I gen analizleri

Özet

Bu çalışma, 2014-2018 yılları arasında Türkiye'nin farklı bölgelerinden elde edilen 20 adet gri kurt örneğinin sitokrom c oksidaz subunit I geninin analizine dayanmaktadır. Bu çalışmadan elde edilen sonuçlar, GenBank'ta bulunan diğer bölgelerdeki *Canis lupus* kayıtlarından elde edilen dizilerle karşılaştırıldı. Bu karşılaştırmada, Türkiye'deki *Canis lupus*'un Gen Bankası'ndaki *Canis lupus* dizileriyle aynı genetik kaynaktan geldiği ve dizilim açısından çok yüksek derecede benzerlik gösterdiği (%97-100) kaydedildi.

Anahtar kelimeler: Mitokondriyal DNA, sitokrom c oksidaz, gri kurt, Türkiye

1-Introduction

With rapid developments in the field of molecular biology in the last fifty years, interspecific and intraspecific genetic relationships are increasingly examined. Mitochondrial DNA is frequently used to examine these relationships due to the reliability of gene sequencing [2]. Because mtDNA is inherited only maternally and there is no recombination of mtDNA, this has led to its intensive use in population genetics, phylogenetics, and studies of intraspecific and interspecific genetic similarity and difference [1].

Mitochondrial genome size in animals is between 16,300-17,000 bp, and the genes in the mitochondrial genome and the sequence of these genes have been very well preserved during evolution [12]. The main reason for choosing the COI gene as the standard barcode gene was its significant discrimination power for more than one species [3]. The first to apply this DNA barcode, stated that the Cytochrome c Oxidase Subunit I (COI) gene can distinguish different species at the molecular level and that this gene is the appropriate DNA barcode for the Animal Kingdom [4].

In a global study examining the phylogeography and population history of gray wolves based on mitochondrial control region sequences, two Turkish gray wolves of unknown origin were included. The analysis revealed that both samples shared a single haplotype, which is identified as the most widely distributed haplotype in the dataset. This haplotype is common across multiple regions, including Portugal, Croatia, Greece, Sweden, and Western Russia.

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Additionally, the Turkish samples were found to cluster within the same phylogenetic group as wolves from Asia, Europe, and the Middle East [11].

In a study aimed at assessing the genetic diversity and elucidating the phylogenetic relationships of gray wolves in Türkiye, 12 wolf samples from central and eastern Anatolia were analyzed. The study identified seven D-loop haplotypes (332 bp), and comparison of these sequences with those recorded in GenBank from Eurasian wolves revealed that two haplotypes were unique to Turkish wolves. The findings also indicated a high level of genetic variation within the wolf populations in Türkiye [5].

Studies based on standard molecular markers, conducted on small samples of gray wolf populations in Türkiye, have reported high genetic variation within these populations. However, it has also been noted that the available genetic data on the species remain limited and insufficient for accurately assessing and monitoring the current and future status of gray wolves in Türkiye [8].

This study aimed to determine the Cytochrome c Oxidase Subunit I gene sequence of grey wolf samples obtained from Türkiye and to compare them with data from the same species in GenBank.

2-Material and Method

2.1. Ethical Scope

For this study a research permit was obtained from the TR Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks (dated 05.04.2016 and numbered 72784983-488.04-75301). In addition, a 5-year cooperation protocol was signed between the same General Directorate and Kırıkkale University Faculty of Arts and Sciences on 15th September 2015.

2.2. Material

Tissues of 20 grey wolves (*C. lupus*) and one dog (*C.familiaris*) were used in the study. Scientific and ethical rules regarding animal rights were complied with in the sampling. All of the samples were taken from dead animals. Sampling from ear cartilage tissues material was placed in Eppendorf tubes, and numbered and stored in a deep freezer at -20˚C until use.

2.3. Oligonucleotide primers

In this study the primer sets shown in the table below were synthesised for the detection of the Cytochrome c Oxidase Subunit I gene [7] (Table 1). Primers were diluted at 100 pm/µl as the storage dilution and 20 pm/µl as the working dilution and stored at -20˚C.

Table 1. Primers and DNA sequences used in the molecular analyses [7]

Primer	DNA Sequence
U_{COI}	(5' - CAC TGC CTT GAG CCT CCT CAT C -3')
D_{COI}	(5' - GGG GAG GTT GCG TCC TGT AAT C -3')

2.4. Nucleic acid isolation

Nucleic Acid isolation was performed using a commercial kit following manufacturer's instructions (Tissue SV Mini, Cat #104-101, Lot #11717B03002, GeneAll Biotech, South Korea). The obtained nucleic acid concentration was measured with a spectrophotometer (Qubit Assay, Thermo Fisher Scientific, USA), and adjusted to 100 pg/µl using molecular-grade water. The obtained DNA was stored at -80°C until sequenced.

2.5. Polymerase Chain Reaction (PCR)

A commercial kit was used for PCR amplification (AmpMaster Kit, GeneAll, Cat no.544-010, Lot no. TM016G19001 GeneAll Biotech, Korea) following the method of Li et al. (2011). The reactions were carried out in a total volume of 20 µl (Table 2).

The results were evaluated on a UV transilluminator (Blook Led Transilluminator BK001) and photographs were taken. expected As an indication of positive PCR amplification, specific bands were expected to form in the approximately 1500 bp regions for the UCOI and DCOI primer pair. The results were recorded by in photographs.

Table 2. Composition of the reagent used in PCR

Reagent	Amount Used (µL)
MasterMix	10
Primer U_{COI} (10 pmol)	1
Primer D_{COI} (10 pmol)	1
dH_2O	4
DNA	4
Total Reaction Volume	20

2.6. DNA sequencing

DNA sequence analysis was done commercially. Positive PCR samples were ordered for duplex DNA sequence analysis. The primer sets used for PCR were used in all DNA sequence analyses. DNA sequence analysis results were received without processing.

2.7. Phylogenetic analyses

The obtained DNA sequences were analysed using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/page2.html) and matching sequences were performed with ClustalX1.83 software [10]. The Blast programme was used to compare the obtained DNA sequences (http://blast.ncbi.nlm.nih.gov/Blast).
In this study it was aimed to determine genetic similarities of the mitochondrial COI gene in the sampled Turkish wolf population compared with other grey wolves. The obtained DNA sequences were recorded in the GenBank database. Sequences previously obtained by other researchers (KU644671.1, KF661055, KU696410, KU696398) were also used to compare these DNA sequences. Dendrograms of these DNA sequences were prepared according to the "neighbor-joining" method.

3- Results

For this study molecular analysis of a total of 21 samples was performed and COI gene sequences of different lengths (1362-1416) were obtained. Appropriately sized gene regions were obtained in a total of 16 samples. PCR was unsuccessful in four samples (4, 11, 12, 22) and one sample did not have a sufficiently long sequence reads (26), so that sequences were unavailable for five of the samples.

Directories matched with the Bio-Edit program were checked with Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi). As a result of matching with the BioEdit program, a partial sequence of the COI gene with a length of 1400 bp was obtained for each useable sample (Table 4). The COI gene sequences obtained in this way were submitted to the “GenBank” (https://www.ncbi.nlm.nih.gov/genbank/) and the following Accession Numbers were obtained (Table 3)As a control for this gene, DNA sequences of wolf samples from different localities (Croatia, Iran, Israel, Canada) were obtained from GenBank and comparisons were made [6], [9].

Table 3. List of grey wolf specimens examined

Sample No	Locality	Accession Number	Reference
Wolf 2	Eskişehir	MK461173	This study
Wolf 3	Çankırı	MK461174	This study
Wolf 6	Bitlis	MK461175	This study
Wolf 7	Çankırı	MK461176	This study
Wolf 8	Bayburt	MK461163	This study
Wolf 9	Bayburt	MK461164	This study
Wolf 10	Bayburt	MK461162	This study
Wolf 11	Bayburt	MK461177	This study
Wolf 16	Çankırı	MK461165	This study
Wolf 17	Ankara	MK461166	This study
Wolf 19	Çankırı	MK461168	This study
Wolf 21	Ankara	MK461167	This study
Wolf 24	Çorum	MK461169	This study

Table 3. Continued

Wolf 25	Çankırı	MK461170	This study
Wolf 27	Çankırı	MK461171	This study
Wolf 28[1]	Çankırı	MK461172	This study
Iran2	Iran	KU644671.1	[6]
Israel2	Israel	KF661055	[9]
WCRO7	Croatia	KU696398	[6]
Can11	Canada	KU696410	[6]

[1] This specimen was identified as a dog according to the results of the DNA sequence analysis.

Table 4. DNA quantification results

Sample No	Concentration
2	22.4 µg/ml
8	11.2 µg/ml
17	6.03 µg/ml
4	0,73 µg/ml
11	1.84 µg/ml
12	0.35 µg/ml
22	0.19 µg/ml

The relationships between these samples are shown in a phylogenetic tree, both without outgroup determination (Figure 1) and with KU644671.1 [6] as an outgroup (Figure 2).

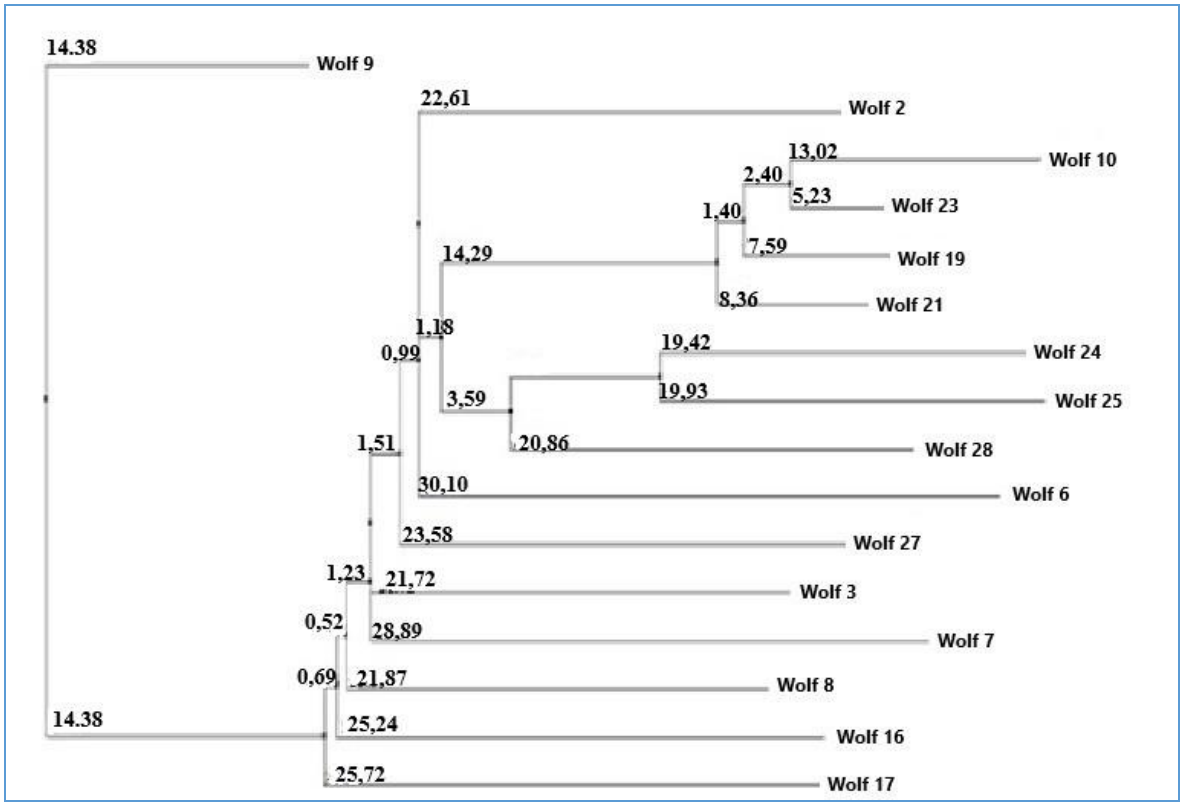

Figure 1. Phylogenetic analysis of samples (without outgroup determination)

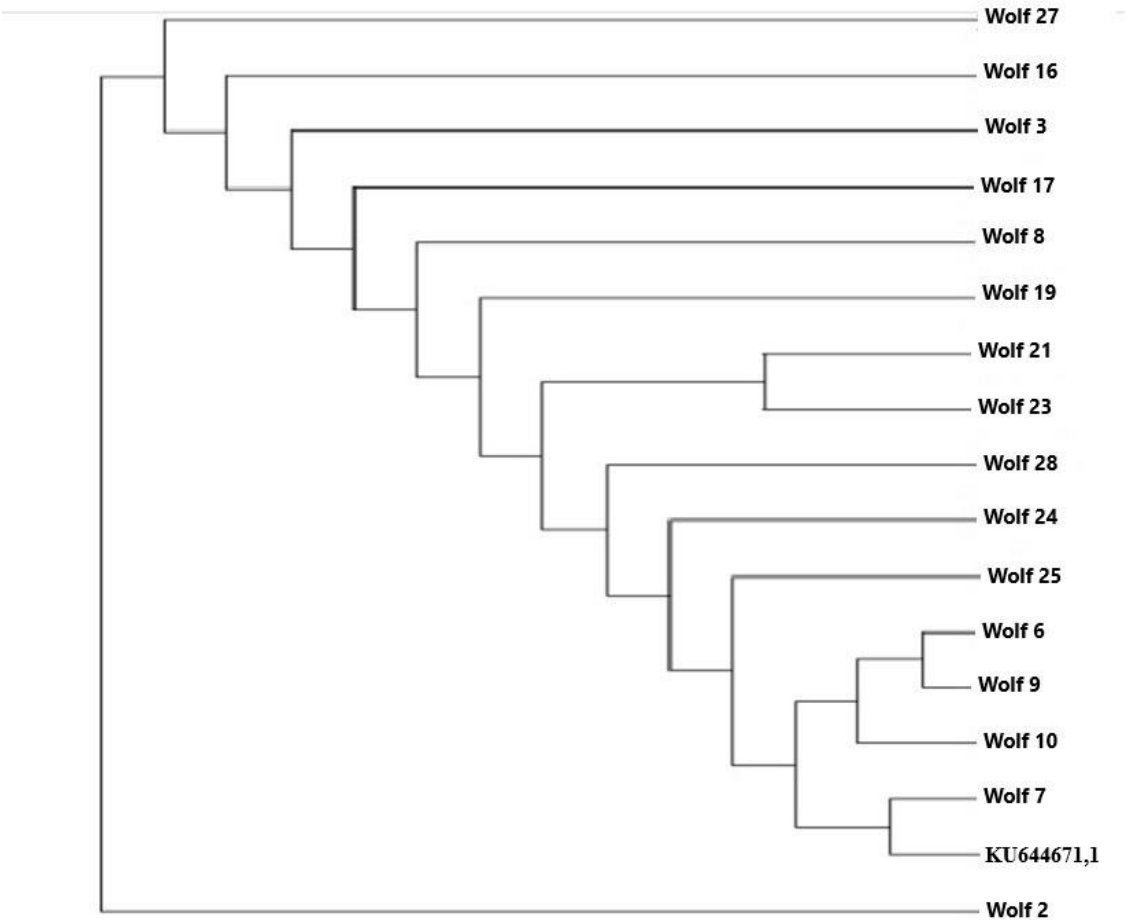

Figure 2. Phylogenetic analysis of the samples (by determining the outgroup)

4- Conclusions and Discussion

In the statistical analysis, the obtained DNA sequences are very similar to the wolf sequences registered in GenBank (97-100%). Again, there is sufficient data to be able to distinguish genetically from dogs. This study is unique in terms of determining the sequence the mitochondrial gene region (COI) and carry out an intraspecific phylogenetic analysis of a wild species in Türkiye.

Genetic information on the gray wolf population in Türkiye has been found to be highly limited and insufficient for effectively monitoring the current and future status of the species [8]. In this context, the contribution of additional genetic data from Türkiye, particularly by uploading the results of this study to the GenBank database, is of significant value. For future research, whole-genome sequencing of Turkish wolf samples is essential to enable more detailed evaluations and comparisons, enhancing our understanding of their genetic diversity and evolutionary history.

