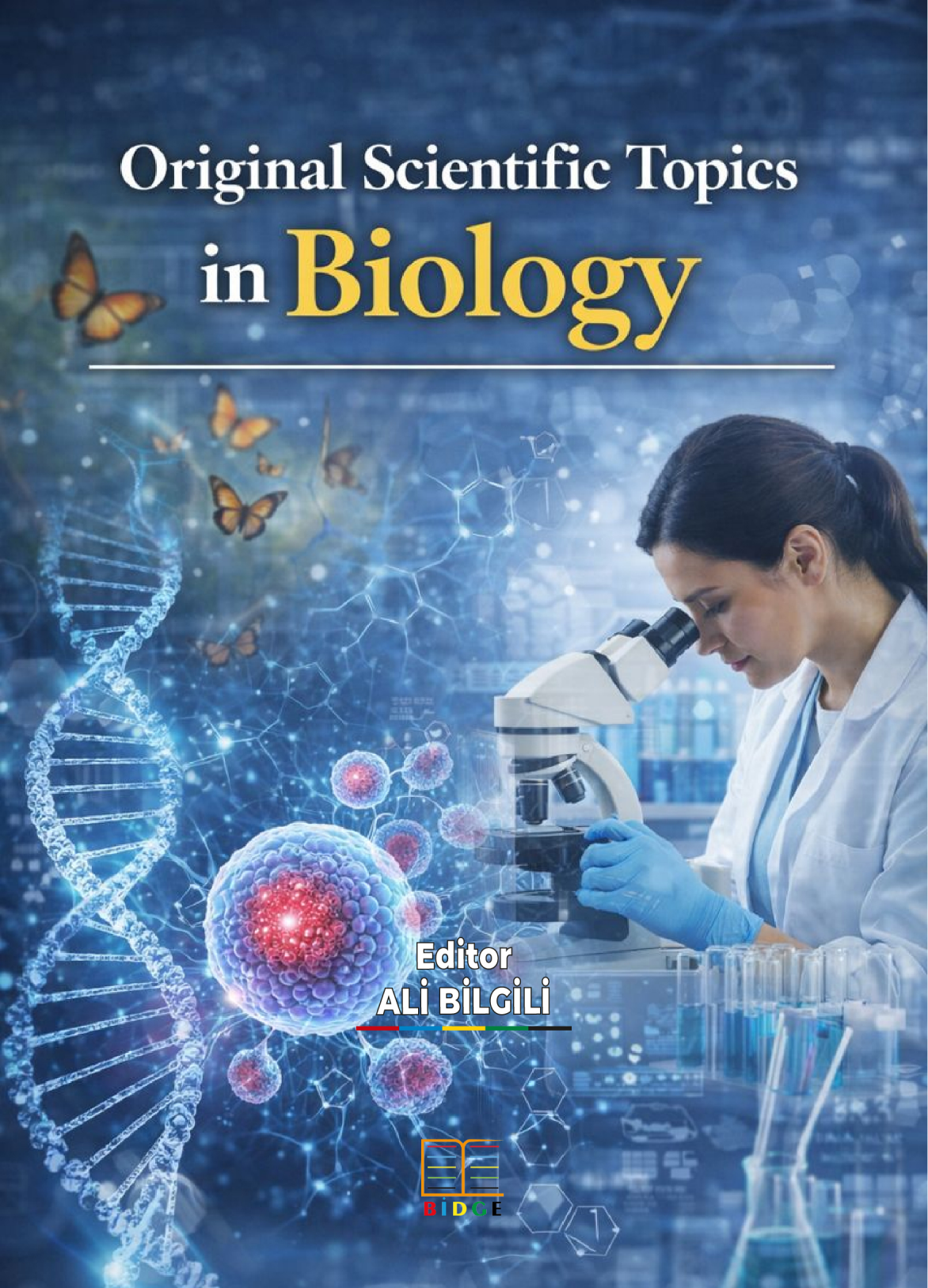
Original Scientific Topics in Biology



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ALİ BİLGİLİ

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PREFACE

Animals play an important role in people's healing processes and in folk rituals. In this book chapter, Pexiganan (MSI-78) (a broad-spectrum antibacterial peptide) obtained from the *Xenopus laevis* frog species, Temporin 10a peptide (a peptide effective against Gram-positive bacteria) obtained from the frog species *Rana ornativentris*, and Temporin A peptide (an antidiabetic peptide) obtained from the frog species *Rana temporaria* are comprehensively discussed in light of scientific sources.

Another chapter of the book provides important information on the geophyte flora of Kırşehir (Central Anatolia, Turkey), which has low species richness and a high endemism rate.

The virulence levels and infection potential of microorganisms are related to their genetic structure. In this context, virulence genes in pathogenic bacteria, their functional roles, phylogenetic utilization and their effects on food safety were explained with reference to scientific sources from recent years.

A wound is generally defined as a disruption of the integrity of body tissue resulting from physical injury. Although wound healing is often overlooked because it is considered a natural feature of the human body, it is an extremely complex and delicate physiological process. This section evaluated studies on wound healing in diabetes, a disease that is very common in humans.

In agriculture, various additives (adjuvants) are used to increase work efficiency and effectiveness when applying pesticides, plant growth regulators and foliar fertilisers. Adhesive-binders are at the top of the list of these additives. Within this scope, this section of the book evaluates the production of natural dispersing-adhesives as alternatives to synthetic substances using the plants *Aesculus Hippocastanum* L. and *Saponaria Officinalis* L.

Angelman syndrome is primarily associated with impaired expression of the UBE3A gene, which is inherited from the mother. This section presents important information from recent scientific sources on the molecular basis of Angelman syndrome, its clinical findings, and current treatment approaches.

Pests represent one of the most critical challenges in global agriculture. Particularly in apple production, which is a product worth billions of dollars annually, these threats can significantly reduce yields if not managed, jeopardizing fruit quality, size and marketability while also increasing production costs. In this context, this chapter of the book also comprehensively addresses the complex of insect pests observed in apple plants and integrated management strategies, along with the most up-to-date scientific data.

According to the Food and Agriculture Organization of the United Nations, integrated pest management means the careful evaluation of all available pest control techniques and the subsequent integration of appropriate measures that prevent the development of pest populations and ensure that pesticides and other interventions are kept at economically justified levels, while reducing or minimizing risks to human health and the environment. This section emphasizes that the adoption of integrated pest management techniques offers numerous advantages, including a reduction in pesticide use and associated risks, increased crop yield and quality, improved biodiversity, and enhanced resilience and profitability of agricultural systems.

Every individual's DNA is unique. DNA determines each person's genetic identity, and this identity, like a fingerprint, cannot belong to anyone else. In this section, the different PCR techniques used in the analysis of forensic cases were comprehensively explained.

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İÇİNDEKİLER

BÖLÜM 1

DIFFERENT PCR TECHNIQUES USED IN THE ANALYSIS OF FORENSIC CASES

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AYSEL VEYISOGLU[3]

Introduction

Every individual's DNA is unique. DNA determines the genetic identity of each person and this identity, like a fingerprint, cannot belong to someone else. A DNA sample found at a crime scene can unambiguously identify the person to whom it belongs. In particular, the uniqueness of DNA constitutes one of the most powerful tools for identifying criminals. In this way, a person's guilt or innocence can be clearly established once their involvement in the crime has been confirmed.

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In forensic science, DNA profiling (especially STR- Short Tandem Repeats) allows a DNA sample from a crime scene to be matched to the identity of a suspect. DNA profiling provides strong evidence about the criminal, and because variations in certain regions of DNA (such as STRs) are different for each individual, this profiling is highly sensitive and accurate. If the offender matches a DNA sample taken from a person of interest, the identification of the offender is greatly enhanced.

Sometimes very small amounts of biological evidence (blood, saliva, hair, skin cells, sweat, etc.) may remain at the crime scene. DNA amplification techniques such as PCR (Polymerase Chain Reaction) amplify these small amounts and make them analyzable. With this method, even destroyed or contaminated samples can be analyzed. For example, at a murder scene, even if the victim's hair or fingerprints of the perpetrator are not found, a DNA sample can be found, which plays a critical role in solving the crime.

The accuracy of DNA tests is extremely high. A person's DNA can be matched in forensic cases with 99.99% accuracy. This increases the power of DNA in solving crimes.

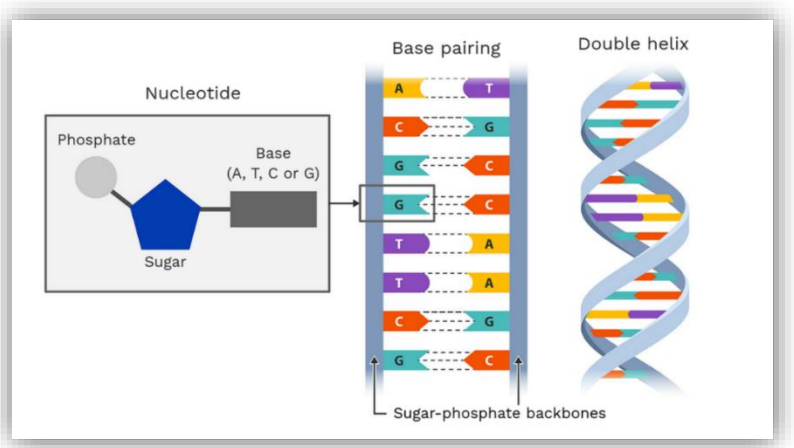
The types of PCR used in forensic science often serve different purposes, such as DNA profiling, offender identification, authentication and evidence analysis. Each PCR variant differs according to specific conditions and targets. Some of the PCR types commonly used in forensic science are Classical PCR (General PCR), RT-PCR (Reverse Transcriptase PCR), Real-Time PCR (qPCR), Multiplex PCR, STR-PCR (Short Tandem Repeat PCR), Nested PCR and Touchdown PCR.

1. Usage of DNA in Forensic Sciences

Individuals leave traces of their DNA wherever they go. DNA can be transferred to surfaces and objects when touched, released into the air and deposited in indoor dust. The presence of individuals in one location is sufficient to facilitate direct or indirect DNA transfer to the surrounding environment (Fantinato, 2024)

DNA consists of nucleotide units. Each nucleotide consists of 3 components: a nucleobase, a sugar and a phosphate. The nucleobase or "base" that binds to the sugar and phosphate moieties that form the backbone of the DNA molecule is the cause of the variability in each nucleotide. The DNA alphabet consists of only 4 characters symbolizing the 4 nucleobases, A (adenine), T (thymine), C (cytosine) and G (guanine) (Fig. 1). Different combinations of these 4 letters, known as nucleotides or bases, make up the differences in humans and all living things (Butler, 2009).

Fig*ure 1. The structure of DNA*



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Since DNA is the molecule that carries all the information necessary for the development and functions of an organism, 99.5% of this molecule is actually the same in all humans. The part that carries the polymorphism used to detect individual differences is only 0.5% of DNA. In this respect, it is this 0.5% of DNA that is of interest to forensic sciences. The method of detecting individual differences on DNA based on polymorphisms in certain regions of DNA is called “DNA Fingerprint analysis”(Jeffrey et al., 1985a).

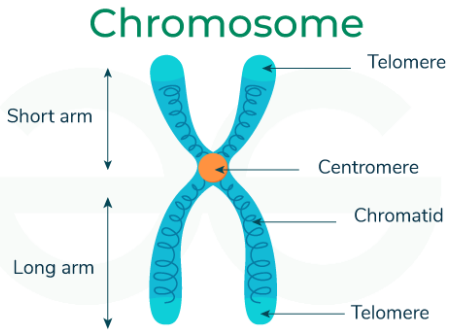
The hybridization of the two strands of DNA is its fundamental property. However, in a process known as “denaturation”, the hydrogen bonds holding the two strands of DNA together can be broken at high temperatures or by treatment with chemicals. The common method used to denature double-stranded DNA is to heat it to near boiling temperature. The DNA double strand can be denatured in salt solution with low ion activity. Chemical denaturants such as urea and formamide can also be used, which prevent the opened DNA from hydrogen bonding with its nucleotides, preventing it from joining with the complementary DNA strand. Denaturation is a reversible event. If double-stranded DNA is heated, the DNA will separate into two separate strands. When the DNA sample is cooled, the single DNA strands will find their complementary strand and rehybridize or bind together. The joining of two complementary DNA strands is called renaturation or reannealing (Butler, 2009).

1.1. Chromosome Structure

The DNA material in chromosomes contains “coding” and “non-coding” regions. The coded regions are known as “genes” and contain information about how to make proteins that are essential for the cell. A gene usually contains several thousand to ten thousand base pairs. The biggest surprise of the Human Genome Project was the discovery that humans are thought to have between 50 000 and

100 000 protein-coding genes, whereas they have fewer than 30 000 (Butler, 2009).

Figure 2. The structure of chromosome



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The centromere is the region where the spindle fibers attach and is the most important structural part of the chromosome. During the anaphase stage of cell division, it plays a role in the movement of chromosomes toward opposite poles of the cell by attaching to the spindle fibers. The centromere divides the chromosome into two arms, which are called chromosome arms. Chromatids are structures formed when chromatin binds to protein-based skeletons. The short and long arms of the chromosome are composed of these chromatids, which are divided into two by the centromere. The short arm is called the p arm, and the long arm is called the q arm. Telomeres are specialized DNA sequences located at the ends of chromosomes (Fig. 2). They play an important role in maintaining chromosome integrity and gradually shorten over time as part of the aging process (Elçi & Sancak, 2013).

Chromosomes are found in a ‘diploid’ state in all somatic cells. This means that both copies of the chromosomes are present in somatic cells. Chromosomes are found in a haploid state in reproductive cells. They contain a single copy of the chromosomes. Genes consist of exons (protein-coding regions) and introns (non-protein-coding regions). Genes account for approximately 5% of human genomic DNA. The non-protein-coding regions of DNA constitute our chromosomal material because these regions are not directly related to protein synthesis. Variable markers that differ between individuals are found in the non-coding regions of the human genome. The position or location of a gene or a DNA marker in a non-coding region on a chromosome is called a locus (plural: loci). As a result of worldwide studies, thousands of loci have been characterized in the Human Genome Project, and their locations on human chromosomes have been mapped. Each pair of chromosomes is called homologous chromosomes because they are the same size and have the same genetic structure. Each copy of a gene is located in the same region (locus) of the homologous parts. One of the homologous chromosomes comes from the person's mother and the other from the father. If a mutation occurs, the DNA chains in the homologous chromosomes will not be identical. Each of the alternative forms found in a specific region of a gene is called an allele. If the two alleles in a genetic locus on homologous chromosomes are different, they are called “heterozygous”; if the alleles are identical in specific regions, they are called “homozygous.” In human identification tests, the differences between alleles are taken into account. Human chromosomes are numbered according to their size, with the largest chromosome being 1 and the smallest being 22. The entire sequence of chromosome 22, which has over 33 million nucleotides, was announced in December 1999. With the completion of the Human Genome Project in April 2003, the lengths and sequences of all 23 pairs of human chromosomes are now known (Butler, 2009).

The first article in the field of forensic genetics was published in 1977 (Stasi, 2023).

2. DNA Applications in the Forensic Field

2.1. DNA profiling

A DNA profile shows whether a biological sample belongs to a specific person. This technique involves examining certain regions of DNA obtained from biological samples in a laboratory to obtain information that acts as a “barcode.” The criminal significance of DNA is as follows: The DNA in every cell of a human being is the same. For example, the DNA in a person's blood is the same as the DNA in their semen, saliva, liver, brain, muscles, bones, etc. Since it is the same in all nucleated cells in the human body, the DNA obtained will be the same regardless of which biological tissue is used. Originally developed for applications such as paternity testing, DNA profiling is conducted in clinical or laboratory environments, where genetic markers are analyzed to establish biological relationships—typically between parents and offspring (Butler, 2005; Jobling & Gill, 2004).

DNA is a very powerful identification tool because, with the exception of identical twins, every person's DNA is unique. This uniqueness makes it possible to identify suspects and victims in forensic science. It can corroborate the victim's statement. It can help the suspect admit to the crime and confess. It helps determine the degree of culpability of those involved in the crime. It is more reliable than eyewitness testimony. In addition, when evidence collected from one crime scene is compared with evidence collected from another location, the perpetrator's local, regional, or national connections can be revealed.

3. Genetic Markers Used in Identification

3.1. Polymorphism

Genetic diversity present in a population is called polymorphism. These natural differences are passed down from generation to generation according to Mendel's Laws (Goodwin et al., 2010).

3.2. DNA polymorphism

DNA polymorphism is defined as nucleotide changes found on DNA sequences that do not cause any disease and often do not encode proteins. Since they are passed down from generation to generation without change, they can be used in genetic typing (Butler, 2011).

These include Restriction enzyme length polymorphism (RFLP), Variable number tandem repeats (VNTR), Short tandem repeats (STR), Single nucleotide polymorphism (SNP) occurring once every 1000 bases.

3.2.1. RFLP Restriction Fragment Length Polymorphism

The first DNA polymorphic markers are called Restriction Fragment Length Polymorphisms (RFLPs). RFLPs are DNA-based polymorphisms caused by base changes, insertions, or deletions that result in variations in fragment lengths when cut by a specific restriction endonuclease. These loci are markers identified through Southern hybridization applications of DNA cut by a restriction endonuclease. The use of RFLP markers is limited due to their requirement for large amounts of unfragmented DNA, the need for tedious and time-consuming Southern hybridization procedures, and their relatively low informativeness. The total number of alleles

observed at loci containing single base changes is low, limiting their ability to distinguish between individuals. Such polymorphisms are of particular importance in the diagnosis of genetic diseases (Strachan & Read, 2010; Sambrook & Russell, 2001; Erlich, 1989; Lander & Botstein, 1989:185).

3.2.2. Variable Number of Tandem DNA Repeats (VNTR)

Another form of DNA polymorphism is VNTR (Variable Number of Tandem Repeats) regions, which are DNA sequences located between restriction enzyme (RE) cleavage sites and contain a variable number of tandem repeats. These sequences are typically 10–100 base pairs in length, and the number of alleles at a locus typically ranges from 2 to 20 (Butler, 2011). VNTR regions are widely distributed throughout the human genome and are not specific to a single locus. Due to these characteristics, VNTRs are frequently used in forensic biology for their high discriminatory power.

The DNA fingerprinting method based on VNTRs, first described in 1985 by Alec Jeffreys in the United Kingdom, represented a revolutionary advancement in individual identification (Jeffreys et al., 1985b:67). Since then, VNTR analyses have been widely adopted in forensic laboratories across many countries, particularly in the United States. The high level of polymorphism in VNTR markers has provided superior differentiation between individuals (Butler, 2011).

However, similar to the RFLP (Restriction Fragment Length Polymorphism) method, VNTR analysis requires intact and high-quality DNA samples. This requirement is considered one of the limitations of the method (Goodwin et al., 2010).

VNTRs with moderate repeat unit lengths are often referred to as “minisatellites.” D1S80, a locus commonly used in forensic medicine, is characterized by a repeat unit length of 16 base pairs and a repeat number ranging from 16 to 41. Special PCR-based kits developed for the D1S80 locus work with ready-to-use reagent mixtures containing primers that amplify the allele region, and the products are analyzed by gel electrophoresis (Inman & Rudin, 2001).

Although VNTRs have high discriminatory power, they have been replaced by STR (Short Tandem Repeat) markers, which contain shorter repeat units. The main reason for this is that STRs have advantageous features such as being easily amplified by PCR and being able to work with low amounts of DNA and degraded DNA samples (Butler, 2011).

3.2.3. Short Tandem Repeats (STR)

STRs are DNA sequences that are 2–7 base pairs long and have a specific base sequence, repeating sequentially in a head-to-tail fashion. These repeats are called microsatellites because they are shorter than minisatellites (VNTRs), which are composed of longer units. They are widely distributed throughout the human genome; on average, there is one STR locus every 6–10 kilobases. Since the number of repeating units at these loci varies from individual to individual, STRs are highly effective for individual identification (Butler, 2009).

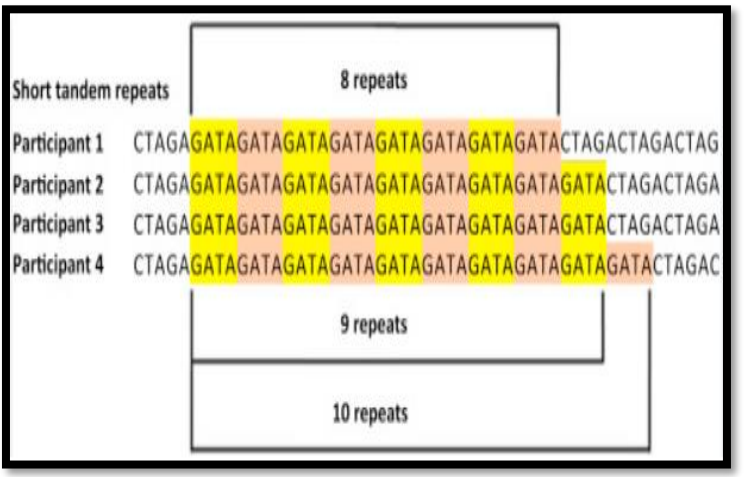
Thousands of polymorphic microsatellites have been identified in the human genome, and it is estimated that they account for approximately 3% of these regions. The length of STRs typically ranges from 100 to 400 base pairs. These sizes are much smaller than the 500–12,000 bp fragments obtained in RFLP analysis. Therefore, STRs are highly advantageous in the analysis of fragmented DNA. Short repeat sequences are more suitable for amplification by PCR,

enabling high-throughput analyses using this technology. Among STR motifs, 4-base repeats (tetramers) are preferred over 2- and 3-base repeats because they are more stable during amplification and separation processes. Five- and six-base repeats are less common but are used in some laboratories. STR loci can be used as genetic markers because genetic information is passed down from generation to generation in accordance with Mendelian inheritance rules (Butler, 2005). Today, STRs are used not only in forensic science but also in a wide variety of fields such as tissue culture, species identification, bone marrow transplantation, chromosome mapping, genetic disease research, and population genetics. The use of STRs in forensic science requires only a limited portion of the thousands of STR loci in the genome. These selected loci must exhibit high variation and be easily distinguishable. STRs showing microvariations are therefore not preferred. STR alleles containing 4-base differences can now be reliably separated using capillary electrophoresis systems. In forensic biology applications, STR loci located in intron regions are particularly preferred because these regions generally do not encode proteins and exhibit greater polymorphism. Over 1,300 STR loci with a heterozygosity rate of over 70% have been identified in the genome. In selecting STR loci, criteria such as discriminatory power, chromosomal location, ability to be amplified in a single PCR reaction with other loci, and reliability are considered. Research has shown that STR analysis can be performed with as little as 50–100 picograms of DNA. This means that identification can be performed even in samples containing very small amounts of DNA or those that are severely degraded. Since STR analysis is PCR-based, only 0.5–1 ng of DNA is required. This means that much less DNA is needed compared to methods such as RFLP and VNTR. For this reason, STRs are preferred for fragmented DNA analysis. The main disadvantage of STRs is that they do not have as high a level of polymorphism as VNTRs. However, this limitation has been addressed through

multiplex PCR systems. With these systems, multiple STR loci can be analyzed simultaneously, resulting in time and cost savings, as well as reduced contamination risk (Butler, 2005).

Short tandem repeats (STRs) are DNA segments consisting of 2–100 nucleotides that are repeated in tandem and in the same direction and can be repeated 5 to more than 200 times (Fig. 3). The repeating unit in Figure 3 is GATA.

Figure 3. Variety in the number of short tandem repeats



Source: URL-3, 2025 https://www.sciencedirect.com/topics/immunology-and-microbiology/short-tandem-repeat 07.07.2025

The lengths of STR loci amplified by PCR must be measured. There is a base pair difference between some STR alleles. STR systems are profiled using PCR and gel electrophoresis, generally with fluorescent or isotopically labeled primers, and then allele profiles are determined by laser, autoradiography, or silver staining. However, the number of loci analyzed in this electrophoresis is limited because the allelic sizes of different loci may overlap. This limitation has been overcome by the forensic science community by

labeling PCR products with different fluorescent dyes. For this purpose, a series of dyes has been developed, and these dyes are added to the 5' end of the PCR primers to enable the identification of DNA fragments during electrophoresis. The ability to use up to five dyes in a single analysis has made it possible to analyze multiple loci simultaneously. Electrophoresis systems have evolved from slab gels to capillary electrophoresis systems, which allow DNA fragments to be detected by laser after being passed through a glass tube with a polymer solution. These systems enable the determination of allele fragment sizes with a resolution of 0.5 base pairs. These systems are routinely used in many DNA laboratories (Butler, 2005).

3.2.4. The Basics of Single Nucleotide Polymorphisms (SNPs)

Differences in a single base pair between individuals are generally referred to as single nucleotide polymorphisms (SNPs) and occupy an important place in the human genome. SNPs are the most common type of genetic variation in the human genome and are widely used in the diagnosis of genetic diseases, the identification of individual differences, and pharmacogenetic studies (Brookes, 1999:177; Syvänen, 2001).

It is estimated that there are approximately 4 to 5 million SNPs in each individual's genome (The International HapMap Consortium, 2005). Such a high SNP density indicates that these variants are an important source of genetic diversity observed among individuals and may play a fundamental role in personalized medicine applications in the future (Butler, 2009).

Numerous technologies have been developed to shorten and automate procedures for SNP analysis. In particular, microarray-based (DNA microarray) methods, which enable the simultaneous analysis of a large number of SNPs, are widely used in both research and clinical settings (Syvänen, 2001).

SNPs exhibit some differences when compared to short tandem repeats (STRs). SNPs are much more prevalent in the human genome than STRs; however, they are not as highly polymorphic as STRs (Butler, 2009). Therefore, while STRs are more suitable for individual identification in forensic science, SNPs offer advantages in determining disease predispositions and population genetics analyses.

4. PCR (Polymerase Chain Reaction)

PCR (Polymerase Chain Reaction) is a molecular biology method that enables the in vitro amplification of specific regions (target loci) in an organism's DNA (Mullis & Faloona, 1987:335). Using this technique, DNA that is initially present in very small amounts can be amplified to millions of copies in a short period of time.

PCR is an enzymatic process in which a specific region of DNA is repeatedly amplified with the help of specially designed primers. This process is typically carried out using thermal cycling, which involves heating and cooling the DNA in cycles of 30 or more (Saiki et al., 1988). This allows DNA to be obtained in quantities sufficient for high-precision analysis from samples containing low amounts of DNA.

A tube or microtiter plate containing the basic components of the PCR process mixed in the correct proportions is used, and this mixture is placed in a thermal cycler. The device automatically performs cyclic heating and cooling processes according to the specified temperature profile (Brown, 2016).

The basic components required for PCR are as follows: DNA polymerase enzyme (usually heat-resistant Taq polymerase), specific PCR primers (short DNA sequences specific to the target DNA

region), dNTPs (dATP, dTTP, dCTP, dGTP), template DNA (DNA to be amplified), Mg^{2+} ions (cofactors required for enzyme activity), sterile deionised water (to complete the reaction volume) (Green & Sambrook, 2012).

After all these components are mixed at appropriate concentrations, the reaction is initiated by applying a specific PCR cycle programme. The amplified DNA products obtained at the end of PCR can be analysed using techniques such as gel electrophoresis for genetic typing (Brown, 2016).

There are three main stages of PCR (Fig. 4):

a. Denaturation (Separation):

The template DNA is exposed to high temperatures (usually 94–96 °C) to convert the double-stranded DNA into single strands.

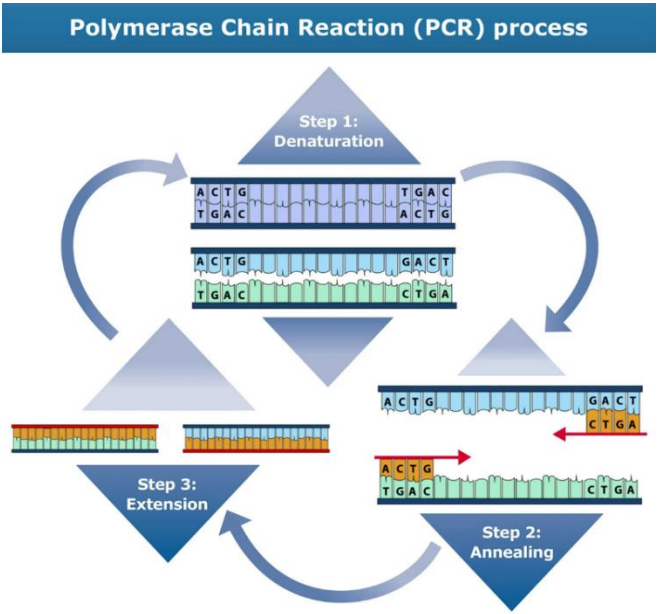
b. Annealing:

The temperature is lowered (typically to 50–65 °C) to allow the DNA primers to bind to the template DNA at the target region.

c. Extension:

The Taq DNA polymerase enzyme synthesises a new DNA strand using dNTPs starting from the 3' end of the primers. This process is typically performed at 72 °C (Saiki et al., 1988:487; Brown, 2016).

Figure 4. Polymerase Chain Reaction Process



Source: URL-4, 2025 *https://www.sciencelearn.org.nz/images/2983-three-steps-of-pcr 15.07.2025*

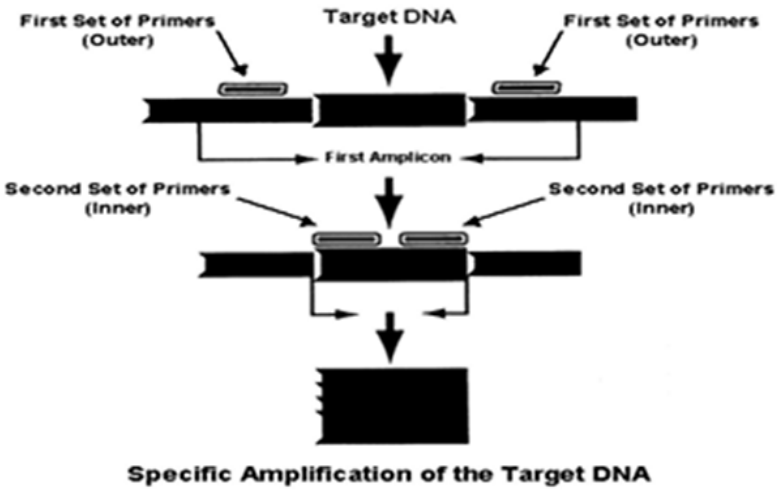
4.1 PCR Types

4.1.1 Nested PCR

This is a highly specific PCR method that prevents the formation of nonspecific products. Two different PCRs are performed in Nested PCR. These are performed using two different primer pairs. In the first PCR, a longer DNA sequence including the target DNA region is amplified, while in the second PCR, the target region is directly amplified. The second PCR is performed using "NESTED" (nested) primers that contain sequences from the internal portions of the DNA amplified from the first PCR. The first PCR products are used as templates for the second PCR, and the desired

target region is amplified to obtain unique products. This prevents nonspecific amplification (Green & Sambrook, 2019) (Fig. 5).

Figure 5. Nested PCR



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4.1.2 Touchdown PCR

High annealing temperatures are applied in the first cycles of the Touchdown PCR technique. In these cycles, primers bind more specifically to target DNA sequences, but sensitivity is low. In subsequent cycles, primer annealing temperatures are gradually reduced until they reach the optimal annealing temperatures for the primers. This prevents non-specific binding in the previous cycles. In subsequent cycles, primers approaching optimal annealing temperatures begin to bind more sensitively to their specific targets. This increases the sensitivity of the test (Korbie & Mattick, 2008:1452).

4.1.3 RAPD-PCR (Random Amplification of Polymorphic DNA)

RAPD-PCR is a PCR method in which DNA segments are amplified randomly. Genomic DNA is amplified using short primers randomly selected to be 8–12 bases in length. The numerous DNA fragments obtained using this method are analysed by gel electrophoresis and used for species identification, determination of genetic differences, or molecular typing (Williams et al., 1990: 6531; Hadrys et al., 1992:55).

4.1.4 Reverse Transcription PCR (RT-PCR)

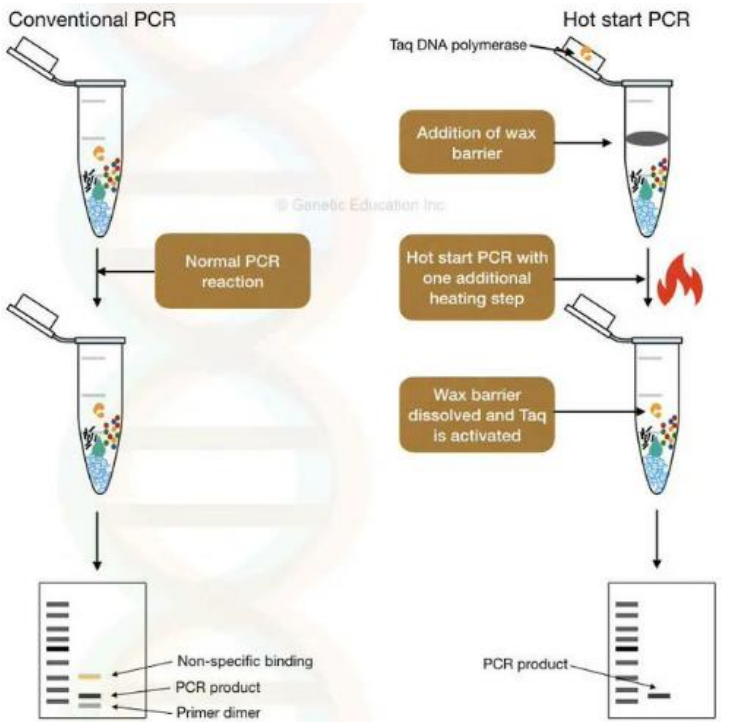
In this type of PCR, the target molecule is RNA rather than DNA. RT-PCR (Reverse Transcription PCR), commonly known as RNA-PCR, consists of two stages. In the first stage, RNA is converted into complementary DNA (cDNA) by the reverse transcriptase enzyme. In the second stage, the obtained cDNA is amplified using standard PCR methods. This technique is particularly used for measuring mRNA or viral RNA levels and for gene expression analyses. RT-PCR can detect even low levels of RNA molecules due to its high sensitivity (Mackay et al., 2002: 1292; Bustin & Nolan, 2004:155).

4.1.5 Hot-Start PCR

Hot-Start PCR is a method developed to prevent non-specific amplification that may occur in the early stages of PCR. This technique minimises non-specific primer binding and primer-dimer formation that may occur at low temperatures. For this purpose, the Taq DNA polymerase enzyme is added to the reaction mixture in an inactivated form using a heat-sensitive inhibitor or antibody and is activated only at high temperatures (typically 94–95°C). In a classic application of this method, DNA samples are first denatured at high temperatures (e.g., 94–95°C). The temperature is then suddenly lowered to approximately 55°C, allowing the primers and Taq

polymerase to enter the reaction (Fig. 6). This prevents the enzyme from being active at low temperatures, ensuring that only target sequences are specifically bound, thereby enhancing the specificity of amplification (Chou et al., 1992:1717; Kellogg et al., 1994:1134).

Figure 6. The difference between conventional and hot start PCR



Source: Anand et al., 2020

4.1.6 Anchored PCR

Anchored PCR is a special PCR technique used when only one end of the DNA sequence is known. In this method, a specific primer is used against the known sequence to target the defined part of the DNA. The unknown sequence at the other end is first bound to an oligonucleotide (this process is typically achieved through 'tailing' or 'adapter ligation'). This bound oligonucleotide serves as a second primer binding site, thereby enabling the unknown region

of the DNA to be amplified via PCR. The method is particularly useful in gene expression analyses, the discovery of new gene regions, or the retrieval of the 5' or 3' ends of transcripts (Loh, 1991:11).

4.1.7 Inverse PCR

Inverse PCR is a PCR technique commonly used to amplify unknown regions at both ends of a known DNA sequence. In this method, the target DNA sample is first cut into small pieces using an appropriate restriction enzyme. These DNA pieces are then converted into circular (ring-shaped) structures through a ligation process. As a result, the sequences adjacent to the known region, which are normally located at the ends of linear DNA, are now close to each other. PCR is then performed using two primers designed in the inverse direction relative to the known region. This allows the unknown regions outside the known sequence to be amplified (Ochman et al., 1988:621).

Inverse PCR is widely used, particularly for identifying transposon insertion sites, genome walking, and determining viral integration sites (Triglia et al., 1988: 8186).

4.1.8 Asymmetric PCR

The asymmetric PCR method is widely used in situations where large amounts of single-stranded DNA (ssDNA) production is required, such as DNA sequencing or some hybridisation-based analyses (Innis et al., 1990). In this technique, one of the two primers used is designed to be at a higher concentration than the other. Due to this imbalance, as PCR cycles progress, one strand is amplified to a much greater extent than the other (Dieffenbach & Dveksler, 1995).

Asymmetric PCR can theoretically be applied to any double-stranded DNA template. However, it is often limited by the inability

to produce sufficient amounts of single-stranded product. Therefore, a two-step method is typically preferred: In the first step, the target DNA region is amplified as a double-stranded template using classical (symmetric) PCR; in the second step, asymmetric PCR is applied to obtain a high yield of single-stranded product from this template (Innis et al., 1990; Dieffenbach & Dveksler, 1995). This two-step approach offers advantages in terms of increasing product yield, particularly in applications based on single-stranded DNA.

4.1.9 In Situ PCR

In situ PCR is a method of amplifying specific nucleic acid sequences within cells, tissues, or tissue fragments on a slide using polymerase chain reaction (PCR) in situ. This technique is used as an extremely sensitive method, particularly for the detection of viral DNA and genes with low copy numbers. Additionally, when combined with reverse transcription PCR (RT-PCR), it enables the determination of the cellular or tissue localisation of viral RNA and gene transcripts (Nuovo, 1994).

This technique offers significant contributions in terms of high specificity and sensitivity for mapping gene expression at the cellular level and determining the localisation of target nucleic acids in pathological samples.

4.1.10 Immuno PCR

Immuno PCR is a combination of polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) methods. This technique enables the detection of antigens with high sensitivity. Instead of the enzymes used in traditional ELISA, a specific DNA sequence covalently or non-covalently bound to the antibody is used as a marker. After the target antigen binds to this DNA-labelled antibody, the DNA is amplified by PCR and quantitative analysis is performed. This method is particularly

effective in detecting biomolecules at low concentrations (Sano et al., 1992: 120).

4.1.11 Multiplex PCR (mPCR)

Multiplex PCR is a method that allows two or more different DNA target regions to be amplified simultaneously in the same PCR reaction tube. This technique proceeds in the same way as the steps in the classic PCR protocol; however, it differs in that it uses multiple primer sets (Chamberlain et al., 1988: 11141). Each primer set is specific to a particular DNA region, and the amplification products formed by these primers are called amplicons.

Multiplex PCR enables simultaneous analysis of different pathogens or genetic variations present in a sample. Therefore, it is widely used in diagnostic applications where evaluation of multiple target DNA regions is required.

Primer design is critical for the success of this method. The melting temperatures (Tm) of the primers used should be close to each other, and cross-linking or dimer formation between primers should be prevented. This increases the specificity and efficiency of the reaction (Elnifro et al., 2000: 559).

4.1.12 Real Time PCR

Real-Time PCR is a PCR technique that allows the amount of product to be monitored simultaneously during each cycle of the DNA amplification process. In this method, since the analysis of amplification products occurs during the reaction, there is no need for additional procedures such as agarose gel electrophoresis or band imaging under ultraviolet light, as is the case with conventional PCR (Kubista et al., 2006:95).

Real-time analysis is performed by measuring the amount of product formed during PCR through fluorescent signals emitted in

each cycle. Two main approaches are used to obtain these fluorescent signals:

Non-sequence-specific fluorescent dyes (e.g., SYBR Green) bind to double-stranded DNA to produce a signal.

Sequence-specific probes (e.g., TaqMan probes) only recognise the target sequence and thus increase specificity (Kubista et al., 2006:95).

Real-Time PCR can be used for both qualitative (presence/absence analysis) and quantitative (copy number determination) purposes. Since the fluorescent signals obtained during each cycle of the reaction directly reflect the amount of product, this technique is widely used in many fields, including gene expression analysis, genetic mutation screening, and microbial load determination.

Conclusion

DNA is extremely powerful evidence in identifying individuals and determining criminals due to its unique and personal structure. DNA analysis provides reliable results with high sensitivity and accuracy rates. Thanks to genetic variations, accurate and meaningful data can be obtained even from very small biological samples. The fact that DNA is a molecule passed down from generation to generation makes it an important tool for determining biological kinship relationships (e.g., parent-child relationships).

In forensic medicine, DNA analysis is used in a wide range of applications, including identifying missing persons, clarifying cases of domestic violence and sexual assault, and detecting certain genetic diseases. This demonstrates that DNA is not only an effective tool in solving criminal cases but also in social and medical fields.

DNA has the ability to remain intact for long periods of time, depending on environmental conditions. Biological samples such as blood, hair, skin cells, and other biological evidence collected from crime scenes can be preserved in a manner suitable for DNA analysis even years later. This property makes DNA an indispensable piece of evidence, particularly in the re-evaluation of ‘cold cases,’ which are incidents that have remained unsolved for extended periods of time.

DNA can be isolated from a wide variety of biological materials. It is possible to obtain DNA from many different sources, such as blood, saliva, hair, tissue, bone, and teeth. This allows DNA profiles to be created from almost any biological trace found at a crime scene.

The PCR (Polymerase Chain Reaction) technique, frequently used in DNA analysis, enables the obtained DNA to be amplified and analysed. Especially in cases where DNA samples are very small or partially damaged, sufficient DNA can be obtained through the PCR method to perform the analysis. The amplification feature provided by PCR plays a fundamental role in the use of DNA as strong evidence in forensic science.

Each type of PCR (e.g., Real-Time PCR, Multiplex PCR, Nested PCR, etc.) offers specific advantages to enhance the accuracy of analyses and achieve effective results in different forensic applications. The choice of which PCR type to use depends on factors such as the type of sample, the quality of the DNA sample, and the genetic information targeted for analysis.

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BÖLÜM 2

THE HIDDEN CURE IN FROG SECRETION: FROM TRADITIONAL TO MODERN MEDICINE

EBRU TANRIVERDİ O [1]

Introduction

Animals play a crucial role in human healing processes, folk rituals, and religious practices **(**Chakravorty et al., 2011). In pre-Hispanic Mexico, many animals were revered as gods because they were seen as symbols of the power of nature. Animals were known as comforters or divine messengers. For example, the owl was considered a harbinger of bad things to come (Saucedo-Sánchez de Tagle, 2007). Likewise, throughout history, people have described frogs as creatures that inspire fear and disgust because of their cold,

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moist skin. They were often imbued with magical powers. Even in legends, frogs were creatures used by witches to cast spells or poison others. They have not failed to appear not only in legends but also in art, literature, and other cultures. In European art and literature, they frequently appear as symbols of evil and agents of the devil (De Graaff, 1991). In other cultures, such as ancient Egypt and the Mayan culture of Central America, they became symbols of abundance, life, and fertility because they appeared during rainy times (Gibbons, 2003).

Biological resources have been used in traditional and folk medicine since ancient times due to their cost-effectiveness and availability. Animals and animal body parts are an important component of traditional medicine (Alves and Alves 2011). The traditional use of animals and their products for medicinal purposes have been documented since ancient times in the civilizations of China, Egypt, Greece, and Mesopotamia (Lev, 2003). Sharaka Samhita, the first work written in India around 900 BC, illustrates the practice of Indian Ayurveda (alternative medicine system) and refers to about 380 species of animal substances (Unnikrisnhan, 1998). Epidemiological research on the use of animal products in the treatment of various ailments indicates that 38.5% are used for rheumatic and other pains, 20.2% for stomach disorders, 14.7% for skin disorders, 18.4% for impotence, 14.7% as an aphrodisiac, and 11.5% for birth control (Mahawar & Jaroli, 2007; Mahawar & Jaroli, 2008). Since ancient times, frogs have been intrigued by the properties of their skin. In many ancient cultures, amphibian skin secretions have been used to make medicines for disease treatment. Secretions from dried frog skin have been used in traditional Chinese medicine to treat arthritis. Chan Su (a traditional Chinese medicine), prepared from the skin glands of the Chinese frog, has been used to

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treat heart disease, leukemia, rhinosinusitis, and other ailments (Gomes et al., 2007). The “Cururu” (Rhinella schneideri), considered the largest toad, is used in Paraguayan traditional medicine to relieve erysipelas, skin infection, skin lesions, wounds, and to treat cancer through syrup or decoction (Schmeda-Hirschmann et al., 2014). The Chinese have traditionally used toad skin and secretions of toad parotid glands to regulate internal body functions and fertility or to treat dog bites (Costa-Neto, 1998). Phyllomedusa bicolor is one of the largest frogs in the Amazon. Known colloquially as "Kambô," "Kampô," or "Kampu," the toxin secreted from its skin is used by indigenous people in traditional medicine. The name "Kambô" is also used to describe the ritual application of the frog's poisonous secretions to the skin (Souza, 2009). Kambô, kapum or frog vaccination (“vacina do sapo” in Portuguese) is a purification ritual and is associated with healing rituals in the Amazon rainforest and urban centers around the world (Lima and Labate, 2007; Haddad and Martins, 2020). The kambô ritual is traditionally performed by shamans to purify the body, increase physical strength and sexual stamina, and ward off “panema” (bad luck and a type of weakness) (Bernarde and Santos, 2009; Labate and Lima, 2014). At dawn, the shaman healer, following the sound of frog calls, stretches the captured frog into an "X" shape on crossed branches, with its hind and front legs tied together, and the poison is collected with a wooden stick (Figure 1A). In this ritual, the shaman healer burns the participant and applies P. bicolor secretion to the wound (Figure 1B) (Daly et al., 1992). The secretion is applied in two to 10 smaller dots on the legs or arms (Lima, 2005). It has been suggested to have benefits for mental and physical health, including treatment of various pathologies such as addictions, depression, chronic pain,

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autoimmune disorders, Hashimoto's thyroiditis, celiac disease, post-traumatic stress disorder, diabetes, infectious diseases, cancer, hypertension, and other health problems (Majić et al., 2021; Thompson and Williams, 2022). In addition, extracts obtained from the skin secretions of P. bicolor are used in Chinese folk medicine to treat cognitive losses seen in disorders such as depression, stroke, seizures, and Alzheimer's disease (Amato, 1992).

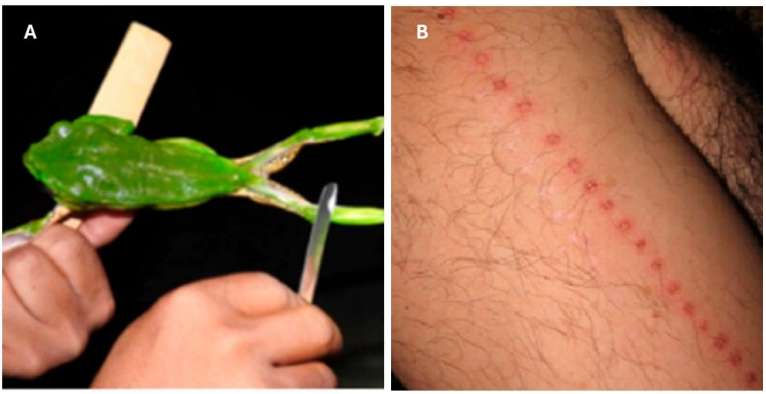

Figure 1. (A) Collection of secretions in a frog. (B) Application of frog secretion. Photograph: Paulo Sérgio Bernarde.

Researchers warn that in areas far from where the vaccine has traditionally been administered, it may be administered by individuals who lack the same level of experience as those in the traditional community that administered the vaccine, and therefore may pose health risks (Silva et al. 2019). Recent findings have revealed side effects of Kambo treatment, including death. A 42-year-old overweight man who showed signs of coronary heart disease due to Kambo use died suddenly (Aquila et al., 2017). In Canada, a 32-year-old female patient was reported to have been hospitalized eight hours after receiving Kambô treatment with

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complaints of prolonged nausea, frequent vomiting episodes, and abdominal discomfort (Li et al., 2018).

In addition to its use in traditional medicine across various cultures and in ancient times, frog skin secretions and their bioactive components have also attracted the attention of modern medicine. Amphibian skin structure and secretions remain a subject of ongoing research among many scientists today.

Frog Skin Structure

Frog skin consists of epidermal and dermal layers. The basal membrane separates the dermis and epidermis. Each layer consists of epithelial and fibroblastic cells (Vitt and Caldwell, 2014). The dermis is a connective tissue that is a thicker layer containing many cell types embedded in its matrix (pigment cells, mucosal and granular glands, blood vessels, nerves) (Figure 2) (Zug, 1993). Granular (serous) glands located in the dermis secrete chemical compounds that play a crucial role in host defense against microbial and fungal infections, as well as predators (Clark et al., 1994).

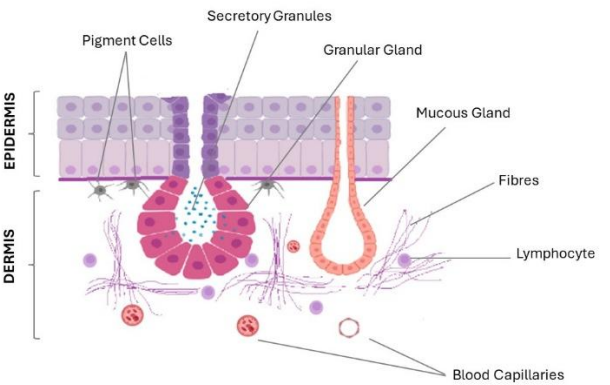

Figure 2. Diagram of frog skin structure (Tanrıverdi o, 2025).

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Granular gland secretions contain large amounts of compounds with biological activities, such as various alkaloids, biogenic amines, steroids, and peptides or proteins. The secretions produced by these glands are of great importance to amphibians in regulating the physiological functions of the skin and protecting it from predators and microorganisms (Barra and Simmaco, 1995). In amphibian skin secretions, biogenic amines, which are peptide precursors, are largely derivatives of catecholamines and indolealkylamines. Adrenaline, noradrenaline, dopamine, and epinin may also be found in the secretion. Biogenic amines are generally vasoconstrictors, and some can lead to transient neurological dysfunction as they possess hallucinogenic and hypertensive properties (Clarke, 1997). Amphibian skin secretions contain numerous peptides and proteins with antimicrobial, antifungal, antiviral, or hemolytic activity; these peptides are generally referred to as antimicrobial peptides (AMPs) (Rollins-Smith, 2009).

Antimicrobial Peptides (AMPs)

AMPs in multicellular organisms were first characterized in the 1980s by researchers who believed that known immune systems could not explain what they were observing: the resistance to bacterial infection of a Cecropia moth pupa lacking antibodies or lymphocytes, the strong microbicidal activity of neutrophils taken from a rabbit, and the healing of a wound on the skin of Xenopus laevis in a non-sterile aquarium without infection (Stenier, 1981; Selsted et al., 1985; and Zasloff, 1987). Since then, many AMPs have

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been discovered in different fungal, plant, and animal species (Fan et al., 2016).

AMPs are central components of an innate network of genetically encoded proteins and peptides that protect animals from microbial, viral, or cellular invaders. Because they are gene-encoded, some AMPs are pre-located in barrier regions, including the skin, or in susceptible areas such as the respiratory, gastrointestinal, and urogenital pathways (Lehrer, 2013). AMPs are typically small molecules, usually consisting of 8–50 amino acid residues, generally in the usual L configuration, with a net positive charge of +2 to +6 (Hancock and Sahl, 2006). They are cationic, mostly due to the presence of arginine, lysine, and histidine amino acids in their structure. Typically, more than 50% of the amino acids in an AMP are hydrophobic (Hancock and Diamond, 2000).

Amphibian AMPs are cationic peptides that adopt an amphipathic α-helix conformation (Boman, 2003). They contain approximately 40–70% hydrophobic amino acids. Due to the presence of multiple positive amino acids, these peptides carry a positive charge between +2 and +6 at pH 7. In aqueous solution, these peptides do not exhibit any stable secondary structure; however, near a phospholipid bilayer, they tend to form an amphipathic alpha-helix structure (Conlon and Mechkarska, 2014). Synthesized in ribosomes, these peptides are ≤5 kDa and are molecules with broad-spectrum microbicidal activity (Hancock and Diamond, 2000).

AMPs Working Mechanism

AMPs exhibit effective activity against a wide range of pathogens, including Gram-negative and Gram-positive bacteria, fungi, viruses, and protozoa. Different species produce different

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peptides with growth-inhibiting activity against various microbes. The main families of AMPs are considered to be amphipathic linear helical peptides. They are cationic, and their structures contain large amounts of positively charged residues and hydrophobic regions. These properties allow them to bind to negatively charged molecules and/or membrane lipids, thereby disrupting the membrane structure (Rollins-Smith et al., 2005).

The cytoplasmic membrane of eukaryotic cells, unlike that of bacterial cells, is composed of electrically neutral phospholipids such as phosphatidylcholine and sphingomyelin. The bacterial outer membrane, on the other hand, consists of negatively charged phosphatidylglycerol and cardiolipin. These differences explain the lethal effects of AMPs on bacteria without significant toxic effects on human cells (Figure 3) (Madigan et al., 1997).

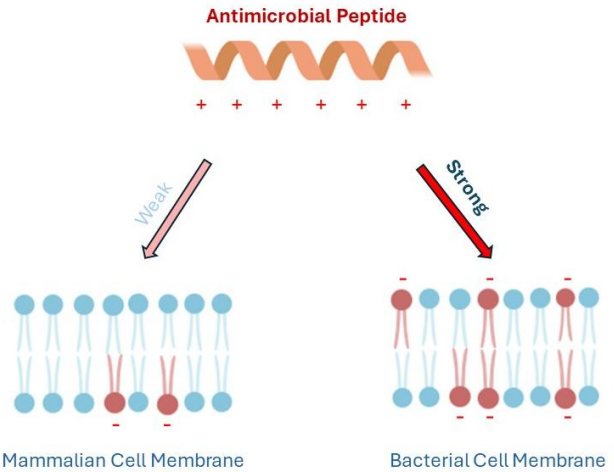

Figure 3. AMPs Working Mechanism (Tanrıverdi o, 2025)

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Current Clinical Studies of Frog AMPs

Antibiotics are considered one of the most significant discoveries in medicine. Alexander Fleming's discovery of penicillin in 1929 ushered in the modern antibiotic era (Levys, 1997). In 1987, Zasloff and colleagues first reported the antibacterial activity of peptides found in the skin secretions of frogs. Widespread commercial interest in the detection of these molecules began with Zasloff's discovery of magain in the skin secretion of Xenopus levis (Zasloff, 1987). Today, antibiotic research and development are progressing rapidly. Antibiotic resistance is developing due to the indiscriminate use of antibiotics, and this necessitates the development of a new generation of antibiotics (Walsh and Wright, 2005). AMPs, in particular, hold promise as precursor compounds for new antibiotics (Govender et al., 2012). To understand the pharmaceutical importance of AMPs, it is essential to emphasize that peptide-based drugs offer numerous advantages, including high efficacy, selectivity, and low toxicity (Sharma et al., 2023; Wang et al., 2016).

The Antimicrobial Peptide Database (APD3) (https://aps.unmc.edu/) contains 3940 antimicrobial peptides and proteins from six kingdoms of life. Of these, 1079 active peptides are found in amphibians (1006 in frogs, 68 in toads, and 5 in others). These peptides possess a wide range of biological activities, including antibacterial, cytotoxic, antimutagenic, antifungal, antiviral, vermicidal, novel antiendotoxic, antidiabetic, and antioxidant properties (Antimicrobial Peptide Database (APD3).

Skin secretions of the African clawed frog Xenopus laevis have been shown to contain high concentrations of various biologically active components, including thyrotropic hormones and

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myotropic peptides such as caerulein, xenopsin, and levitide (Lazarus and Attila, 1993).

Intracellular accumulation of the Gram-positive bacterium Staphylococcus aureus can cause many inflammatory skin diseases, as well as skin lesions and severe cellular damage due to wounds or implanted medical devices. S. aureus can also become more invasive and cause life-threatening infections such as bacteremia, pneumonia, meningitis, osteomyelitis, endocarditis, and sepsis (Grazia et al., 2014). Some members of the Temporin peptide family obtained from Rana frogs show very high efficacy in vitro against Gram-positive bacteria, including clinical isolates of methicillin-resistant staphylococci and antibiotic-resistant enterococci (Mangoni, 2006; Conlon et al., 2007).

Currently, cationic α-helix antimicrobial peptides are understood to be multifunctional, exhibiting immunomodulatory, chemotactic, and insulinotropic properties as well as cytotoxic activities (Yeung et al., 2011). Analogues of amphibian peptides, elements of innate immunity, have been developed that show selective cytotoxicity against tumor cells and therefore have the potential to be transformed into anticancer agents (Lu et al., 2008). Similarly, some peptides in frog skin secretions have shown potent antiviral activity by directly inactivating viral particles or by interfering with the initial steps of the viral replication cycle, such as binding to specific cell surface receptors and subsequent entry into the cytoplasm. These properties, combined with the short contact time required to trigger killing, have led to their evaluation as candidates for the development of novel antiviral agents (Lord and Ashworth, 2013).

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Anti-cancer peptides have also been detected in the skin secretions of many frog species. Brevinin-1BYa peptide from Rana boylii is effective against human breast adenocarcinoma (Conlon et al., 2014), Temporin-CEa peptide from Rana chensinensis against human breast cancer (Wang et al., 2013), Magainin-2 peptide from Xenopus laevis against human lung cancer and bladder cancer (Lehmann et al., 2006, Cruciani et al., 1991), and Dermaseptin B2 peptide from Phyllomedusa bicolor against prostate adenocarcinoma cells (Van et al., 2012).

Studies on frog skin are not limited to peptides with antimicrobial effects. Recent studies have also yielded gelatin like commercial bovine gelatin from frog skin (Karnjanapratum, et al., 2017). Gelatin is widely used in the food, pharmaceutical, and medical industries due to its biodegradability and biocompatibility properties (Balakrishnan and Jayakrishnan, 2005). It exhibits low affinity during the digestive process and produces harmless metabolic products. Because of these properties, gelatin is used as a drug delivery agent (Deshmukh et al., 2017). Gelatin suitable for human consumption has been obtained from the skin of the Asian bullfrog Rana tigerina, providing high yield and good gelling, as a biocompatible material (Karnjanapratum and Benjakul, 2020).

A series of frog skin peptides, initially identified based on their ability to inhibit the growth of microorganisms, have subsequently been shown to have the ability to induce insulin secretion from BRIN-BD11 cells and improve glucose tolerance in mice at low concentrations that are not cytotoxic to the cells (Conlon et al., 2014).

During the Vietnam War in the 1960s, the lack of adequate medical supplies for treating napalm burns led surgeons to

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investigate traditional Vietnamese treatment methods. They found that amphibian skins from the genus Rana were a successful treatment method as temporary grafts for patients with severe skin loss (Lee, 1992). When these grafts were tested on Wistar rats, experimental wounds wrapped in frog skin healed much faster than those wrapped in cotton gauze. Biochemical assessments of wound granulation were performed every 2 days until complete healing was achieved. These experiments showed that the group of rats treated with frog skin produced higher levels of the amino acid hydroxyproline compared to the control group (Purna et al., 1995).

Pexiganan (MSI-78) is a broad-spectrum antibacterial peptide derived from magainin, a species of frog called Xenopos laevis. This peptide is currently in phase III of clinical trials (clinicaltrials.gov). Temporin 10a peptide, derived from the frog species Rana ornativentris, is an effective peptide against Gram-positive bacteria and is in the preclinical stage of trials (Kim et al., 2001). Temporin A peptide, derived from the frog species Rana temporaria, is an antidiabetic peptide and is also in the preclinical stage of trials (Musale et al., 2018).

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BÖLÜM 3
ANGELMAN SYNDROME: MOLECULAR BASIS, CLINICAL FINDINGS, AND CURRENT THERAPEUTIC APPROACHES

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Introduction

This review was conducted using PubMed, Web of Science, and Scopus databases with the keywords “Angelman syndrome,” “UBE3A,” “genomic imprinting,” and “neurodevelopment.” Articles published between 2000 and 2025 in English were reviewed. Case reports were excluded. Reviews and original research focusing on genetic mechanisms, clinical manifestations, diagnosis, and emerging therapies were included

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Angelman syndrome (AS) is a rare neurodevelopmental disorder characterized primarily by severe intellectual disability, speech impairment, movement disorders, and distinctive behavioral features. It was first described in 1965 by the British pediatrician Harry Angelman (Angelman, 1965). The fundamental cause of the syndrome is the functional loss of the *UBE3A* gene located in the chromosomal region 15q11-q13. The characteristic behavioral manifestations generally include:

- tremors in the limbs,
- frequent laughter,
- prominent smiling and episodes of excitement reflecting a happy demeanor,
- microcephaly, and seizures.

Developmental delays are usually observed within the first six months of life; however, the typical clinical features of AS emerge after the age of one year.