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Using remote sensing to map the occurrence of *Cistus salviifolius* L. (Cistaceae) in Armutlu, Yalova, Türkiye

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Abstract

By scientifically grounded nature management, the biodiversity of medicinal plants can be preserved, providing a foundation for the research and development of new therapeutics. Monitoring the condition and distribution of plant communities is one of the contemporary responsibilities of botanical resource research. *Cistus salviifolius* used to mange various ailments in both modern and traditional medicine. Sentinel-2 based on remote sensing techniques, offer promising alternatives to accurately detect, map and monitor the extent of plants. The aim of this study was to investigate the utility of Sentinel-2 data to detect and map *Cistus salviifolius* as a case study in Armutlu. The obtained results reveal the significance of the red-edge and shortwave infrared regions of the spectrum, as well as the inclusion of vegetation indices in the classification for *C. salviifolius* discrimination. Here, we demonstrate the potential of Sentinel-2 data for mapping of medicinal plants.

Keywords: remote sensing, Sentinel-2, *Cistus salviifolius*, classification, spectral signature

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Cistus salviifolius L. (Cistaceae) oluşumunun haritalandırılması için uzaktan algılamanın kullanılması, Armutlu, Yalova, Türkiye

Özet

Bilimsel temelli doğa yönetimiyle tıbbi bitkilerin biyolojik çeşitliliği korunabilir ve yeni tedavi yöntemlerinin araştırılması ve geliştirilmesi için bir temel oluşturulabilir. Bitki topluluklarının durumunu ve dağılımını izlemek, botanik kaynak araştırmasının çağdaş sorumluluklarından biridir. *Cistus salviifolius* hem modern hem de geleneksel tıpta çeşitli rahatsızlıkların tedavisinde kullanılır. Uzaktan algılama tekniklerine dayanan Sentinel-2, bitkilerin dağılımını doğru bir şekilde tespit etmek, haritalamak ve izlemek için umut verici alternatifler sunuyor. Bu çalışmanın amacı, Armutlu'da bir vaka çalışması olarak *Cistus salviifolius*'un tespit edilmesi ve haritalandırılmasında Sentinel-2 verilerinin kullanılabilirliğini araştırmaktır. Elde edilen bulgular, spektrumun kırmızı-kenar ve kısa dalga kızılötesi bölgelerinin öneminin yanı sıra bitki indekslerinin *C. salviifolius* ayrımcılığı sınıflandırmasına dahil edildiğini ortaya koyuyor. Burada Sentinel-2 verilerinin tıbbi bitkilerin haritalandırılmasındaki potansiyeli gösterilmiştir.

Anahtar kelimeler: uzaktan algılama, Sentinel-2, *Cistus salviifolius*, sınıflandırma, spektral imza

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1. Introduction

Due to the fact that the traditional methods for land mapping, especially the preparation of vegetation distribution maps on a regional scale, require detailed field investigations. In addition, allocating a budget and considerable time are required. In the last decade, with the advancement of imaging technologies and the availability of high resolution images, the use of remote sensing methods to produce spatial information for management and micro- and macro-planning has become a common procedure (Figure1). Remote sensing data for vegetation classification and mapping provides accurate results in a time- and cost-effective manner.

Figure 1: Combination of Spectral signatures belonging to different classes and sentinel-2 bands [1], with modification

The use of remote sensing technology to study phenomena on earth is based on different reflectance values of different chemical compounds. Receiving the reflected and emitted waves from the surface of the earth and converting these electromagnetic signals into information that can be used in computers is the basis for preparing these data. In simple words, remote sensing data are the result of measuring energy reflected from the Earth's surface at different wavelengths. The electromagnetic spectrum refers all possible frequencies of electromagnetic energy, which vary from very long wavelengths (such as electric waves) to very short wavelengths (gamma rays). Generally, the spectral behavior of phenomena refers to reflections that occur in the visible and infrared range. In general, electromagnetic waves in any form of the spectrum that we have come across in the types of waves have different characteristics such as how they are produced or radiated, how they interact with the environment, and how they are used. In fact, the electromagnetic spectrum is the frequency range of electromagnetic radiation which includes low radio frequencies to gamma rays. An object's temperature has a direct correlation with the quantity of electromagnetic radiation it emits. The higher the temperature of an object, the shorter its wavelength and the higher its frequency, and vice versa. The visible light includes a very small range of the electromagnetic spectrum, which is between the wavelengths of 380 to 740 nm. The invisible ranges are only detected and recorded by special sensors and cannot be seen by the naked eye [2].

Spectroscopy is one of the methods used to obtain scientific and practical information, using the interaction of electromagnetic energy and the subject of spectroscopy. Because the absorption of electromagnetic energy related to each element or chemical compound occurs a certain wavelength, recording objects surface reflection spectrum and examining their absorption characteristics, it is possible to be helpful in identifying elements and compounds that make up these surfaces. By knowing the spectral behaviors, we can study changes in phenomena that cannot be seen and extracted using the naked eye. The spectral response curve or the so-called spectral signature is a curve that displays the reflectance values of a material at different wavelengths. The behavior of this curve is directly related to the physical and chemical properties of the constituents of the material. As different materials have different components, the spectral response curve of each material is unique. By measuring these curves, we can identify different materials and compounds without expending too much effort or resources (time, money, and effort). One of the main and common

methods of identifying and purifying different materials is to create a spectral library and analyze their reflection spectra [3].

The spectral library is considered as a reference for identifying the spectral behavior of various phenomena on the earth's surface and distinguishes them from each other. A spectral library is an assortment of reflectance spectra, often obtained from materials prepared in the field or in a laboratory with compositons. Most researchers collect spectral library materials as part of a project and to facilitate the analysis of multispectral images from the project site. Several high-quality spectral libraries have been prepared for various materials such as minerals, plants and artificial materials, which help us in the spectral classification of images or can be used in the identification of targets for spectral analysis of images. One of the most basic applications of the spectral library is to extract the spectral behaviors of various phenomena. Suppose that we want to extract the spectral behavior of a healthy plant from a reliable reference and use it in our research. In this regard, the spectral library covering thousands of diverse phenomena is the best source from which information can be extracted [4-9]. A spectral signature can be defined as a unique pattern of wavelengths emitted by an object. Function of wavelength (e.g. features and temporal spatial variations, etc.) can be classified as spectral diversity. Each characteristics of electromagnetic radiation may depend on different times or different spectral bands. The measurement of these changes and their correlation with the known characteristics of an object provide the signature of the corresponding object. Knowledge of the polarization state of the reflected radiation plus the spectral signature of various objects in remote sensing adds another dimension to the analysis and interpretation of remote sensing data. These parameters are used in providing valuable data for object recognition and classification. Comprehending and analyzing a remote sensing image requires an awareness of spectral fingerprints. Spectral reflectance is the attribute that is utilized to measure these spectral characteristics. This represents the energy ratio as a function of wavelength between the incident and reflected energy [10].

In recent years, remote sensing and spectrometry data have been widely used in various feilds, especially for natural resource management and sustainable development. This technology has become very important due to providing the possibility of accessing useful information in a desired time frame using non-destructive, cheap and accurate methods. Among these data is the preparation of distribution maps for various important medicinal plants on both large scale and regional scales, which can be one of the important tools in planning and studies of these plants. The Cistaceae family includes the *Cistus* genus, which has 66 accepted species. The native range of this species is South Central Europe, Mediterranean to North-West Iran. It is a shrub and grows primarily in the subtropical biome [11,12]. One of the important species belonging to this genus is *C. salviifolius*, commonly known as Laden Otu (Turkish), Sage-leaf Rock Rose (English). This plant and related species used since ancient times in traditional medicine as antidiarrheic, sedative, expectorant, anti-wound and anti-microbial [13-15].

2. Materials and methods

2.1. Study site

The study area is located to the middle parts of the Armutlu Peninsula in the eastern Marmara region and its territory was investigated. This area geologically is overlain by sedimentary rocks and andesitic volcanic rocks of the Late Cretaceous-Early Tertiary. The investigated area is located near the middle part of the Armutlu Peninsula in the eastern Marmara region. Geologically the area is overlain by sedimentary rocks and andesitic volcanic rocks of the Late Cretaceous-Early Tertiary [16]. Armutlu climate as a subtropical climate is a transition between the Mediterranean climate and the Black Sea. In this province, which has a continental climate in some parts, the summers are dry and hot, and the winters are cool and rainy (Figure 2).

2.2. Plant materials

Field specimens of *C. salviifolius* were collected in Armutlu District, Yalova (40°31'35.7"N 28°47'23.3"E) in June 2022. Authenticated plants were deposited at Istanbul University Faculty of Pharmacy herbarium (voucher number: ISTE-118583) (Figure 3).

Figure 2. Location of the study area, Land-cover classification (S2B_MSIL2A_20230423T085559_N9999_R007_T35TPE_20231108T115522)

Figure 3. The photos of *C. salviifolius* by Ahmet Beyatlı

2.3. Satellite data processing

In this study, the Sentinel-2 satellite images were used, which is one of the most valuable free remote sensing images. This product is a surface reflection, and all necessary pre-processing has already been carried out. The Sentinel satellite is designed to improve missions such as surveying land cover changes, environmental and agricultural monitoring, forest management, and natural disaster management, etc. Sentinel 2 provides images with different spatial resolution, depending on its spectral band, images with a spatial resolution of 10, 20, and 60 meters are provided. This spatial resolution makes it widely used in various fields. The Sentinel-2 satellites are equipped with high-resolution multispectral cameras that record data in several electromagnetic spectrum bands, including visible wavelengths, short-wave infrared and infrared. This feature makes it possible to examine characteristics of plants that exist on the surface of earth with high precision (Figure 4).

Figure 4. The flow chart of research

To process images Snap software has been used. In the first step, we unify the data of different bands that are under different resolutions (Resampling) and then we rectify the image again in the global coordinate system. The

processed images required for preparing maps. Image processing steps performed before image classification. The commands used for this step are as follows:

Open Optical from the navigation bar at the top --> Open Processing --> Open S2 Resampling --> Given an Appropriate name to your S2 Resample Result --> Select the Main Imagery as the Input --> Open the Parameters Section from the Menu --> Set the Resolution to 10m --> Run.

The multispectral data of Sentinel-2 images provide information about the reflective behavior of various objects on Earth at 13 different bands. As mentioned earlier, different wavelengths of energy have unique behaviors when they hit the target (object). This difference in spectral behavior is called a spectral signature and is one of the important tools in identifying and distinguishing between different objects in the analysis of digital data of satellite images [17]. The study of spectral signatures has helped to identify different vegetation and non-vegetation classes to understand their spectral resolution, which in turn will give a clue for the final grouping of reflectance spectra during the classification of Sentinel-2 data with SNAP free software. Hence, spectral signature can be considered as one of the important tools in identifying different land covers and distinguishing them in remote sensing studies that deal with digital data. In standard reference-free data collection, field calibration and ground-based spectral measurements are usually performed using portable hand-held spectrometers [9]. Typically, environmental studies and agriculture employ this kind of spectrometer. For the purposes of managing and operating water resources, the acquired spectral fingerprints can be utilized for vegetation classification and mapping, ecosystem productivity, crop kind, and plant stress detection.

It is also possible to obtain the range of statistical changes for each group by studying the spectral data of different dates and generalize obtained information to classify unknown areas of land and vegetation. Image classification is the process of assigning land cover classes to pixels. These classes include the diversity of soil, water, and plants, etc. In Snap software, classification of vegetation and other land surface effects is done using supervised and unsupervised classification methods. Supervised classification involves identifying regions of specific spectral features for each land cover or land use group of interest to the analyst. In comparison, unsupervised image classification into spectral classes is based solely on natural groupings of image values. In addition, land use or vegetation classification is done along with some kind of ground truthing or reference data collection. Unsupervised classification is one of the most basic techniques. Since we don't need samples for unsupervised classification, it is an easy way to segment and understand an image. Indeed, this classification is not considered as an output. It's used only for the general knowledge of study area. In this method, we do not interfere in the classification process except for determining the number of classes, i.e. sampling. In this method, firstly clustering carried out based on the number of pixels and mathematical relations between them, and then each cluster is generalized into classes upon their characteristics. Then, we assign each cluster with a land cover class. By using Snap software, K-mean and ISODATA algorithms are used for image clustering.

After choosing the clustering algorithm [18], we identify the number of groups we want to create. For example, we can create 8, 20 or 42 clusters. Fewer clusters are more like pixels within groups. But more clusters increase the diversity within groups. Because spectral similarities between pixels prevent clusters from being correctly and easily separated from each other. In one of the most common strategies, the user determines the maximum possible number of clusters for an image. Accordingly, the software or computational systems define different statistical information for each class. For example, cluster centers are used in the determination of pixel characterization. Then, each pixel in the feature space that has the minimum distance from the center of the clusters is added to that cluster. At this stage, each pixel is considered a label for that class. Then the calculation process is repeated, and the center of the clusters is continuously recalculated until the best possible clustering is formed. The clustering process stops when the cluster center and the created cluster do not change during subsequent processing (sensitivity analysis). In this regard, by using a threshold, clusters that have less than a certain number of pixels can be removed. After the end of the clustering process, the degree of similarity and separation of the clusters from each other is calculated using the average intra-cluster distance and divergence. The integration of clusters is done to reduce some unnecessary and useless clusters so that the obtained result will be more expressive in this regard. The percentage and amount of consolidation that takes place in this context is a function of the threshold set by the user.

In classification process, we determine the maximum distance between clusters (classes), the distance between the centers of two clusters, the radius of the cluster and the minimum number of pixels in a cluster. Analysis of the distribution of clusters around the average is determined by the average standard deviation of each recorded spectral band. The narrower the clusters created in the feature space are, the more likely they are to be separated in the feature space. The proximity analysis of the clusters in the feature space is determined by measuring the distance between their centers. If the distances between the centers of the clusters are less than the desired threshold, they are combined with each other. Generally, the last cluster obtained from repeating calculations is the best.

Supervised classification is more commonly used for medium resolution images such as Sentinel-2. In this method, we have full control over the classification process. Firstly, samples are taken, and these samples should be taken from homogeneous pixels with the same distribution, and number. Then, after taking the samples, we specify our classifications to the software. In supervised classification, separate samples should be taken for each class. Then the samples are introduced to the software and classified based on their spectral behavior [19] or spectral signature.

3. Results

The area coverage was classified into 7 classes using the K-means method, the results of which are given as a map in the figure 5. As can be seen in the aerial image, these classes include water, soil, forest, cultivated areas, urban areas, asphalt, clouds. To control and validate the prepared map, spectrometry of the classes was done in the order shown in the figure 5. The obtained spectral signatures, except for vegetation areas, do not show significant differences in reflection curves. The observed changes in the recorded vegetations are considered as evidence for the diversity in the plants of the region. According to the results of this classification, the studied area was reclassified in several stages using supervised classification algorithms.

This procedure was used as control points in the supervised classification by considering the sampling and field surveys of the geographical coordinates of the samples. Based on the results of maximum likelihood and random forest methods, at least 8 main classes are classified in vegetation areas. These classes include forest areas with pine trees (e.g. *Pinus pinea*, *Taraxacum turcicum*), cypress trees, deciduous trees, shrubs, and grassland. Based on the surveyed GPS points, *C. salviifolius* is mainly observed in grassland areas and fields roadsides. Grasslands and bush cover areas around Yalova, which were distinguished in the classification, were also evaluated in terms of spectral reflection pattern. The reflection patterns indicate similar vegetation characteristics. *C. salviifolius* reflection was also higher than other species reflection in the whole spectrum (Figure 5).