Additional findings, observed in fewer than 80% of individuals with AS, include:

- wide mouth, protruding tongue, widely spaced teeth, flat occiput, occipital groove, and prognathism,
- sucking and swallowing difficulties, feeding problems during infancy, tongue thrusting, drooling, excessive chewing or mouthing behaviors,
- strabismus,

- hypopigmented skin, light hair, and eye color in cases with 15q11.2-q13 deletion,
- exaggerated deep tendon reflexes in the lower extremities,
- uplifted, flexed arm position during ambulation,
- broad-based gait with ankles in pronated or valgus position,
- hypersensitivity to heat,
- abnormalities in sleep–wake cycles and reduced need for sleep,
- excessive attraction to and fascination with water,
- fascination with crinkly objects such as paper or plastic,
- obesity in cases without 15q11.2-q13 deletion,
- scoliosis, constipation (Williams et. al., 2001).

The estimated global prevalence of Angelman syndrome varies across geographic regions, ranging from approximately 1:10,000 to 1:24,000 individuals worldwide, with the highest reported detection rates in Europe and North America (Mertz et. al., 2013; Bird, 2014; Clayton-Smith, & Laan, 2003; Stanurova, 2022) (Table 1).

Tablo 1: Global prevalence of Angelman syndrome

Region	Estimated prevalence
Europe	1 / 12,000 – 20,000
USA	~1 / 15,000
Asia	Unknown – underreported
Global estimate	1 / 10,000 – 24,000

Genetic and Molecular Mechanisms

Angelman syndrome is primarily associated with the disruption of the expression of the maternally inherited *UBE3A* gene. This gene encodes ubiquitin-protein ligase E3A, which plays a critical role particularly in the central nervous system (Kishino et. al., 1997). Four major genetic mechanisms have been identified:

- maternal deletion of chromosome 15q11-q13 (≈70%),
- mutations in the *UBE3A* gene (≈10–15%),
- paternal uniparental disomy (UPD) (≈3–7%),
- imprinting center defects (≈3–5%) (Table 2) (Tan & Bird, 2016). The common consequence of these mechanisms is the loss of expression of the maternal *UBE3A* allele. In the brain, the paternal *UBE3A* allele is silenced due to imprinting; thus, only the maternal allele is active. Loss of this function results in the neurological manifestations of the disorder (Figure 1) (Stanurova, 2022).

Table 2. Genetic mechanisms underlying Angelman Syndrome

Genetic mechanism	Approximate frequency	Clinical implications
Maternal 15q11-q13 deletion	~70%	Most common mechanism; associated with typical AS phenotype, often severe clinical presentation

Genetic mechanism	Approximate frequency	Clinical implications
UBE3A gene mutations	~10–15%	Variable phenotype; some cases may exhibit milder cognitive and motor impairments
Paternal uniparental disomy (UPD)	~3–7%	May present with relatively milder seizures and less pronounced microcephaly
Imprinting center defects	~3–5%	Phenotypic variability; can resemble either deletion or UPD cases depending on imprinting disruption

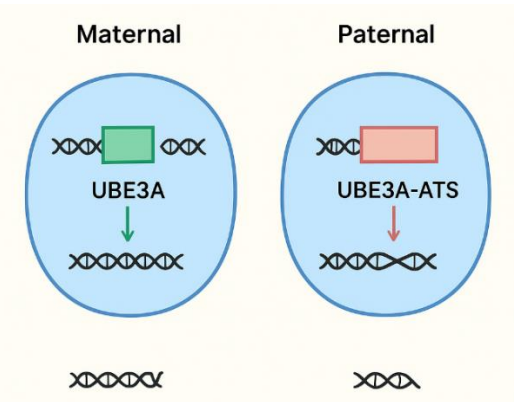

Figure 1. Epigenetic imprinting of the UBE3A gene in neurons.

In neuronal cells, the maternal UBE3A allele is actively expressed and produces functional UBE3A protein, whereas the paternal allele is transcriptionally silenced by the UBE3A antisense transcript (UBE3A-ATS). Loss of maternal UBE3A expression results in the absence of functional UBE3A protein, leading to the neurological manifestations characteristic of Angelman syndrome.

Genotype–Phenotype Correlations in Angelman Syndrome:

Increasing evidence suggests that different genetic mechanisms underlying Angelman syndrome are associated with distinct clinical phenotypes. Patients with the typical 15q11–q13 maternal deletion frequently present with more severe developmental delay, early-onset epilepsy, hypopigmentation, and pronounced microcephaly. In contrast, individuals harboring UBE3A point mutations or paternal uniparental disomy often show milder seizure activity and less severe neurodevelopmental impairment. Imprinting center defects demonstrate greater phenotypic variability and may resemble either deletion or UPD phenotypes depending on the extent of imprinting disruption (Clayton-Smith, & Laan, 2003; Tan & Bird, 2016; Bindels-de Heus et. al., 2020).

Clinical Manifestations

The core clinical features of Angelman syndrome include (Table 3):

- **Neurodevelopmental delay:** severe intellectual disability with profound impairment in speech development,
- **Motor disturbances:** ataxic gait, tremor, and impaired motor coordination,
- **Epilepsy:** seizures occur in approximately 80% of cases, typically with onset in early childhood,
- **Behavioral features:** inappropriate or unprovoked laughter, happy demeanor, and hyperactivity,

- **Physical characteristics:** microcephaly, prognathism, open-mouthed appearance, and hypopigmentation (Clayton-Smith, & Laan, 2003).

In later life, secondary complications such as scoliosis, obesity, and loss of mobility may also develop (Tan & Bird, 2016).

Table 3. Clinical Features and Prevalence of Angelman Syndrome (Bird, 2014; Bindels-de Heus et. al., 2020).

Clinical feature	Prevalence (%)	Description
Intellectual disability	~100	Severe mental retardation
Speech impairment	~100	Markedly limited development of speech
Happy demeanor and laughter	>90	Inappropriate bouts of laughter, positive social behavior
Motor coordination deficits	>90	Ataxia, gait disturbances, tremor
Epilepsy	80–90	Refractory epileptic seizures with onset in early childhood
Sleep disturbances	70–80	Irregular sleep, frequent nocturnal awakenings
Microcephaly	~70	Head circumference below age-appropriate norms
Hypopigmentation	~50	Reduced pigmentation of skin, hair, and eyes

Clinical feature	Prevalence (%)	Description
Scoliosis	10–30	Progressive spinal curvature developing in adolescence

Diagnostic Approaches

The diagnosis of Angelman syndrome is established through clinical evaluation in combination with molecular genetic testing. Methods such as DNA methylation analysis, *UBE3A* mutation testing, SNP array, or chromosomal microarray are commonly employed (Mertz et. al., 2013). DNA methylation analysis can confirm approximately 80–90% of cases (Stanurova, 2022; Buiting, 2010).

The diagnostic yield of currently used molecular techniques varies depending on the underlying genetic mechanism; a comparative overview of detection rates is presented in Table 4.

Table 4 – Diagnostic methods vs detection rate

Method	Detection Rate
Methylation analysis	80–90%
UBE3A sequencing	10–15%
SNP array	Structural + UPD cases
Whole genome sequencing	Emerging

Treatment and Management

Currently, there is no curative therapy for Angelman syndrome. Available treatment strategies are symptomatic and include seizure control, speech and physical therapy, as well as behavioral support interventions (Bird, 2014). For epilepsy, valproate, levetiracetam, and clobazam are frequently used medications; however, certain antiepileptic drugs, such as carbamazepine and vigabatrin, are not recommended as they may exacerbate clinical symptoms (Thibert, 2009).

Experimental therapeutic strategies, including gene therapy, antisense oligonucleotides, and epigenetic reprogramming, hold promise for the future (Lee & Walker, 2016).

Current Clinical Trials and Emerging Therapies:

Recent advances have focused on disease-modifying approaches aimed at reactivating the paternal UBE3A allele. Antisense oligonucleotides (such as GTX-102) are currently under Phase I/II clinical trials and aim to suppress the UBE3A-antisense transcript, thereby restoring functional UBE3A expression. Preliminary data indicate target engagement and favorable safety profiles (Lee & Walker, 2016). In parallel, preclinical studies using adeno-associated virus (AAV)–mediated gene replacement and CRISPR-based epigenetic editing strategies are under investigation, offering potential long-term therapeutic benefits for Angelman syndrome.

Future Directions

Future research in Angelman syndrome is expected to focus on disease-modifying therapeutic strategies aimed at restoring UBE3A expression in neurons. Advances in antisense oligonucleotide–based therapies to unsilence the paternal UBE3A allele represent one of the most promising approaches and are currently under early clinical evaluation. Viral vector-mediated gene replacement therapy and CRISPR-based epigenetic editing techniques also show substantial preclinical potential for achieving long-term UBE3A reactivation. (Fiumara, 2023; Sztainberg, & Zoghbi, 2023; Kaczmarczyk, et al. 2022; Wolter et al., 2020).

In addition to therapeutic development, improving early diagnosis through expanded newborn screening programs and increasing access to genetic testing are critical priorities (Wolter et al., 2020). Establishment of international patient registries and real-world evidence platforms will be essential to define the natural history of Angelman syndrome, refine genotype–phenotype correlations, and optimize treatment outcomes. Furthermore, future studies should explore combination approaches integrating gene-targeted interventions with behavioral and neurorehabilitative strategies to maximize functional improvement and quality of life in affected individuals.

Conclusion

This review provides a holistic overview of the genetic and molecular basis, clinical findings, diagnostic approaches, and emerging treatment strategies of Angelman syndrome, highlighting the relationship between molecular pathology and clinical phenotype.

Angelman syndrome represents a well-defined neurogenetic disorder with clearly established molecular mechanisms centered on loss of maternal UBE3A expression. Advances in molecular diagnostics have significantly improved early identification of affected individuals and have enabled a more refined understanding of genotype–phenotype correlations. Despite the availability of symptomatic management strategies, effective disease-modifying treatments remain limited.

The emergence of targeted molecular therapies, including antisense oligonucleotides, viral gene replacement platforms, and CRISPR-based epigenetic editing technologies, offers renewed optimism for restoring UBE3A activity and modifying disease progression. However, successful clinical translation will require earlier diagnosis, standardized outcome measures, expanded patient registries, and long-term safety monitoring. Continued multidisciplinary collaboration between molecular biologists, clinicians, and geneticists will be essential to transform promising experimental strategies into effective clinical therapies and improve

long-term outcomes and quality of life for individuals with Angelman syndrome.

Kaynakça

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BÖLÜM 4

A BIBLIOMETRIC ANALYSIS WITH VOSVIEWER ON OCCUPATIONAL SKIN DISEASES

KAAN KALTALIOGLU[1]

Introduction

The discipline of protecting the health of workers in various working environments is based on identifying and preventing occupational diseases and hazards before accidents occur (Alli, 2008). The International Labour Organisation (ILO) estimates that approximately 2.9 million work-related deaths occur worldwide each year, most of which are due to chronic diseases. This situation shows an urgent need for more effective prevention strategies (Hamzaoui, 2024; Armenteros-Cosme et al., 2025). Still, understanding the real burden of occupational diseases is not very easy, since issues like the long incubation periods of many chronic conditions, the high mobility in flexible labour markets, and the need for trustworthy exposure information make the process quite complicated (Rushton, 2017; Lee et al., 2025).

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Occupational skin diseases (OSDs) are especially common in industrial work environments, and they are estimated to make up roughly 30–45 percent of all occupational diseases (Srinivas & Sethy, 2023). Contact dermatitis constitutes the majority of OSD cases. Contact dermatitis is generally classified as irritant or allergic. Irritant contact dermatitis accounts for around 80 per cent of occupational cases (Sasseville, 2008; Lee et al., 2025). Furthermore, hand eczema is one of the most common occupational skin diseases, occurring in up to 40% of high-risk occupations involving wet work or mixed exposures, such as healthcare workers, metal workers, hairdressers, and cleaners (Thyssen et al, 2022; Weisshaar, 2024). A range of chemical agents may lead to OSDs; for example, organic compounds, acids, alkalis and different metals can all trigger skin problems such as allergic or irritant dermatitis, and even chemical burns in some cases (Won, 2010; Lee et al., 2025).

As the skin is the first line of defence against external factors, scientific research into the prevention of OSD, the provision of appropriate personal protective equipment, and the improvement of working environments is of great importance in terms of occupational health and safety (OSH).

In recent years, bibliometric analyses conducted systematically visualize the evolving research landscape in the field of occupational diseases and OSH, highlighting critical trends and emerging thematic clusters in global and regional contexts. A comprehensive examination of the literature published between 2014 and 2024 revealed that core concepts such as occupational exposure, risk factors, and the human factor remain central to the field, with a distinct increase in interest toward infectious diseases and global health threats, particularly following the COVID-19 pandemic (Karakurt & Özbakır, 2025). More focused bibliometric studies have also delineated research trends in specific high-risk populations and environments. For example, Zhang et al. (2023)

conducted an analysis specifically on occupational exposure among nurses in Asia, identifying key research hot topics within this critical professional group. Addressing health challenges in the farming sector, Petit & Vuillerme (2025) explored the increasing value of administrative health databases for studying health outcomes like cancer, mortality, and injuries in farming populations. Furthermore, the broader OSH literature has been analyzed to delineate conceptual structures, such as the prominent cluster linking leadership, the work environment, and employee well-being (Prihatsanti et al., 2025). Academic trends have been mapped, including a bibliometric analysis of domestic theses in Türkiye that specifically focused on OSH in schools (Demir & Gök, 2025). Thus, these recent bibliometric works provide essential visualization and conceptual mapping to guide future research and policy in occupational health.

This study conducted a bibliometric analysis to explore research trends and the scientific structure related to occupational skin diseases. This study will contribute to future research by mapping developments in the field, identifying research trends and gaps, and highlighting international collaboration networks.

Methodology

Bibliometric analysis is useful in identifying developments, gaps and possible directions for future research in the field (Varshabi, Arslan Selçuk, & Mutlu Avinç, 2022). Data were collected from the Web of Science (WoS) Core Collection database, which is widely used for quantitative literature evaluations due to its large coverage of peer-reviewed journals (Yan & Zhiping, 2023).

For mapping and visualization, the software VOSviewer (v 1.6.20) was used. VOSviewer is commonly applied in bibliometric studies because it allows easy construction of co-occurrence networks and clustering maps. In this research, keyword co-

occurrence, co-authorship, citation, co-citation, and bibliographic coupling networks were generated. The minimum threshold values were decided after trying several options, and we aimed to get a clearer network visualization in this way. Before creating the maps, data cleaning was performed manually, particularly for authors and keywords, and obvious spelling errors were corrected. However, as is a known limitation in bibliometric studies, not all inconsistencies could be fully resolved. The overall approach of the methodology was mainly to keep the workflow systematic and also as transparent as possible, although not every step was perfect.

The search was carried out on 29 November 2025 in the WoS Core Collection, using the keywords “occupational skin disease” and “occupational skin diseases” with the option set to “all fields”. This first produced 757 records, and later these were filtered according to the specific criteria of the study.

Results and discussion

General information and findings about regarding publications

The oldest publication dates back to 1980, and the most recent dates back to 2025. In total, there are 583 articles, including 103 review articles, 23 meeting abstracts, 23 editorial materials, 20 proceeding papers, 16 letters, 8 book chapters, 2 early access articles, 1 note, and one book compilation. As Figure 1 shows, the majority of studies were published as articles related to occupational skin diseases. It is observed that the vast majority of publications are in English (589 publications).

Figure 1 Analysis of academic studies conducted on occupational skin diseases

According to the WoS index, 610 studies are indexed in the Science Citation Index (SCI), 119 in the Emerging Sources Citation Index (ESCI), 43 in the Social Sciences Citation Index (SSCI), 23 in the Conference Proceedings Citation Index-Science (CPCI-S), 8 in the Conference Proceedings Citation Index-Social Science (BKCI-S), 1 in the Conference Proceedings Citation Index-Social Science (CPCI-SSH), and 1 in the Arts & Humanities Citation Index (AHCI) (Figure 2).

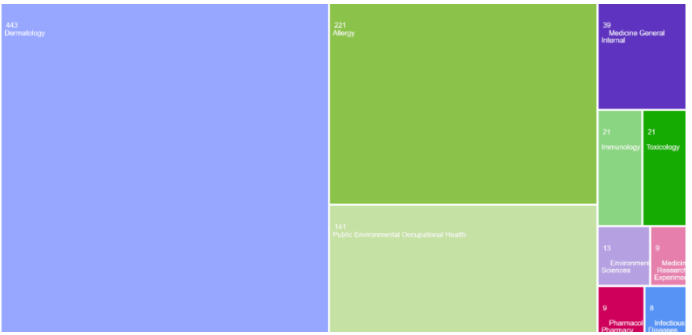
Figure 2 Analysis of Studies Related to Occupational Skin Diseases in Terms of Indexes

In general, studies that fall within the scope of the United Nations Sustainable Development Goals relate to Good Health and Well-being (Goal 3) (134 studies). Apart from these, one study relates to Quality Education (Goal 4), one to Clean Water and Sanitation (Goal 6), one to Responsible Consumption and Production (Goal 12), and one to Climate Action (Goal 13) (Figure 3).

Figure 3 Analysis of Studies Related to Occupational Skin Diseases in Terms of Sustainability Goal Compliance Indexes

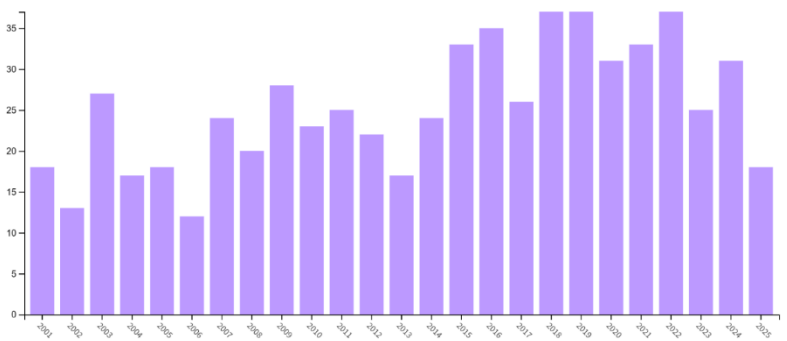
When evaluated in terms of WoS categories, the majority of studies in this field fall under the following categories: Dermatology (443), Allergy (221), Public Environmental and Occupational Health (141), Medicine General Internal (39), Immunology (21), Toxicology (21), Environmental Sciences (13), Medicine Research Experimental (9), and Pharmacology Pharmacy (9) (Figure 4).

Figure 4 Analysis of Studies Related to Occupational Skin Diseases in Terms of WoS Categories



Examining the publication years of articles resulting from a WoS Core Collection search using the keywords "occupational skin disease" and "occupational skin diseases" reveals that the highest number of publications occurred in 2022, 2019, and 2018 (37 each), followed by 2016 (35), 2021 (33), 2015 (33), 2020 (31), and 2024 (31). Thus, it can be concluded that studies in this field have increased in frequency over the last 10 years, but that interest has declined in recent years (Figure 5).

Figure 5 Analysis of Studies Related to Occupational Skin Diseases in Terms of Publication Year (VOSviewer Screenshot)



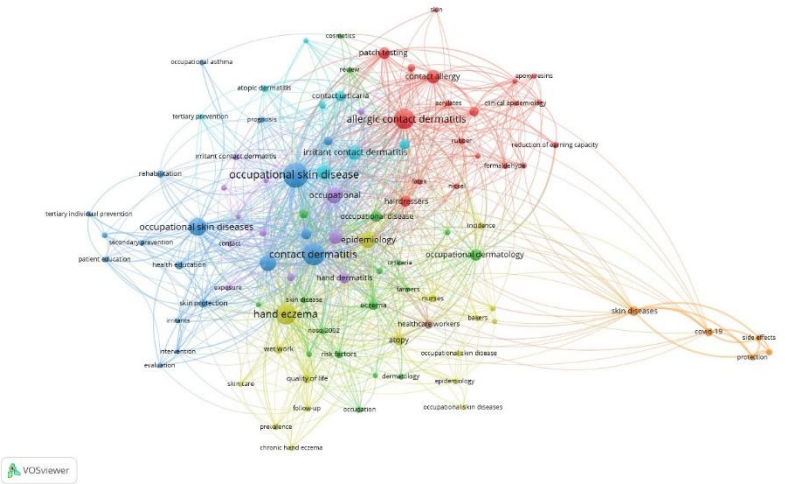
Co-occurrence of all keywords

A total of 98 keywords were identified from a pool of 1436 keywords when examining the keyword analysis for the terms "occupational skin disease" and "occupational skin diseases," with a minimum number of occurrences of 5 for each keyword. The most frequently used words among these were contact dermatitis (88 occurrences), hand eczema (76 occurrences), allergic contact dermatitis (72 occurrences), prevention (47 occurrences), epidemiology (43 occurrences), and irritant contact dermatitis (34 occurrences) (Table 1). These 98 words formed 8 clusters related to each other (Figure 6). Examining Table 1, it is evident that contact dermatitis, eczema, and their prevention are key areas of research in studies related to occupational skin diseases.

Table 1 Analysis of the most frequently used keywords in studies on occupational skin diseases

Keyword	Occurrences	Total Link Strength
contact dermatitis	88	240
hand eczema	76	193
allergic contact dermatitis	72	198
prevention	47	129
epidemiology	43	114
irritant contact dermatitis	34	106

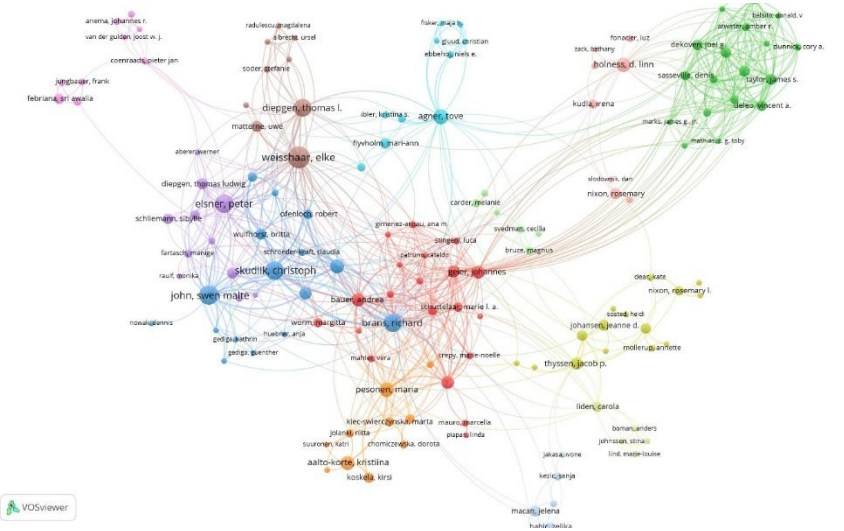
Figure 6 Analysis of the most frequently used keywords in studies on occupational skin diseases using VOSviewer



Co-authorship of authors

After filtering by the criterion that each author in the field should have 2 publications and at least 15 citations, 317 connected authors were found out of 2071 total authors. The analysis identified 143 items, 13 clusters, 747 connections, and a total connection strength of 1398. Diepgen, T.L., John, S.M., Weisshaar, E., Elsner, P. and Skudlik, C. were identified as the authors with the most publications (65, 61, 52, 39, and 38, respectively). These authors were found to have the highest total connection strength. Figure 7 is a network visualization of authors with a high number of publications.

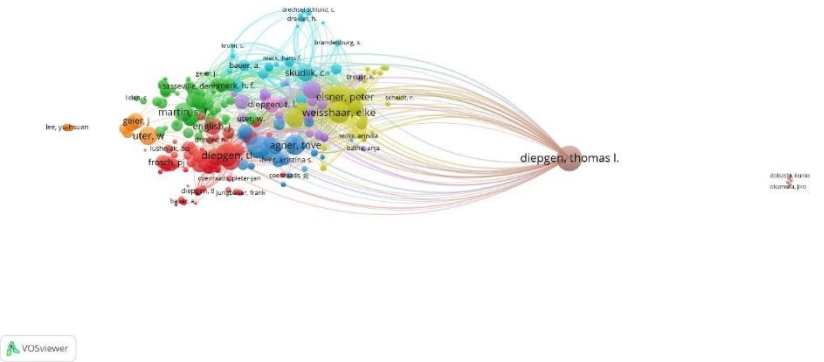
Figure 7 Analysis of the co-authorship of authors in studies on occupational skin diseases using VOSviewer



Citation of authors

To identify the authors' citation networks, a network map was created based on the criterion that each author had 2 publications and at least 15 citations. Accordingly, 317 out of 2071 authors were found to be connected (Figure 8). Analysis of the 317 connected authors revealed 9 clusters of link strength. The total connection strength was 23431. The top four authors with the highest connection strength were John, S.M., Skudlik, C., Diepgen, T.L., and Weisshaar, E., with 3021, 2651, 2477, and 2239 citations, respectively. Diepgen, T.L. had the most citations, with 2279. He was followed by John, S.M. (1392 citations) and Skudlik, C. (914 citations).

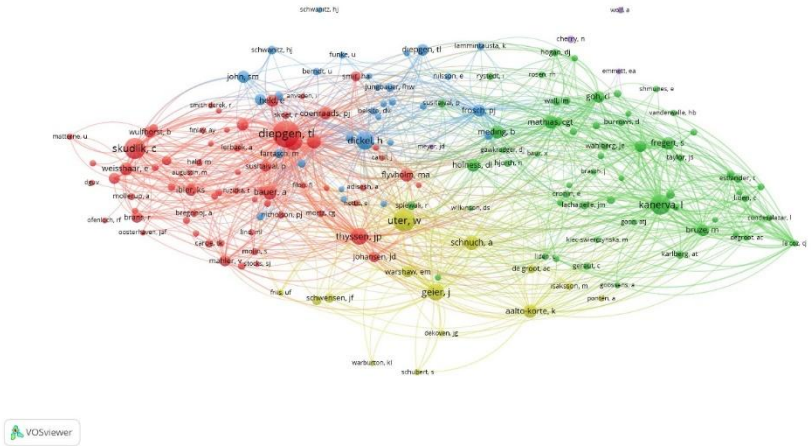
Figure 8 Analysis of the citation of authors in studies on occupational skin diseases using VOSviewer



Co-citation of authors

In this analysis, we examined the common citation counts of cited authors assuming each had at least 20 citations. Connections were established for 173 out of 9457 authors (Figure 9). Among the authors with the highest number of common citations, Diepgen, T.L. had 460 citations and a connection strength of 8330. Uter, W. follows with 292 common citations and a connection strength of 7134. Skudlik, C. comes in third with 260 common citations and a connection strength of 3913.

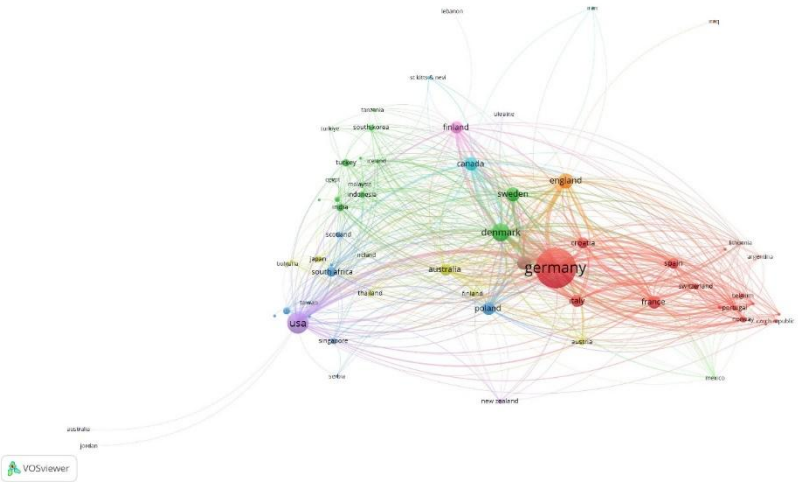
Figure 9 Analysis of the co-citation of authors in studies on occupational skin diseases using VOSviewer



Citation of countries

In this analysis, we attempted to create a map of citations by country of publication. The analysis was based on the criterion that each country had at least 1 publication and 1 citation. Accordingly, Germany ranks first with 276 publications and 6883 citations (Figure 10). The USA ranks second with 81 publications and 3616 citations; Denmark ranks third with 55 publications and 2389 citations; the UK ranks fourth with 41 publications and 1862 citations; and the Netherlands ranks fifth with 35 publications and 1307 citations. The results indicate that European countries are more dominant in this field.

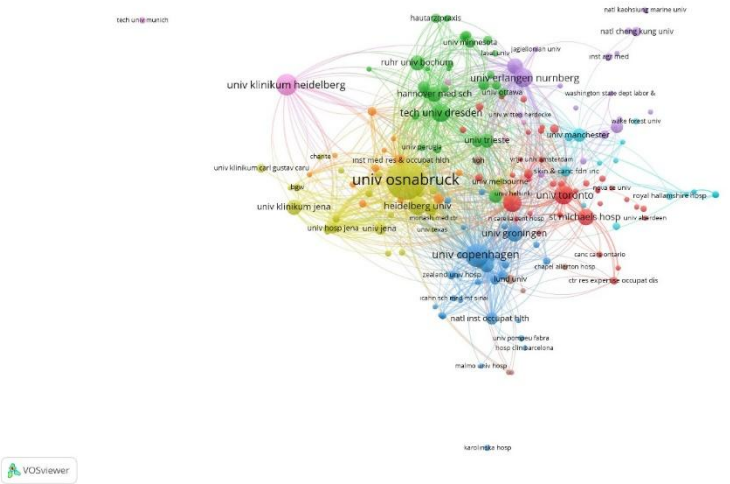
Figure 10 Analysis of the citation of countries in studies on occupational skin diseases using VOSviewer



Citation of organizations

In this filtering process, which aimed to establish network connections for inter-institutional citations, the analysis was conducted based on the criterion of at least 2 publications and 15 citations from each institution. Accordingly, 190 observation units emerged from 951 organizations. The University of Osnabrück in Germany (98 publications/1882 citations) and the University of Copenhagen in Denmark (34 publications/1509 citations) are at the top based on the number of publications. A total of 9 clusters and 4124 connections were identified (Figure 11).

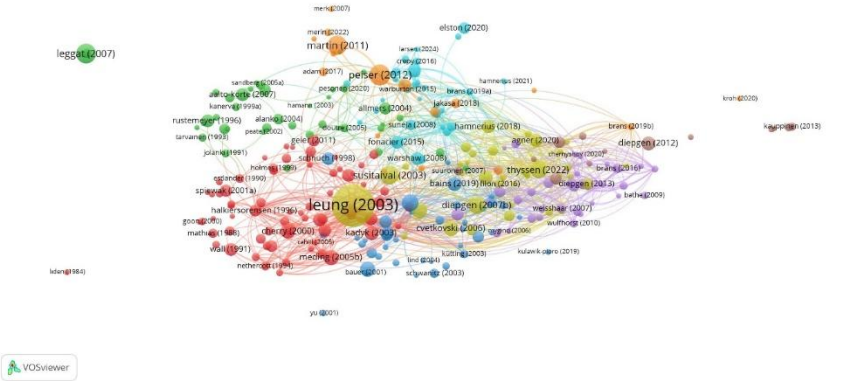
Figure 11 Analysis of the citation of organizations in studies on occupational skin diseases using VOSviewer



Bibliographic coupling of documents

Bibliographic matching refers to the citation of a common publication by two independent sources. The analysis of 296 works, selected based on having received at least 15 citations and connections, yielded 8 clusters, 8774 connections, and a total connection strength of 18859. Figure 12 shows that the publications with the most bibliographic matching were Leung (2003) with 1109 citations, Peiser (2012) with 261 citations, and Leggat (2007) with 235 citations. The works with the highest total connection strength were Clark (2009) with 743 and Thyssen (2022) with 724.

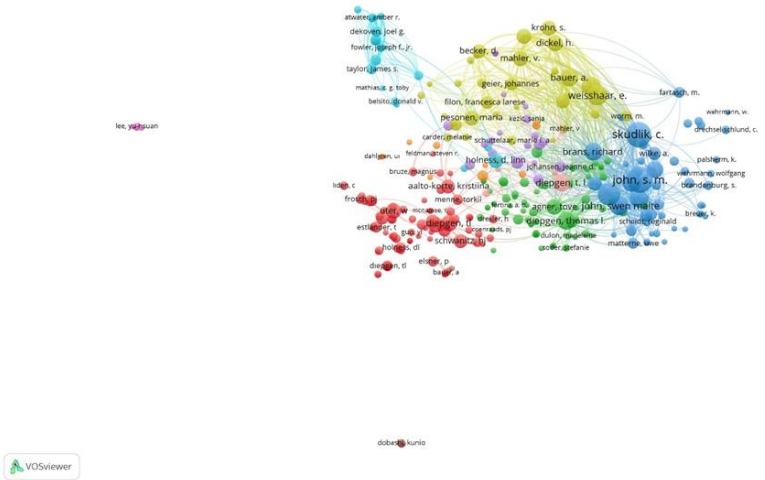
Figure 12 Analysis of the bibliographic coupling of documents in studies on occupational skin diseases using VOSviewer



Bibliographic coupling of authors

The analysis of 317 meeting units, which were selected based on the criterion of having published at least 2 works and received at least 15 citations, revealed 10 clusters, 30585 connections, and a total connection strength of 481384. The authors with the most bibliographic matches were John, S.M. and Diepgen, T.L. (Figure 13).

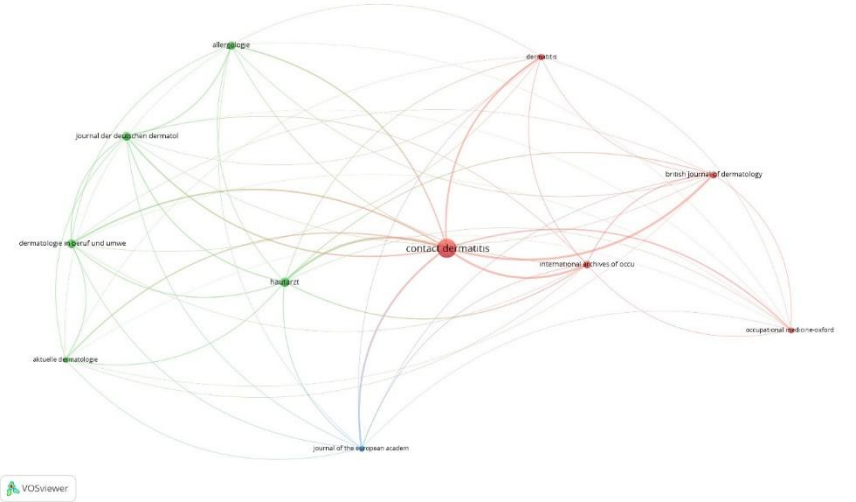
Figure 13 Analysis of the bibliographic coupling of authors in studies on occupational skin diseases using VOSviewer



Bibliographic coupling of sources

This filtering process examined the most frequently cited sources among all analysed publications. The analysis was conducted based on the criterion that a source must consist of at least 15 documents and 15 citations. Figure 14 shows that the most cited sources were Contact dermatitis (5095 citations), British journal of dermatology (1239 citations), and International archives of occupational and environmental health (897 citations).

Figure 14 Analysis of the bibliographic coupling of sources in studies on occupational skin diseases using VOSviewer



Conclusions

This study employs bibliometric approaches to evaluate the scientific influence of publications on occupational skin diseases, as well as their development trends and the collaborations between countries. The results show that research in this field has grown especially in the last ten years, with strong attention on dermatology, allergy and also immunology topics, but that interest has declined in recent years. The keyword analysis indicates that terms like “contact dermatitis”, “eczema” and “prevention” appear quite often, which suggests that these subjects make up the main focus in the present research landscape. Studies on proactive preventative strategies are becoming a major area of interest because these disorders are frequently observed in occupational health and safety settings. Analyses of author interaction show that some academic groupings

have established solid worldwide connections. In this field, publications based in Germany are the most numerous, with The University of Osnabrück in Germany ranking first among institutions. This result may be due to Germany's long-standing industrial development and subsequent greater exposure to occupational risk factors. Furthermore, well-established monitoring and reporting systems may create a favourable environment for working in this field. Only including studies listed in the WoS database in the analysis is a significant limitation. In future, it is recommended that various databases are included to minimise such limitations. Although interest in the subject appears to have waned during the period of the COVID-19 pandemic, it can be said that new studies could focus on topics such as side effects, healthcare workers and reduced earning capacity.

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BÖLÜM 5

RESEARCH ON WORKS RELATED TO DIABETIC WOUND HEALING: A BIBLIOMETRIC ANALYSIS

KAAN KALTALIOGLU[1]

Introduction

Wound is generally described as a disruption of the integrity of the body tissue caused by physical injury. Although wound healing is frequently neglected as it is naturally assumed to be a characteristic of the human body, however, it is an extremely intricate and sensitive physiological action. This mechanism may be impaired by a number of pathological conditions such as diabetes mellitus and oxidative stress (Werner & Grose, 2003; Lee et al., 2012). DM, a systemic metabolic disease involving chronic hyperglycemia, is known to be one of the key diseases that disrupt normal healing of wound (Kaltalioglu, 2023).

The healing mechanism for diabetic wounds is a complex and multifactorial pathological process, which occurs accompanied by the decreased activity of cells, prolonged inflammation, and the increased susceptibility to infection. Chronically, these deep veins

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become much more likely to be involved in the flow of chronically generated complications, such as foot ulcers (Armstrong et al., 2023; Zhao et al., 2023; Lang et al., 2024). These diseases severely affect the quality of life for patients in terms of chronic pain and high risk of lower extremity amputation, doing significant economic damage to health systems globally (McDermott et al., 2023;Wen & Nie, 2025; Shang et al., 2025).

Bibliometrics was first introduced by Pritchard (1969) as a statistical and quantitative method for analysing large volumes of scientific literature. Adopted as an analytical frame, bibliometrics is employed to efficiently explore the research landscape of a given field by analyzing its related literature to identify its knowledge structures, system development history and emerging patterns (Chen & Song, 2019).

The bibliometric approach becomes a more and more prominent tool to illustrate the evolution of particular fields of research, as demonstrated in relation to wound healing and diabetes mellitus. For instance, Wen & Nie (2025), Lang et al. (2024), using visualization mapping method, obtained the global research hotspots and dynamic trends of diabetic wound treatment, indicating an increasing demand for efficient targeted intervention. Similarly, Zhao et al. (2023) was devoted for mapping the knowledge in diabetic foot research and someone like Shang et al. (2025) offered a comprehensive bibliometric analysis of the contribution of growth factors in tissue healing.

The use of novel biological treatments is also becoming more popular according to the literature. This tendency is consistent with Sun et al. (2024) who illustrated the stem cell-based strategies in wound repair and She et al. (2022), who provided a twenty-year bibliometric overview of Fibroblast Growth Factor 21 (FGF21) studies. And, natural and alternative therapies have become the focus of increased energies in recent times. Farhat et al. (2023)

studied the bibliometrics of research on curcumin, and there are some studies have conducted scientometric analyses for natural product use in wound healing (Yang et al., 2025). Last, from a clinical and applied point of view, Taylan & Küçükakça Çelik (2025) explored the literature on nursing and outlined management, preventive strategies and holistic care as guiding themes in all the matter of wound and ostomy care.

The aim of this study is to present an overview of the international literature on diabetic wound healing using bibliometric analysis and visual mapping; we sought to systematically grasp main research contents, publication patterns as well as overall scientific development trends in the field.

Methodology

Bibliometric analysis is useful in showing research trends, recognizing current gaps and suggesting future directions in a scientific field (Varshabi, Arslan Selçuk, & Mutlu Avinç, 2022). The data used in the present study were based on Web of Science (WoS) Core Collection, which is commonly applied in quantitative literature analysis as it covers broad ranges of high-quality peer-reviewed journals (Yan & Zhiping, 2023).

For the visualization and mapping processes, VOSviewer software (version 1.6.20) was employed. This tool is widely used in bibliometric research as it enables the construction of co-occurrence networks and clustering maps in a relatively straightforward manner. Within the scope of this study, networks based on keyword co-occurrence, co-authorship, citation, co-citation, and bibliographic coupling were generated. Minimum threshold values were determined after several trial runs in order to obtain clearer and more interpretable network structures.

Before generating the map, manual cleaning of data was performed (they were primarily focused on author names and keywords, where visible spelling mistakes were corrected). However, due to the typical limitations of bibliometric measurements, some outliers could not be fully removed from the database. Overall, the methodological workflow was designed to be systematic and transparent, although some minor imperfections were unavoidable.

The literature search was conducted on 15 December 2025 using the WoS Core Collection database. The keyword “diabetic wound healing” was searched with the “all fields” option selected. This initial search yielded 3575 records, which were subsequently refined based on the predefined inclusion criteria of the study.

Results and discussion

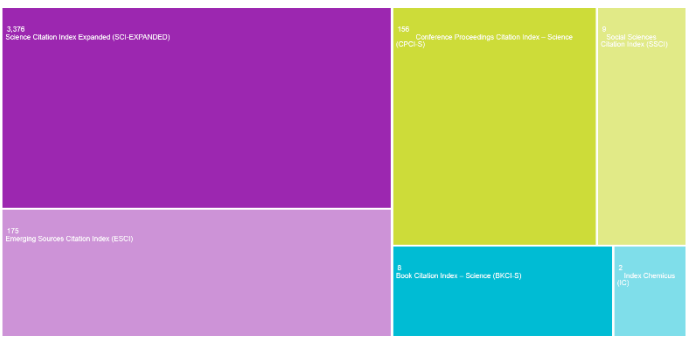
General information and findings about regarding publications

The earliest publication included in this analysis dates back to 1993, while the most recent one was published in 2025. Overall, the dataset consists of 2859 articles, along with 382 review papers, 224 meeting abstracts, 28 editorial materials, 29 proceedings papers, 13 letters, 8 book chapters, and 59 early access publications. As illustrated in Figure 1, most of the studies in the field of diabetic wound healing were published in the form of original research articles. In addition, it was observed that almost all publications were written in English, accounting for 3573 records.

Figure 1 Analysis of academic studies conducted on diabetic wound healing

Based on the Web of Science classification, the majority of publications are indexed in the Science Citation Index (SCI), comprising 3376 studies. In addition, 175 records are included in the Emerging Sources Citation Index (ESCI), the Conference Proceedings Citation Index–Science (CPCI-S) with 156 studies, while smaller numbers are indexed in the Social Sciences Citation Index (SSCI) with 9 studies, the Conference Proceedings Citation Index–Social Science (BKCI-S) with 8 studies, and the Index Copernicus (IC) with 2 studies, as presented in Figure 2.

Figure 2 Analysis of studies related to diabetic wound healing in terms of indexes



Overall, the majority of the studies linked to the United Nations Sustainable Development Goals are associated with Good Health and Well-being (Goal 3), accounting for 2179 publications. In addition to this main category, a limited number of studies are related to other goals, including Zero Hunger (Goal 2) with 8 studies, Industry, Innovation and Infrastructure (Goal 9) with 7 studies, Gender Equality (Goal 5) with 3 studies and Affordable and Clean Energy (Goal 7) with 3 studies, as illustrated in Figure 3.

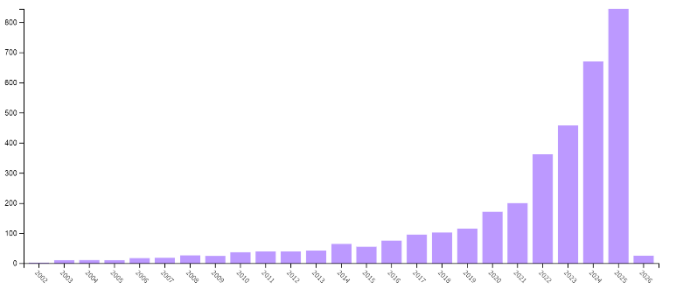
Figure 3 Analysis of studies related to diabetic wound healing in terms of sustainability goal compliance indexes

An evaluation based on Web of Science categories shows that most publications in this area are concentrated in several major fields. These include Materials Science Biomaterials (528 studies), Nanoscience and Nanotechnology (520), Pharmacology and Pharmacy (448), Biochemistry and Molecular Biology (441), and Materials Science–Multidisciplinary (402). In addition, notable contributions are observed in Engineering–Biomedical (401), Medicine–Research and Experimental (368), Cell Biology (366), Chemistry–Multidisciplinary (342), and Polymer Science (335), as presented in Figure 4.

Figure 4 Analysis of studies related to diabetic wound healing in terms of WoS categories

An analysis of publication years based on the Web of Science Core Collection search for the term “diabetic wound healing” shows that the largest numbers of studies were published in 2025 (844), followed by 2024 (669), 2023 (457), 2022 (362), and 2021 (200). These findings indicate a clear increase in research activity in this field, particularly over the last five years, as illustrated in Figure 5.

Figure 5 Analysis of studies related to diabetic wound healing in terms of publication year (VOSviewer Screenshot)



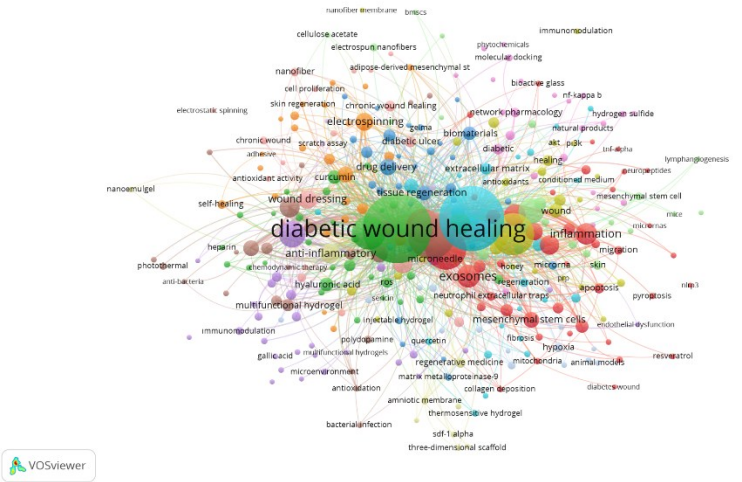
Co-occurrence of all keywords

In the keyword analysis related to “diabetic wound healing” a total of 333 keywords were selected from an initial set of 5556 keywords by applying a minimum occurrence threshold of five. The most frequently used words are given in Table 1. These 333 words formed 14 clusters related to each other (Figure 6). When Table 1 is examined, it becomes clear that angiogenesis, hydrogel, and antibacterial-related topics are among the most studied areas in diabetic wound healing research.

Table 1 Analysis of the most frequently used keywords in studies on diabetic wound healing

Keyword	Occurrences	Total Link Strength
Diabetic wound healing	869	1454
Angiogenesis	299	778
Hydrogel	209	530
Antibacterial	131	325
Diabetes mellitus	120	273
Exosomes	95	242

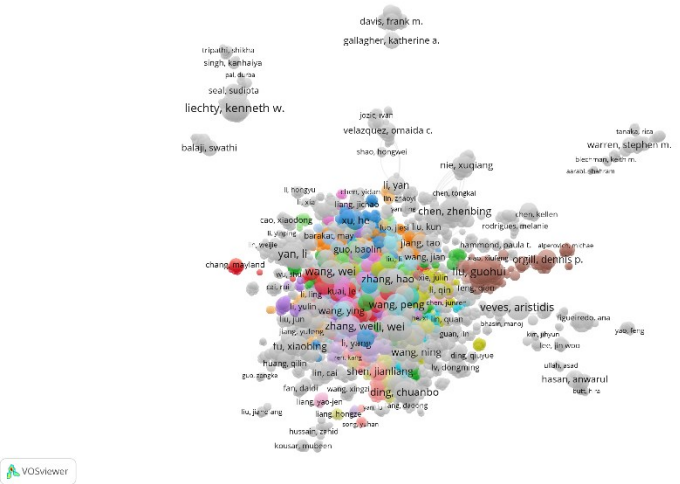
Figure 6 Analysis of the most frequently used keywords in studies on diabetic wound healing using VOSviewer



Co-authorship of authors

After applying the inclusion criteria of a minimum of two publications and at least fifteen citations per author, 3346 connected authors were identified from a total of 18200 authors. The resulting network consisted of 2717 items grouped into 55 clusters, with 15460 links and an overall total link strength of 27871. Among these, Liechty K.W., Zgheib C., Li W., Yan L. and Tan Q. were the most productive authors. Figure 7 presents the network visualization highlighting authors with higher publication output.

Figure 7 Analysis of the co-authorship of authors in studies on diabetic wound healing using VOSviewer



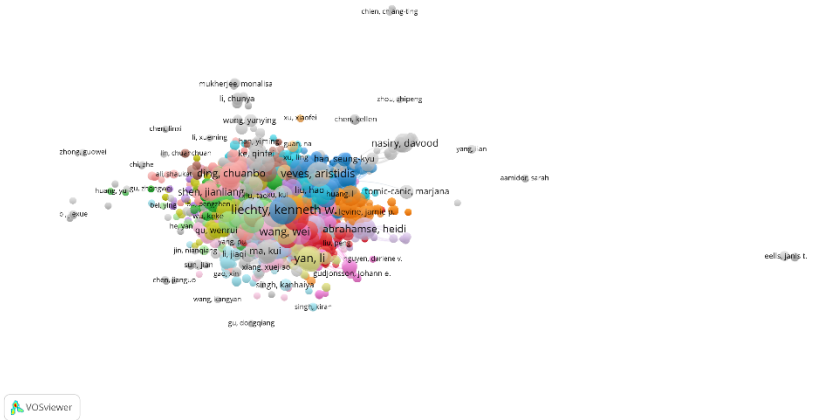
Citation of authors

To examine citation relationships among authors, a citation network map was generated by applying the threshold of at least two publications and a minimum of fifteen citations per author. Based on these criteria, 3346 authors were identified as connected within the network out of a total of 18200 authors (Figure 8). Further analysis of these connected authors revealed a structure consisting of 29 clusters with varying link strengths, and the overall total link strength reached 172354.

In terms of connectivity, Liechty K.W., Zgheib C., Mao C., and Lin C. showed the highest link strength values, with 1751, 1545, 1407, and 1402, respectively. When citation counts were considered,

Mao C. appeared as the most cited author with 1873 citations, followed by Lin C. with 1744 citations and Lei B. with 1737 citations.

Figure 8 Analysis of the citation of authors in studies on diabetic wound healing using VOSviewer

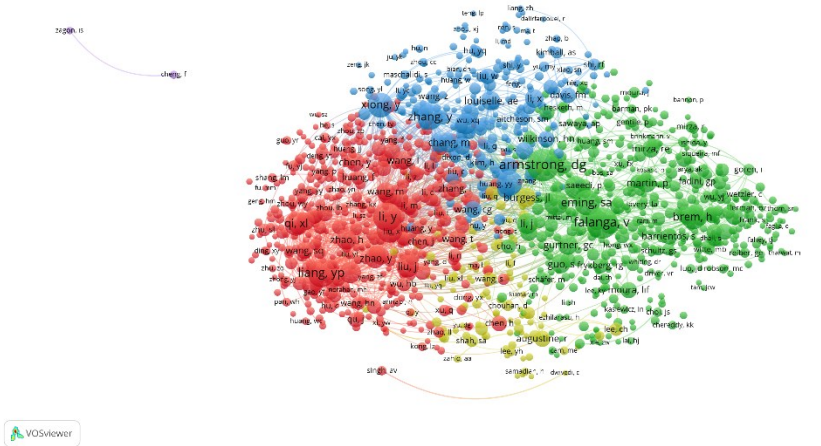


Co-citation of authors

In this analysis, co-citation patterns of cited authors were evaluated by applying a minimum threshold of 20 citations per author. Based on this criterion, 1397 authors were found to be connected out of a total of 6687 cited authors (Figure 9). Among those with the highest co-citation frequencies, Armstrong D.G. ranked first with 624 citations and a total link strength of 17365. Falanga V. followed with 574 co-citations and a link strength of

13771, while Li Y. ranked third with 479 co-citations and a link strength of 17359.

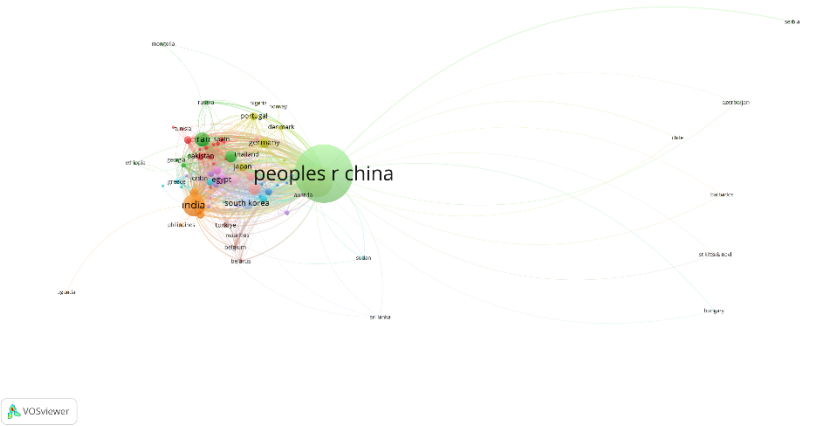
Figure 9 Analysis of the co-citation of authors in studies on diabetic wound healing using VOSviewer



Citation of countries

In this part of the analysis, a citation map based on countries of publication was generated by applying the minimum requirement of at least one publication and one citation per country. Under these criteria, the People's Republic of China emerged as the leading country with 2086 publications and 65733 citations (Figure 10). The United States followed in second place with 581 publications and 30495 citations. India ranked third, contributing 293 publications and receiving 7123 citations, while Iran placed fourth with 132 publications and 2893 citations. Taiwan was ranked fifth, with 85 publications and a total of 2360 citations.

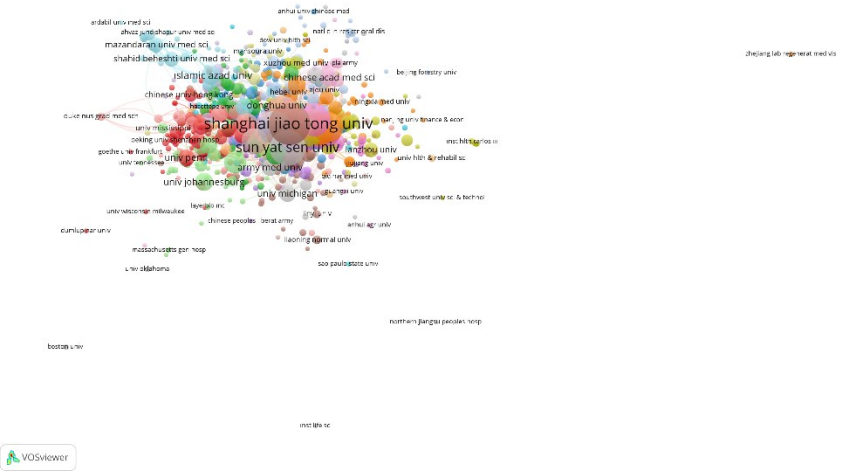
Figure 10 Analysis of the citation of countries in studies on diabetic wound healing using VOSviewer



Citation of organizations

To establish inter-institutional citation networks, a filtering process was applied using the criteria of a minimum of two publications and at least fifteen citations per institution. Based on these thresholds, 2999 units were identified from a total of 867 institutions. In terms of publication output, Shanghai Jiao Tong University in the People's Republic of China ranked first with 156 publications and 6203 citations, followed by Sun Yat-Sen University, also from the People's Republic of China, with 115 publications and 4025 citations. Overall, the analysis revealed 22 clusters and 25390 connections within the institutional network, as shown in Figure 11.

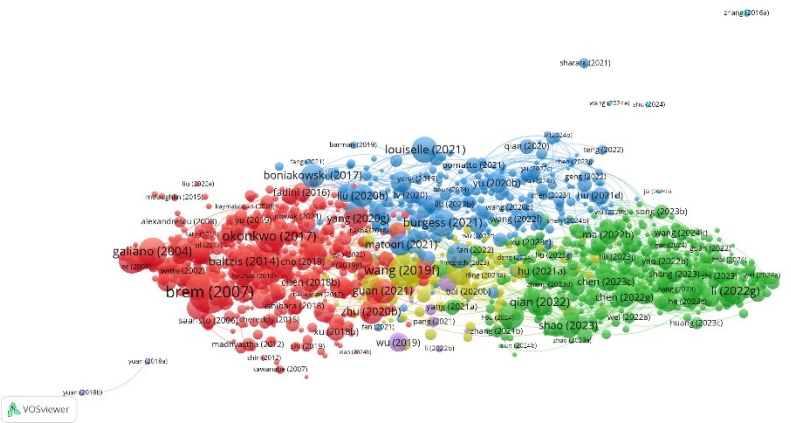
Figure 11 Analysis of the citation of organizations in studies on diabetic wound healing using VOSviewer



Bibliographic coupling of documents

Bibliographic coupling describes a situation in which two independent publications cite the same reference. In this analysis, 1613 studies meeting the criteria of at least 15 citations and established connections were included. The results revealed a structure consisting of 6 clusters, 293956 links, and an overall total link strength of 446446. As illustrated in Figure 12, the publications showing the highest levels of bibliographic coupling were Brem (2007) with 1331 citations, followed by Okonkwo (2017) with 764 citations and Wang (2019) with 717 citations. In terms of total link strength, Xiang (2024) ranked first with a value of 4883, while Khattak (2025) followed with 3955.

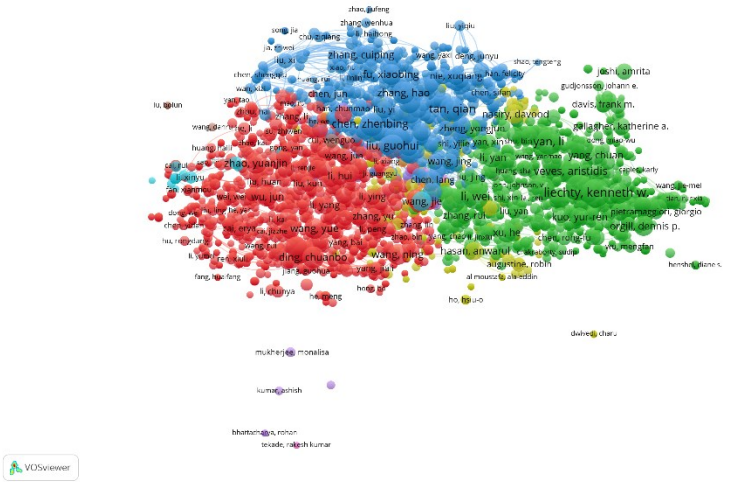
Figure 12 Analysis of the bibliographic coupling of documents in studies on diabetic wound healing using VOSviewer



Bibliographic coupling of authors

An analysis was carried out on 3346 units that met the criteria of having at least two publications and a minimum of fifteen citations. This analysis resulted in a network composed of 9 clusters, 3652338 links, and an overall total link strength of 20401641. The results indicate that Mao C. and Lin C. were the authors with the highest levels of bibliographic coupling, as illustrated in Figure 13.

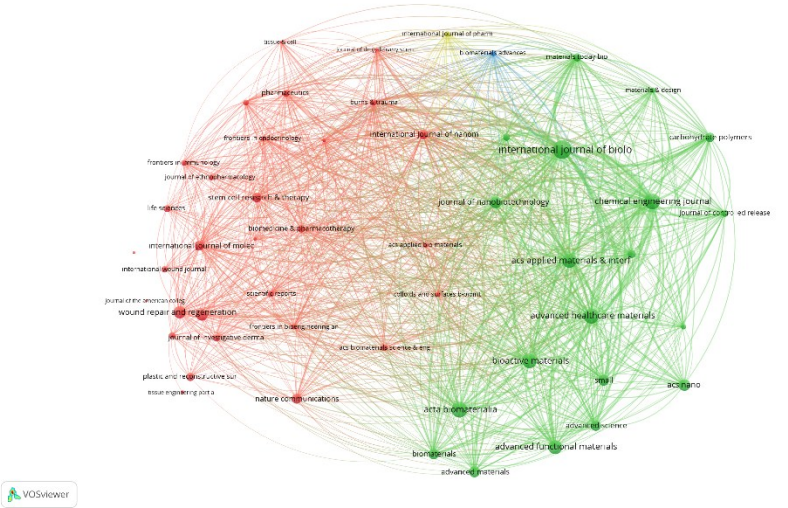
Figure 13 Analysis of the bibliographic coupling of authors in studies on diabetic wound healing using VOSviewer



Bibliographic coupling of sources

This filtering step focused on identifying the most frequently cited sources across all publications included in the analysis. The evaluation was performed using the threshold of at least 15 documents and a minimum of 15 citations per source. As illustrated in Figure 14, the International Journal of Biological Macromolecules emerged as the most cited source with 5085 citations, followed by the Chemical Engineering Journal with 3689 citations and Acta Biomaterialia with 3557 citations.

Figure 14 Analysis of the bibliographic coupling of sources in studies on diabetic wound healing using VOSviewer



Conclusions

This bibliometric analysis provides a comprehensive overview of the intellectual structure and evolving research trends in the field of diabetic wound healing between 1993 and 2025. The results show that research in this field has grown especially in the last five years, with strong attention on material and nano science topics. The keyword analysis indicates that terms like “angiogenesis”, “hydrogel”, “antibacterial” and “exosomes” appear quite often, which suggests that these subjects make up the main focus in the present research landscape. Research on angiogenesis, drug delivery systems and nanostructured materials suggests that regenerative and tissue engineering-based approaches have become an important area of interest in the field of diabetic wound healing. Analyses of co-authorship patterns indicate that several research

groups have developed strong international collaborations. In this research area, institutions from the People’s Republic of China contribute the highest number of publications, with Shanghai Jiao Tong University ranking as the leading institution. This outcome may be partly explained by China's enormous population and high prevalence of diabetes provide researchers with a large patient pool; in addition, Traditional Chinese Medicine's deep history and research into medicinal plants may be increasing the number of scientific publications on diabetes and its complications (such as wound healing). Future studies are recommended to explore additional databases and utilise various software applications, enhancing the ability to analyse literature content comprehensively. These findings provide valuable insights for future research and potential treatments in diabetic wound management.