Figure 5. A) Classified on cover map of Armutlu region using supervised classification, number 1, 2, 3, 4 and 5 vegetation types, 6 build up and 7 water, B) supervised classification methods for grassland of area

The infrared spectral bands (700–1300 nm) showed the highest spectral reflectance, the spectral region (1600–2300 nm) showed the lowest reflectance, and the 1300–2300 nm wavelengths showed very low reflectance. It is noteworthy that there is a great similarity in the spectral reflection pattern between *C. salviifolius* and *T. turcicum*. The reason for this may be the close characteristics and plant structure of these two species. The results of the statistical analysis explained that the near-infrared (NIR) spectral region (700-1150) is the best spectral region to differentiate between the two species. Only one specific spectral region, (710:900 nm for *C. salviifolius* and 730:950 nm for T. turcicum), can be used in the separation between them. At the same time, three spectral bands were sufficient to separate between plants (450:750, 1350:1600, 1900:2350 nm) while three spectral bands were sufficient to separate grassland 480:710, 810:1100, 1300: 1500, 1550:2300 was enough. Simultaneously, three spectral bands were used to separate between plants (450:720, 800:, 1550:2300 nm), while three spectral bands were sufficient to distinguish grassland (450:720, 830:950, 1550:2400 nm) (Figure 6). All measurements were performed at the maximum vegetative growth stage for most plants. Close spectral bands were identified, which can be used to classify grassland. Results show that in the case of classification of close structure plants, using the spectral features of the maximum vegetative growth stage may not be sufficient. There will be a need to evaluate the spectral features in all growth stages.

Figure 6. Spectral view of the *C. salviifolius* in the 4 diferent locaions

Table 1 shows the results from the process of classified images validation using the confusion matrix of classified region matrix and the obtained parameters: producer's accuracy, user's accuracy, and kappa coefficient.

Table 1. Accuracy evaluation results for supervised classification

	Ground Truth (Pixels)											
Class	**ROI:water**	**ROI:veg-1**	**ROI:veg-2**	**ROI:veg-3**	**ROI:veg-4**	**ROI:veg-5**	**ROI:build up**	**Total**	**Prod. Acc. (%)**	**User Acc. (%)**	**Commission (%)**	**Omission (%)**
Unclassified	0	0	0	0	0	0	0	0	0	0	0	0
Water	**22044**	0	0	0	0	0	0	22044	99.57	100.00	0.00	0.43
Veg-1	0	**10368**	662	301	202	0	0	11533	82.61	89.90	10.10	17.39
Veg-2	0	1370	**11592**	0	30	0	0	12992	93.96	89.22	10.78	6.04
Veg-3	0	370	0	**9751**	1	0	0	10122	96.48	96.33	3.67	3.52
Veg-4	0	407	83	24	**4389**	0	0	4903	94.22	89.52	10.48	5.78
Veg-5	0	35	0	31	36	**10469**	273	10844	96.63	96.54	3.46	3.37
Build up	95	1	0	0	0	365	**3471**	3932	92.71	88.28	11.72	7.29
Total	22139	12551	12337	10107	4658	10834	3744	76370	**Overall accuracy = 94.3878%**		**Kappa Coefficient= 0.9316**	

4. Conclusions and discussion

The spectral and spatial characteristics of Sentinel-2 images were used as a standard model to describe and interpret the results and general mapping of the region. Typical spectral reflectance curves for five main types of land features, include vegetation, soil, water, cloud and urban areas (Figure 5). As can be seen from the results, the spectral reflectance of vegetation changes with wavelength. The chlorophyll in the leaf has a strong absorption at 450 nm and 670 nm, and in contrast to the plant structure and the covering of the leaves, it contributes to the high reflectance of the near-infrared region (700-900 nm), which proves the effect of pigments and leaf structure on absorption and reflctivity.

The spectral reflectance curves of healthy vegetation have characteristic shapes that are determined by different plant characteristics. This curve is governed by the absorption properties of chlorophyll and other leaf pigments in the visible part of the spectrum. Stress conditions can produce changes in leaf structure, which vary greatly throughout plant species. Therefore, the species variance, stress, and canopy condition can affect the near infrared reflectance and can be used as an indicator to distinguish between different types of vegetation [20].

In general, the spectral differences of the vegetation covering the study area can be identified in three main reflectance regions: visible region (VIS, 400–680 nm), the near-infrared (NIR, 750–1200 nm), and the shortwave-infrared (SWIR, 1200–2500 nm). For SWIR region is within the range of absorption and physical control of leaf internal structures. Reflectance and transmittance often fall from moderate to low as wavelength increases, whereas absorption typically rises from low to high. Plant water content is the main physical factor controlling vegetation at these mid-infrared wavelengths.

In plants, the curve of the visible spectrum is determined by the plant pigments. For example, the blue (450 nm) and red (670 nm) regions are strongly absorbed by chlorophyll, which is known as chlorophyll absorption [21]. The difference between plant species is slight, but the result of the spectral reflectance shows a significant increase in the red edge (680-800 nm), the plant leaf shows approximately 40-60 % sudden reflectance. The transition zone between red and near-infrared is shown to have a high information content for the vegetation spectrum. This area is generally known as the "red edge". Red edge region represents the region of sudden change in leaf reflectance between 680-780 nm, which is caused by the combined effects of strong absorption of chlorophyll in red wavelengths and high reflectance in bands 5 and 6 (red edge bands) due to the scattering of the internal structure of the leaf. The increased of chlorophyll amount, for example, leads to broadened absorption feature to the center around 680 nm, causing a shift of the red edge slope and the wavelength of the maximum slope towards longer wavelengths, which is mentioned as red edge position (REP). The shift of REP to longer or shorter wavelengths has been used as a means of estimating changes in leaf chlorophyll content and as an indicator of plant stress. Since REP is defined as the turning point of the NIR slope, as can be seen in the figures (6), the shape and slope of the curve change, that includes the bands (5, 6, 7 and 8) is very high and these changes are directly related to the diversity of different species. The correlation and similarity of the GPS points in the curves of this part of the spectral signatures is remarkable. In the NIR region, which includes B7, B8, B8a, and B9 bands a very slight amount of waves is absorbed (about 5%). The structural variation in the leaves in these ranges allows us to distinguish between different species. There are small changes both within and between types of plants due to their phenology, vigor, canopy pattern, and other ecological parameters. These changes are the basis for classifying and distinguishing plant species from each other.

Plants absorb or reflect most incident radiation after 1300 nm, transferring relatively little energy. Three prominent water absorption bands at wavelengths of 1400, 1900, and 2700 nm were found. From NIR to SWIR, the amount of reflection decreases. The reason for the reduction of reflection in SWIR is because of the moisture content of plants. IR waves and SWIR are highly sensitive to water because water strongly absorbs SWIR and thermal waves. The presence of moisture in the soil has caused significant absorption centers to be created in the range between IR and SWIR [4].

Compared to covered soils, the reflectance variance in the bare soil spectral curve is significantly smaller. This is caused by elements that alter soil reflectance in less focused spectral bands, such as moisture, soil texture, surface roughness, iron oxide content, and organic matter content (Figure 1) [22]. Compared to other objects, soil has a spectral behavior without oscillation or low oscillation. The spectral behavior of soil shows a logarithmic trend that increases from short to long wavelength. In other words, as we move from the blue band range to the short wavelength IR band, the amount of wave reflection increases. This increasing process continues with increasing wavelength until a small absorption center is created in the near IR range, which is caused by the moisture that presents. After this absorption center, the increasing process of reflection continues with increasing wavelength. The effect of minerals in the soil can generally be seen in the SWIR range. Accordingly, many satellite sensors designed for mineralogical studies have multiple spectral bands in the SWIR range. Many minerals have similar spectral behavior to each other, but the difference in their absorption centers in the SWIR range is a method to identify and separate them from each other. In general, moisture absorbs electromagnetic waves. The presence of water in the soil reduces reflection and increases absorption of electromagnetic waves. As the amount of water in the plant increases, the reflection ratio decreases in different bands. So, maximum absorption ratio occurs with increasing moisture in the SWIR band. The presence of organic matter in the soil creates an effect like moisture (reducing reflection and increasing absorption) in the soil.

Band ratios or spectral indices have been utilized in a number of studies to highlight particular physiological traits and differentiate between various plant [23-25]. Normalized difference vegetation index (NDVI) and soil adjusted vegetation index (SAVI) are two frequently used indicators. Indices are also used in the classification process of covered areas from remote sensing images. Principal component analysis (PCA) has been used as a data augmentation method when analyzing remote sensing images to separate vegetated and non-vegetated areas [26].

The accuracy rate of the classification calculated by the Maximum likelihood classification (MLC) method. According to this calculations, the accuracy rate of the water class is the highest and the Veg-1 class is the lowest. This can be due to the water class being homogeneity and the Veg-1 class consisting of different plant species. In addition, the Veg-1 control points are fewer than the other plant classes. Our calculations shows the classification agreement between the kappa coefficient result (0.93) and the control points.

Upon the results, it can be concluded that the vegetation indices based on the ratio between NIR, SWIR-I and SWIR-II may be sufficient for grassland separation compared to other indices based on the visible composition. On the other hand, in the case of *C. salviifolius* differentiate, the use of plant indices based on the ratio between the NIR, and any other spectral region (visible or invisible) may give good results.

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Science teacher candidates' awareness and behaviors regarding biodiversity: Scale development study

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Abstract

The aim of this study was to develop a scale to determine the awareness and behavior levels of pre-service science teachers regarding biodiversity. A pool of 60 items for the measurement tool was created through a literature review conducted by the researcher. After obtaining expert opinions, the number of items was reduced to 56 and revised accordingly. The prepared scales were administered to 280 students at two public universities. Exploratory factor analysis (EFA) was conducted to assess the construct validity of the scale. The 'Biodiversity Awareness Measurement Tool' (BAMT) was finalized with 24 items based on the EFA results, while the 'Biodiversity Behavior Measurement Tool' (BBMT) was finalized with 25 items. After the reliability study, the overall Cronbach's alpha coefficient for BAMT was found to be .72, and for BBMT, it was .75. As a result of all these studies, a 5-point Likert-type scale consisting of 24 items prepared in 6 sub-dimensions to measure biodiversity awareness levels and a scale consisting of 25 items prepared in 7 sub-dimensions to measure biodiversity behavior levels were developed.

Keywords: biodiversity, awareness, behavior, teachers candidate

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Fen bilgisi öğretmen adaylarının biyolojik çeşitlilik konusundaki farkındalık ve davranışları: Ölçek geliştirme çalışması

Özet

Bu araştırmanın amacı, fen bilgisi öğretmen adaylarının biyolojik çeşitlilik konusundaki farkındalık ve davranış düzeylerini belirlemeye yönelik bir ölçek geliştirmektir. Araştırmacı tarafından ilgili alan taraması yapılarak ölçme aracı için 60 maddelik madde havuzu belirlenmiştir. Alan uzman görüşü alındıktan sonra, madde sayısı 56 maddeye indirilerek yeniden düzenlenmiştir. Hazırlanan ölçek iki devlet üniversitesindeki 280 öğrenciye uygulanmıştır. Ölçeğin yapı geçerliliği için açımlayıcı faktör analizi yapılmıştır. “Biyolojik Çeşitlilik Farkındalık Ölçme Aracı” (BÇFÖA), yapılan açımlayıcı faktör analiziyle 24 madde olarak, “Biyolojik Çeşitlilik Davranış Ölçme Aracı” (BÇDÖA) ise yapılan açımlayıcı faktör analiziyle 25 maddelik son halini almıştır. Güvenirlik çalışması sonrasında da BÇFÖA’nın genel cronbach alfa katsayısının .72 olduğu, BÇDÖA’nın geneli için cronbach alfa katsayısının .75 olarak bulunmuştur. Tüm bu çalışmalar sonrasında biyolojik çeşitlilik farkındalık düzeylerini ölçmek için 6 alt boyutta hazırlanmış 24 maddeden oluşan ölçek ve biyolojik çeşitlilik davranış düzeylerini ölçen 7 alt boyutta hazırlanmış 25 maddeden oluşan 5’li likert türü ölçek geliştirilmiştir.

Anahtar kelimeler: biyolojik çeşitlilik, farkındalık, davranış, öğretmen adayları

1. Introduction

Biodiversity in a region is formed by the totality of ecosystems and ecological relationships. This diversity is vital for the existence of natural habitats, human life, and species in general. Turkiye is quite rich in terms of

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biodiversity, which constitutes an important part of our natural heritage. This natural wealth is thought to be due to the country's division into seven geographical regions. Each region has its unique climate, vegetation, elevation, landforms, soil diversity, lakes, rivers, and wetlands. Our biodiversity offers many benefits, including those related to health, medicine, nutrition, pharmacy, forestry, fishing, economy, industry, tourism, cultural values, and education. Most importantly, biodiversity is essential for the continuation of life. Therefore, the preservation of biodiversity is of great importance for future generations to witness and understand the species and ecosystems that constitute it. Preserving biodiversity is vital for leaving a balanced and healthy natural environment for future generations to develop.

Since humanity understood that it is the ruler of the world, the relationship that must be harmonious with its natural environment has been disrupted. In recent years, agricultural lands, forests, and consequently biodiversity have been destroyed to meet the housing needs of the growing population. Biodiversity has high cultural, economic, aesthetic, social, and ecological value and is under social and economic pressure [1]. Human demographic, financial, and political movements have caused changes, and these changes have affected biodiversity. As a result, biodiversity erosion has occurred. The extinction rate indicates biodiversity erosion. Research shows that an average of 10,000 to 30,000 species become extinct every year and that by 2050, 11% of the natural areas the world had in 2000 could be lost, 40% of agricultural land is at risk of overuse, and 60% of coral reefs could be gone by 2030 [2]. Humanity must slow down and/or stop biodiversity erosion to survive.

In Türkiye, factors threatening biodiversity include opening 2/B lands for construction, deforestation, and dams. Most 2/B areas are either forested or have natural vegetation, and since these lands are a boundary point between urbanization and natural habitats, opening them for construction further restricts the area of natural life and poses a significant threat to living organisms. At the same time, industrialization rapidly pollutes nature. For these reasons, there is a decrease and even extinction in plant and animal species whose natural habitats have been destroyed. The decrease and extinction of plant and animal species, which are of great importance to humans, indicate that biodiversity is not being preserved. Parallel to this, biodiversity has reached dangerous levels globally due to improvements in developing countries, rapid population growth, and the destruction of forests and arable land to raise the average standard of living [4]. Moreover, the desire of people to benefit from both the living and non-living elements of their natural environment for their nutrition and security needs has created various effects on the natural environment and biodiversity. As a result, environmental problems have increased rapidly. It is seen that the emergence of irreversible damage occurs by disrupting the food chain among living beings, the processes in the inanimate environment, and the interactions between living and inanimate elements. Among these are the known and unknown extinction of many plants, animals, and microorganisms, the migration of species to other areas, and the increase in spatial struggles among species in that area. As can be understood from this situation, one of the important environmental problems of our time is the loss of biodiversity, and one of the most significant reasons for this problem is humanity's indifference to this extinction. However, the damage caused by humans to their environment through conscious or unconscious actions does not appear all at once, so the danger is not realized. This situation increases the threat awaiting humanity [5].

Individuals remain indifferent to biodiversity issues unless these problems affect them directly and do not seek any solutions. Individuals must be aware that the damage they cause to nature will negatively affect biodiversity, show behaviors aimed at taking precautions against the problems that may arise, and develop solutions. For this, it is essential to raise individuals who are aware of biodiversity and exhibit positive behaviors towards it. The most effective solution for preserving biodiversity is to raise awareness about this issue among individuals and create a foundation for turning it into positive behavior. All of this is possible through education. Raising individuals who are aware of biodiversity issues and exhibit positive behaviors towards preserving and properly using biodiversity is among the aims of biodiversity education. At this point, it becomes increasingly important to use the results and recommendations from studies aimed at preserving biodiversity in biology education and environmental education to create awareness about biodiversity and eliminate behavioral pollution towards biodiversity [6].

To raise a generation that respects biodiversity preserves it, understands the need to sustain it, and passes it on from generation to generation, it is necessary first to instill awareness and positive behaviors in people. In this way, individuals who are aware of, respect, and ethically behave towards biodiversity can be raised. Biodiversity cannot be preserved only through behaviors. To protect biodiversity, it is necessary to establish bioethics-based, environment-centered biopolicies. Behaviors such as being aware of biodiversity and protecting and using it properly can be achieved through bioeducation. Since the factors negatively affecting biodiversity are human awareness and behavior patterns, these are an education problem. The primary activity in the learning process, which humans are continuously involved in throughout their lives, is education and training. In Turkiye, the primary aim of education should be to develop models and educational environments that will raise individuals who are creative, inquisitive, think critically, research, learn to learn, communicate, are proficient in technology, are friendly with knowledge, are sensitive to society and their environment, and have lifelong learning skills [7].