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BÖLÜM 6

BÖLÜM I

SOME VIRULENCE-ASSOCIATED GENES IN PATHOGENIC BACTERIA: FUNCTIONAL ROLES, PHYLOGENETIC UTILITY, AND IMPLICATIONS FOR FOOD SAFETY AND DIAGNOSIS

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Introduction

Microorganisms' virulence levels and infection potential are related to their genetic structures. Identification in different bacterial

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species of genes associated with virulence and cellular activities provide information about pathogens disease development strategies and also their adaptation processes to environmental conditions (Y. Chen et al., 2025: 173). A detailed examination of the relevant genes to be mention in this review is better understanding the infection processes as well as a great matter in developing identification approaches, reducing foodborne risk and contributing to the fundamentally resolve of antibiotic resistance.

A meticulous examination of these critical genetic elements serves a dual purpose. First, it deciphers the complex biological pathways underlying infection, from initial adhesion and invasion to immune evasion and toxin production. Second, and of equal importance, this genetic knowledge forms the cornerstone of modern public health and diagnostic science. By pinpointing key virulence determinants, researchers can develop more precise and rapid molecular identification tools, directly contributing to enhanced food safety surveillance and outbreak traceability. Furthermore, understanding the genetic basis of pathogenesis and bacterial fitness is indispensable for addressing the global crisis of antibiotic resistance. It enables the identification of novel, pathogen-specific therapeutic targets that could circumvent conventional resistance mechanisms, moving us toward more fundamental and sustainable solutions.

To illustrate this critical junction of virulence genetics and practical application, this review examines a curated set of genes from high-consequence foodborne and zoonotic pathogens. The *bceT* and *cytK* genes reported in *Bacillus cereus* attract attention with their toxic activities in cells, the *prs*, *iap* and *prfA* genes in *Listeria* spp. takes critical roles both in processes associate to cellular activities and in regulation of virulence (Hansen et al., 2020: e00115; Ishino et al., 1987: 5429; Lund, De Buyser, & Granum, 2000: 254; Ugbogu, Schweizer, & Schweizer, 2022: 1909; Vazquez-Armenta et al., 2020: 103377; Zhao & Sun, 2022: 305). Smilarly, the *iacP*, *iroN*, and *avrA* genes in *Salmonella* Typhimurium play role in pathogenesis processes such as invasion, biofilm formation and the maintenance of epithelial integrity (Arrieta-Gisasola et al., 2024: 110753; Ben-Barak et al., 2006: 25; Eom et al., 2012: 4332; Salam

et al., 2023: 431; Viala et al., 2013: 19). Additionally, while the *bigA* gene functions in host cell adhesion in *Brucella abortus*, more evolutionary conserved genes such as *gyrB* and *tuf* are used for the phylogenetic classification of bacteria and for diagnostic purpose (Berdejo et al., 2020: 6; Czibener et al., 2016: 500; Liu et al., 2021: 763359; Misra et al., 2024: 100229).

1. *Bacillus cereus* Genes

Bacillus cereus is a Gram-positive, spore-forming bacterium of significant concern in food safety, responsible for both diarrheal and emetic types of illnesses (Yang et al., 2023: 1). Its pathogenicity is largely attributed to a wide range of enterotoxins and cytotoxins, the genetic determinants of which are critical for understanding its virulence. Among these, the *bceT* and *cytK* genes have been extensively studied for their roles in cellular toxicity and contribution to foodborne disease (Castiaux et al., 2015: 79; Hansen et al., 2003: 21; Heckler et al., 2024: 110813; Jovanovic et al., 2021: 3719).

1.1. The *bceT* Gene

The *bceT* gene was initially identified and characterized as a putative enterotoxin gene in *Bacillus cereus*, with early studies reporting its expression product to exhibit cytotoxic effects on cultured mammalian cells (Agata, Ohta, Arakawa, & Mori, 1995). This activity suggested a potential role in the diarrheal syndrome caused by the bacterium, drawing significant research interest toward *bceT* as a key virulence marker (Dietrich et al., 2021: 98). However, the direct etiological link between the *bceT* gene product and clinical foodborne poisoning has remained elusive and controversial. Key research revealed that the toxic activity originally ascribed to the *bceT* gene may not originate from *bceT* itself. Instead, evidence points to the possibility that the observed phenotype was due to the product of a fusion gene or the expression of a closely related homologous sequence present in the bacterial strains studied (Burtscher et al., 2021: 234). This implies that *bceT* might be part of a larger, more complex genetic locus or that its function has been misinterpreted due to genetic linkage with other toxic determinants.

This ambiguity underlines a critical challenge in microbial pathogenesis: distinguishing between correlation and causation at the genetic level. The case of *bceT* highlights the necessity for comprehensive genetic deletion studies, purified protein analysis, and robust epidemiological correlation to definitively assign a role in disease. Consequently, while *bceT* remains a genetic marker of interest within the *B. cereus* group, its status as a primary diarrheal enterotoxin is questionable, and its utility as a standalone diagnostic target for virulent strains is limited (Choma & Granum, 2002: 115).

The scientific discourse surrounding *bceT* serves as a cautionary tale on the importance of validating genomic predictions with direct functional evidence. Although the *bceT* gene identified in *B. cereus* has been reported in various studies to shown toxic effects in cells, its association with foodborne poisoing has not been clearly proven subsequently research revealed that the report activity was originates from a possible fusion gene or a homologous sequence (Hansen et al., 2003: e00115; Lund et al., 2000: 254).

1.2. The *cytK* Gene

In contrast to the ambiguous role of *bceT*, the *cytK* gene encodes a well-characterized and potent virulence factor called Cytolysin K (CytK) (Koné et al., 2021: 698). This protein belongs to the β-barrel pore-forming toxin family and is a major contributor to the necrotic enteritis and diarrheal symptoms associated with *Bacillus cereus* foodborne outbreaks (Hardy, Lund, & Granum, 2001: 47). CytK exerts its pathogenic effect by binding to specific receptors on the host intestinal epithelial cell membrane, oligomerizing, and forming transmembrane pores. This disrupts cellular ion gradients, leading to rapid cell lysis, loss of epithelial barrier integrity, and severe tissue damage.

The *prs* gene, which is used with high specificity and sensitivity in the identification of *Listeria* spp. and is responsible for phosphoribosyl pyrophosphate (PRPP) synthesis in cellular metabolism has been reported to undertake vital functions with different gene complexes in *Saccharomyces cerevisiae* and its overactivity has been associated with various diseases in humans

(Murdoch, Schweizer, & Schweizer, 2024: 6; Schneiter et al., 2000: 3269; Sharma et al., 2024: 696; Ugbogu, Schweizer, & Schweizer, 2022: 1909).

Genetic and functional analyses have identified two principal variants of this toxin, CytK-1 and CytK-2, which exhibit significant differences in their pathogenic potential. CytK-1 is recognized as the more virulent variant. CytK-1 possesses superior membrane-binding affinity and forms more stable, larger pores compared to CytK-2, resulting in markedly higher cytotoxic activity (Fagerlund et al., 2004: 2689). This enhanced toxicity translates to a more serious risk to human health, as strains harboring the cytK-1 allele are associated with outbreaks of greater clinical severity, including cases of fulminant, life-threatening necrotic enteritis. The unknown link between the *cytK* gene, its protein product's mechanism of action, and clinical illness solidifies its status as a critical molecular marker for assessing the virulence potential of *B. cereus* isolates in both food safety surveillance and epidemiological investigations.

2. *Listeria* Genes

Listeria is a genus of Gram-positive, rod-shaped, facultatively anaerobic bacteria. It is ubiquitous in nature and can be found in soil, water, vegetation, and the intestinal tracts of some animals (Manyi-Loh & Lues, 2025: 1266). The most notable species is *Listeria monocytogenes*, which is a major foodborne pathogen responsible for the disease listeriosis. Other species, such as *L. innocua*, *L. ivanovii*, and *L. seeligeri*, are generally considered non-pathogenic or rarely cause illness in humans (Wang et al., 2017: e44).

2.1. The *prs* Gene: A Metabolic Housekeeper with Diagnostic and Pathogenic Significance

The *prs* gene, encoding phosphoribosylpyrophosphate synthetase (PRS), occupies a unique and critical niche at the intersection of core bacterial metabolism, diagnostic microbiology, and broader cellular pathophysiology (Nilsson & Hove-Jensen, 1987: 247). Its primary and essential biochemical function is the synthesis of phosphoribosyl pyrophosphate (PRPP), a pivotal

metabolite that serves as a fundamental building block in the de novo and salvage pathways for nucleotides, amino acids (histidine and tryptophan), and cofactors (NAD and NADP). This places the prs gene product at the very heart of cellular biosynthesis and energy management. In the context of *Listeria* spp., this indispensable metabolic role has been strategically leveraged for diagnostic purposes. Because the *prs* gene sequence contains regions that are highly conserved within the genus yet distinct from other bacteria (Hove-Jensen, 1985: 269), it serves as an excellent target for DNA-based detection methods, such as polymerase chain reaction (PCR). Assays targeting the *prs* gene are renowned for their high specificity and sensitivity, enabling the accurate identification and differentiation of *Listeria* species from complex food and environmental samples (Chen et al., 2017: 39).

Beyond its utility as a diagnostic beacon, research in model organisms reveals the profound cellular consequences of PRPP synthetase activity. Studies in *Saccharomyces cerevisiae* (baker's yeast) have shown that PRS does not operate in isolation but forms essential protein complexes with other enzymes (Murdoch et al., 2024: 6). These complexes are crucial not just for efficient PRPP production but also for maintaining cell wall integrity and proper subcellular localization, indicating a direct link between central metabolism and cellular structure. This connection to cellular integrity finds a startling parallel in human medicine. While *Listeria* utilizes *prs* for survival, dysregulation of the homologous human *PRPS1* gene is pathogenic (Moran et al., 2012: 455). Overactivity of human PRS, often due to gain-of-function mutations, leads to PRPP overproduction.

Consequently, it can be said that the prs gene, which is used with high specificity and sensitivity in the identification of *Listeria* species and has been reported to undertake vital functions with different gene complexes its overactivity has been associated with various diseases in humans (Murdoch et al., 2024: 6; Sharma et al., 2024: 696)

2.2. The *iap* Gene: A Vital Autolysin

In *Listeria monocytogenes*, the *iap* gene encodes p60, a membrane-associated autolysin essential for cell wall remodeling, division, and viability. Beyond its role in maintaining cellular vitality, p60 contributes to pathogenesis by facilitating host cell invasion and bacterial surface modification (Wuenscher et al., 1993: 3491). Due to its conserved sequence, the iap gene is also a key target for sensitive and specific DNA-based detection of *L. monocytogenes* in diagnostic and food safety testing. To sum up, this gene also encodes a proteolytic enzyme that plays significant role in izosyme conversion in *L.* monocytogenes with the role of maintenance of cellular vitality and in diagnostic purposes DNA amplification, it expresses a membrane associated protein (Ishino et al., 1987: 5429; Wuenscher et al., 1993: 3491).

2.3. The *prfA* Gene

The *prfA* gene (positive regulatory factor A) in *Listeria monocytogenes* functions as the master transcriptional regulator of its virulence program (Johansson & Freitag, 2019: 1). It encodes the PrfA protein, which activates the expression of a suite of essential virulence genes – including those encoding internalins (*inlA* and *inlB*), listeriolysin O (*hly*), and phospholipases (*plcA* and *plcB*) – required for host cell invasion, phagosomal escape, and intracellular replication (Renzoni, Cossart, & Dramsi, 1999: 552).

The activity of PrfA is tightly controlled by multiple regulatory mechanisms, primarily at the post-transcriptional and allosteric levels. Its expression is thermoregulated, with optimal activation occurring at 37°C (host body temperature). Functionally, PrfA activity is modulated by the binding of specific cofactors, such as glutathione, which enhance its DNA-binding affinity. Additionally, certain point mutations can lead to a constitutively active form of the PrfA protein, resulting in hypervirulence in some strains.

Notably, the expression and activity of PrfA can be pharmacologically influenced. Studies have shown that quercetin, a naturally occurring flavonoid, downregulates *prfA* expression in a dose-dependent manner, subsequently reducing the transcription of

key virulence genes (Vazquez-Armenta et al., 2020: 103377). This highlights the potential of targeting the PrfA regulon for the development of novel anti-infective strategies against listeriosis.

3. Determinant genes for *Salmonella* spp.

Salmonella enterica serovar Typhimurium is a leading cause of foodborne gastroenteritis. Its pathogenicity relies on a sophisticated arsenal of virulence genes that coordinate host invasion, immune evasion, and persistence. Among these are the *iacP*, *iroN*, and *avrA* genes, which govern distinct yet complementary stages of the infectious cycle (Fàbrega & Vila, 2013: 308).

3.1. The *iacP* Gene

The *iacP* gene is integral to the function of *Salmonella* Pathogenicity Island 1 (SPI-1), the primary genetic locus responsible for host cell invasion. It encodes a protein essential for the proper acylation and activation of effector proteins delivered by the SPI-1 Type III Secretion System (T3SS) (Egan, Barret, & O'Gara, 2014: 34). Consequently, mutations in *iacP* severely impair the bacterium's ability to invade intestinal epithelial cells and diminish overall virulence. Beyond its role in effector secretion, *iacP* has also been implicated in modulating the expression of flagellin, linking the regulation of motility with the invasive machinery, a coordination critical for effective host colonization (Eom et al., 2012: 4332).

3.2. The *iroN* Gene

The *iroN* gene is basically an iron-capturing biofilm promoter. Iron acquisition is a critical challenge for pathogens in vivo. *S.* Typhimurium employs the *iroN* gene, which encodes the outer membrane receptor for salmochelin, a catecholate siderophore that scavenges ferric iron from the host (Bjarnason, Southward, & Surette, 2003: 4973). Additionally, *iroN* plays a surprising role in biofilm formation. Deletion of *iroN* leads to a significant reduction in biofilm biomass, suggesting that efficient iron sequestration is crucial for building these protective, surface-associated communities. The expression of *iroN* is finely tuned by the small

regulatory RNAs RyhB1 and RyhB2, which activate its transcription under iron-limiting conditions, exemplifying a sophisticated link between environmental sensing and virulence (Massé, Vanderpool, & Gottesman, 2005: 6962).

3.3. The *avrA* Gene

This gene is can be found in the majority of *Salmonella enterica* serovars, the *avrA* gene encodes an effector protein with a nuanced, dual role in pathogenesis. Secreted into the host cell, AvrA acts to maintain epithelial integrity by inhibiting pro-inflammatory NF-κB and JNK signaling pathways (Ye et al, 2007: 882). This anti-inflammatory activity paradoxically reduces the initial host immune response, facilitating bacterial colonization and systemic spread. However, the loss of *avrA* leads to increased epithelial cell apoptosis and a more severe inflammatory infection, demonstrating its role in perfection the host-pathogen interaction to achieve a balance favorable for *Salmonella* persistence. Although actively expressed in most strains, genomic deletions of *avrA* can be seen in certain serovars, highlighting the dynamic nature of *Salmonella* virulence collections.

4. *Brucella abortus* Gene

4.1. *bigA*

Brucella abortus, the causative agent of brucellosis, establishes chronic infection through intimate interaction with host cells (Ahmed, Zheng, & Liu, 2016: 30). In *Brucella abortus* the *bigA* gene which plays a role in adhesion to host cells encodes a strong adhesin, mutation analyses and cell culture experiments have shown that BigA alters focal adhesion processes but the identified single nucleotide variants don't directly contribute to antibiotic resistance (Berdejo et al, 2020: 937; Czibener et al., 2016: 500).

5. Evolutionary and Phylogenetic Markers

Conserved housekeeping genes provide a universal framework for bacterial identification and evolutionary study,

offering powerful tools for diagnostics and epidemiology (Meganathan, Vishwakarma, & Ramya, 2022: 103981).

5.1. The *gyrB* Gene

The *gyrB* gene, encoding the β-subunit of DNA gyrase (a type II topoisomerase), has emerged as a superior phylogenetic marker compared to the traditional 16S rRNA gene (Liu et al., 2021b: 763359). Due to its essential function in DNA replication and a higher evolutionary mutation rate, *gyrB* provides significantly greater discriminatory power for distinguishing between closely related bacterial species and strains (La Duc et al., 2004: 383). This makes it a vital tool for precise phylogenetic analysis, the classification of cryptic species, and the development of rapid, specific molecular assays for pathogen detection in clinical and environmental samples.

5.2. The *tuf* Gene

The *tuf* gene encodes elongation factor Tu (EF-Tu), a GTPase essential for protein synthesis by delivering aminoacyl-tRNAs to the ribosome. Like *gyrB*, it exhibits a level of sequence variability that offers high resolution in phylogenetic studies, often exceeding that of 16S rRNA (Pourahmad, 2025: 594). Its utility extends across diverse bacterial groups, including fastidious organisms like phytoplasmas, where it is used to investigate disease transmission pathways. In most Gram-negative bacteria, the presence of two functional *tuf* copies (*tufA* and *tufB*) further enhances its reliability and provides an internal control, solidifying its role as a robust genetic marker for population genetics and diagnostic identification (van der Meide et al., 1983: 398).

6. Conclusion

The systematic investigation of the genes detailed in this chapter (spanning dedicated virulence factors like *cytK*, *prfA*, and *iroN*, and conserved phylogenetic markers like *gyrB* and *tuf*) together advances our understanding of bacterial pathogenesis at a

molecular level. This knowledge is not merely academic; it is fundamentally translational. Elucidating these disease-causing mechanisms directly informs the rational design of novel diagnostic methods, enhances food safety surveillance through precise pathogen detection and source tracking, and identifies potential targets for next-generation antimicrobials and therapeutic interventions. Ultimately, the integration of functional virulence genetics with evolutionary phylogenetics provides a powerful, dual-strategy framework for combating bacterial infections and safeguarding public health and play important roles in the development of diagnostic methods and strategies related to food safety.

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BÖLÜM 7

SPECIES-POOR BUT ENDEMISM-RICH: THE GEOPHYTE FLORA of KIRŞEHİR (CENTRAL ANATOLIA, TURKEY)

ŞENER ÖZCAN[1]

Introduction

Geophytes are vascular plants that lose all their aerial parts at the end of the growing season, yet survive through metamorphosed undergFround organs such as corms, rhizomes, bulbs, or tubers (Raunkiaer, 1934; Rees, 1989). These subterranean structures allow plants to store energy and preserve their life forms during unfavorable environmental conditions. Consequently, geophytes have developed an adaptive strategy against stress factors such as drought, low temperatures, and fire, by separating their active growth phase from a passive dormancy period. When conditions become favorable again, new shoots rapidly emerge from these storage organs, reinitiating vegetative activity (Dafni, Cohen & Noy-Meir, 1981).

At the global scale, the geophytic life form is particularly concentrated within the monocotyledonous families (Monocotyledonae), among which Orchidaceae, Iridaceae, Asparagaceae, and Amaryllidaceae stand out in terms of species richness. The Orchidaceae, with more than 30,000 taxa worldwide, represents the most species-rich geophytic family. It is followed by the Iridaceae, which is predominantly centered in the Mediterranean and South African floristic regions, and subsequently by the Asparagaceae and Amaryllidaceae families. This evaluation was conducted according to the APG IV (Angiosperm Phylogeny Group, 2016) classification system, and the data on species richness and distribution were verified using the WFO (World Flora Online, 2025) and POWO (Plants of the World Online, Kew, 2025) databases.

Although geophytes occur in nearly all parts of the world, the majority are naturally distributed within the Mediterranean Basin. This basin, whose eastern margin includes Türkiye, harbors the second richest geophyte flora globally (Özhatay et al., 2013) and represents a major biogeographic center where high species diversity and endemism coexist. In the flora of Türkiye, geophytes hold a remarkable position not only in terms of species number but also for their intensity of endemism and diversity of ecological adaptations. According to recent evaluations, approximately 2,500 geophytic taxa have been recorded, of which about 34% are endemic and confined to Türkiye (Ekim et al., 1991; Demir & Eker, 2015). This high rate of

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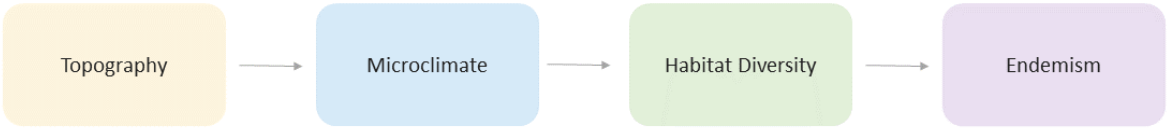
endemism is closely linked to the pronounced geomorphological heterogeneity, rugged mountainous topography, fine-scale microclimatic variation, and the complex geological mosaic characteristic of Anatolia (Figure 1). These interacting landscape features collectively generate a dense network of fine-scale ecological units shaped by localized variation in moisture, temperature, substrate, or topographic factors (Bennie et al., 2008), which in turn enhance ecological niche diversity and facilitate the persistence and diversification of narrowly distributed geophytes in the Kırşehir region.

Figure 1. Conceptual Framework Illustrating How Topographic Heterogeneity Generates Fine-Scale Microclimatic Variation and Habitat Diversity in Central Anatolia



Source: This framework explains how geomorphological complexity in the Kırşehir province promotes microrefugial conditions that sustain high geophyte endemism. Figure prepared by Özcan (2025).

Under the Mediterranean transitional climate, characterized by mild and rainy winters, hot and dry summers, and the onset of rainfall in autumn, the Southeastern Anatolia Region stands out for its species richness. The increased humidity and microclimatic stabilization created by the Atatürk Dam have led the region to partially acquire a Mediterranean transitional character according to the Emberger climatic index. Under these conditions, autumn precipitation restores soil moisture and triggers the flowering period of bulbous and cormous taxa such as *Crocus, Colchicum, Scilla*, and *Sternbergia* (Özcan, 2024a).

The Mediterranean Region represents one of the richest geophyte zones in Türkiye in terms of species diversity; however, its endemism rate (20%) remains below the national average (33%) (Topal et al., 2022). Similarly, high species diversity is observed in the Aegean, Marmara, and Black Sea regions due to the influence of maritime climatic conditions (Öz & Akan, 2019; Sefalı, 2022). In contrast, the Eastern Anatolia Region exhibits both high species richness and a high rate of endemism (Babacan & Eker, 2017; Fırat et al., 2015), where prolonged snow cover and late-melting snow masses support the persistence of cold-tolerant taxa.

The Kırşehir Province in Central Anatolia is shaped by a continental climate, featuring hot and dry summers, cold winters, and low annual precipitation. In combination, the heterogeneous topography and geo-edaphic variation under strong continental climatic influence create a fine-scale mosaic of ecological microhabitats—small, environmentally buffered habitat patches that enable species persistence under local climatic stress (Rull, 2009; Dobrowski, 2011). These microhabitats function as shelter areas for narrowly distributed geophyte taxa and provide a critical context for the targeted inventory and conservation approach adopted in this study.

Indeed, the fact that 15 out of the 56 geophyte taxa identified in this study are endemic (ca. 27%) indicates that, despite the overall low species diversity in the region, the rate of

endemism remains close to the national average for Türkiye. This suggests that the prevailing continental stress conditions may serve as an important ecological driver in promoting microhabitat-scale adaptation and the emergence of local endemism.

One of the most characteristic morpho-physiological adaptations developed in response to these selective pressures is the presence of contractile root systems (CRSs). By pulling their renewal organs deeper into the soil, these structures provide protection against stress factors such as frost, drought, and surface erosion (Pütz, 1996; North, Brinton & Garrett, 2008; Brown & Mies, 2012). This mechanism represents one of the fundamental strategies enhancing the persistence and resprouting success of geophytes under the harsh steppe conditions of Central Anatolia.

Situated in Central Anatolia, the Kırşehir Province represents a transition zone along Türkiye's humidity–aridity axis, where maritime and continental climatic influences meet over short spatial distances. This climatic interplay strongly shapes the distribution and endemism patterns of geophytes observed in the area.

Materials and Methods

Geographical Features of the Study Area

The province of Kırşehir is located in the Central Anatolia Region of Türkiye, within the Central Kızılırmak Section, between 38°50'–39°50' N latitude and 33°30'–34°50' E longitude. The province covers an area of approximately 6,570 km² and exhibits a distinctly rugged topography (Republic of Türkiye, Kırşehir Governorship, 2008). Major mountain ranges include Kervansaray Mountain (1,679 m), Çiçek Mountain (1,691 m), and Aliöllez Mountain (1,528 m). In addition, several smaller elevations such as Kargasekmez, Cemele, Naldöken, and Buzluk Mountains further diversify the province's geomorphology.

The main river of the province is the Kızılırmak, while the Kılıçözü, Deliceırmak, and Kaman Kılıçözü streams also pass through the region. The principal lakes in Kırşehir include Seyfe Lake, Hirfanlı Reservoir, Çoğun Reservoir, and Obruk Lake (HGM, 2025). Seyfe Lake is among the few wetlands protected under the Ramsar Convention. However, in recent years it has faced a severe risk of desiccation due to unregulated irrigation and prolonged drought. Similar degradation has been observed in the Kılıçözü River, which flows through the city center, and in the Hılla pond, which was formerly sustained by nearby thermal springs (Turkish: *Ilıca*). These hydrological disruptions have weakened local ecosystem integrity and compromised the stability of fine-scale ecological microrefugia across the region.

Geomorphological and Geological Structure of the Study Area

The province of Kırşehir is situated on the Kırşehir Massif, which lies within the Central Anatolia Region of Türkiye. The Kırşehir Massif is composed of Paleozoic and Mesozoic metamorphic rocks and represents a key geological domain of the region. Major formations within the massif include the Kalkanlıdağ and Bozçaldağ Formations, the Santonian–Campanian-aged Karahıdır Volcanic Member, and the Cretaceous-aged Buzlukdağ Syenitoid. The geological composition of the massif is characterized by the presence of crystalline phyllites, marbles, calcareous schists, green schists, mica schists, and fine-grained quartz. In

addition, neotectonic structures in the region comprise the Late Miocene–Pliocene Kızılırmak Formation and the Kozaklı Member, along with Quaternary-aged sedimentary deposits (Temiz, 2004; Beyazpirinç et al., 2022).

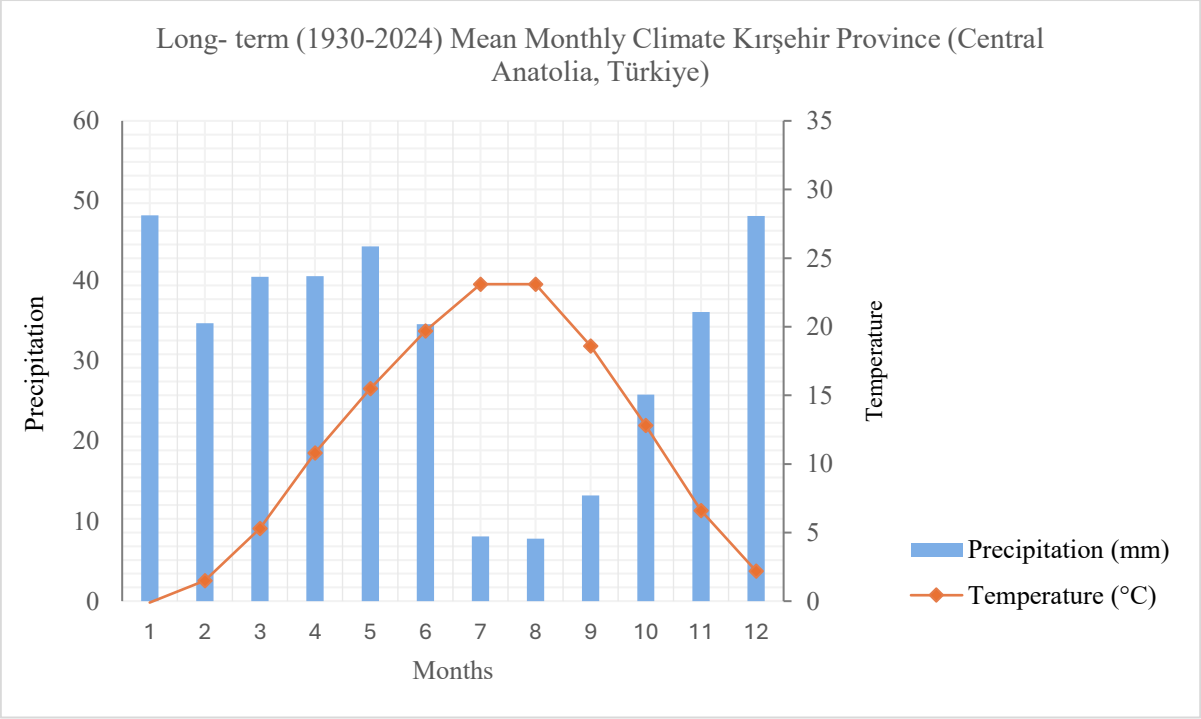
Soil Characteristics and Diversity of the Study Area

The soil structure of Kırşehir exhibits considerable diversity, primarily influenced by the region's climatic conditions and underlying bedrock composition. The dominant soil types are brown soils (339,624 ha) and red-brown soils (139,799 ha). In addition, several other soil groups are represented, including alluvial soils (28,587 ha), hydromorphic–alluvial soils (7,158 ha), and saline–alkaline soils (12,794 ha). In forested areas, brown forest soils (33,061 ha) and non-calcareous brown soils (9,486 ha) are prevalent (Kıymaz, 2011). This diversity of soil types significantly influences agricultural potential and land-use dynamics across the province.

Climate Data Integration

Kırşehir is characterized by a typical continental climate regime of the Central Anatolia Region. According to long-term meteorological data from the Kırşehir station of the Turkish State Meteorological Service (Station Code: 17160), the mean minimum temperature in January is –4.2 °C, while the mean maximum temperature in August reaches 30.2 °C. According to the Turkish State Meteorological Service (MGM, 2024), the annual total precipitation averages 382.0 mm, with the majority of rainfall occurring during the winter and early spring months (Figure 2).

Figure 2. Long-term (1930–2024) mean monthly air temperature and precipitation patterns for Kırşehir Province (Central Anatolia, Türkiye)



Source: Data are derived from the Turkish State Meteorological Service (MGM) station 17160 and illustrate the continental climate regime characterized by cold winters, hot summers, and limited annual precipitation. Figure prepared by Özcan (2025).

Phytogeographical Position of the Study Area

The study area lies within the Irano-Turanian floristic region, an important phytogeographical zone characterized by a high endemism rate of approximately 25–30% (Muratgeldiev et al., 2000). The vegetation of Central Anatolia is largely influenced by both the Irano-Turanian and Eastern Mediterranean floristic elements (Takhtajan, 1974, 1986).