Any organism's activities, including its responses and movements in a particular situation, are defined as behavior. Behavior is the result of continuous interaction between personal and environmental variables. Environmental conditions shape behavior through learning, and the person's behavior also shapes the environment [8]. As individuals gain knowledge about the functioning of ecosystems and how human activities affect these systems, they exhibit more positive behaviors toward the environment [7]. Individuals who do not have sufficient knowledge about the

environment exhibit negative behaviors towards the environment, disrupting the natural balance and harming the environment [9]. Environmental behaviors can be influenced by socioeconomic conditions, the living environment, education level, gender, profession, and ecological knowledge [10]. A review of the literature shows that a series of studies have been conducted on stakeholders' knowledge levels and behavior tendencies towards biodiversity [11]. However, it is noticeable that studies related to awareness and behaviors towards biodiversity are more commonly conducted abroad, while such studies are scarce in our country. "Creating awareness" can lead to change if it is supported and combined with knowledge. Therefore, public awareness and knowledge level play a significant role in both combating climate change, which affects human life in various environmental, social, and economic ways, and preserving biodiversity, which is under severe threat. Increasing the awareness and knowledge level of individuals is very effective in achieving success in combating climate change and solving environmental problems on a global, national, and local scale. Individuals with increased consciousness and awareness levels can create public opinion on environmental and climate change issues, stand against anti-environmental decisions made by policymakers, and become one of the main actors in these issues [3].

Individuals who comprehend the concept of biodiversity are expected to protect, develop, and use the natural environment sustainably. To create awareness about biodiversity and encourage positive behaviors, educational practices should be designed to attract students' interest and encourage active participation in the learning process. Continuing the development of our country's biological wealth without causing harm is of great importance for future generations, with teacher candidates who will address large masses being trained with a consciousness of protection. The plant, animal, and microorganism species that makeup biodiversity, along with their varieties and communities, have a significant impact on maintaining the natural balance [12]. Educating conscious generations can ensure that such organisms continue to live well in the future. Therefore, studies that contribute to raising awareness about biodiversity and developing positive behaviors are important. It has been stated that the preservation of biodiversity is a promising frontier for behavioral sciences. To reduce the global (climate change) and local (economic development) threats to biodiversity caused by human activities, it is extremely important for people to have basic knowledge about biodiversity and its value and to exhibit appropriate behavior tendencies towards biodiversity and its values. Biodiversity can only be protected by people who can understand its importance, perceive the factors causing negative impacts, and know what can be done to protect it. The educators who ensure the formation of awareness about biodiversity and increase positive behavior levels are undoubtedly educators. It is also a fact that an ideal nature education that will effectively teach the impacts of damaging biodiversity, the importance of this biodiversity for our planet, and what can be done to protect it, and contribute to raising sensitive and conscious citizens, can only be provided by educators with sufficient knowledge on these subjects. It is stated that knowledge and sensitivities gained without awareness of biological and ecological diversity and without turning them into positive behaviors cannot be put into practice.

The awareness and behavior of teacher candidates who play a key role in educating young children are important. For students to be aware of the ecological and use values of biodiversity and to adopt behaviors that protect biodiversity, teachers who educate and prepare students for the future must be prepared and have the necessary and sufficient knowledge to be aware of the ecological and use values of biodiversity and to adopt behaviors that protect biodiversity. Since the extinction of biodiversity can have significant effects in various fields now and in the future, increasing individuals' awareness of these issues from primary education, even preschool, and having individuals in society who can make correct decisions about preserving biodiversity is undoubtedly closely related to the efforts of science teachers [13, 14]. In this context, this study is extremely important in determining the awareness and behavior levels of science teacher candidates who will work in middle schools regarding biodiversity and contributing to the preservation of sustainable biodiversity.

In the accessible sources, no scales have been developed for teacher candidates regarding Biodiversity Awareness and Behavior [7, 9, 10]. Thus, the primary aim of this research is to develop sufficient and reliable measurement tools to determine biodiversity awareness and behavior levels. In subsequent research, it is thought that using these measurement tools will contribute significantly to the studies [7-14]. Studies conducted with these aims can contribute to identifying science teachers' awareness and behavior levels on biodiversity issues and determining what needs to be done to address any deficiencies or misinformation during the professional education of teacher candidates.

2. Material and method

2.1. Preparation of the Measurement Tool

In the development of the Biological Diversity Awareness Measurement Tool (BAMT) and Biological Diversity Behavior Measurement Tool (BBMT) ; (1) creating the item pool for the measurement tool, (2) seeking expert opinions, (3) pilot study phase, (4) calculating validity and reliability. Literature was reviewed on measurement tools related to biodiversity awareness and behavior levels suitable for the study's purpose. However, no measurement tool was found to determine the awareness and behavior levels of teacher candidates regarding biodiversity. From the literature, research generally focused on determining knowledge levels, attitudes, and literacy levels, but very few studies addressed the awareness and behavior dimensions. In addition, the sections of the questionnaires related to

biodiversity and the sections related to biodiversity in high school and middle school textbooks were examined. The 60 items prepared for the measurement tool were reduced to a total of 56 items, 30 items related to awareness and 26 items related to behavior, by consulting field experts. It was decided to use a Likert-type scale. Positive and negative items related to the attitude to be measured, developed by Likert, were presented to individuals.

The items of the biodiversity awareness measurement tool and biodiversity behavior measurement tool in the trial form were evaluated for content validity and suitability to the student level by consulting the opinions of three faculty members in elementary science education. Additionally, the measurement tools were presented to two faculty members in Turkish education to determine its comprehensibility and grammatical correctness. Long items were shortened, and sentences with grammatical errors were revised. Thirty (30) items related to awareness and 26 items related to behavior were used for the application.

2.2. Study Group and Data Collection Process

The designed measurement tool form was applied face to face to a total of 280 randomly selected students studying at two state universities.

2.3. Data Analysis

Exploratory factor analysis was conducted to reveal the construct validity of the BAMT and BBMT. For reliability, Cronbach α internal consistency coefficients were examined.

The prepared measurement tool consists of three parts. The first section includes 12 items covering demographic information. The second section contains a 30-item biodiversity awareness scale designed to measure participants' levels of awareness about biodiversity. The third section includes a 26-item biodiversity behavior scale to measure participants' levels of behavior related to biodiversity. A 5-point Likert scale format was chosen, with response options of 'Strongly Disagree,' 'Disagree,' 'Neutral,' 'Agree,' and 'Strongly Agree,' scored from 1 to 5, respectively (Table 1). Reverse coding was applied for negative items.

Table 1. Likert-type scale ıtem scoring key

	Positive Questions	Negative Questions
Strongly Disagree	1	5
Disagree	2	4
Neutral	3	3
Agree	4	2
Strongly Agree	5	1

3. Results

3.1. Content and Construct Validity

Content validity is concerned with whether the measurement tool fully meets the quantitative and qualitative aspects of measuring the target behaviors [15]. The content validity of the measurement tool was evaluated considering the opinions of three faculty members in elementary science education. The suitability of content validity was decided. Then, exploratory factor analysis was applied to identify the factors among the variables. Item analysis was performed to test the relationship between the items in the measurement tool and the attitude to be measured. Pearson product-moment correlation coefficients were calculated to determine the relationship between each item in the measurement tool and the total score. Finally, the reliability coefficients (Cronbach Alpha) of the measurement tool and each factor were calculated separately [16].

3.1.2. Development Study of the Biological Diversity Behavior Level Measurement Tool

Analyses were conducted on 280 individuals in the biological diversity behavior level measurement tool development application study. First, it was examined whether the sample showed a normal distribution. The findings are presented in Table 2. The data obtained from the pilot application of the BBMT were analysed. The scoring for responses to negative items was adjusted as “1-5; 2-4; 3-3; 4-2; 5-1”. The total score obtained by each student with the measurement tool was calculated, and the normal distribution graph was examined (Figure 1).

Table 2. Skewness and kurtosis values of the biological diversity behavior level measurement tool

Biological Diversity Behavior Level		
Skewness	-.02	.14
Kurtosis	-.12	.29

Table 2 shows the skewness and kurtosis values of the biological diversity behavior level measurement tool. Skewness values range from -.02 to .14, and kurtosis values range from -.12 to .29. According to George and Mallery (2003), skewness and kurtosis values between (+2) /(-2) indicate normal distribution. In addition, to test the normality of the distribution, a histogram graph is provided in Figure 1.

Figure 1. Normal distribution graph of the biological diversity behavior level

The average value calculated according to the given answers shows a normal distribution. In the research, first, the construct validity of the biological diversity behavior measurement tool was performed. Based on the literature and expert opinions, it was decided to perform construct validity (EFA) for the item pool. Before performing EFA, it is necessary to investigate whether the data set is suitable for factor analysis. It is necessary to test the sample size for EFA. One of the methods used to determine whether the sample is suitable for data analysis is the Kaiser-Meyer-Olkin (KMO) test of sampling adequacy and Bartlett Sphericity test of sphericity (Table 3).

Table 3. Results of the kaiser-meyer-olkin and bartlett sphericity tests

Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO): .77		
Bartlett's Test of Sphericity	X^2	1564.70
	sd	300
	p	.000

The KMO measure of sampling adequacy value was 0.77. The calculated Bartlett Sphericity Test for the same data was 1564.70 and was significant at the 0.001 level ($X^2(300)=1564.701$). These findings show that the sample size and correlations between items were adequate, thus the data can be subjected to factor analysis [17].

After determining the sample size's suitability for factor analysis, a "Scree Plot" graph was drawn to see the number of factors in the measurement tool. The Scree plot is shown in Figure 2.

Figure 2. Scree plot graph

When the Scree Plot graph is examined, it is seen that the eigenvalue of the measurement tool falls below eigene value of 1 after the 7th factor. This situation shows that the measurement tool consists of 7 factors.

The data of the 26 items in the biodiversity behavior level measurement tool were subjected to exploratory factor analysis to identify the primary factors measured. In determining the primary factors, there was no restriction on the number of factors, and the principal components factor analysis and varimax rotation method were used. Validity studies revealed that the measurement tool had a seven-factor structure with an eigenvalue greater than 1. The results are presented in Table 4.

Table 4. Analysis for determining the factor structure of the measurement tool

Factor No	Eigenvalues	Percentage of Variance	Cumulative Percentage of Variance
1	3.64	14.57	14.57
2	3.51	14.06	28.64
3	1.88	7.53	36.17
4	1.46	5.85	42.03
5	1.22	4.88	46.91
6	1.15	4.60	51.52
7	1.01	4.05	55.57
8	.98	3.95	

The measurement tool consists of a seven-factor structure explaining 55.58% of the total variance. The varimax rotation method was used to determine which item fell under which factor, and the distribution of the items was determined. The findings are presented in Table 5.

Table 5. Post-rotation load values of ıtems in the measurement tool and ıtems in the factors

Item No	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
16	.892						
15	.873						
13	.753						
14	.461						
24		.772					
25		-.755					
8		.615					
18		.611					
19			.669				
20			.606				
26			.589				
17			-.564				
11				.763			
12				.659			
9				.564			
3				.408			
22					.712		
21					.666		
23					.485		
6						-.646	
7						.629	
2						.528	
4						.460	
1							.752
5							.742

From the results, the factor loadings of the biological diversity behavior level measurement tool range from .40 to .89. According to Büyüköztürk [18], the load values of the items in the factors they belong to should be high. The difference between the highest load value of an item in the factors and the next highest load value should be as large as possible. It is recommended that this difference between the two high values be at least .10. Otherwise, the item is removed from the measurement tool. In this study, the 10th item was removed from the measurement tool due to the difference between the load values under two different factors being less than .10. According to the literature, items can be used if the factor loadings are greater than .30 [19]. Thus, the total number of items became 25.The names of the factors resulting from the interpretation of the items that constituted them are given in Table 6.

Table 6. Names of the sub-factors of the biological diversity behavior level measurement tool

Factor No	Sub-Factor Name	Number of Items
1	Biodiversity Protection Behavior	4
2	Economic Value of Biodiversity	4
3	Ethical Value of Biodiversity	4
4	Threat Elements of Biodiversity	4
5	Political and Legal Behaviors of Biodiversity	3
6	Usage Value of Biodiversity	4
7	Individual and Societal Persuasion of Biodiversity	2
	Total	25

In the first factor, items 12, 13, 14, and 15; in the second factor, items 8, 17, 23, and 24; in the third factor, items 16, 18, 19, and 25; in the fourth factor, items 3, 9, 10, and 11; in the fifth factor, items 20, 21, and 22; in the sixth factor, items 2, 4, 6, and 7; and in the seventh factor, items 1 and 5 are included.

3.1.3. Development Study of the Biological Diversity Awareness Level Measurement Tool

The data obtained from the pilot application of the BAMT were analysed. The scoring for responses to negative items was adjusted as "1-5; 2-4; 3-3; 4-2; 5-1". The total score obtained by each student with the measurement tool was calculated, and the normal distribution graph was examined (Figure 3).

Analyses were conducted on 280 individuals in the biological diversity awareness level measurement tool development application study. First, it was examined whether the sample showed a normal distribution. The findings related to this situation are presented in Table 7.

Table 7. Skewness and kurtosis values of the biological diversity awareness level measurement tool

	Biological Diversity Awareness Level	
Skewness	-.15	.14
Kurtosis	-.43	.29

The skewness values range from -.15 to .14, and kurtosis values range from -.43 to .29 According to [20], skewness and kurtosis values between (+2) /(-2) indicate normal distribution. In addition, to test the normality of the distribution, a histogram graph is provided.

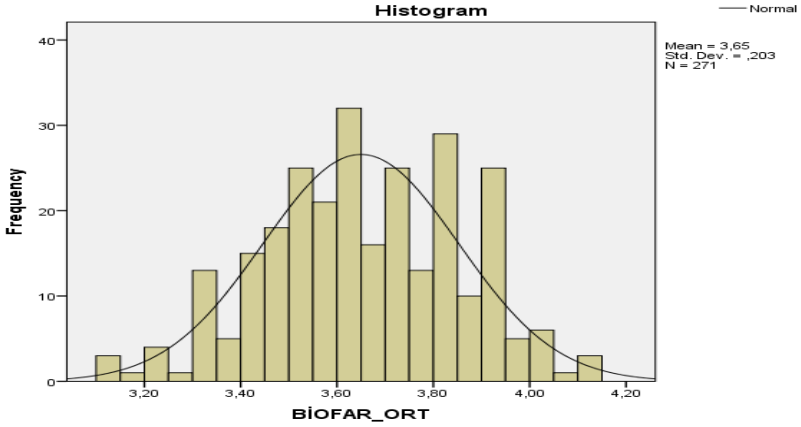

Figure 3. Normal distribution graph of the biological diversity awareness level

The biodiversity awareness data approximately shows a normal distribution in the histogram. After determining that the data showed a normal distribution, it was tested whether the sample was suitable for factor analysis. The results of the Kaiser-Meyer-Olkin (KMO) test of sampling adequacy and Bartlett Sphericity test of sphericity are presented in Table 8.

Table 8. Results of the kaiser-meyer-olkin and bartlett's test of sphericity

Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO): .79		
Bartlett's Test of Sphericity	X^2	1333.97
	sd	276
	p	.000

From the results, the calculated KMO suitability measure value was 0.79. The calculated Bartlett's Test of Sphericity for the same data was 1333.97 and was significant at the 0.001 level (X2276=1333,974). These findings show that the data can be subjected to factor analysis [17].

After determining the sample size's suitability for factor analysis, a "Scree Plot" graph was drawn to see the number of factors in the measurement tool. The Scree plot is shown in Figure 4.