The region is shaped by a continental climate, featuring hot and dry summers, cold winters, and low annual precipitation. In combination, the heterogeneous topography, climatic continentality, and geo-edaphic variation create a fine-scale mosaic of ecological microrefugia, which function as shelter habitats for narrowly distributed geophyte taxa and provide a critical context for the targeted inventory and conservation approach adopted in this study.

According to the national desertification sensitivity map of Türkiye, the Kırşehir Province is classified among the high-risk zones, indicating that the area is highly vulnerable to desertification and ecosystem degradation (ÇMUSEP, 2019).

Position of the Study Area within the Global Hotspots Framework

The province of Kırşehir, which constitutes the study area, is located within the Irano-Anatolian Biodiversity Hotspot, recognized as a region of critical importance for biological diversity (Myers et al., 2000). On a global scale, the increasing human population, climate change, habitat degradation, and overexploitation of species have intensified ecological pressure, particularly in areas with extensive agricultural activity (Sanderson et al., 2002; Burgman et al., 2007). In this context, biodiversity hotspots—where more than 70% of the original natural vegetation has been lost—are considered among the most vulnerable regions in terms of biodiversity decline (Myers et al., 2000).

Kırşehir Province is exposed to high ecological risk due to multiple interacting pressures, including habitat degradation (*e.g.*, active quarrying and planned mining activities), overgrazing, agricultural intensification, and increasing climatic stress associated with drought and desertification. These drivers correspond to regionally prioritized threat categories within the Conservation Measures Partnership classification system (CMP, 2024; Salafsky et al., 2025).

As a critical bio-geological buffer zone in Central Anatolia, Kırşehir still maintains notable floristic resilience under these pressures. However, the synergistic effect of anthropogenic disturbance and climatic aridification underscores the need for targeted conservation strategies. In this context, the establishment of Plant Micro-Reserves (PMRs) in areas harboring narrowly distributed taxa could play a pivotal role in safeguarding remaining microrefugial habitats (Laguna et al., 2004).

Sampling and Observations

Field studies were conducted between 2019 and 2025, covering the active vegetation periods. Sampling sites were selected based on elevation, bedrock type, habitat characteristics, and moisture-retention potential. Data were compiled by integrating literature sources with direct field observations. Plant taxa were identified according to the Flora of Turkey series (Davis 1984; Davis, Mill & Tan, 1988; Güner et al., 2000), and nomenclatural updates were

verified through the Plants of the World Online (POWO, 2025) and International Plant Names Index (IPNI, 2025) databases. The IPNI database was particularly used to confirm original publication sources, author citations, and nomenclatural validity, thereby supporting the taxonomic verification process.

Phenological records were monitored through at least four observation periods each season (early, mid, late, and very late flowering), allowing the determination of species-specific phenological windows. Species that are rare and/or represented by a single population within the province were classified as Single Micro-Populations (SMPs). Detailed information on SMP taxa is provided in Appendix 1. SMP indicates taxa represented by a single known micro-population within the province, whereas SMPs refers to taxa occurring in more than one isolated micro-population.

Low-Impact Field Documentation for Rare and Sensitive Plant Taxa

For taxa occurring in small populations or within ecologically sensitive microhabitats, field documentation was conducted using a low-impact and non-destructive sampling strategy. This approach prioritizes minimizing disturbance to rare plants and their habitat, following best-practice recommendations for rare plant conservation and non-destructive documentation techniques developed by the Center for Plant Conservation (CPC, 2019).

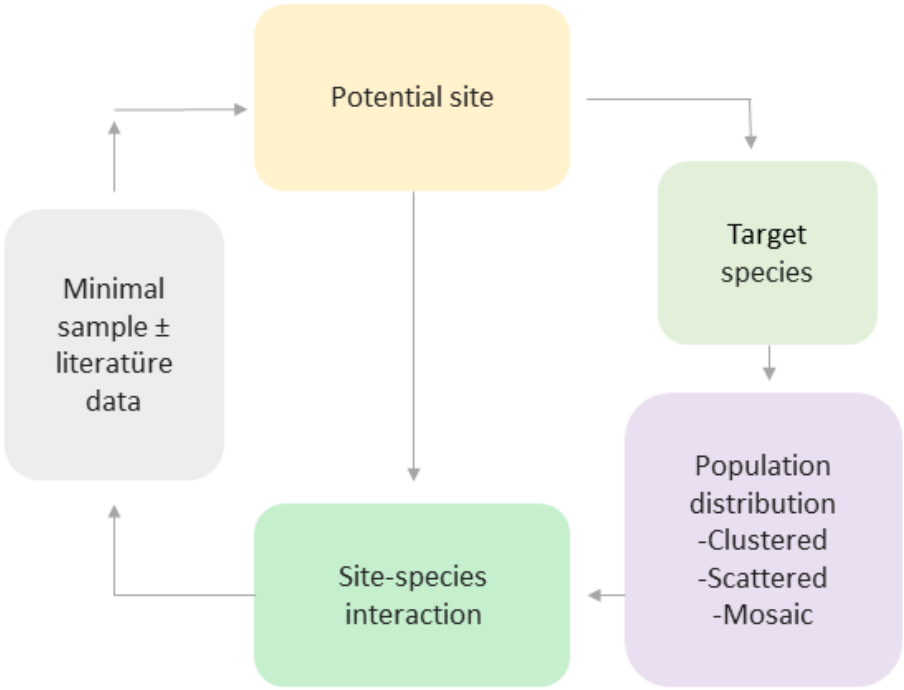
In accordance with the general guidelines of the International Union for Conservation of Nature (IUCN, 2024) for threatened taxa, all recording activities were designed to avoid unnecessary collection and to prevent any actions that might negatively affect population viability. Within this framework, only observational, photographic, and minimal diagnostic data (*e.g*., leaf morphology, tepal size measurements, and stigma–style proportional ratios) were recorded, ensuring that habitat integrity and population continuity were fully maintained, particularly for narrowly distributed geophytes such as *Tulipa, Hyacinthella, Iris,* and *Arum*.

Floristic Data Verification and Targeted Inventory Approach

A two-stage methodological design was applied: (1) literature-based preliminary inventory, and (2) field verification to confirm existing records and identify new sites. In the first stage, Flora of Turkey (Davis, 1984; Davis, Mill and Tan, 1988; Güner et al., 2000) and the current floristic studies published for the province of Kırşehir (Bahar & Özkan, 2022; Karavelioğulları et al., 2005) were thoroughly reviewed to identify the potential geophyte taxa occurring in the region.

Unlike conventional broad-scale floristic inventories, which require extensive manpower, time, and involve high financial and logistical costs to survey large areas, the present study employed a Microhabitat-Oriented Targeted Inventory (MOTI) Strategy, here proposed as a practical and fine-scale approach designed for efficiency under limited field resources (Figure 3).

Figure 3. Conceptual workflow of the Microhabitat-Oriented Targeted Inventory (MOTI) Strategy (example from Kırşehir, Central Anatolia, Türkiye)



Source: The model integrates literature data with pre-assessment of potential habitats, followed by targeted field observations to maximize time and cost efficiency. This workflow represents the core principle of the Microhabitat-Oriented Targeted Inventory (MOTI) Strategy proposed in this study. Figure prepared by Özcan (2025).

In this approach, previously studied localities and the geophyte taxa already reported from Kırşehir were first compiled from existing floristic studies. In addition to these records, supplementary MOTI sampling areas were established in microhabitats selected to maximize efficiency and habitat diversity across the province. This targeted method was developed to enable effective field verification within a large and topographically complex region, where conducting a complete province-wide inventory by a single researcher would otherwise be unfeasible.

Accordingly, several key localities—including the central district of Kırşehir and the Kervansaray Mountain Range, which spans the districts of Mucur and Boztepe; the Özbağ village–Kervansaray Mountain Range corridor; the surroundings of Lake Seyfe and the Kılıçözü Stream riparian corridor; the oak woodlands around Boğazevci Reservoir; the Çiçekdağ oak woodlands; the Akçakent oak woodlands; Mahmutlar village and its vicinity; and the Obruk Lake area—were systematically assessed using GPS-assisted verification transects.

Conceptual Position of the Plant Micro-Reserve (PMR) Model within the Study

Through this digital pre-assessment process, no random scanning was conducted across the province; instead, areas known to possess high geophyte diversity were prioritized. This approach aligns with the principles of targeted sampling recommended for detecting rare and

habitat-dependent species (Laguna et al., 2004; Gaston & Fuller, 2009; Guisan et al., 2013). Accordingly, fieldwork was scheduled exclusively during active vegetation periods, and potential microhabitat foci were verified in situ to ensure optimal use of field time.

Laguna et al. (2004) demonstrated that legally designated Plant Micro-Reserves (PMRs), typically ranging between 2–20 ha, function as highly effective conservation units for rare, threatened, and endemic plant species by enabling long-term monitoring and site-based management. The definition and conceptual framework of PMRs used in this study follow Laguna et al. (2004), who established PMRs as legally designated, small-scale conservation units aimed at protecting rare, endemic, or threatened plant species and their habitats.

In the present study, however, the PMR framework was not implemented as a formal conservation tool. Instead, a pre-PMR, habitat-based exploratory approach was applied to identify ecologically distinctive microhabitats that may potentially qualify for future PMR designation. This pre-PMR approach differs from the formal PMR model in that it does not create statutory protection zones; rather, it focuses on early-stage reconnaissance designed to detect microrefugia where geophyte diversity is concentrated. Through this strategy, field efforts were directed toward localities expected to yield the highest floristic richness and conservation value.

The pre-PMR framework applied in this study therefore provides a field-based foundation for potential future implementation of the PMR concept in Türkiye, bridging the gap between exploratory site identification and legally designated conservation networks.

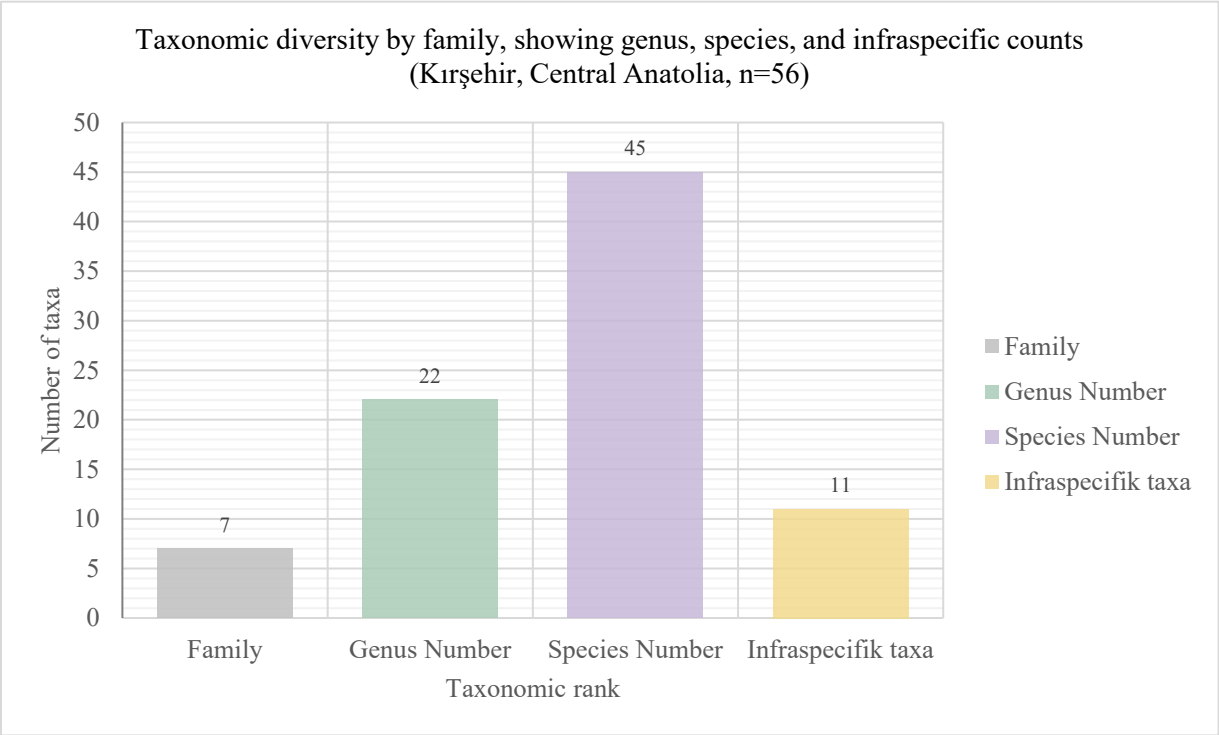
Results and Discussion

The assessment of field surveys and published records revealed a total of 56 geophyte taxa documented throughout the province of Kırşehir during the period 2019–2025. The species exhibited a high level of morphological and ecological diversity not only across steppe, oak woodland, and mountainous habitats, but also within the region’s moisture-retentive riparian and lakeside microhabitats.

Taxonomic Composition of the Gfeophyte Flora

The geophyte flora of Kırşehir exhibits a well-defined taxonomic structure, comprising 7 families, 22 genera, 45 species, and 11 infraspecific taxa (subspecies and varieties). This hierarchical distribution reflects the ecological heterogeneity of the province and underscores the floristic importance of the steppe–mountain transition zone, including the oak scrublands and lacustrine margins that act as effective moisture-retaining microrefugia (Figure 4).

Figure 4. Taxonomic framework of the geophyte flora of Kırşehir (Central Anatolia, Türkiye)

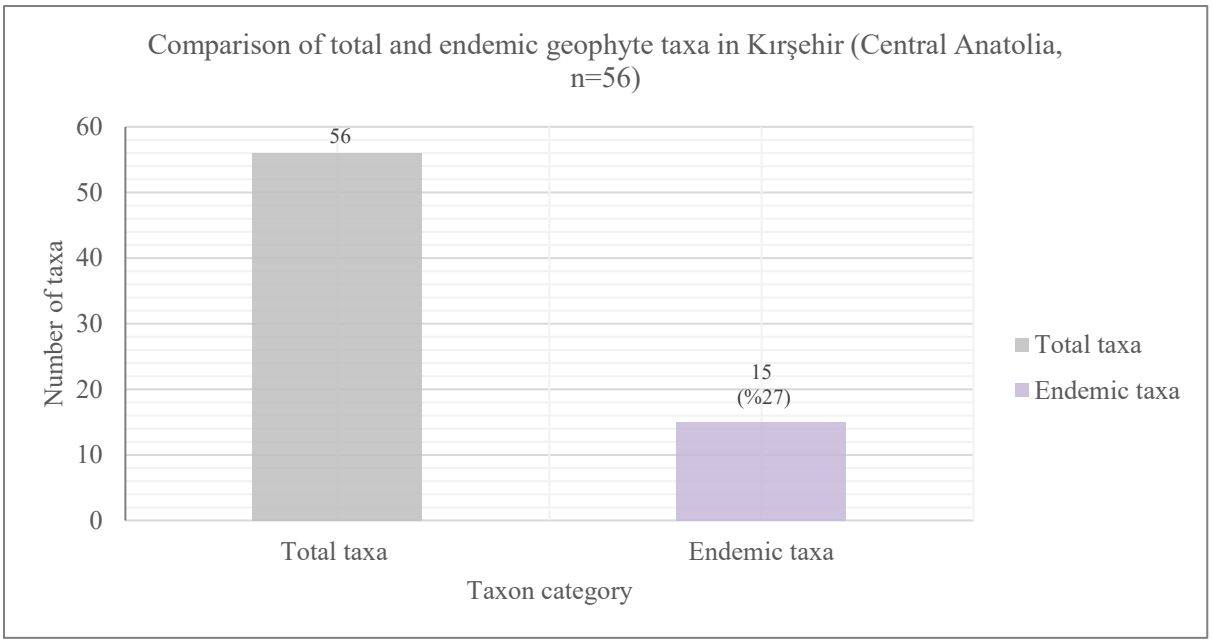


Source: The diagram summarizes hierarchical diversity across families, genera, species, and infraspecific ranks based on field observations and floristic assessments conducted between 2019 and 2024. Figure prepared by Özcan (2025).

Geophyte Diversity and High Endemism

A total of 56 geophyte taxa belonging to 7 families and 22 genera were recorded across the province of Kırşehir (Figure 5). Among these, 15 taxa are endemic to Türkiye, corresponding to an endemism rate of approximately 27%. This ratio indicates a remarkably strong endemism signal within the steppe–mountain transition zone of Central Anatolia. Although slightly lower than Türkiye's national average endemism level (ca. 34%), the observed value nonetheless highlights both a high degree of local biodiversity continuity and the presence of a regional core area of endemism.

Figure 5. Overall diversity and endemism ratio of the geophyte flora of Kırşehir (Central Anatolia, Türkiye)



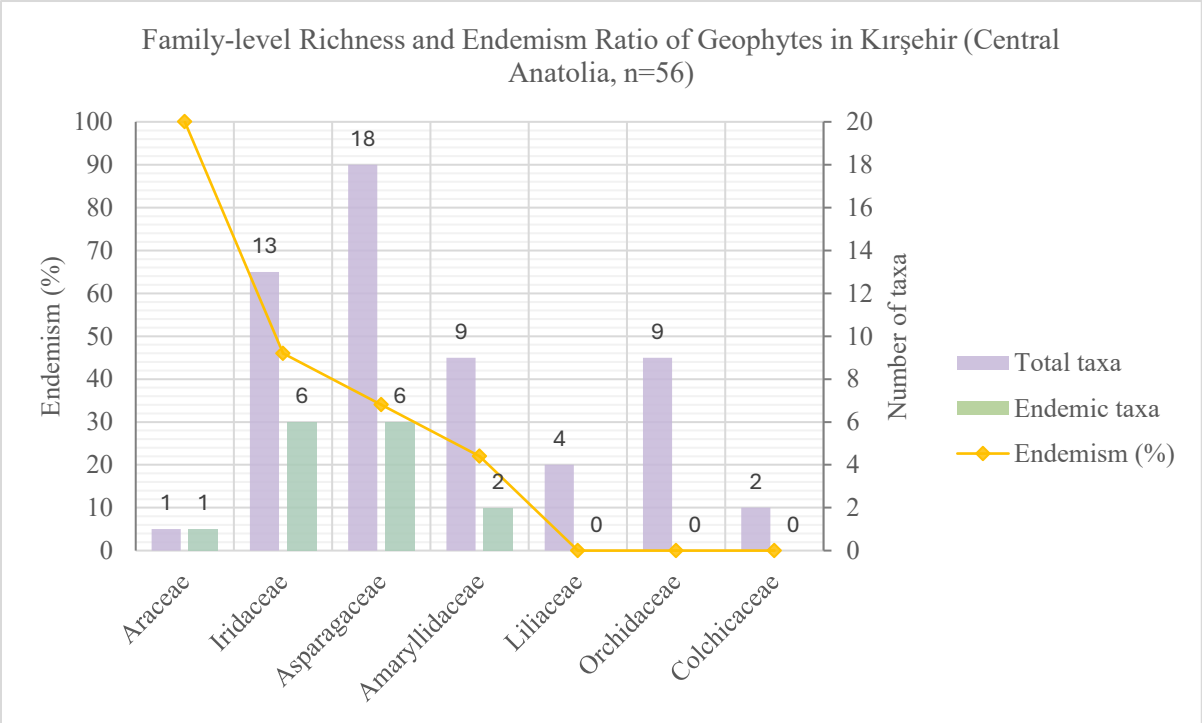
Source: A total of 56 geophyte taxa belonging to 7 families and 22 genera were recorded, of which 15 (ca. 27%) are endemic to Türkiye. The diagram summarizes the richness and endemism patterns revealed by field and literature data. Figure prepared by Özcan (2025).

The recorded species exhibited a broad phenological and morphological variation across multiple habitat types, including the steppe environments of the Kervansaray Mountains (Kırşehir city center, Boztepe district, and Özbağ village), pine plantations, mixed woodlands dominated by *Crataegus* and wild *Prunus* species (Kırşehir city center and Boztepe district), oak forests and remnant oak patches (Çiçekdağı and Akçakent districts), and moisture-retentive microhabitats such as temporary pools, streamside zones, and areas surrounding Seyfe Lake and the Boğazevci Reservoir.

Family-level Geophyte Species Richness and Endemism Ratios

Family-level assessment (Figure 6) reveals a distinct hierarchy of richness within the geophyte flora of Kırşehir. Asparagaceae is by far the most species-rich family (18 taxa, 6 endemic; 33%), standing out not only in overall diversity but also as the principal contributor of endemic taxa in the region. Iridaceae (13 taxa, 6 endemic; 46%) exhibits a pronounced concentration of local endemics and primarily includes genera (e.g. Crocus, Iris) strongly associated with geo-edaphic microrefugia. Amaryllidaceae (9 taxa, 2 endemic; 22%) provides a moderate contribution, whereas Liliaceae (4 taxa), Orchidaceae (9 taxa), and Colchicaceae (2 taxa) contain no endemic representatives. In contrast, Araceae, although represented by a single species, is remarkable for exhibiting 100% endemism, indicating its highly restricted distribution within the study area. Overall, the relationship between family richness and endemism is non-linear, suggesting that endemism is shaped not by taxonomic abundance but rather by habitat specialization within narrow ecological ranges and dependence on microrefugial conditions.

Figure 6. Family-level richness and endemism ratio of geophyte taxa recorded from Kırşehir Province (Central Anatolia, Türkiye)

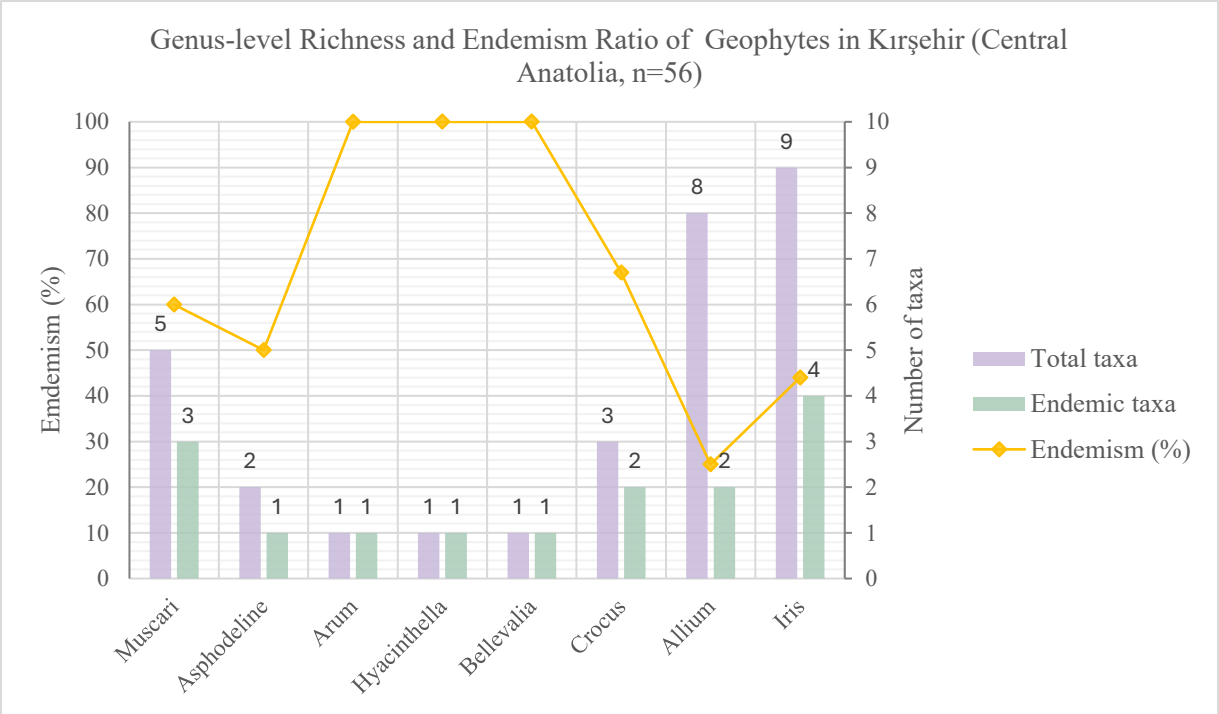


Source: The bar chart shows total taxa (purple) and endemic taxa (green) per family, while the orange line represents the endemism percentage. Asparagaceae and Iridaceae are the dominant families, reflecting strong geo-edaphic differentiation and microrefugial specialization. The overall pattern highlights a non-linear relationship between richness and endemism, emphasizing habitat specialization as a key driver of localized diversity. Figure prepared by Özcan (2025).

Genus-level Geophyte Species Richness and Endemism Ratios

The genus-level pattern of species richness and endemism within the geophytic flora of Kırşehir reveals a distinct mosaic of biological diversity in the study area. *Hyacinthella, Bellevallia*, and *Arum* are represented by a small number of species; however, all of these species are endemic, which is particularly noteworthy. Despite their low species numbers, the 100% endemism rates indicate that these genera include narrowly distributed and conservation-sensitive taxa in Kırşehir. In contrast, *Muscari* (5 taxa, 3 endemic; 60%) and *Iris* (9 taxa, 4 endemic; 44%) stand out in terms of both total species richness and the presence of endemic taxa. In particular, in *Muscari* and *Crocus*, endemic species constitute more than 50% of all recorded species, whereas Iris displays high species diversity accompanied by a considerable proportion of endemic taxa (Figure 7). Conversely, genera such as *Gagea, Colchicum*, and *Ornithogalum* exhibit high species richness but contain no endemic taxa, illustrating the opposite trend (see Appendix 1 for the full list of taxa).

Figure 7. Genus-level richness and endemism ratio of geophyte taxa recorded from Kırşehir Province (Central Anatolia, Türkiye)



Source: The bar chart displays total taxa (purple) and endemic taxa (green) per genus, with the orange line representing endemism percentage. The overall pattern indicates that endemism is primarily driven by habitat specialization and ecological isolation rather than species number. Figure prepared by Özcan (2025).

These two contrasting patterns—(i) genera represented by few species but showing complete or high endemism, and (ii) genera containing a larger number of species but exhibiting low or zero endemic intensity—together demonstrate that the geophytic flora of Kırşehir encompasses both narrowly distributed, habitat-sensitive taxa and species-rich genera that contribute substantially to overall regional diversity. Collectively, these findings indicate that the study area functions as a regionally important center of geophytic diversity and endemism.

Phenological Patterns by Underground Organ Type

Pattern 1 – Dominance of Bulbous Taxa

Bulbous species represent the dominant underground organ type within the Kırşehir geophyte flora (Figure 8). Of the 56 taxa recorded, 31 are bulbous, and the majority of these concentrate within the mid-season flowering window (April–May) (n = 19). This distribution reflects a life-history strategy optimized for the region's brief, moisture-dependent spring growth period.

Pattern 2 – Broad Phenological Range in Rhizomatous Taxa

Rhizomatous taxa (n = 13) exhibit the broadest phenological spread, flowering during both the mid-season (April–May) and late-season (June–July) phases. These species were predominantly found in semi-shaded, moisture-retentive microhabitats, which provide moderate environmental buffering.

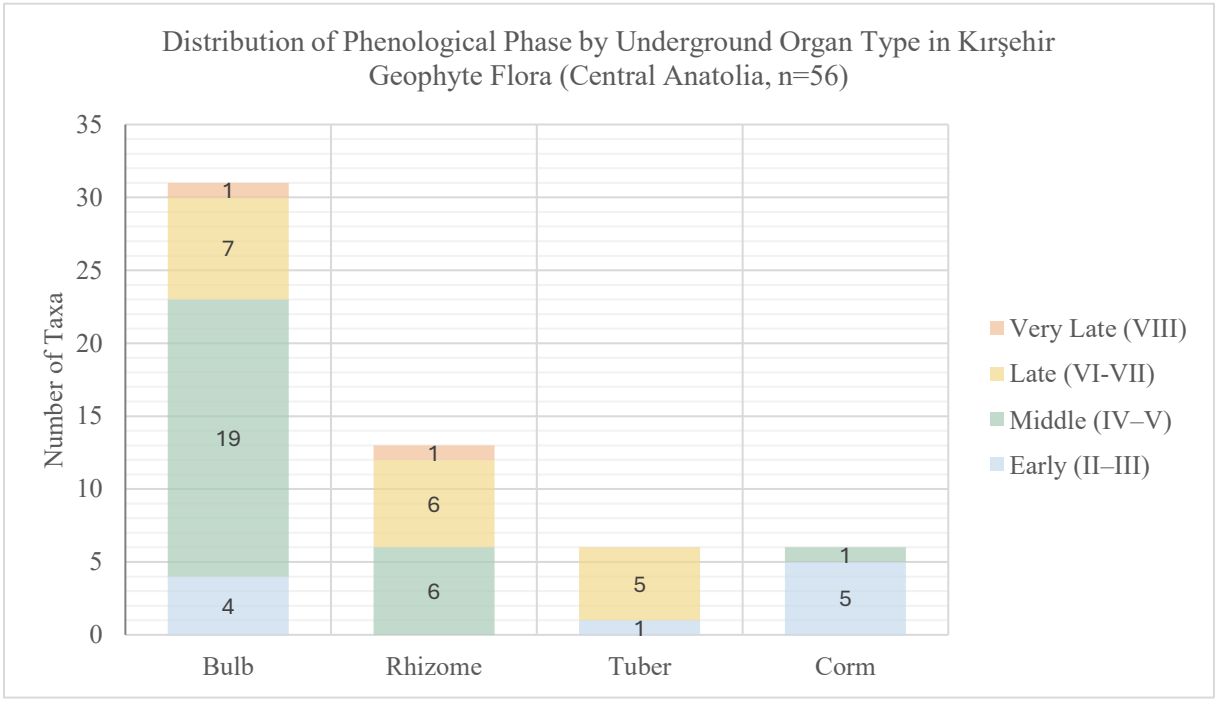
Pattern 3 – Late-Season Flowering in Tuberous Taxa

Tuberous taxa (n = 6) show a pronounced shift toward late-season flowering (June–July), with the majority (5 out of 6) flowering during this period. They are chiefly associated with oak-woodland clearings and riparian moist habitats. Their limited diversity suggests that both phenological timing and edaphic stress tolerance operate within relatively narrow ecological constraints.

Pattern 4 – Early-Season Flowering in Cormous Taxa

Cormous taxa (n = 6) predominantly flower within the early-season window (February–March), with five of six taxa flowering during this period, and only one species extending into April–May. They appear to be adapted to moist riparian microhabitats and sub-canopy environments within pine or almond–hawthorn woodland systems.

Figure 8. Phenological patterns by underground organ type in the geophyte flora of Kırşehir (Central Anatolia, Türkiye) (n = 56)



Source: The diagram shows the distribution of flowering periods—early (Feb–Mar), mid (Apr–May), late (Jun–Jul), and very late (Aug)—across four underground organ types: bulb, rhizome, tuber, and corm. Bulbous taxa dominate the assemblage with a pronounced mid-season peak, whereas rhizomatous, tuberous, and cormous taxa exhibit distinct phenological preferences linked to their habitat specialization. Figure prepared by Özcan (2025).