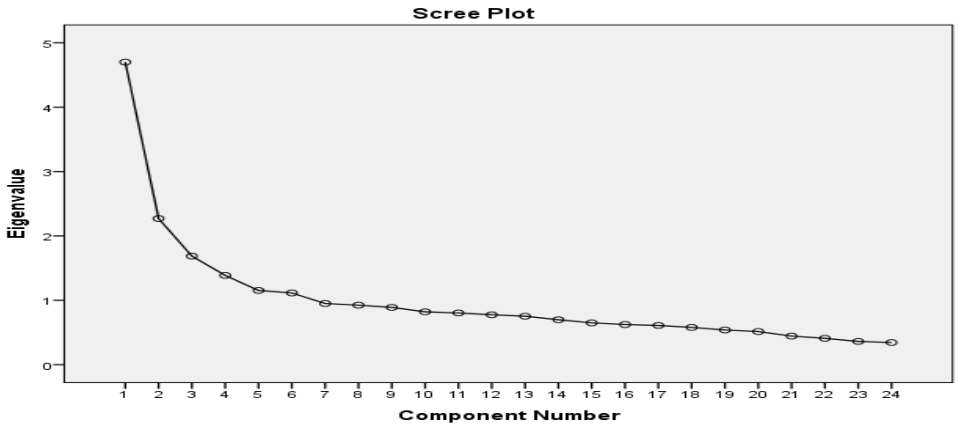

Figure 4. scree plot graph

When the Scree Plot graph is examined, it is seen that the eigenvalue of the measurement tool falls below eigene value of 1 after the 6th factor. This situation shows that the measurement tool consists of 6 factors.

For the development study of the biological diversity awareness level measurement tool, data related to the 30 items in the pilot application were subjected to exploratory factor analysis to identify the primary factors measured. Principal components factor analysis and varimax rotation method were used. Validity studies revealed that the measurement tool had a six-factor structure with an eigenvalue greater than 1. The results are presented in Table 9.

Table 9. Analysis for determining the factor structure of the measurement tool

Factor No	Eigenvalues	Percentage of Variance	Cumulative Percentage of Variance
1	4.69	19.58	19.58
2	2.27	9.458	29.03
3	1.68	7.02	36.06
4	1.38	5.77	41.84
5	1.15	4.80	46.64
6	1.11	4.64	51.28
7	.95		

The measurement tool consists of a six-factor structure explaining 51.29% of the total variance. The varimax rotation method was used to determine which item fell under which factor, and the distribution of the items was determined. The findings related to this situation are presented in Table 10.

The factor loadings of the biological diversity awareness level measurement tool range from .372 to .752. Items 5, 9, 17, 25, 28, and 29 were removed from the measurement tool due to loadings below .30. Thus, the total number of items became 24. The names of the factors resulting from the interpretation of the items that constituted them are given in Table 11.

In the first factor, items 14, 15, 17, 18, 19, 20, and 21; in the second factor, items 6, 7, 8, 12, and 24; in the third factor, items 2, 3, and 5; in the fourth factor, items 1, 4, 11, and 22; in the fifth factor, items 9, 10, and 23; and in the sixth factor, items 13 and 16 are included.

Table 10. Post-Rotation load values of ıtems in the measurement tool and ıtems in the factors

Item No	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
21	.616					
20	-.614					
22	.589					
18	.583					
23	.570					
16	.494					
24	.429					
7		.675				
10		.657				
8		.657				
30		.599				
14		.554				
3			.749			
2			.735			
6			.608			
4				-.705		
1				.627		
26				.413		
13				.372		
11					.752	
12					.634	
27					-.436	
19						.704
15						-.614

Table 11. Names of the sub-factors of the biological diversity awareness measurement tool

Factor No	Sub-Factor Name	Number of Items
1	Ecological Function	7
2	Species Diversity	5
3	Usage Value of Biodiversity	3
4	Elements of Biodiversity	4
5	Biodiversity Loss	3
6	Current State of Biodiversity	2
	Total	24

3.2. Reliability

Cronbach Alpha reliability coefficients were examined for the reliability of both measurement tools. The Cronbach Alpha coefficients for each factor and the overall measurement tool from the pilot application results are given in Table 12 and Table 13.

Table 12: Cronbach alpha values of biodiversity behavior factors

Sub-Factor Name	Number of Items	Cronbach Alpha Value
Biodiversity Protection Behavior	4	.76
Economic Value of Biodiversity	4	.73
Ethical Value of Biodiversity	4	.78
Threat Elements of Biodiversity	4	.78
Political and Legal Behaviors of Biodiversity	3	.71
Usage Value of Biodiversity	4	.73
Individual and Societal Persuasion of Biodiversity	2	.76
Total	25	
Overall Measurement Tool		.75

The reliability coefficients for the overall biodiversity behavior measurement tool and its sub-dimensions indicate that the measurement tool is reliable. The general Cronbach Alpha coefficient for the biological diversity behavior level measurement tool was found to be .75, with factor coefficient values ranging from .71 to .78.

Table 13: Cronbach Alpha Co-Efficients of Biodiversity Awareness Factors

Factor Name	Number of Items	Cronbach Alpha Value
Ecological Function	7	.70
Species Diversity	5	.72
Usage Value of Biodiversity	3	.71
Elements of Biodiversity	4	.73
Biodiversity Loss	3	.72
Current State of Biodiversity	2	.74
Total	24	
Overall Measurement Tool		.72

The reliability coefficients for the overall biodiversity awareness measurement tool and its sub-dimensions indicate that the measurement tool is reliable. A single α value can be determined for each item, or an average α value can be obtained for all items in the measurement tool. The α value obtained for all items indicates the total reliability of the questionnaire. The general Cronbach Alpha coefficient of the biodiversity awareness level measurement tool was found to be .72, with factor coefficient values ranging from .70 to .74.

4. Discussion and Conclusion

A literature review was conducted on measurement tools related to biodiversity awareness and behavior levels suitable for the study's purpose. However, no measurement tool was found to determine the awareness and behavior levels of teacher candidates regarding biodiversity. Therefore, previous studies on the environment and biodiversity were reviewed and evaluated. Additionally, the sections of the questionnaires related to biodiversity and the parts related to biodiversity in high school and middle school textbooks were examined. As a result of all these reviews, a total of 56 items, 30 related to awareness and 26 related to behavior, were prepared by the researcher, and a Likert-type measurement tool was prepared.

According to Büyüköztürk [18], factor analysis is a statistical technique that aims to bring together a large number of related variables and express them with a much smaller number of conceptually meaningful variables such as factors or dimensions. The sample size for exploratory factor analysis was determined using the Kaiser-Meyer-Olkin (KMO) sample adequacy test. According to Şencan [21], if the value obtained from the KMO test is less than .50, factor analysis cannot be performed.

In the analyses conducted within the scope of the Biological Diversity Awareness and Behavior Scales, the calculated KMO suitability measure value for the Biological Diversity Behavior Scale was 0.77. Bartlett's Test of Sphericity was used to determine whether the data set was suitable for factor extraction. If the significance value in Bartlett's Test of Sphericity is greater than .05, it is interpreted that no factors can be extracted from the data set, and factor analysis cannot be performed [22]. The result of Bartlett's Test for the Biological Diversity Behavior Scale showed that the data set was suitable for factor analysis ($p<.001$). After determining that the sample size was sufficient and the data set was suitable for factor extraction, exploratory factor analysis was performed using the principal components analysis method. According to Tabachnick and Fidell [23], principal components analysis allows for the extraction of the highest variance from the data set.

For the BBMT scale, the calculated Bartlett's Test of Sphericity for the same data was 1564.70 and was significant at the 0.001 level. These findings show that the data can be subjected to factor analysis [21]. Data related to the 26 items in the biodiversity behavior level measurement tool were subjected to exploratory factor analysis to identify the primary factors measured. There was no restriction on the number of factors in determining the primary factors, and the principal components factor analysis and varimax rotation method were used. Validity studies revealed that the measurement tool had a seven-factor structure with an eigenvalue greater than 1. The factor loadings of the biodiversity behavior level measurement tool ranged from .40 to .89. According to Büyüköztürk [22], the load values of the items in the factors they belong to should be high. The difference between the highest load value of an item in the factors and the next highest load value should be as large as possible. It is recommended that this difference between the two high values be at least 10. Otherwise, the item is removed from the measurement tool. In this study, the 10th item was removed from the measurement tool due to the difference between the load values under two different factors being less than.10. According to the literature, items can be used if the factor loadings are greater than .30 [23]. Thus, the total number of items became 25.

Seven factors were determined for the biodiversity behavior measurement tool. These are: the first factor, biodiversity protection behavior; the second factor, economic value of biodiversity; the third factor, ethical value of biodiversity; the fourth factor, threat elements of biodiversity; the fifth factor, political and legal behaviors of

biodiversity; the sixth factor, usage value of biodiversity; and the seventh factor, individual and societal persuasion of biodiversity.

For the Biological Diversity Awareness Scale, the calculated KMO suitability measure value was 0.79. The calculated Bartlett's Test of Sphericity for the same data was 1333.97 and was significant at the 0.001 level ($X^2(276)=1333.974$). These findings show that the data can be subjected to factor analysis [21]. When the Scree Plot graph was examined, it was seen that the eigenvalue of the measurement tool fell below 1 after 6 factors. This situation shows that the measurement tool consists of 6 factors.

Data related to the 30 items were subjected to exploratory factor analysis to identify the primary factors measured. Principal components factor analysis and varimax rotation method were used. Validity studies revealed that the measurement tool had a six-factor structure with an eigenvalue greater than 1. The measurement tool consists of a six-factor structure explaining 51.29% of the total variance. The varimax rotation method was used to determine which item fell under which factor, and the distribution of the items was determined. The factor loadings of the biological diversity awareness measurement tool ranged from .372 to .752. Items with loadings below .30 were removed from the measurement tool. Thus, the total number of items became 24.

Six factors were determined for the biodiversity awareness measurement tool. These are: the first factor, ecological function; the second factor, species diversity; the third factor, usage value; the fourth factor, elements of biodiversity; the fifth factor, biodiversity loss; and the sixth factor, current state of biodiversity. Measurement tools with high consistency among the items that constitute them are reliable measurement tools [24]. For the reliability of both measurement tools, Cronbach Alpha coefficients were examined. The α value obtained for all items indicates the total reliability of the questionnaire, and the general acceptance is that this value should be .70 or higher.

In the research, the general Cronbach Alpha coefficient for the biodiversity awareness measurement tool was found to be .72. Additionally, the Cronbach Alpha coefficients for the six dimensions of the scale were as follows: ecological function dimension, .70; species diversity dimension, .72; usage value dimension, .71; elements of biodiversity dimension, .73; biodiversity loss dimension, .72; and current state of biodiversity dimension, .74.

In the research, the general Cronbach Alpha coefficient for the biodiversity behavior measurement tool was found to be .75. Additionally, the Cronbach Alpha coefficients for the seven dimensions of the scale were as follows: biodiversity protection behavior dimension, .76; economic value of biodiversity dimension, .73; ethical value of biodiversity dimension, .78; threat elements of biodiversity dimension, .78; political and legal behaviors of biodiversity dimension, .71; usage value of biodiversity dimension, .73; and individual and societal persuasion of biodiversity dimension, .76.

The developed scale does not have to be used as a whole scale, as done in similar scales [25]. Each sub-dimension of the scale allows for separate evaluation within itself.

As a result, it was concluded that both the Biological Diversity Awareness Measurement Tool and the Biological Diversity Behavior Measurement Tool are valid and reliable measurement tools.

The primary purpose of this study is to enable other researchers to use the developed scale, whether with all dimensions or preferred sub-dimensions, for any region or study group. Measuring the awareness and behavior of the chosen study group on biodiversity, and then making recommendations based on the findings to conduct effective studies and find solutions, is seen as a very important step in addressing the issue.

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Ek 1. Biyolojik Çeşitlilik Farkındalık ve Davranış Ölçme Araçları

KİŞİSEL BİLGİ FORMU	
Tanımlayıcı Bilgiler	**Açıklamalar ve seçenekler**
1. Cinsiyetiniz	☐ Kadın ☐Erkek
2. Yaşınız:	
3.Sınıfınız:	☐ 1. Sınıf ☐2. Sınıf ☐3. sınıf ☐4. sınıf
4.Ailenizin Gelir durumu:	☐1000 TL"den az☐1000-1999 TL arası ☐2000-2999 TL arası ☐3000-3999 TL arası ☐4000 TL"den fazla
5. Lisans öğreniminiz süresince biyolojik çeşitlilik ile ilgili bir ders aldınız mı? (Cevabınız hayır ise7. 8. ve 9. soruyu geçiniz).	☐ Evet ☐Hayır
6.Lisans öğreniminizde biyolojik çeşitlilik ile ilgili dersi kaçıncı sınıfta aldınız?	☐ 1. sınıf ☐2. Sınıf ☐3. sınıf ☐4. sınıf
7. Lisans öğreniminizde gördüğünüz biyolojik çeşitlilik ile ilgili dersin niteliğinden ve neler öğrendiğinizden kısaca bahsediniz.	
8. Biyolojik çeşitlilik konuları ile ilgili en çok yararlandığınız kaynak	☐ İnternet ☐Dergi-Gazete ☐Kitap ☐ TV- Radyo ☐Diğerleri .....
9. Biyolojik çeşitlilik ile ilgili olarak yapılan herhangi bir etkinliğe katıldınız mı? Yanıtınız evet ise kısaca bilgi veriniz (Etkinliğin adı. yeri. tarih, düzenleyen kurum vb.)	☐ Evet ☐Hayır
10. Biyolojik çeşitlilik konularının verildiği bir hizmet içi eğitim almak ister misiniz?	☐ Evet ☐Hayır
11- Biyolojik çeşitliliğin dört temel öğesini yazınız.	a). ..... b). ..... c). ..... d). .....
12- Biyolojik çeşitlilik ile alakalı ilgi ve bilgi durumunuzu aşağıdaki cümlelerden hangisi karşılamaktadır.	a- Biyolojik çeşitlilik ve faydaları hakkında az da olsa bilgim var. b- Biyolojik çeşitlilik hakkında bilgi edinmeye istekliyim ve biyolojik çeşitliliğin faydaları hakkında yeterli bilgiye sahip olduğumu düşünüyorum. c- Biyolojik çeşitlilik ve faydaları hakkında yeterince bilgim yok. d- Biyolojik çeşitlilik ile bilgi sahibi olmak istemiyorum e- Fikrim yok

1. Her bir ifadeyi okuduktan sonra, buna ne derecede katıldığınızı ya da katılmadığınızı liste üzerinde ayrılan yere uygun şekilde işaretleyiniz. Bir ifadeyi okuduktan sonra aklınıza ilk geleni işaretleyiniz. İşaretsiz ifade bırakmayınız. Size verilen liste üzerine adınızı yazmayınız, kimliğinizi belirtecek herhangi bir işaret koymayınız.