Contractile root systems (CRSs) constitute one of the most fundamental adaptive traits determining the survival and spatial persistence of geophytes in the continental steppe–mountain transition of Central Anatolia. The strength and functional efficiency of CRS development vary systematically among underground organ types, revealing a clear morpho-ecological gradient that mirrors both phenological timing and microhabitat preference. This relationship underscores that CRS efficiency, rather than taxon abundance, governs the long-

term persistence of geophytes under the extreme edaphic and climatic stress regimes of Central Anatolia (Figure 8).

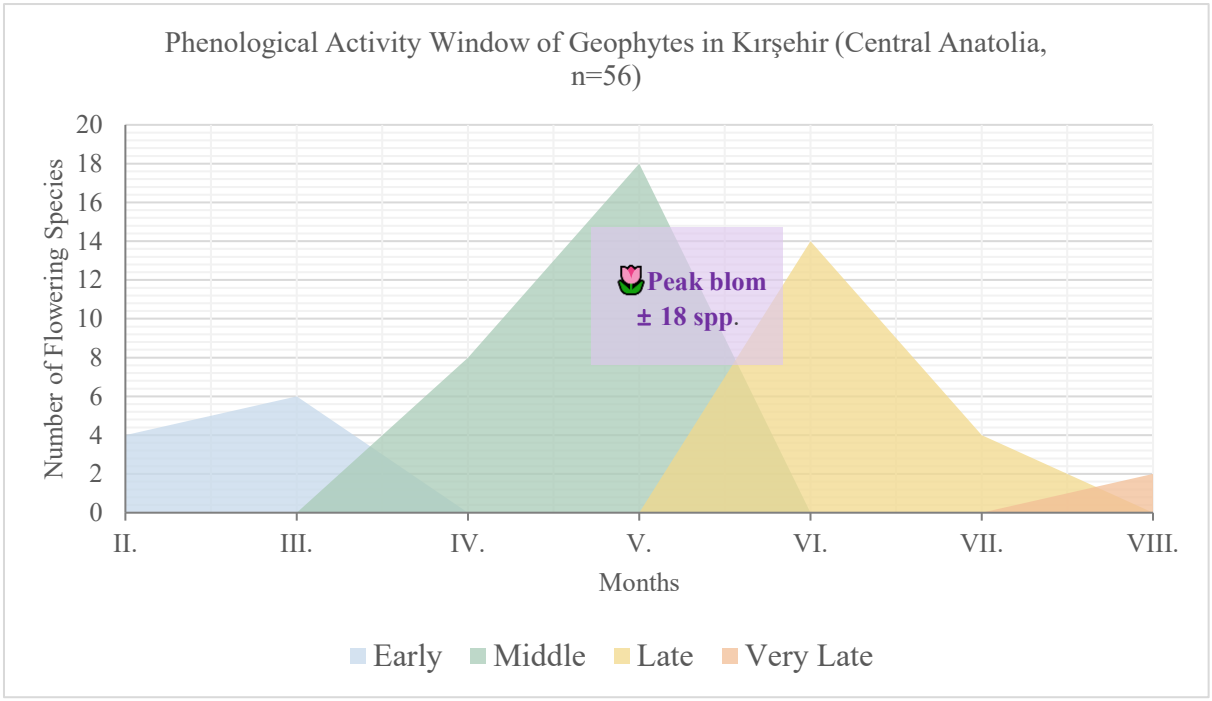
Flowering Period and Phenological Activity Window

The geophyte flora of Kırşehir displays a distinctly spring–to–early-summer phenological pattern, with most species flowering between April and June (Figure 9). Flowering activity reaches a clear peak in May, during which approximately 18 taxa are simultaneously in bloom. This dominant pattern reflects a growth strategy finely synchronized with the region's spring precipitation regime and short, moisture-dependent optimal growth period.

A limited number of species flowering in the early season (February–March)—predominantly bulbous and cormous taxa—indicate adaptations to low-temperature tolerance and early-season resource availability. In contrast, the few species blooming during the late (June–July) and very late periods (August) represent drought-adapted phenological strategies, largely associated with bulbous and rhizomatous forms that tolerate high summer temperatures and reduced soil moisture.

Overall, geophytes in Kırşehir remain in a dormant state for nearly half of the year (approximately September to January), a pattern consistent with the region's strong climatic seasonality and prolonged dry summer period.

Figure 9. Annual flowering activity window of geophytes recorded from Kırşehir (Central Anatolia, Türkiye) (n = 56)



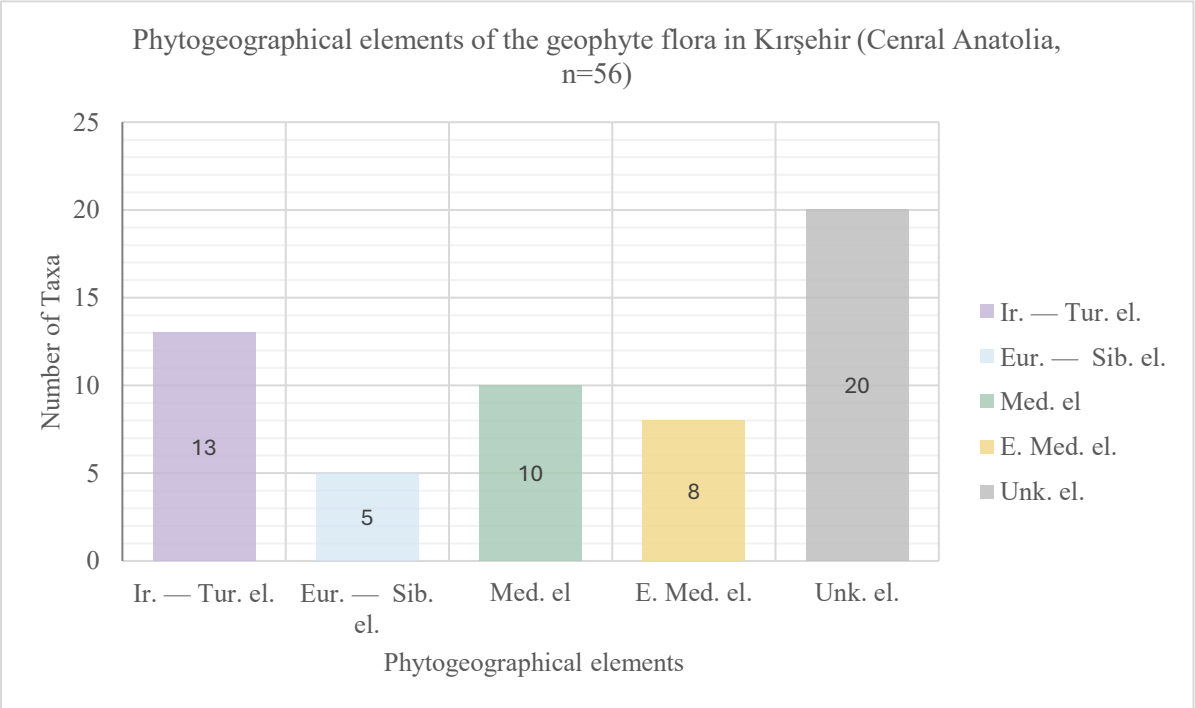
Source: The stacked area chart illustrates the monthly distribution of taxa across four phenological phases: early (Feb–Mar), mid (Apr–May), late (Jun–Jul), and very late (Aug). Flowering intensity peaks in May (ca. 19 spp.), indicating a strong spring-to-early-summer concentration that mirrors the region's climatic and topographic seasonality. Figure prepared by Özcan (2025).

Phytogeographical Elements

The phytogeographical composition of the Kırşehir geophyte flora (Figure 10) clearly reflects the region's position within the Irano–Turanian steppe–mountain transition zone. Of the 56 recorded taxa, 13 (23%) belong to the Irano–Turanian element, representing species adapted to high thermal amplitude and arid-season regimes characteristic of steppe and montane environments. This pattern highlights the location of Kırşehir within the biodiversity core of Central Anatolia and underscores the strong role of geo-edaphic isolation.

Mediterranean (n = 10; 18%) and Eastern Mediterranean (n = 8; 14%) elements together account for 34% of the flora, indicating an openness to southern floristic influxes. These taxa are typically associated with moisture-retentive microhabitats and microrefugia, where they successfully establish under locally buffered conditions. European–Siberian elements have limited representation (n = 5; 9%) and were mostly recorded in shaded, humid microrefugial habitats, suggesting that north-facing microclimates play a minor but complementary role in shaping the regional flora. Taxa classified as unknown or broadly distributed elements (n = 20; 36%) indicate substantial phytogeographical transition, ecological flexibility, and adaptive plasticity within the local geophyte assemblage.

Figure 10. Phytogeographical composition of the geophyte flora of Kırşehir (Central Anatolia, Türkiye)



Source: Among the recorded taxa (n = 56), 23% belong to the Irano–Turanian element, 9% to the Euro–Siberian, 18% to the Mediterranean, and 14% to the East Mediterranean elements, while 36% are transitional or broadly distributed taxa. This pattern reflects the province's position within the Irano–Turanian steppe–mountain transition zone and the influence of geo-edaphic isolation on floristic composition. Figure prepared by Özcan (2025).

Site-Specific Habitat Significance

Across the study area, a series of irreplaceable (non-substitutable) microhabitat nodes were identified for endemic geophyte species. Among these, the Kervansaray Mountains stand out with their rock-base steppes, xeric rock-crevice habitats, moist semi-shaded understorey of Pinus nigra forests, and shaded deciduous woodland floors.

The surroundings of Boğazevci Reservoir, together with the oak woodlands and remnant stands in the districts of Akçakent and Çiçekdağ, are characterized by north-facing cool and stony slopes, moist alluvial soils along stream margins, and semi-shaded open areas. In addition, the humid–saline transition zones around Lake Seyfe, the sub-shrub habitats under Astragalus communities near Mahmutlar Village — where subsurface thermal water influence creates distinctive microrefugial conditions — and the shaded moist clearings around Harmanpınar spring represent other key microrefugia zones for endemic species.

These habitats collectively host endemic taxa belonging to the genera *Arum, Crocus, Iris, Asphodeline, Muscari, Hyacinthella,* and *Bellevalia*, forming a complex microrefugia mosaic of high conservation value (see Appendix 1). Overall, these areas represent ecological units that are truly irreplaceable within the geography of Kırşehir, each maintaining unique microrefugial conditions critical for the persistence of endemic geophytes. The preservation of these local microrefugia is essential for sustaining biodiversity resilience within the steppe–mountain transition zone of Central Anatolia.

This site-specific evaluation underlines how geographically restricted microrefugia function as last refuges for endemic geophytes. By integrating geological, hydrological, and vegetation-structure variables, these fine-scale habitats ensure the persistence of sensitive taxa under regional climatic stress. Therefore, maintaining habitat integrity at these irreplaceable nodes should be regarded as a cornerstone of future conservation planning across Central Anatolia.

Conservation Status (IUCN)

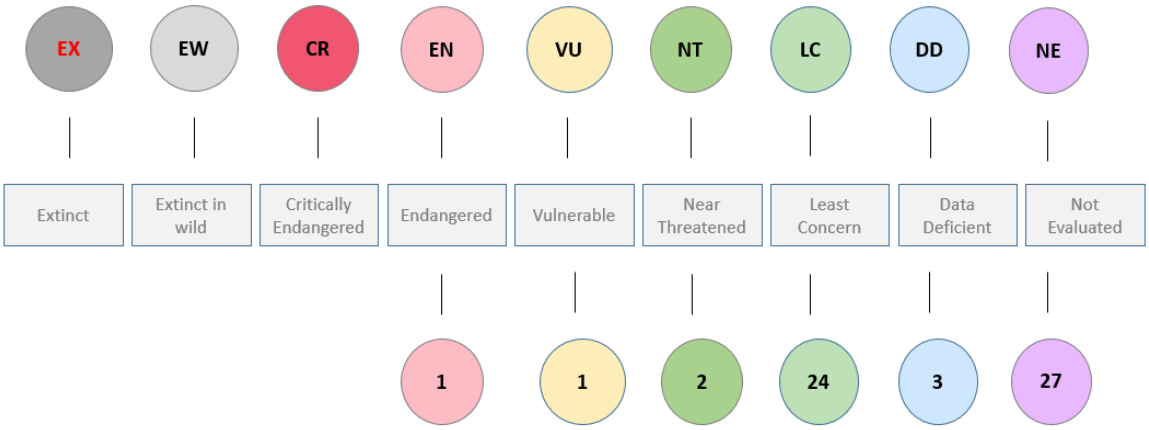
Conservation status (IUCN Red List) assessments were based on the Red Data Book of Turkish Plants (Ekim et al., 2000) and the global IUCN Red List criteria (IUCN, 2024), and were further verified using recent taxonomic and conservation literature.

According to the IUCN assessments of the geophyte flora of Kırşehir (Figure 11), a substantial portion of the 56 recorded taxa fall within the Least Concern (LC) category (n = 24; ca. 40%). However, this seemingly secure pattern should be interpreted with caution, given the habitat fragmentation and microrefugia loss observed at the local scale. The presence of one taxon in the Endangered (EN) and Vulnerable (VU) categories indicates that narrowly distributed, habitat-dependent species face a significant risk of local extinction.

Taxa classified as Near Threatened (NT) (two species in total) represent groups that may exhibit medium-term population declines under climatic variability and anthropogenic pressures. Conversely, the combined total of Data Deficient (DD) (n = 3) and Not Evaluated (NE) (n = 25) categories—28 taxa overall— reveals that a considerable portion of the Kırşehir flora remains insufficiently assessed or poorly understood in terms of conservation status. This

underscores the urgent need for microrefugia-scale monitoring, population-based conservation planning, and ecological sensitivity-focused field studies in the region.

Figure 11. IUCN Red List categories and the number of geophyte taxa assessed in each category (EX–NE) for the flora of Kırşehir Province (Central Anatolia, Türkiye)



Source: Categories follow the IUCN (2024) system: EX – Extinct; EW – Extinct in the Wild; CR – Critically Endangered; EN – Endangered; VU – Vulnerable; NT – Near Threatened; LC – Least Concern; DD – Data Deficient; NE – Not Evaluated. The chart summarizes the conservation status distribution of the recorded taxa, highlighting the predominance of LC and NE categories and the limited representation of threatened classes. Figure prepared by Özcan (2025).

Figure 11 presents the IUCN Red List categories of the endemic taxa, based on Ekim et al. (2000) and the updated global assessments (IUCN, 2024). In several cases, recent IUCN assessments have modified the national threat categories initially proposed by Ekim et al. (2000), reflecting changes in data availability and evaluation criteria. For instance, *Iris sari* was previously assessed as Least Concern (LC) at the national level but is currently listed as Endangered (EN) in the global Red List. Özcan (2024b) emphasizes that the IUCN categories of taxa occurring in Türkiye should be periodically updated to reflect the most recent conservation data and to maintain consistency with global assessment standards.

CMP Threat Codes, Affected Species, and Conservation Measures for Habitats

The "Classification of Direct Threats to the Conservation of Ecosystems and Species v4.0", developed by the Conservation Measures Partnership (CMP), provides an internationally recognized framework for defining and categorizing direct threats to biodiversity. It organizes direct threats into 11 major Level 1 categories, each subdivided into a series of Level 2 sub-categories, thereby providing a standardized framework to assess anthropogenic pressures on ecosystems and species (Salafsky et al., 2025).

A total of 15 Level 2 threat categories, belonging to nine major CMP Level 1 classes, were identified for the Kırşehir geophyte flora (Table 1). These threats encompass both direct anthropogenic pressures (*e.g.*, grazing, mining, infrastructure development) and indirect climatic drivers (*e.g.*, drought and temperature anomalies).

Table 1. Conservation threat categories and corresponding CMP codes applied in the assessment of geophyte taxa from Kırşehir (Central Anatolia, Türkiye)

CMP Threat Category (Level 2)	Description / Conservation Recommendation
1.1 Housing & urban areas	Increasing urbanization pressure on the foothills of the Kervansaray Mountains is fragmenting the natural habitats and reducing overall ecological integrity. Conservation recommendation: It is recommended to establish ecological buffer zones and to ensure that the boundaries of natural areas are strictly maintained in urban development plans.
2.3 Livestock farming & ranching	Intensive grazing during early spring and summer prevents flowering and seed set, thereby limiting the natural regeneration of geophyte populations. Conservation recommendation: It is recommended that a local grazing management plan be implemented, with grazing restrictions during the flowering season to ensure successful regeneration.
3.2 Mining & quarrying	Quarrying and proposed mining activities have the potential to disrupt habitat integrity through blasting and associated disturbance. Conservation recommendation: It is recommended that ecological buffer zones be established around extraction sites, and that blasting operations be minimized or, where feasible, replaced with lower-impact techniques.
3.3 Renewable energy	The installation of wind and solar power facilities and their associated infrastructure causes habitat fragmentation and ecosystem degradation. Conservation recommendation: It is recommended that ecological sensitivity be considered during route planning, that shared service roads be used whenever possible, and that vegetation restoration be conducted in disturbed areas after construction.
4.1 Roads & railroads	New road construction and widening projects within forest areas cause habitat fragmentation and disrupt wildlife movement corridors. Conservation recommendation: It is recommended to improve existing roads instead of building new ones, to restore roadside vegetation, and to integrate ecological corridor planning into transportation projects.
5.2 Gathering terrestrial plants	The collection of bulbs and tubers by local people leads to population declines and threatens the long-term viability of native geophyte species. Conservation recommendation: It is recommended to provide community education programs, strengthen enforcement and inspection activities, and develop *in-situ* propagation initiatives for sustainable use.

5.3 Logging & wood harvesting	The cutting of willow and poplar trees along riverbanks weakens riparian habitats and reduces their ecological functions such as shading and bank stabilization. Conservation recommendation: It is recommended to limit tree cutting, replant native species along riparian zones, and maintain the ecological functions of these habitats.
7.2 Dams & water management/use	Groundwater extraction and well drilling around Seyfe Lake are causing the drying of wetland areas and the degradation of marsh ecosystems. Conservation recommendation: It is recommended to limit groundwater withdrawal levels, strengthen the control of drilling permits, legally protect wetland buffer zones, and establish long-term hydrological monitoring stations.
8.1 Invasive non-native/alien plants & animals	Invasive herbaceous species suppress the root development of native geophytes, leading to reduced growth and regeneration potential. Conservation recommendation: It is recommended to remove invasive plant species mechanically and to monitor recolonization dynamics in restored areas.
8.2 Problematic native plants & animals	The population increase of wild boars and moles exerts pressure on tuberous plants, causing physical damage and reducing their survival and regeneration capacity. Conservation recommendation: It is recommended to establish ecological barriers, apply controlled trapping where necessary, and implement population monitoring programs to assess impact levels.
9.1 Household sewage & urban wastewater	The direct discharge of untreated sewage into rivers reduces water quality and leads to the accumulation of toxic loads in aquatic ecosystems. Conservation recommendation: It is recommended to establish local wastewater treatment facilities, develop natural filtration systems, and create riparian buffer zones to improve water quality and ecosystem health.
9.3 Agricultural & forestry effluents	Soil runoff and fertilizer residues cause chemical pollution in surface waters, leading to eutrophication and deterioration of aquatic habitats. Conservation recommendation: It is recommended to designate controlled disposal areas, establish vegetative buffer strips, and provide farmers with training on waste management and nutrient control practices.

9.4 Garbage & solid waste	The uncontrolled disposal of household and solid waste causes habitat degradation and long-term soil and water pollution. Conservation recommendation: It is recommended to establish regular waste collection and recycling systems, restore soil and vegetation in former dumping areas, and conduct community education programs on proper waste management.
11.3 Changes in temperature regimes	Increasing temperatures and the onset of earlier springs reduce seed-set success and disrupt the phenological cycles of native geophytes. Conservation recommendation: It is recommended to establish low-cost drought and soil-temperature monitoring plots using portable data loggers, to maintain long-term phenological observation sites for key geophyte taxa, and to apply localized shading measures (*e.g*., natural stone barriers or temporary shade nets) in exposed microhabitats.
11.4 Changes in precipitation & hydrological regimes	Decreasing precipitation and irregular hydrological patterns disrupt the moisture balance of microrefugia habitats and limit natural regeneration. Conservation recommendation: It is recommended to improve micromoisture retention by covering soil surfaces with organic mulch (*e.g*., dry leaves, straw, or plant residues) or by placing small stone clusters that trap rainfall and reduce evaporation. Integrating microrefugia management into local water-use planning is therefore essential. In parallel, groundwater use should be regulated by limiting uncontrolled well drilling and promoting sustainable irrigation practices to maintain the hydrological integrity of natural habitats.

Source: Threat categories follow the CMP Unified Classification of Threats (Version 4.0; Salafsky et al., 2025).

For the geophyte flora of Kırşehir, the most prominent pressures include agricultural and livestock activities (CMP 2.3), mining and quarrying operations (CMP 3.2), alterations in the hydrological regime (CMP 7.2), temperature regime shifts associated with climate change (CMP 11.3), and a decline in precipitation (CMP 11.4).

Field investigations conducted across Kırşehir Province revealed that the endemic taxa *Arum euxinum, Tulipa undulatifolia* var. *undulatifolia, Hyacinthella micrantha*, and *Iris sari* occur with small population sizes and within narrow distribution ranges. Multiple threat factors were found to act synergistically on these taxa. The identified CMP codes are 5.2–5.3–11.4 for *Arum euxinum*, 2.3–5.2–5.3–8.2 for *Tulipa undulatifolia* var. *undulatifolia,* 2.3–5.2–8.1–11.3–11.4 for *Hyacinthella micrantha*, and 1.1–2.3–4.1–5.2 for *Iris sari*.

Single populations of *Limodorum abortivum* var. *abortivum, Cephalanthera rubra,* and *Platanthera bifolia* were recorded within the province, all being threatened under CMP 5.3. *Allium cyrilli* and *Galanthus elwesii* are exposed to 2.3–5.2–11.3–11.4, while *Muscari azureum, M. coeleste, and M. aucheri* are mainly affected by 2.3–5.2. *Bellevalia clusiana* faces threats classified under 1.1–2.3.

For Iris orientalis, Gagea fibrosa, G. granatellii, G. bohemica, Colchicum triphyllum, and C. szovitsii subsp. szovitsii, major pressures correspond to CMP 2.3–4.1–5.2–5.3. Crocus ancyrensis, C. danfordiae, and C. oliveri subsp. oliveri are influenced by CMP 5.2–8.2–11.3–11.4, whereas geophytes collected for salep extraction are predominantly affected by CMP 8.2, representing the most intense anthropogenic pressure across the region.

Active quarrying and planned mining activities around the Kervansaray Mountains are expected to become one of the most significant pressures on the geophyte microrefugia network of Kırşehir in the coming years. Such operations may lead to direct habitat loss through excavation, road expansion, and material stockpiling, as well as indirect degradation caused by dust accumulation, soil compaction, and disruption of local hydrological balance. Within microrefugial habitats that host narrow-ranged and phenologically sensitive taxa, a portion of the populations is expected to overlap with potential mining zones, which could result in reduced flowering success and regeneration capacity.

Projected increases in temperature (CMP 11.3), together with a continuing decline in precipitation and increasing hydrological irregularities (CMP 11.4), are projected to exert profound stress on the geophyte microrefugia network of Kırşehir Province. The shallow basins of Seyfe Lake and Boğazevci Reservoir, along with the Kılıçözü Stream, the Kızılırmak River, and a network of seasonal creeks and small ponds, currently sustain numerous hygrophilous and seasonally moist-habitat geophytes dependent on stable soil moisture and shallow groundwater conditions. These hydrological systems are highly sensitive to climatic anomalies, and reduced rainfall coupled with earlier spring warming accelerates soil desiccation, shortens the effective growth period, and disrupts the regeneration cycles of endemic bulbous taxa that rely on localized wetness. Under these scenarios, the contraction of temporary wet meadows, drying of small ponds, and irregular flow of seasonal creeks may lead to the local retreat or disappearance of moisture-dependent microrefugia, while xeric slope populations experience additional stress from prolonged summer droughts and rising soil temperatures. Consequently, maintaining the hydrological integrity of these wetland-adjacent and riparian systems is essential to prevent fragmentation of geophyte refugia and to ensure long-term population persistence under future climatic conditions.

In light of these findings, it becomes evident that multiple anthropogenic and climatic pressures are simultaneously acting upon the geophyte flora of Kırşehir. The integration of field-based microrefugia observations with CMP threat codes demonstrates that conservation strategies must address both habitat-specific and process-driven stressors. Therefore, the establishment of locally adapted management actions—particularly in hydrologically sensitive areas such as Seyfe Lake, Boğazevci Reservoir, Obruk Lake, and adjacent stream corridors—will be critical for ensuring the persistence of endemic geophyte populations under future environmental change.

Conclusion

The Kırşehir Province, located inland in Central Anatolia and far from maritime climatic influence, forms a key Irano-Turanian transition within the Irano-Anatolian hotspot. Under semi-arid conditions and marked geomorphological heterogeneity, the geophyte flora shows a clear paradox: modest overall richness yet remarkably high endemism. This pattern reflects

selective pressures from climatic stress and geo-edaphic variation; notably, Iridaceae (*e.g*., *Iris, Crocus*) and Asparagaceae (*e.g*., *Muscari, Bellevalia*) exhibit strong topo-climatic sensitivity. Life-form strategies form a continuous phenological sequence from early spring to midsummer, with bulbous taxa forming the core, cormous emerging earliest, tuberous flowering latest, and rhizomatous taxa maintaining broad seasonal activity under xeric stress. In addition to above-ground microhabitat heterogeneity, below-ground adaptations play a key role in geophyte persistence under continental conditions. CRSs contribute to survival by maintaining optimal soil depth and reducing exposure to winter cold and frost penetration, whereas taxa lacking such adaptations may be constrained by thermal thresholds. The failure of Mediterranean climate adapted genus level taxa (*e.g., Ophrys* and *Prospero*) under winter conditions in Kırşehir — despite their successful growth in transitional climates such as Besni (Adıyaman)— suggests that microhabitat suitability alone cannot compensate for limited cold tolerance.

Field evidence shows that prioritizing habitat recognition before species detection Microhabitat-Oriented Targeted Inventory (MOTI) Strategy improves efficiency under limited time, manpower, and budget, and reveals micro-habitats of high conservation value. Field sampling was conducted with strict attention to non-destructive protocols, focusing solely on diagnostic characters required for accurate identification and avoiding any removal that could compromise population viability. Within this framework, a pre-PMR (Plant Micro-Reserve) perspective proved effective for identifying sites of localized endemism and potential microrefugia. Yet major pressures persist: agricultural and grazing impacts (CMP 2.3), mining and quarrying (CMP 3.2), hydrological alteration (CMP 7.2), and climate-driven temperature/precipitation shifts (CMP 11.3–11.4). These factors threaten microhabitat stability and emphasize the urgency of habitat-based conservation planning. Collectively, our results highlight micro-scale ecological heterogeneity as the organizing principle of geophyte persistence and provide the conceptual foundation for the forthcoming MicroRefugia Cluster-Based Sampling Framework (MiCS-Framework), which will expand upon the MOTI and pre-PMR approaches in future studies.

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Appendix 1.

Floristic Inventory of Geophytes in the Study Area (According to the APG IV System). This appendix presents the systematic list of geophyte taxa observed and recorded from the study area. Species are arranged by family according to the APG IV classification, with indications of endemism status, phytogeographical element, underground organ type, observation or literature source, IUCN assessment, and habitat notes.

ARACEAE (Alismatales)

1. *Arum euxinum* R.R.Mill. — **End**. — Eur-Sib. — tuber — 12.VI.2024 — Loc. obs. Özcan leg. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — Humid streamside — (SMP).

LILIACEAE (Liliales)

2. *Tulipa undulatifolia* Boiss. var. *undulatifolia* — bulb — 25.V.2023 — Loc. obs. Özcan leg. — Not assessed (IUCN, 2024) — Rocky crevices (xeric) — (SMP).
3. *Gagea fibrosa* (Desf.) Schult. & Schult.fil. — Med. el. — bulb — 24.V.2025 — Loc. obs. Özcan leg.; Lit. rec. — LC (IUCN, 2024) — Seasonally moist riverbank.
4. *Gagea granatellii* (Parl.) Parl. — Med. el. — bulb — 24.V.2025 — Loc. obs. Özcan leg.; Lit. rec. — LC (IUCN, 2024) — Seasonally moist riverbank.
5. *Gagea bohemica* (Zauschn.) Schult. & Schult.fil. — Med. el — bulb — 24.V.2025 — Loc. obs. Özcan leg.; Lit. rec. — LC (IUCN, 2024) — Seasonally moist riverbank.

COLCHICACEAE (Liliales)

6. *Colchicum triphyllum* G.Kunze — Med. el. — corm — 24.III.2025 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Seasonally moist riverbank.
7. *Colchicum szovitsii* Fisch. & C.A.Mey. — Ir.-Tur. el. — corm — 24.III.2025 — Loc. obs. Özcan leg.; Lit. rec. — DD (IUCN, 2024) — Seasonally moist riverbank.

AMARYLLIDACEAE (Asparagales)

8. ***Allium cappadocicum*** Boiss. — **End**. — Ir.-Tur. el. — bulb — 24.VII.2019 — **Lit. rec.** — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — Rock-base steppe.
9. *Allium borszczowii* Regel (syn. *A. callidictyon* C.A.Mey. ex Kunth) — Ir.-Tur. el. — bulb — 18.V.1995 — Lit. rec. — Not assessed (IUCN, 2024) — Halophytic steppe.