Biyolojik Çeşitlilik Farkındalık Ölçeği	**Tamamen Katılıyorum**	**Katılıyorum**	**Kararsızım**	**Katılmıyorum**	**Tamamen Katılmıyorum**
1. Biyolojik çeşitliliğin, madde değişimi, suyun temizlenmesi, toprağın oluşumu vb. ekolojik işlevlerinin de olduğunun farkındayım.					
2. Biyolojik çeşitliliğin, besin için biyolojik kaynaklar, tıp ve insanların günlük hayattaki ihtiyaçlarının ham maddelerinin oluşturulması vb. görevlerinin de olduğunu bilirim.					
3. Biyolojik çeşitliliğin, türlerin yaban formlarının yanı sıra ıslah edilmiş türlerin çeşitliliğinden de oluştuğunu bilirim.					
4. Biyolojik çeşitliliğin, genetik, tür ve ekosistem çeşitliliği olmak üzere üç kısımdan oluştuğunun farkındayım.					
5. Ziraat alanında kullanılan bitkilerin genetik çeşitliliğinde büyük oranda kayıp olduğunu bilirim.					
6. Nesli tükenen bir türün tekrar yeniden oluşacağının farkındayım.					
7. Bitki ve hayvan tür çeşitliliğinin yeryüzünde eşit bir şekilde dağılım gösterdiğini bilirim.					
8. Biyolojik çeşitliliğin sadece canlıların sınıflandırılmasını kapsamadığını bilirim.					
9. Bazı canlıların insanlar için faydalıyken bazılarının insanlar için zararlı olduğunu bilirim.					
10. Bilinçsiz tarımsal faaliyetlerin (bilinçsiz sulama yöntemleri, kimyasal ve gübrelerin kullanılması vb.) toprak ekosistemini olumsuz etkileyeceğinin farkındayım.					
11. Türlerin yarıdan fazlasının tropikal ormanlarda yaşadığının farkındayım.					
12. Bir tür içerisindeki bireylerin her birinin genetik yapılarının aynı olmadığını bilirim.					
13. Mercan kayalıklarının biyolojik çeşitlilik açısından tropikal ormanlar kadar zengin olmadığını bilirim.					
14. Tür çeşitliliğindeki azalmadan dolayı ekosistemdeki işlevsel çeşitliliğin azalacağı hakkında bilgi sahibiyim.					
15. Kendi aralarında çiftleştiklerinde sağlıklı ve doğurgan bireyler veren benzer özellikteki canlıların tür olduğu hakkında bilgi sahibiyim.					
16. Kelaynak, yaz ördeği ve yılan kartalının nesli tükenme tehlikesiyle karşı karşıya olan canlı türlerinin arasında olduğunun farkındayım.					
17. Bir popülasyonda birden fazla tür olabileceğinin bilincindeyim.					
18. Bir ekosistemin canlı faktörlerini mikroskobik canlılar, mantarlar, bitkiler ve hayvanların oluşturduğunu bilirim.					
19. Kaktüs bitkisinin çölde; kutup ayısının ise kutuplarda yaşamasına etki eden faktörlerin başında sıcaklık ve suyun geleceğinin farkındayım.					
20. Bir canlı türünün doğal olarak yaşadığı ve ürediği yerin habitat olduğu hakkında bilgi sahibiyim.					
21. Deniz ekosistemlerinde tuz oranının, sıcaklığın ve ışık miktarının hayvan çeşitliliğini belirleyeceğini bilirim.					
22. Birçok besin zincirinin bir araya gelerek daha karmaşık yapılı olan besin ağını oluşturacağını bilirim.					
23. Doğal seçilim ile ortama uyum sağlayamayan bireylerin yaşama şansının olmadığının farkındayım.					
24.Dünya yüzeyindeki türlerin toplam sayısının azalmakta olduğunu bilirim.					

2. Her bir ifadeyi okuduktan sonra, buna ne derecede katıldığınızı ya da katılmadığınızı liste üzerinde ayrılan yere uygun şekilde işaretleyiniz. Bir ifadeyi okuduktan sonra aklınıza ilk geleni işaretleyiniz.

İşaretsiz ifade bırakmayınız. Size verilen liste üzerine adınızı yazmayınız, kimliğinizi belirtecek herhangi bir işaret koymayınız.

Biyolojik Çeşitlilik Davranış Ölçeği	**Tamamen Katılıyorum**	**Katılıyorum**	**Kararsızım**	**Katılmıyorum**	**Tamamen**
1. Daha pahalı olsa da organik yiyecekleri almayı tercih ederim.					
2. Sulak alanların, çeşitli sektörlerde (ör: tarım, turizm vb.) o alanda yaşayan halka geçim kaynağı sağlaması amacıyla kurutulmasını desteklemem.					
3. Ekonomik sıkıntılar nedeniyle doğal kaynakların (ör: kardelen) aşırı kullanımına karşı tepki gösteririm.					
4. Doğal kaynakları doğru bir şekilde kullansak bile biyolojik çeşitliliği korumak mümkün değildir.					
5. Tüketilen ürünler daha pahalı olsa bile biyolojik çeşitliliğe zarar vermeyen ürünleri kullanmayı tercih ederim.					
6. Çok sık rastlanmayan bitkilerden elde edilen ürünleri kullanmam.					
7. Milli park ve ormanlık alanlarda turizmin gelişmesi amacıyla bina yapımı için devletin izin vermesi beni rahatsız etmez.					
8. Türkiye biyolojik çeşitlilik açısından zengin olduğundan dolayı doğal kaynakların tükenmesi konusunda rahatsız olmam.					
9. Tüketim alışkanlıklarımız, toprağın kalitesine ve tarım topraklarının kaybına neden olacağından dolayı bitkilerin yetişmesine engel olacaktır.					
10. İlaçların özenle seçimiyle tehlike altındaki tıbbi bitkilerin korunmasına katkıda bulunulur.					
11. Kozmetik ürünleri dikkatli seçerek tehlike altında bulunan türlerin korunmasına katkıda bulunurum.					
12. Türlerin korunması için çeşitli projelerin yürütüldüğü bir çevre grubuna katılırım.					
13. Bahçemin bir kısmını kendi haline bırakarak biyolojik çeşitliliğin korunmasına doğrudan katkıda bulunurum.					
14. Türkiye"de nesli tehlike altında olan türlerin yeterli bir şekilde korunması için kulüp çalışmalarına katılırım.					
15. Biyolojik çeşitliliği korumak için toplumsal olarak sorumluluk projelerinde görev alırım.					
16. İnsanları biyolojik çeşitliliğe zarar veren davranışlarından dolayı uyarmam.					
17. Biyolojik çeşitlilik, günlük faaliyetleri doğaya uyumlu olarak yaşamakla ve en yakın çevreden başlamakla korunmaz.					
18. Doğal kaynakları, gereksiz kullanmayarak biyolojik çeşitlilikle ilgili sorunlar çözülebilir.					
19. İnsan faaliyetleri, dünyanın iklimini değiştirerek biyolojik çeşitliliğe zarar vermektedir.					
20. Fauna, flora ve habitatlar, yaşanabilirlik açısından elverişli hale getirilmelidir.					
21. Vahşi hayvan türlerinin ticari amaçlı kullanımı konusunda sıkı kontroller yapılmalıdır.					
22. Tarımsal ürünlerin yalnızca insanlara faydalı olan çeşidi değil, tüm ürün çeşitleri korunmalıdır.					
23. Orman özelliğini kaybetmiş arazilerin, ülkeye gelir getirmesi amacıyla satılması beni rahatsız etmez.					
24. İnsanların yararlanmadığı doğal alanların korunması için harcanan para gereksizdir.					
25. Biyolojik çeşitliliğe zarar veren davranışlardan kaçınırım.					

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Investigating pollinator dynamics and regional variations in Doda, J&K, INDİA: challenges, monitoring and conservation perspectives

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Abstract

This study explores the plant-pollinator network in district Doda, Jammu & Kashmir, India, a Himalayan region with limited research on biodiversity and pollination ecology. Pollinators like bees, butterflies, and beetles are vital for ecosystem health and agricultural productivity, yet Doda lacks comprehensive data on its pollinator species and interactions, unlike the Kashmir Valley and Jammu region. Drawing insights from similar regions such as Kashmir, Himachal Pradesh, and Uttarakhand, this research highlights the ecological role of pollinators and emphasizes the need for extensive field exploration in Doda. The study advocates for an interdisciplinary approach combining ecological, agricultural, and local knowledge, recommending detailed surveys, long-term monitoring, and socio-economic studies to understand pollination's impact on agriculture. It calls for collaborative efforts among researchers, agricultural departments, and conservationists to gather baseline data, promote biodiversity preservation, and enhance agricultural sustainability. These efforts are crucial for supporting local livelihoods and establishing a foundation for future research and conservation initiatives in Doda's unique ecological networks.

Keywords: pollinator networks, biodiversity conservation, plant-ınsect ınteractions, agricultural sustainability, environmental ımpacts

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Doda, J&K, HİNDİSTAN'da tozlayıcı dinamiklerinin ve bölgesel farklılıkların araştırılması: zorluklar, izleme ve koruma perspektifleri

Özet

Bu çalışma, biyolojik çeşitlilik ve tozlaşma ekolojisi üzerine sınırlı araştırmalara sahip bir Himalaya bölgesi olan Hindistan'ın Jammu ve Keşmir, Doda bölgesindeki bitki tozlayıcı ağını araştırıyor. Arılar, kelebekler ve böcekler gibi tozlaştırıcılar, ekosistem sağlığı ve tarımsal üretkenlik açısından hayati önem taşıyor; ancak Keşmir Vadisi ve Jammu bölgesinin aksine Doda, tozlayıcı türleri ve etkileşimleri hakkında kapsamlı verilere sahip değil. Keşmir, Himaşal Pradeş ve Uttarakhand gibi benzer bölgelerden bilgiler alan bu araştırma, tozlayıcıların ekolojik rolünü vurguluyor ve Doda'da kapsamlı saha araştırmalarının gerekliliğini vurguluyor. Çalışma, ekolojik, tarımsal ve yerel bilgileri birleştiren disiplinler arası bir yaklaşımı savunuyor; tozlaşmanın tarım üzerindeki etkisini anlamak için ayrıntılı araştırmalar, uzun vadeli izleme ve sosyo-ekonomik çalışmalar öneriyor. Temel verileri toplamak, biyolojik çeşitliliğin korunmasını teşvik etmek ve tarımsal sürdürülebilirliği geliştirmek için araştırmacılar, tarım departmanları ve korumacılar arasında işbirlikçi çabalar çağrısında bulunuyor. Bu çabalar, yerel geçim kaynaklarının desteklenmesi ve Doda'nın benzersiz ekolojik ağlarında gelecekteki araştırma ve koruma girişimleri için bir temel oluşturulması açısından çok önemlidir.

Anahtar kelimeler: tozlayıcı ağları, biyolojik çeşitliliğin korunması, bitki-böcek etkileşimleri, tarımsal sürdürülebilirlik, çevresel etkiler

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1. Introduction

Pollination is a critical ecological service, essential for the reproduction of many flowering plants and the production of a significant portion of the world's food crops [1]. In agricultural ecosystems, pollinators play a pivotal role in enhancing crop yields and quality, thereby supporting both food security and economic stability [2]. Among the most vital pollinators are insects, including bees, butterflies, beetles, and others, which facilitate the transfer of pollen from one flower to another, enabling fertilization and seed production [3]. Plant-insect pollinator networks represent complex and dynamic interactions between plant species and their pollinators [4]. These networks are crucial for maintaining biodiversity and ecosystem health, as they ensure the survival and proliferation of both plants and pollinators [5]. In recent years, there has been growing recognition of the importance of understanding these networks, particularly in the context of environmental changes such as habitat loss, climate change, and pesticide use, which threaten pollinator populations globally [6].

The Kashmir Valley, known for its rich biodiversity and unique agro-climatic conditions, has been a focal point for ecological and agricultural research in India [7]. However, the region of Doda, located within Jammu and Kashmir, remains comparatively under-researched. Despite its similar climatic and ecological characteristics, Doda's plant-pollinator interactions have not been studied extensively, leaving a gap in our understanding of the region's ecological networks. The primary objective of this review is to bring attention to the underexplored plant-insect pollinator networks in Doda, J&K, India, and to articulate the ecological and agricultural significance of these networks. While the Kashmir Valley has been easily accessed by researchers and government policy framework for ecological research, Doda's unique sub-temperate environment remains unexplored and unobserved, particularly concerning its pollination systems. This review aims to fill this gap by synthesizing existing literature from similar climatic regions and providing a comparative analysis with the well-documented Kashmir Valley. Furthermore, the review seeks to identify the key pollinator species within these networks and their roles in local agriculture, thus laying the groundwork for future research. Additionally, this review aims to highlight the critical need for establishing baseline data on pollinator species and their interactions with native and cultivated plants in district Doda. This review will also address the challenges and gaps in current research, such as the impact of environmental changes and anthropogenic activities on pollinator populations. Ultimately, this review proposes a framework for future interdisciplinary research, combining ecological, agricultural, and socio-economic studies. This approach is essential for developing targeted conservation strategies and promoting sustainable agricultural practices in the region. By encouraging collaboration between local researchers, agricultural departments, and conservationists, the review seeks to foster a more comprehensive understanding of the ecological dynamics in district Doda and support the resilience of its agricultural systems..

2. Materials and methods

2.1 Literature search strategy

This review synthesized research on plant-insect pollinator networks with a specific focus on the understudied region of Doda, Jammu & Kashmir, India. A comprehensive literature search was conducted across various scientific databases, including Google Scholar, PubMed, and Web of Science, using keywords such as "pollinator networks," "plant-pollinator interactions," "biodiversity conservation," "Himalayan pollinators," and "agricultural pollination." Studies conducted in similar sub-temperate and temperate regions, like the Kashmir Valley, Himachal Pradesh, and Uttarakhand, were also included to provide comparative insights.

2.2 Topography of the region

The Doda District, situated in the eastern part of the Jammu Region (33°08'N, 75°3'E) within the outer Himalayan Range, has an average elevation of 1,107 meters above sea level [8]. This district was selected for study due to its critical role in temperate fruit production in the state of Jammu and Kashmir, based on both the area cultivated and the yield. While the focus for temperate fruit cultivation predominantly is in the Kashmir valley, the Doda district shares similar agro-climatic conditions, making it a suitable region for growing high-value fruit crops as done in Kashmir. Currently, the district is engaged in the cultivation of significant temperate fruit species, including *Malus domestica* (apple), *Pyrus* (pear), *Prunus armeniaca* (apricot), *Prunus persica* (peach), *Prunus domestica* (plum), *Olea europaea* (olive), *Juglans regia* (walnut), *Carya illinoinensis* (pecan), and *Prunus dulcis* (almond) (https://hortikashmir.gov.in/Area%20Production%20data.html). However, no studies on plant-insect pollinators or pollination ecology have been reported from the area. Further the described area has the potential to become a fruit and vegetable region, but the locals seems having no information about economic importance of these fruits and its produce. So there is a communication lacunae between horticulture department of J&K and locals of District Doda.

2.3 Comparative regional analysis

To draw parallels with Doda's ecological conditions, we compared data from neighboring regions with similar agro-climatic conditions, such as Himachal Pradesh and Uttarakhand. Comparative analysis involved evaluating existing pollinator species lists, documented interaction patterns, and identified threats to pollinators. This approach provided a broader understanding of Doda's potential pollinator diversity and network structure, allowing us to infer likely challenges and conservation needs.

2.4 Network structure and pollinator ecology

The review included studies utilizing network analysis to assess the structure of plant-pollinator interactions, particularly metrics such as network modularity, nestedness, and connectance. These parameters, as reported in relevant studies, were used to compare network stability and resilience in Himalayan pollinator networks. The synthesis involved summarizing findings on dominant pollinator groups, interaction patterns, and implications for ecosystem stability.

2.5 Conservation, socio-economic perspectives, data extraction and synthesis

A section of the review was dedicated to examining socio-economic studies that assessed pollinator impact on agriculture in similar regions. These studies provided insights into how pollination services benefit local communities economically, emphasizing the need for pollinator conservation in sustaining agricultural productivity. Additionally, this review analyzed the effectiveness of conservation initiatives from neighboring regions to propose potential frameworks for Doda.For each selected article, data on pollinator species, interaction types, environmental threats, and conservation strategies were extracted and organized thematically. Key findings were synthesized into comparative summaries and tables to highlight regional differences and similarities in pollinator diversity, network dynamics, and conservation challenges.This review aims to consolidate the available knowledge on Doda's pollinator networks and underscore gaps in research, encouraging further studies and conservation efforts in this understudied Himalayan region.

3. Results

3.1 Area and production of fruit crops in Doda

Based on the data available from Directorate of Horticulture, Jammu and Kashmir (https://hortikashmir.gov.in/Area%20Production%20data.html), the area under fruit cultivation in district Doda has shown fluctuations over the years. It started at 13,052 hectares in 2015-2016 and peaked at 15,064 hectares in 2018-2019. The lowest recorded area was 12,054 hectares in 2020-2021. On the contrary, the production of fruits, measured in metric tonnes, also shows significant variability. The highest production was in 2015-2016 with 43,321 metric tonnes, while the lowest was in 2017-2018 at 18,372 metric tonnes. There is a notable increase in production in 2018-2019, reaching 35,916 metric tonnes, after which it dropped again. This suggests that factors such as climatic conditions, agricultural practices, or market conditions might have influenced production levels. While the area under cultivation and production levels generally move in tandem, this is not always the case. For instance, despite a relatively high area in 2019-2020, the production was lower compared to 2018-2019, indicating that other factors beyond just the area might affect production yields (Figure 1).

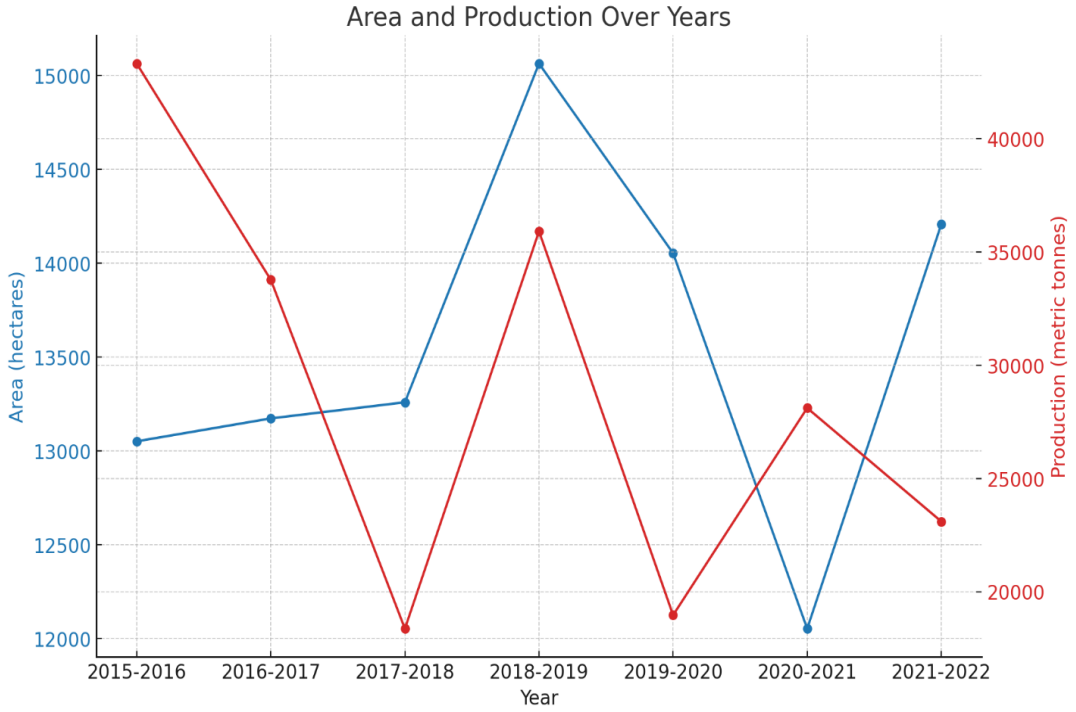

Figure 1. Area under fruit crop and production of fruit crops in district Doda from 2015-2022

3.2 Comparative analysis between Doda and Srinagar Districts

The comparative analysis between Districts Doda and Srinagar from 2015-2022 reveals significant differences in agricultural area and production. Doda has a larger agricultural area, ranging from 12,054 to 15,064 hectares, but its production fluctuates, peaking at 43,321 metric tonnes in 2015-2016 and dipping to 18,372 metric tonnes in 2017-2018. In contrast, Srinagar, with a smaller and more stable agricultural area (around 3,000 to 3,354 hectares), shows a steady increase in production, reaching a high of 31,266 metric tonnes in 2021-2022. This disparity suggests varying factors influencing productivity, such as crop types, soil quality, or agricultural practices, emphasizing the need for tailored strategies to optimize agricultural output in each district. Overall, the District Srinagar shows a promising trend in fruit crop production, with increasing outputs despite relatively stable cultivation areas. This highlights the potential for further optimization and development in the region's agricultural practices.

The observed fluctuations in agricultural yields in District Doda, compared to the more stable outputs in Srinagar, may stem from several critical factors (Figure 2). One key issue is the limited understanding of pollinator dynamics in Doda, as no comprehensive studies have been conducted to assess their role in supporting agricultural productivity. Pollinators are vital for the reproductive success of many crops, and insufficient pollinator activity due to habitat loss, pesticide use, or climate variability could be a significant factor behind Doda's fluctuating yields. Additionally, land use patterns in Doda, characterized by fragmented agricultural plots and limited irrigation infrastructure, may restrict farming efficiency and reduce crop resilience. Climatic variability in the region further exacerbates these issues, disrupting both crop cycles and pollinator activity. By comparison, Srinagar benefits from more stable climatic conditions, better-managed agricultural practices, and potentially healthier pollinator populations, which contribute to consistent agricultural outputs. To address these disparities, targeted research on pollinator dynamics, habitat restoration, reduction of pesticide use, and the adoption of integrated pest and land management practices in Doda are necessary to enhance pollination services, stabilize yields, and improve overall agricultural sustainability.

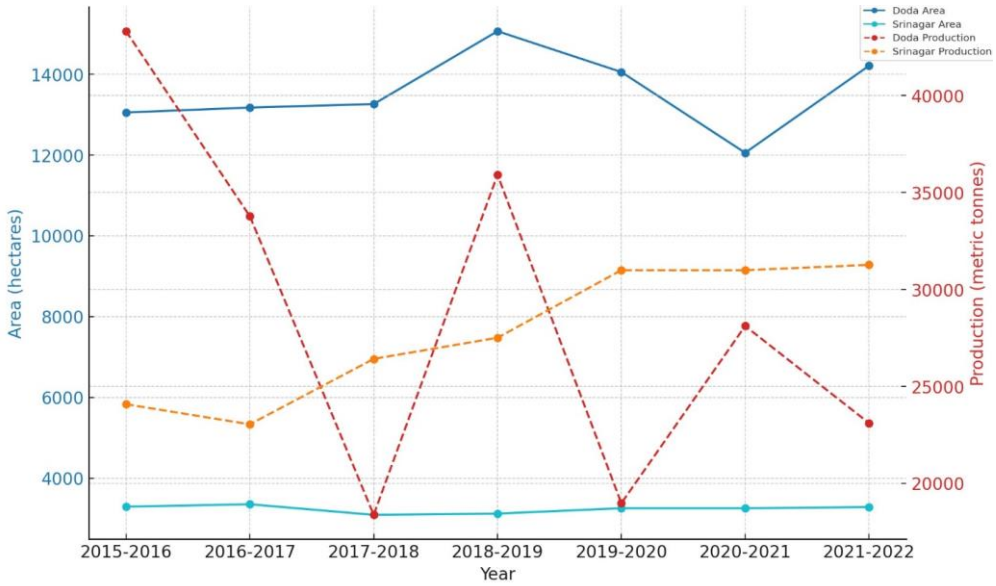

Figure 2**.** Comparative Analysis of Area under fruit crops and fruit Production between Districts Doda and Srinagar

3.3 Plant-insect pollinator networks in Kashmir division

The Kashmir Himalaya, located in the northwestern part of the Himalayas, is famous for its natural landscape, which features a diverse and unique biodiversity [9]. Despite this rich biodiversity, there have been relatively few research studies focused on the diversity of flowering plants and their insect visitors in this region. In the study conducted by Rather *et al.* (2017), a comprehensive survey of the Kashmir Himalaya's flora revealed 227 plant species spanning 182 genera and 58 families, identified as key foraging sources for various pollinators. The research indicated that annual herbaceous plants were the predominant contributors of pollen and nectar, followed by perennial herbs, shrubs, trees, and biennial herbs. The family Asteraceae emerged as the leading family in terms of pollen and nectar production. The study documented 70 species of flower visitors from 43 genera and 23 families, with Apidae being the most dominant family. A significant diversity in floral traits was noted, including variations in floral display size, inflorescence architecture, and flower shape. These traits were found to significantly influence pollinator attraction and visitation rates, with larger and more conspicuous floral displays attracting more pollinators. Actinomorphic flowers, which are symmetrical and accessible to a wide range of pollinators, were more prevalent compared to zygomorphic flowers, which are more specialized. The study identified *Xylocopa valga*, along with *Apis mellifera* and *Apis cerana*, as highly effective pollinators in terms of pollen deposition, with *Xylocopa valga* being particularly noted for its efficiency. The analysis highlighted a predominance of generalized plant-pollinator interactions, with a substantial number of plant species being visited by multiple pollinator species. This generalist strategy indicates a resilient plant-pollinator network capable of sustaining biodiversity under varying environmental conditions.

A further study by Rather *et al.* (2023) investigated a plant-pollinator meta-network in the Kashmir Himalaya, highlighting its structure and stability. This research documented interactions between 230 plant species and 80 pollinator species, with Malus domestica and Apis bees being particularly influential. The network showed significant modularity, including eight distinct modules comprising both native and alien species, indicating a well-integrated community. Notably, alien species contributed to 40% of the interactions. Extinction simulations revealed that the network is more vulnerable to the loss of key pollinators than plants, emphasizing the critical role of pollinators in maintaining ecological stability [10]. A detailed list of plat-Insect pollinators associated with different agro-horticultural crops of Kashmir Valley is discuused in Table 1.

3.4 Plant-insect pollinator networks in Jammu division

Shankar *et al.* (2017) conducted a study in the Jammu region to examine the species composition of pollinators on *Pongamia pinnata* L., a perennial flowering plant commonly used as an avenue tree. The study identified 21 pollinator species from the orders Hymenoptera, Diptera, Thysanoptera, and Lepidoptera, as well as birds. Among these, megachilid bees were the most abundant, constituting over 55% of the insect visitors. The foraging activity of these pollinators peaked between 10:00 and 12:00 hours. The study suggests that Pongamia flowers could be important floral sources, providing a reservoir for pollinators during hot summers [16]. Ganai *et al.* (2017) studied the insect pests, pollinators, and natural enemies associated with marigold crops in the Jammu division and recorded several pollinators visiting marigold flowers, including *Apis mellifera*, *Apis dorsata*, *Pieris brassicae*, and the thistle butterfly (*Vanessa cardui*). Among natural enemies, the syrphid fly (*Syrphus spp*.), ladybird beetle (*Coccinella septumpunctata*), spider (*Oxyopes javanus*), and big-eyed bug (*Geocoris spp.*) were noted [17].

Table 1. Insect pollinators associated with different agro-horticultural crops of Kashmir Valley

Order	Species	References
Hymenoptera	*Bombus funerarius*	[10, 11, 12, 13, 14, 15]
	Thyreus nitidulus	
	Melissodes bimaculata	
	Lasioglossum moroi	
	Ceratina calcarata	
	Ceratina hieroglyphica	
	Halictus confuses	
	Polistes maculipennis	
	Vespa auraria	
	Andrena gravida	
	Pison punctifrons	
	Athalia proxima	
	Megascolia haemorrhoidalis	
	Eusaphyga verticalis	
	Anthophora sp.	
	Polistes sp.	
	Odynerus sp.	
	Pimpla sp.	
	Megachile sp.	
	Osmia sp.	
	Pepsis sp.	
	Rhynchium sp.	
	Xylocopa valga	
	Xylocopa violacea	
	Bombus simillmus	
	Bombus tunicatus	
	Andrena patella	
	Andrena cineraria	
	Sphecodes tantalus	
	Sphecodes lasimensis	
	Lasioglossum himalayense	
	Lasioglossum nursei	
Diptera	*Sphaerophoria bengalensis*	
	Metasyrphus bucculatus	
	Episyrphus balteatus	
	Eristalodes paria	
	Eristalis tenax	
	Eoseristalis cerealis	
	Eristalis soliatus	
	Didea fasciata	
	Scaeva pyrastri	
	Syrphus balteatus	
	Syritt orientalis	
	Eristalis tenax	
	Eristalis arbustorum	
	Sphaerophoria scripta	
	Hermetia illucens	
	Helophilus trivittatus	
	Bombylus major	
	Musca domestica	
	Dryomyza flaveola	
	Eristalinus sp.	

Table 1. Continued

	Syritt sp.
	Sphaerophoria sp.
	Eristalis sp.
	Bibio sp.
	Chrysomyia sp.
	Pseudopyrellia sp.
	Bombylidae sp.
	Machinus sp.
	Fanna sp.
	Sarcophaga sp.
Lepidoptera	*Pieris brassicae*
	Cynthia cordui
	Colias romonovi
Coleoptera	*Coccinella septumpunctata*
	Hippodamia variegate
	Altica cyanea
Odonata	*Ischnura pumilio*
	Libellula quadrimaculata

3.5 Plant-insect pollinator networks in Jammu division

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Jamwal *et al.* (2019) conducted a study in the Jammu division to investigate the diversity of insect pollinators on citrus flowers. The study identified 44 insect species across 5 orders and 16 families. The distribution of these species included 15 from Hymenoptera, 13 from Diptera, 6 from Lepidoptera, 8 from Coleoptera, and 2 from Hemiptera. Among the hymenopterans, the Indian honeybee, *Apis cerana*, was the most abundant, accounting for 33.07% of insect visitors to citrus crops [18]. Kumari & Sidhu (2020) studied the grasslands in Jammu, part of the union territory of Jammu and Kashmir, highlighting their ecological importance as habitats for diverse species, including Hymenoptera. These insects are crucial pollinators in grassland ecosystems and help control pest populations, thus maintaining ecological balance. Over two years, the researchers identified 13 species of Apoidea from 6 genera and 9 subgenera, with two species newly recorded for the region. The study underscores the role of Hymenoptera in pollination and the ecological significance of grasslands [19]. Mondal *et al.* (2022) investigated the diversity of bumblebee species across sub-tropical, intermediate, and temperate regions in the Jammu division, adjacent to District Doda. The study, conducted over two years (2016-2017), recorded varying species distributions across different altitudes. In the sub-tropical zone, two species, *Bombus haemorrhoidalis* and *Bombus trifasciatus*, were identified. The intermediate zone hosted three species: *B. haemorrhoidalis*, *Bombus simillimus*, and *Bombus tunicatus*. The temperate zone, ranging from 1000 m to 2214 m above mean sea level, supported five species, including the aforementioned three and *Bombus pyrosoma*. Overall, seven bumblebee species were documented, highlighting a greater diversity in higher altitude regions [20].

Abrol & Chatterjee (2022) documented a diverse range of Megachilid bees in the Jammu division of Jammu and Kashmir, India, many of which were newly recorded for the region. The study identified sixteen morpho-species, with most found in plains and foothills, and one species in both hilly and plain regions. These bees showed peak foraging activity in the forenoon and were efficient pollinators, suggesting their importance in sustainable agriculture. The species included ten morpho-species of Megachile, three of *Osmia*, two of *Coelioxys*, and one of *Anthidium*, highlighting the rich biodiversity of the area [21]. Shankar & Mukhtar (2022) conducted a two-year study on the diversity and foraging behavior of pollinators on Sesamum indicum L. flowers across different altitudes in Jammu. They identified 26 native pollinator species from the families Apidae, Megachilidae, Halictidae, and Sphingidae. The dominant pollinators, in descending order, were Megachilid bees, Honeybees, Halictid bees, Ceratina bees, Amegilla bees, *Xylocopa* bees, and Bumblebees. Pollinator abundance followed a polynomial distribution, suggesting an even

distribution among groups, with some dominant in flower visitation. The study found that Megachilid and Honeybees had longer foraging bouts compared to other pollinators [22].

3.6 Existing research from similar regions

Sub-temperate regions such as Himachal Pradesh and Uttarakhand have been the focus of numerous studies on plant-insect pollinator networks due to their rich biodiversity and significant agricultural landscapes [23]. These areas are characterized by diverse ecosystems ranging from alpine forests to subtropical woodlands, each supporting a unique assemblage of pollinator species [24]. The research conducted in these regions has been pivotal in understanding the complex interactions between plants and their pollinators, offering valuable insights into the functioning of these ecosystems [25]. In Himachal Pradesh, extensive studies have been conducted recently on the role of native bee species, particularly in apple orchards, which are a major agricultural crop in the region [26]. These studies have shown that native bees, including various species of solitary bees and bumblebees, are crucial for pollination, contributing to fruit set and quality. The research also highlights the importance of maintaining natural habitats adjacent to agricultural lands, as these areas provide nesting sites and floral resources for pollinators throughout the year. Uttarakhand, with its diverse topography and climate, offers a wide range of habitats for pollinators. Research in these localities has focused on both managed pollinators, such as the Indian honey bee (*Apis cerana*), and wild pollinators, including a variety of butterflies, moths, and beetles [27]. Studies have documented the critical role these insects play in pollinating a wide range of crops, from traditional staples like millets and pulses to horticultural crops such as tomatoes and cucumbers [28]. Additionally, Uttarakhand's forests support a rich variety of flowering plants that rely on insect pollination, further underscoring the ecological importance of these networks.

3.7 Implications for Pollinator montioring in Doda Based on regional studies

The studies conducted in neighbouring districts of Doda in both Jammu and Kashmir divisions, Himachal Pradesh, Uttarakhand, and other sub-temperate regions offer valuable insights and lessons that can be directly applied to understanding and managing plant-insect pollinator networks in Doda, J&K, India.One of the key takeaways from the research in these regions is the critical need for comprehensive baseline data on pollinator species and their interactions with plants [29]. In Doda, similar efforts are necessary to catalog the diversity and abundance of pollinators and to identify the plant species they pollinate. This baseline data is crucial for understanding the ecological roles of different pollinators, assessing their contributions to crop production, and establishing conservation priorities [30]. Moreover, In Doda, conducting preliminary field studies is crucial for gathering baseline data on the diversity and behavior of pollinator species, as well as their interactions with local flora [31]. Suggested methods include direct observation, where researchers systematically observe pollinator activities and behaviors; net sampling, which involves capturing pollinators to accurately identify and study them; and photographic documentation, which serves as a visual record for later analysis and species identification (Figure 3). These methods provide essential data that can help establish a foundational understanding of the pollination dynamics in Doda, informing both conservation strategies and agricultural practices. The research in neighbouring localities of Doda has highlighted the significant role that native pollinators, such as solitary bees and bumblebees, play in pollinating crops and wild plants. For Doda, this underscores the importance of identifying and conserving native pollinator species, which may be adapted to local environmental conditions and plant species. Protecting these native pollinators can enhance crop yields and biodiversity, particularly in a region with diverse agricultural practices and natural habitats [32]. Studies from these regions have demonstrated how environmental changes, including habitat loss, pesticide use, and climate change, can negatively affect pollinator populations [33]. In Doda, similar threats are likely present, and the lessons learned from these other regions emphasize the need for sustainable agricultural practices and habitat conservation. For instance, reducing pesticide use, planting diverse floral resources, and preserving natural habitats can help mitigate the impacts of environmental changes on pollinator health and diversity [34].

Research in neighbouring localities of Doda has shown that involving local communities in conservation efforts is crucial for the success of pollinator protection strategies. In Doda, community education and involvement can be equally important. Raising awareness about the importance of pollinators, promoting practices that protect these species and encouraging local participation in monitoring and conservation efforts can lead to more sustainable outcomes [35]. Community-based initiatives, such as establishing pollinator-friendly gardens or reducing pesticide use, can play a vital role in supporting pollinator populations. For Doda, adopting a similar interdisciplinary approach can help address the complex challenges related to pollinator conservation and agricultural productivity. Collaboration between researchers, agricultural extension services, local farmers, and conservation organizations can lead to more holistic and effective strategies for managing pollinator networks. The experiences from neighbouring localities of district Doda suggest that policies supporting sustainable agricultural practices and habitat conservation are essential for protecting pollinator networks. Moreover, advocating for policies that promote the use of pollinator-friendly practices, protect natural habitats, and support research and education initiatives can help ensure the long-term health of pollinator populations and the ecosystems they support. By applying these insights and lessons to Doda, stakeholders can develop targeted

conservation strategies that protect pollinator diversity, enhance agricultural sustainability, and support the overall health of the region's ecosystems.

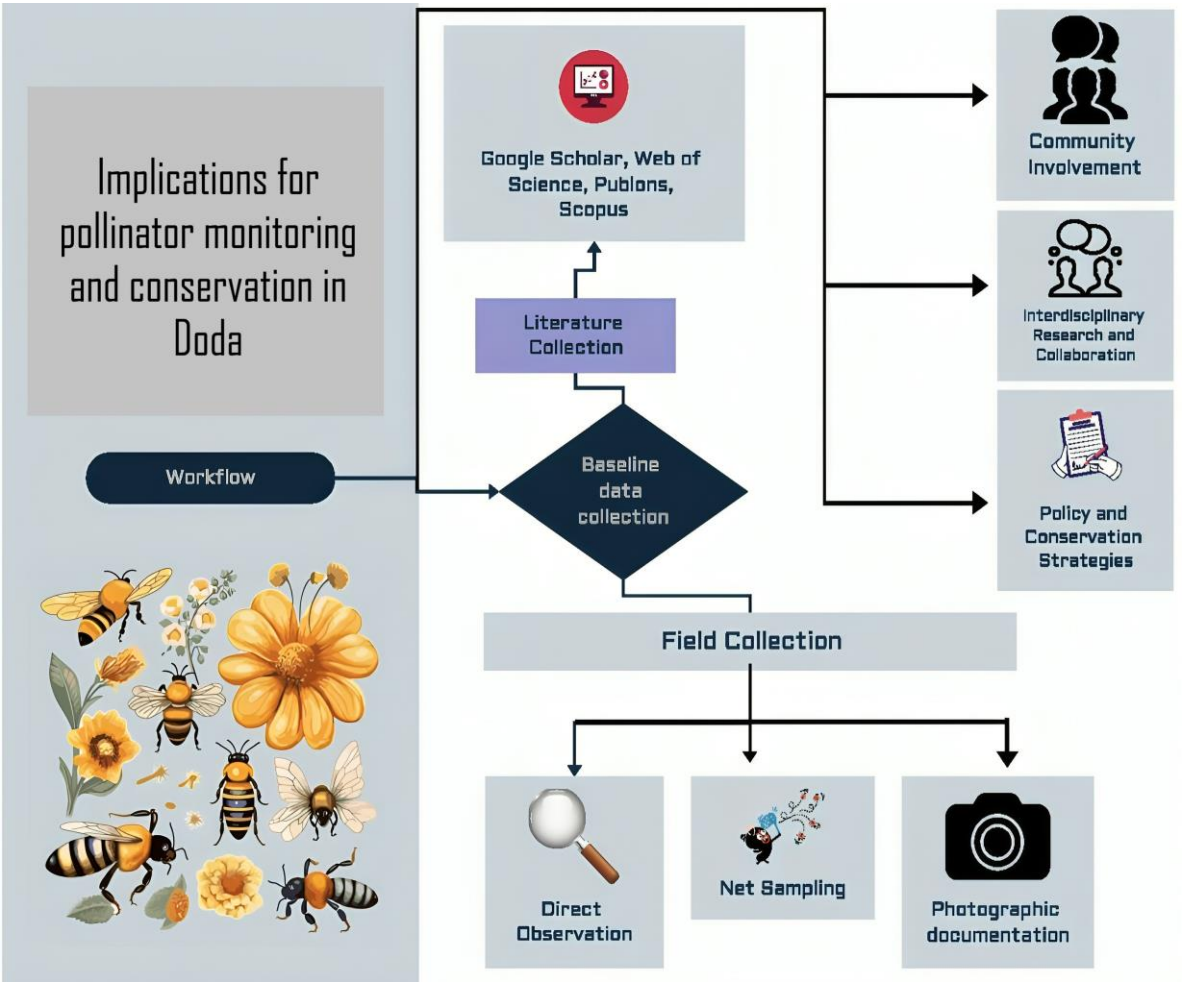

Figure 3. A workflow of implications for pollinator monitoring in district Doda on regional studies

4. Conclusions and discussions

The proposed research framework emphasizes the importance of interdisciplinary approaches in studying plant-insect pollinator networks. Integrating perspectives from ecology, agriculture, and local knowledge is crucial for developing a comprehensive understanding of these networks in Doda. Ecological studies can provide insights into the biodiversity and behavior of pollinator species, while agricultural research can explore the implications of pollinator activity on crop yields and quality. Local knowledge, including traditional practices and observations from farmers and indigenous communities, can offer valuable context and enhance the relevance and applicability of research findings. To address the gaps in current knowledge and support the conservation and management of pollinator networks in Doda, the following studies and surveys are proposed:

4.1. Detailed surveys and long-term monitoring

Conducting detailed surveys to document the diversity and abundance of pollinator species in various habitats across Doda is essential. These surveys should include the identification of key pollinator species and their interactions with native and cultivated plants. Long-term monitoring is also crucial to track changes in pollinator populations and behaviors over time, providing data on the effects of environmental changes, habitat loss, and climate change.

4.2. Socio-economic studies

Pollination has significant socio-economic implications, particularly in agricultural regions. Studies should investigate the impact of pollination services on local agriculture, including crop yields, quality, and economic benefits to farmers. These studies can also explore the socio-economic factors that influence pollinator health, such as land use

practices, pesticide application, and conservation attitudes. Understanding these factors can help develop targeted interventions and policies that support both pollinator conservation and agricultural sustainability. By combining ecological, agricultural, and socio-economic research, this proposed framework aims to create a robust foundation for understanding and enhancing pollinator networks in Doda. Such an integrated approach will not only contribute to the conservation of biodiversity but also support sustainable agricultural practices and improve the livelihoods of local communities.

This review synthesizes findings from regions with similar sub-temperate climates, such as neighbouring districts, and states like Himachal Pradesh and Uttarakhand, highlighting the rich diversity of plant-insect pollinator networks and their critical roles in both natural ecosystems and agricultural systems. These studies emphasize the importance of native pollinators, the impact of environmental changes, and the benefits of community involvement in conservation efforts. By applying these insights to Doda, it becomes evident that there is a significant gap in baseline data and understanding of local pollinator species and their interactions with plants. The applicability of these findings to Doda suggests that similar ecological patterns and challenges are likely present, underscoring the need for comprehensive research and targeted conservation strategies.

Given the critical role of pollinators in maintaining biodiversity and supporting agricultural productivity, there is an urgent need for focused research and conservation efforts in Doda. Establishing baseline data on pollinator species, conducting detailed ecological and socio-economic studies, and monitoring environmental impacts are essential steps toward understanding and protecting these vital networks. Additionally, fostering collaboration between local researchers, agricultural departments, and conservationists is crucial. Such partnerships can facilitate the sharing of knowledge, resources, and expertise, leading to more effective conservation strategies and sustainable agricultural practices. By engaging with local communities and integrating traditional knowledge, these efforts can be further strengthened, ensuring the resilience and health of Doda's ecosystems and agricultural systems. This collective approach is not only beneficial for biodiversity conservation but also vital for the long-term sustainability of local livelihoods dependent on agriculture.

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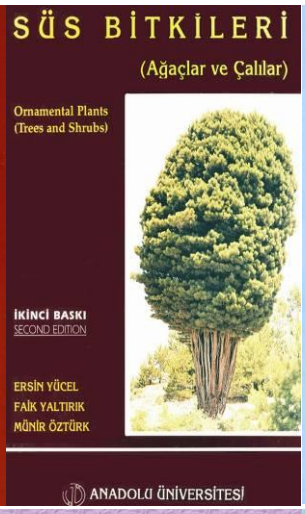

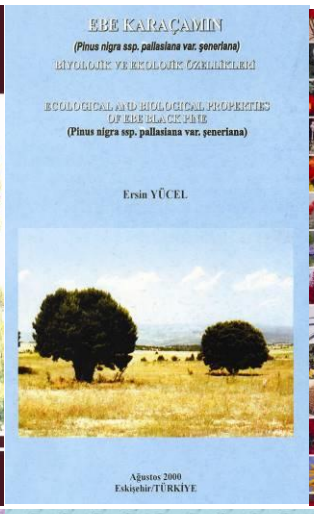

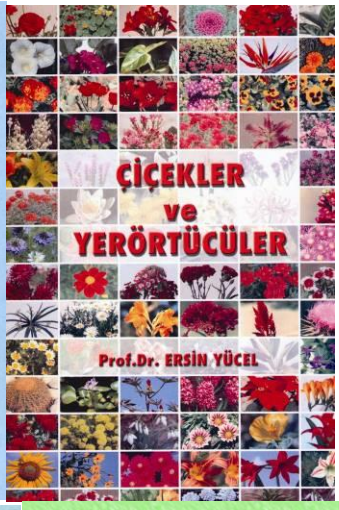

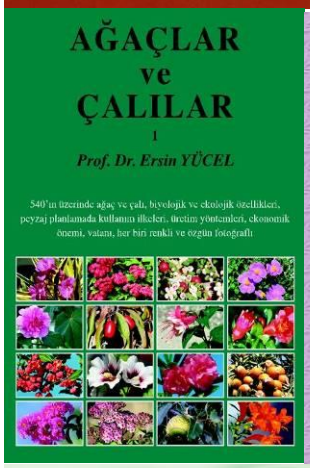

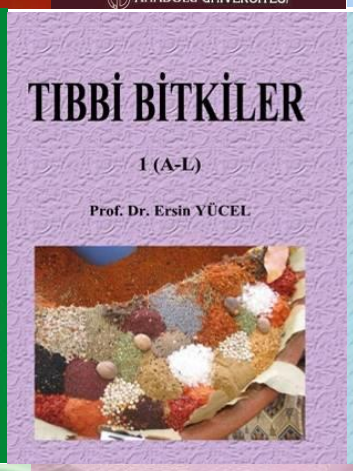

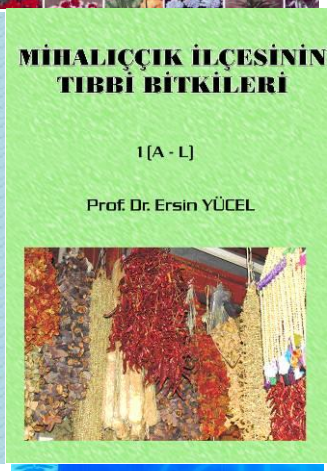

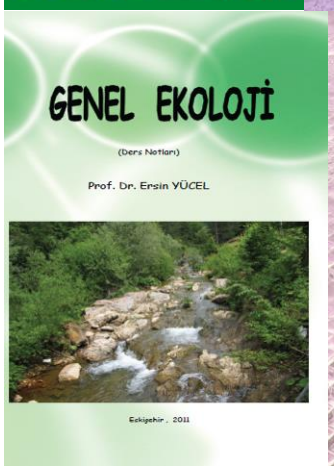

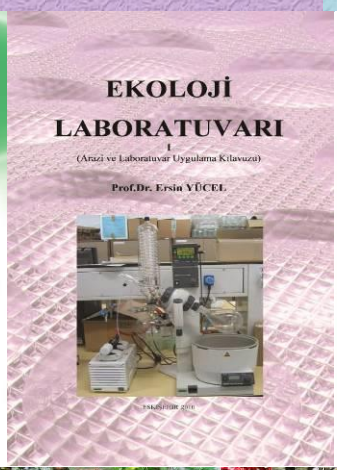

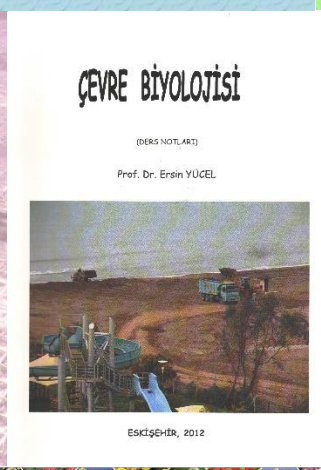

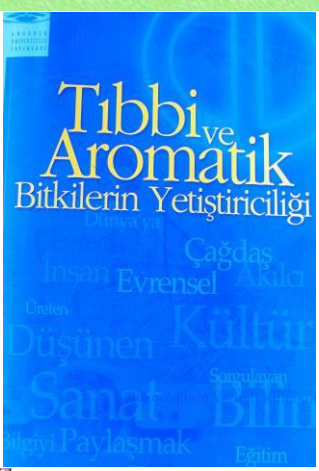

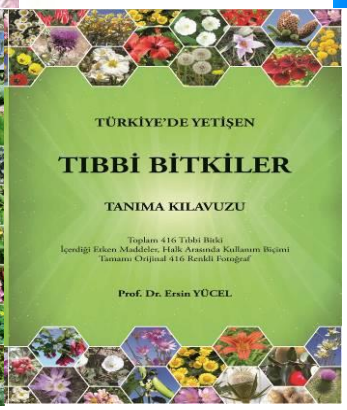

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