10. *Allium paniculatum* L. subsp. *paniculatum* — Med. el. — bulb — 24.V.2025 — Loc. obs. Özcan leg.; Lit. rec.; Not assessed (IUCN, 2024) — *Astragalus* shrub bases – sandy soils.
11. *Allium pseudoflavum* Vved. — Ir.-Tur. el. — bulb — 24.V.2025 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — *Astragalus* shrub bases – sandy soils.
12. *Allium huber-morathii* Kollm., Özhatay & Koyuncu — **End**. — Ir.-Tur. el. — bulb — 5.VIII.1995, Lit. rec. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — *Quercus* scrub.
13. *Allium rotundum* L. (syn. *A. scorodoprasum* L. subsp. *rotundum* (L.) Stearn) — Med. el. — bulb — 30.VI.1993 — Lit. rec. — Not assessed (IUCN, 2024) — Rock-base steppe.
14. *Allium lycaonicum* Siehe — Ir.-Tur. el. — bulb — 15.V.1995 — Lit. rec. — Not assessed (IUCN, 2024) — Halophytic steppe.
15. *Allium cyrilli* Ten — E. Med. el. — bulb — 25.V.2023, Loc. obs. Özcan leg. — DD (IUCN, 2024) — Rocky crevices (xeric) — (SMP).
16. *Galanthus elwesii* — E. Med. el. — bulb — 14.II.2023 — Loc. obs. Özcan leg.; DD (IUCN, 2024) — Shaded deciduous woodland floor — (SMP)

ASPARAGACEAE (Asparagales)

17. *Asparagus officinalis* L. — Med. el. — rhiz. — 19.V.1995 — Lit. rec. — LC (IUCN, 2024).
18. *Asphodeline damascena* (Boiss.) Baker subsp. *damascena* — Med. el. — rhiz. — 19.VI.2024 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Depression or sinkhole edge – moist.
19. *Asphodeline damascena* subsp. *rugosa* E.Tuzlacı & M.Saraçoğlu — **End**. — E. Med. el. — rhiz. — 19.VI.2024 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — Xeric rock-crevice.
20. *Ornithogalum sphaerocarpum* Kerner — bulb — 22.V.1994 — Lit. rec. — Not assessed (IUCN, 2024) — Rock-base steppe.
21. *Ornithogalum pyrenaicum* L. — bulb — 18.V.1995 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Forest edge – moist litter.
22. *Ornithogalum narbonense* L. — Med. el. — bulb — 28.V.1993 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Forest edge – moist litter.

23. *Ornithogalum oligophyllum* E.D.Clarke — bulb — 6.VII.1993 — Lit. rec. — Not assessed (IUCN, 2024) — *Quercus* scrub – stony slopes.
24. *Ornithogalum neurostegium* Boiss. & Blanche subsp. *neurostegium* (syn. *O. ulophyllum* Hand.-Mazz.) — bulb — 28.VI.1993 — Lit. rec. — Not assessed (IUCN, 2024) — *Quercus* scrub edge – dry sandy soils.
25. *Ornithogalum orthophyllum* Ten. — E. Med. el. — bulb — 9.V.1993 — Lit. rec. — Not assessed (IUCN, 2024) — Streamside steppe – seasonally moist soils.
26. *Leopoldia tenuiflora* (Tausch) Heldr. (syn. *Muscari tenuiflorum* Tausch) — bulb — 30.IV.1993 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Geothermal semi-shaded ground.
27. *Leopoldia longipes* (Boiss.) Losinsk. (syn. *Muscari longipes* Boiss.) — bulb — 9.V.1993 — Lit. rec. — Not assessed (IUCN, 2024) — Ploughed field margin – disturbed steppe soils.
28. *Muscari neglectum* Guss. — bulb — 21.IV.1993 — Lit. rec. — Not assessed (IUCN, 2024) — Open steppe–forest transition.
29. *Muscari azureum* Fenzl — **End**. — bulb — 21.IV.1993 — Lit. rec. — Not assessed (IUCN, 2024) LC (Ekim et al., 2000) — North-facing cool moist stony ground.
30. *Muscari coeleste* Fomin — **End**. — Ir.-Tur. el. — bulb — 21.IV.1993 — Lit. rec. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — North-facing cool moist stony ground.
31. *Muscari aucheri* (Boiss.) Baker — **End**. — bulb — 5.VII.2019 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024), LC (Ekim et al., 2000) — Streambed margin – moist alluvial soils.
32. *Muscari tenuiflorum* Tausch — bulb — 3.VI.1993 — Lit. rec. — Not assessed (IUCN, 2024). Open steppe slope area.
33. *Hyacinthella micrantha* (Boiss.) Chouard — **End**. — bulb — 25.III.2024 — Loc. obs. Özcan leg. — Not assessed (IUCN, *2024*) — NT (Ekim et al., 2000), North-facing cool moist stony ground. (SMP).
34. *Bellevalia clusiana* Griseb. — **End**. — Ir.-Tur. el. — bulb — 6.VII.1993 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — Dense semi-shaded dry meadow.

IRIDACEAE (Asparagales)

35. *Iris sintenisii* Janka — Eur.-Sib. el. — rhiz. — 5.VIII.1995 — Lit. rec. — Not assessed (IUCN, 2024) — *Quercus* scrub edge – moist semi-shaded.
36. *Iris schachtii* Markgr. — **End**. — Ir.-Tur. el. — rhiz. — 19.V.1995 — Lit. rec. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — Sandy alluvial streambed.
37. *Iris sari* Schott ex Baker — **End**. — Ir.-Tur. el. — rhiz. — 23.V.1995 — Loc. obs. Özcan leg.; Lit. rec. — EN (IUCN, 2024) — LC (Ekim et al., 2000) — Sandy alluvial streambed — (SMPs).
38. *Iris caucasica* Hoffm. subsp. *turcica* B.Mathew — Ir.-Tur. el. — bulb — 9.V.1993 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Under *Pinus nigra* forest – moist semi-shaded.
39. *Iris stenophylla* subsp. *stenophylla* Hausskn. ex Baker — **End**. — Med. el. — bulb — 9.III.2023 — Not assessed (IUCN, 2024) — semi-shaded moist open areas.
40. *Iris orientalis* Mill. — E. Med. el. — rhiz. — 28.IV.2024 — Loc. obs. Özcan leg. — Not assessed (IUCN, 2024) — Forest edge – moist streambeds.
41. *Iris haussknechtii* Bornm. ex Baker — **End**. — rhiz. — 27.IV.2024 — Loc. obs. Özcan leg. — LC (Ekim et al., 2000) — Under *Pinus nigra* forest – moist semi-shaded.
42. *Iris persica* L. — bulb — 3.III.2023 — Loc. obs. Özcan leg. — Not assessed (IUCN, 2024).
43. *Iris* × *germanica* L. — rhiz. — 12.IV.2024 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Old settlement surroundings – semi-shaded moist open areas.
44. *Crocus ancyrensis* (Herb.) Maw — **End**. — Ir.-Tur. el. — corm — 15.II.2023 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000) — Shaded deciduous woodland floor.
45. *Crocus danfordiae* Maw — **End**. — corm — 15.II.2023 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — LC (Ekim et al., 2000). Shaded deciduous woodland floor.
46. *Crocus oliveri* Gay subsp. *oliveri* — corm — 15.II.2023 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — NT (Ekim et al., 2000) — Under autumn-dried invasive grasses – semi-shaded moist openings.
47. *Gladiolus atroviolaceus* Boiss. — Ir.-Tur. el. — corm — 14.IV.2023 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Open steppe slope area.

ORCHIDACEAE (Asparagales)

48. *Epipactis helleborine* (L.) Crantz — rhiz. — 3.VI.2018 — Loc. obs. Özcan leg.; Lit. rec. — LC (IUCN, 2024) — Decayed litter–humus layer.
49. *Orchis purpurea* Huds. — Eur.-Sib. el. — tuber — 3.VI.2018 — Loc. obs. Özcan leg.; Lit. rec. — LC (IUCN, 2024) — Wet–moist meadow.
50. *Orchis mascula* (L.) L. subsp. *pinetorum* (Boiss. & Kotschy) G.Camus — E. Med. el. — tuber — 24.III.2023 — Loc. obs. Özcan leg.; Lit. rec. — Not assessed (IUCN, 2024) — Forest–shrub transition line – light–shade balance.
51. *Orchis anatolica* Boiss. — E. Med. el. — tuber — 14.VI.2018 — Loc. obs. Özcan leg.; Lit. rec. — LC (IUCN, 2024) — Decayed litter–humus layer.
52. *Dactylorhiza iberica* (M.Bieb. ex Willd.) Soó — E. Med. el. — tuber — 14.VI.2018 — Loc. obs. Özcan leg.; Lit. rec. — VU (IUCN, 2024) — Wet–moist meadow.
53. *Limodorum abortivum* (L.) Sw. var. *abortivum* — rhiz. — 13.VI.2018 — Loc. obs. Özcan leg. — LC (IUCN, 2024) — Root-base – organic moist — (SMP).
54. *Cephalanthera rubra* (L.) Rich. — rhiz. — 14.VI.2018 — Loc. obs. Özcan leg. — LC (IUCN, 2024) — Decayed litter–humus layer — (SMPs).
55. *Cephalanthera longifolia* (L.) Fritsch — Eur.-Sib. el. — rhiz. — 3.VI.2018 — Loc. obs. Özcan leg. — LC (IUCN, 2024) — Decayed litter–humus layer.
56. *Platanthera bifolia* (L.) Rich. — Eur.-Sib. el. — tuber — 12.VI.2018 — Loc. obs. Özcan leg. — LC (IUCN, 2024) — Humid streamside — (SMPs).

Abbreviations: Loc. = Locus; obs. = Observatio; leg. = legit; End. = endemic; Ir.–Tur. el. = Irano-Turanian element; Med. el. = Mediterranean element; E. Med. el. = Eastern Mediterranean element; Eur.–Sib. el. = European-Siberian element; Unk. el. = Unknown element; SMP/SMPs = Single MicroPopulation(s).

Notes: *Allium cepa* and *A. sativum* were recorded in managed agricultural habitats, but they are cultivated as annuals and do not form self-sustaining or naturalized populations. Therefore, they were excluded from quantitative analyses (species richness, diversity indices, family composition, etc.).

BÖLÜM 8

PRODUCTION OF A NATURAL SPREADER-ADHESIVE ALTERNATIVE TO SYNTHETICS USING AESCULUS HIPPOCASTANUM L. AND SAPONARIA OFFICINALIS L. PLANTS

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Introduction

When applying pesticides, plant growth regulators, and foliar fertilizers in agriculture, various additives (adjuvants) are used to increase work efficiency and effectiveness (Bernacki & Haman, 1972:891; Harrison, 1998; Muller et al., 2002:1243). Spreaders-adhesives are at the forefront of these additives. In foliar spray applications, components that help or modify the movement of the main active ingredient are called spreader-adhesives (Hochberg, 1996:203). In general, spreader-adhesives reduce the surface tension of the solution used as a pesticide or fertilizer, increase the surface coverage (contact diameter) on the leaf surface, and enhance the

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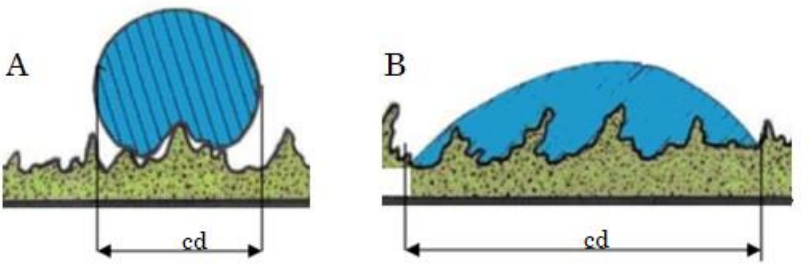
adhesion of the solution to the leaf and its penetration into the plant (Castro et al., 2013:476). In applications where the carrier is water, the pesticide droplet hitting the target surface may roll-off due to the high pressure of water. Spreader-adhesives are used to prevent this rolling-off and to make the round shape more flat.

The spread of pesticides on leaf surfaces is influenced by factors such as surface tension, density, viscosity, and temperature. Substances that reduce surface tension when dissolved in water or an aqueous solution are called surfactants (Işık et al., 2018:75). As surface tension decreases, droplets spread better, contact diameter increases, contact angle decreases, and coverage rate increases (Bernacki et al., 1972:891) (Figure 1). In other words, when the contact angles and heights of droplets are minimum, their contact diameters are maximum (Toraman & Bayat, 2019:1).

Figure 1. Contact diameter (cd) of water droplet (A) and spreader-adhesive droplet (B) on a hairy leaf surface

(Temeldaş, 2007)



Additionally, surfactants can maximize the spreading, penetration, and absorption efficiency of pesticides on leaf surfaces (Hess & Foy, 2000:807; Ramsey et al., 2005:162, 2006:205). On the other hand, as the density of the applied pesticides increases, viscosity decreases, and the spreading-coverage rate also decreases (Zeren & Bayat, 1999). Considering these properties, studies have been conducted using various vegetable and petroleum oils to increase the spreading properties of additives by reducing surface tension (Bernacki et al., 1972:891; Harrison, 1998; Zeren & Bayat,

1999). In their study, Muller et al. (2002:1243) stated that vegetable oils are more effective than petroleum oils. In studies conducted with various commercial spreader-adhesives, it was determined that organic silicone-based spreader-adhesives reduced surface tension more than others (Knoche, 1994:221; Sanyal et al., 2006:627). Today, various organic and inorganic substances such as non-ionic polyoxyethylene octyl phenyl ether (Triton X-100), pentaethylene glycol monododecyl ether (C12E5), polysorbate 20 (Tween-20), cationic dodecyltrimethylammonium bromide (DTAB), and anionic sodium dodecyl sulfate (SDS) are used as surfactants (Zhang et al., 2023:22121).

In order for the application to be more effective, in addition to the good spreading of the pesticide on the surface, it is also necessary for the droplets to adhere so that they do not run off. In interviews carried out with farmers about spreader-adhesives, it has been observed that the use of sugar, which was widely used in the 18^{th} and 19^{th} centuries, still continues. In interviews conducted with production, sales, and marketing companies, and in the examination of the labels of various products, it has been determined that synthetic materials such as CMC (Carboxymethyl Cellulose), synthetic resin, synthetic cellulose, glyphosate, and acrylic polyoxyethylene glycol phosphate are used predominantly. Studies investigating some properties of various commercial spreaders-adhesives have revealed that spreader-adhesives are produced from fatty acid + polyalcohol, sodium carboxylmethyl celulose, sodium dicotyl sulfosuccinate, trisiloxane alkoxylate + Polyalkaleneoxide – heptamethyl, trisiloxane + Allyloxy polyethylene glycol, alkyl aryl polyethoxy ethanol, and alkyl polyglycol ether (Toraman & Bayat, 2015:187, 2016:308). Various studies have been conducted to investigate the effects of using organic silicon and organic resin, in addition to synthetic materials, in the production of spreader-adhesives (Knoche, 1994:221; Sanyal et al., 2006:627; Türkseven et al., 2011:25; Toraman & Bayat, 2016:308; Kaptan, 2021:15). The proteins, natural rubber, starch, natural gums, and cellulose, which are abundant in nature, including the organic silicon and resin used in these studies, are renewable polymers. These materials, called

biopolymers or green polymers, are also used as adhesives and surfactants (Heinrich, 2019:1866; Gürkok & Özdal, 2021:7).

In the literature, the effects of commercial spreader-adhesives together with various pesticides or foliar fertilizers on different plants have been generally investigated and compared (Bernacki et al., 1972:891; Hall & Fox, 1996:31; Harrison, 1998; Webb et al., 1999:382; Holloway & Western, 2003:1237; Gaskin et al., 2005:179; Temeldaş, 2007; Türkseven et al., 2011:25; Toraman & Bayat, 2016:308; Peirce et al., 2019:1532; Kaptan, 2021:15). Leaper et al. (2000:313) used glyphosate as a spreader-adhesive in their study. Some studies have investigated the wetting properties of the materials used (Zhang et al., 2023:22121), the coverage area on the leaf depending on leaf morphology (Januszkiewicz et al., 2019:1), concentration levels (Xu et al., 2010:58; Lin et al., 2016:42), and the relationships between leaf surface and droplet size, evaporation time, humidity, and wetting area (Yu et al., 2009a, 2009b:324). However, no studies that tested natural spreader-adhesives made from plant-based sources or food waste biopolymers have been encountered in the literature. Moreover, interviews with farmers before the study revealed that they used substances such as sugar and dish detergent as spreader-adhesives, and sugar led to the use of more chemical pesticides by attracting other pests.

In the study conducted taking into account sustainability, a natural spreader-adhesive was aimed to be produced by using *A. hippocastanum* L. (Kuşçu et al., 2022:117) and rice as adhesives and *Saponaria officinalis* L. (Bozkurt, 2019:66) as a spreader. *A. hippocastanum* L. is a plant which is native to the Balkan Peninsula, well-adapted to our country's climate, fast-growing, abundant in nature, especially in parks, and rich in starch. In addition, *Saponaria officinalis* L. is found in nature and widely used in various health fields. Moreover, *rice* is commonly found as leftover rice paste in almost every restaurant and canteen. The objectives of this study were: 1) to produce a natural spreader-adhesive as an alternative to synthetic or organic spreader-adhesives that do not have the desired properties, 2) to prevent some spreader-adhesives from negatively affecting plants, the environment, and other living organisms, 3) to

find healthier solutions instead of substances used as spreader-adhesives that attract other pests to the plant and lead to the use of more chemical pesticides, and 4) to expand the use areas of biopolymers obtained from natural and sustainable sources.

1.Materials and Methods

1.1. Materials

Horse chestnut (*Aesculus hippocastanum* L.), soapwort (*Saponaria officinalis* L.), leftover rice, olive oil, hazelnut oil, castor oil, jojoba oil, sunflower oil, tartaric acid, bean (*Phaseolus vulgaris* L.), black bean aphid (*Aphis fabae*), commercial spreader-adhesive (X), and an insecticide for black bean aphid (Y) were used as materials in the study.

1.2. Preparation of Adhesives to be Used in the Creation of Sample Spreader-Adhesive

Green polymer starch to be used as adhesive was obtained from *A. hippocastanum* L. and leftover rice. Fruits of *A. hippocastanum* L. collected from school gardens and parks in Tekirdağ city center (Tekirdağ, Türkiye, 40°59'38.5"N 27°35’08.6” E) were peeled and seeds were removed. Dried seeds were separated from their husks and passed through a grinder. The seeds turned into flour were kept in distilled water at room temperature for 24 hours. 100 g was taken from the material that was dried after filtration and boiled with 200 ml of distilled water. Leftover rice obtained from the canteen was thoroughly washed to remove oil and salt. 100 g of the dried and ground rice residue was also boiled with 200 ml of distilled water.

1.3. Preparation of Spreader to be Used in the Creation of Sample Spreader-Adhesive

Saponin, which had a surface tension-reducing effect, was extracted from the root parts of *S. officinalis* L. using the Soxhlet extraction method. As solvents, petroleum ether and ethanol were used. After concentrating the ethanol solution in a rotary evaporator,

it was placed in an ice bath and acetone was added dropwise to it. After a while, it was observed that saponin precipitated as a white solid. The saponin was filtered using filter paper and dried at room temperature (Özek, 1987). A 1% solution was prepared from the obtained saponin. In addition to saponin, sunflower, olive, jojoba, hazelnut, and castor oils obtained from Zeytinburnu Medicinal Plants Garden (Istanbul, Türkiye) were used as spreaders in the preparation of the sample mixtures.

1.4.Testing the Spreader-Adhesive Properties of the Prepared Samples

A large number of mixtures with different compositions were obtained using the prepared adhesives, saponin, vegetable oils, and distilled water. The mixtures were homogenized by centrifugation. The spreading and adhesion properties of these mixtures (spreader-adhesive samples) were investigated on hairy leaves of *Viola* sp. and hairless leaves of *Citrus limon* L. Spreader-adhesive samples (1 µl, 10 drops) were dropped (from 4 cm height) onto the leaf surfaces of plants by using a micropipette, and their spreading and adhesion properties were examined (Gaskin et al., 2005:179). Three different leaves were taken from each plant and the procedures were repeated three times. The spreading properties of the prepared samples were compared with pure water (- control) and a commercial spreader-adhesive (+ control), and results were evaluated by giving scores between 0 and 5. Although numerous experiments were conducted until the results were obtained, only a portion of the experiments are shown in Table 1.

1.5. Adjustment of PH and Concentration in Selected Sample Spreader-Adhesives

After evaluating the observations based on spreading properties, the selected samples were coded and their pH and concentration were adjusted. Tartaric acid (10 %, pH = 2.2), a natural acid, was used to adjust the pH of the samples. The concentrations of commercial spreader-adhesives were taken as a basis for adjusting the concentration. The general recommendation for the use of commercial spreader-adhesives is 0.2-2.5 ml L^{-1} (Toraman & Bayat,

2015:187). Therefore, the sample spreader-adhesives were prepared at two different concentrations: 1 ml L^{-1} (100 µl 100 ml^{-1}) and 2 ml L^{-1} (200 µl 100 ml^{-1}). The commercial spreader-adhesive was applied as 1 ml L based on the product manual.

1.6. Measurement of the Contact Diameter of Sample Spreader-Adhesive Droplets

In previous studies, it was stated that there was an inverse relationship between the contact angles and heights of droplets and their contact diameters; therefore, in this study, spreader-adhesives were evaluated according to their droplet contact diameters (Bernacki et al., 1972:891; Toraman & Bayat, 2019:1). Droplets (1 µL, 10 drops) were dripped (from a height of 4 cm) onto the leaves of the selected samples and allowed to stand for a while (average 7 seconds). Then, the contact diameter of the droplets was measured using a LYK 2710-8105 precision digital caliper (0-15 mm 0.005 mm^{-1}). The process was repeated three times, and the averages of the measurements were calculated. In the experiments, distilled water (- control) and X commercial spreader-adhesive (+ control) were used as control groups.

1.7. Investigation of the Effect of Sample Spreader-Adhesives on Stomata

The effect of the samples on stomata, whose pH and concentration were adjusted, was investigated using leaves of *Citrus limon* L. and *Triticum aestivum* L., which are important in agriculture. One microliter of 5 drops of each sample and commercial spreader-adhesive was dripped onto the upper surface of *T. aestivum* L. leaves and the lower surface of *C. limon* L. leaves. Each sample was applied to 3 different leaves taken from the plants. After the samples were allowed to spread on the leaf surface and the leaves were completely dry, nail polish was applied to the leaf surfaces and dried. These operations took approximately 2 hours. The dried nail polish was removed using a scalpel and forceps, and preparations were prepared. Preparations were examined under a light microscope (Olympus BX51) and images of the stomata were

taken using a CCD digital camera (Spot RT Slider) attached to the microscope.

1.8.Testing of the Sample Spreader-Adhesive on *P. vulgaris* L.

The prepared sample spreader-adhesive was tested on bean plants in combination with insecticide Y, which was used for the control of black bean aphids (*Aphis fabae*). Following the manufacturer's instructions written on insecticide Y, three 100 ml samples were prepared (50 g of insecticide per 100 L). 40 ml of a commercial spreader-adhesive (X) was added to one sample, and the same amount of the prepared spreader-adhesive was added to the other sample. Water was used as a control group. Bean aphids collected from the university were infested onto laboratory-grown bean seedlings (20 aphids per plant). The care of the infested bean plants was maintained for one week. The four experimental groups (water + Y, commercial spreader-adhesive + Y, sample spreader-adhesive + Y, water) were applied to the bean plants using a hand pump (twice, with a 3-day interval). Three potted bean plants were used for each experimental group. The growth of the bean plants was monitored, and the number of aphids was counted one day after each application.

1.9. Statistical Analysis

The data obtained from the study were subjected to analysis of variance in leaf types and applications according to the split-plot experimental design in coincidence plots using the MSTAT-C package program. The differences between the mean values were statistically evaluated using LSD test.

2. Results and Discussion

Based on the experiments conducted to evaluate the spreading and adhesion properties of the prepared spreader-adhesives, six samples that scored 3 or 4 were selected (Table 1).

Table 1. Evaluation results of spreading-adhesion properties of spreader-adhesive samples prepared using two different adhesives and 6 different spreaders and selected samples

Adhesive 1 (A1) (µl)	Adhesive 2 (A2) (µl)	Hazelnut oil (µl)	Sunflower oil (ml)	Olive Oil (µl)	Castor Oil (µl)	Jojoba oil (µl)	Saponin (ml)	Distilled water (µl)	Spreading
1000		500						2000	0
1000		1500						2000	1
1000		1500					500	1000	2
1000		1500					1000	1000	2
1000		1000						1000	1
	1000	1000						2000	1
	1000	1000					1000	2000	3
	1000	500					500	1500	3*
1000			2000					1000	0
1000			1000					1000	1
1000			2000					2000	1
1000							500	1000	0
1000							1000	2000	3
1000			1000				500	2000	3*
	1000		2000					2000	0
	1000		3000					2000	4
	500		2000					2000	4*
	1500		3000					3000	2
	1000		1000				1000	1000	4*
1000				1000				1000	0
1000				1500				1500	3
1000				1000			500	1000	1
1000				500			500	1000	4*
1500				500			500	1000	5
	1000			1500				2000	0
	1500			2000				3000	1
	1500			3000				3000	2
	1000			3000				3000	3
	1000			1000			1000	1000	3*
1000					1000			1000	0
1000					500		500	1000	2
	1000				1000			1000	0
	1000				1500			1500	0
	1000				1000		500	1500	2
1000						1000		1000	1
1000						500	500	1000	2
	1000					1000		1000	1
	1000					1500		2000	2

	1000					1500		1000	2
	1000					2000		2000	2
	1000					500	500	1000	2
Water (-Control)									0
Commercial spreader-adhesive= X (+ Control)									4
A1: Adhesive obtained from rice, A2: Adhesive obtained from *A. hippocastanum* 0: not spread, 1: very little spread, 2: less spread, 3: good spread, 4: very good spread, 5: too much spread (flowed), *: selected samples									

The samples prepared at two different concentrations (100 µl 100 ml-1 and 200 µl 100 ml-1) and given code numbers were applied to hairy (*Viola* sp.) and hairless (*Citrus limon* L.) leaves. In order to statistically evaluate the spreading-adhesive properties of the samples, variance analysis was performed on leaf types and applications (Table 2).

Table 2. Results of variance analysis performed on leaf types and applications for spreading-adhesive properties of the samples prepared in the study

Variation Source	**Degrees of Freedom**	**Sum of Squares**	**Average of Squares**	**Ratio F**	**Table value**	
					% 5	**% 1**
Leaf type	1	0.001	0.001	0.092 ns	7.710	21.200
Error-1	4	0.047	0.012			
Application	13	1.601	0.123	18.831 **	1.700	2.120
Leaf type x application interaction	13	0.247	0.019	2.909 **	1.700	2.120
Error	52	0.340	0.007			
General	83	2.236	0.027			
* : Significant at 5 % alpha level **: Significant at 1 % alpha level						

In the variance analysis, it was determined that the difference between leaf types was not statistically significant, but the applications and leaf type x application interaction were statistically significant ($P< 0.01$). In this study, the spreader-adhesive properties of the samples were evaluated according to droplet contact diameters on hairy and hairless leaves. Water (- control) and commercial spreader-adhesive (+ control) were used as control groups and the study was carried out in 3 replications. The mean values and

significance groups of the droplet diameters of the samples are given in Table 3. In addition, LSD test was performed since treatments and leaf type x treatment interactions were statistically significant (Table 3).

Table 3. Results of Duncan and LSD tests. Also, code numbers, densities, pH values, mean droplet diameters, standard deviations, and significance groups of the prepared spreader-adhesive samples.

The code number	Content of the sample spreader-adhesive	Density	pH		Measurements of the mean diameter (mm)		
			Spreader-adhesive	SA + Tartaric Acid	Hairless Leaf $\overline{X}$ ± SD, SG	Hairy Leaf $\overline{X}$ ± SD, SG	Hairy + Hairless Leaf $\overline{X}$ ± SD, SG
SA1	A2+Sunflower oil+water	100 µl 100 ml^{-1}	7.8	5.7- 5.6	1.30± 0.10^{d-g}	1.47±0.11 $^{a-d}$	1.38±0.12 $^{b-c}$
SA2	A2+Sunflower oil+water	200 µl 100 ml^{-1}	7.4	5.7- 5.6	1.30±0.10 $^{d-g}$	1.33±0.04 $^{c-g}$	1.32±0.02 $^{b-c}$
SA3	A2+ Water + sunflower oil + Saponin	100 µl 100 ml^{-1}	6.6	5.6	1.57± 0.05^{a}	1.53± 0.06 $^{a-b}$	1.55±0.02^{a}
SA4	A2+ Water + sunflower oil+ Saponin	200 µl 100 ml^{-1}	6.1	5.6	1.47± 0.05^{a-d}	1.37±0.06 $^{b-g}$	1.41±0.07^{b}
SA5	A2+ Water + zeyin of oil + Saponin	100 µl 100 ml^{-1}	6.5	5.9	1.43± 0.05^{a-e}	1.27±0.07 ^{efg}	1.35±0.11 $^{b-c}$
SA6	A2+ Water + olive oil + Saponin	200 µl 100 ml^{-1}	6.5	5.8	1.40± 0.10^{a-f}	1.20±0.10 ^{g}	1.30±0.14 $^{b-c}$
SA7	A1+ Saponin + Water + olive oil	100 µl 100 ml^{-1}	6.5	5.7	1.30± 0.02^{d-g}	1.23± 0.05 $^{f-g}$	1.27±0.04^{c}
SA8	A1 + Saponin + Water + olive oil	200 µl 100 ml^{-1}	6.3	5.6	1.40± 0.10^{a-f}	1.33±0.05 $^{c-g}$	1.37±0.04 $^{b-c}$
SA9	A1+ Saponin + Water + sunflower oil	100 µl 100 ml^{-1}	7.0	5.5	1.30±0.03^{d-g}	1.50±0.01 ^{abc}	1.40±0.14^{b}
SA10	A1+ Saponin + Water +	200 µl 100 ml^{-1}	7.1	5.8	1.37±0.10 $^{b-g}$	1.43±0.06 $^{a-c}$	1.40±0.04^{b}

	Sunflower Oil						
SA11	A2 + Saponin + Water + coconut oil	100 µl 100 ml^{-1}	5.9	5.5	1.27±0.06 ^{efg}	1.33± 0.14 $^{c-g}$	1.30±0.04 $^{b-c}$
SA12	A2 + Saponin + Water + hazelnut oil	200 µl 100 ml^{-1}	5.6	5.6	1.40±0.10 $^{a-f}$	1.40±0.10 $^{a-f}$	1.40±0.00 ^{b}
SA13	(+) Control	100 µl 100 ml^{-1}	5.6		1.37±0.06 $^{b-g}$	1.37±0.04 $^{b-g}$	1.37±0.00 $^{b-c}$
SA14	(-) Control		to 7.8 - 7.7		0.90±0.10 ^{h}	0.90±0.10 ^{h}	0.90± 0.00^{h}
Mean:					1.34±0.15	1.33±0.15	1.34±0.14
LSD (0.01)			Applications = 0.125 Leaf type x Ap. Int.= 0.183				
A1:Adhesive obtained from rice, A2: Adhesive obtained from *A. hippocastanum*, SA: Spreader-Adhesive, $\overline{X}$: Mean droplet diameters, SD: standard deviation, SG: Significance groups, (-) control: water, (+) control: Commercial spreader-adhesive X							

When the diameter measurement results of the samples obtained in the applications are examined, it was observed that the contact diameter was 0.9 – 1.57 mm ($\overline{X}$=1.34 mm) on hairless leaves and 0.9 – 1.53 mm ($\overline{X}$=1.33 mm) on hairy leaves. The variance analysis showed that there was no statistically significant difference between leaf types.

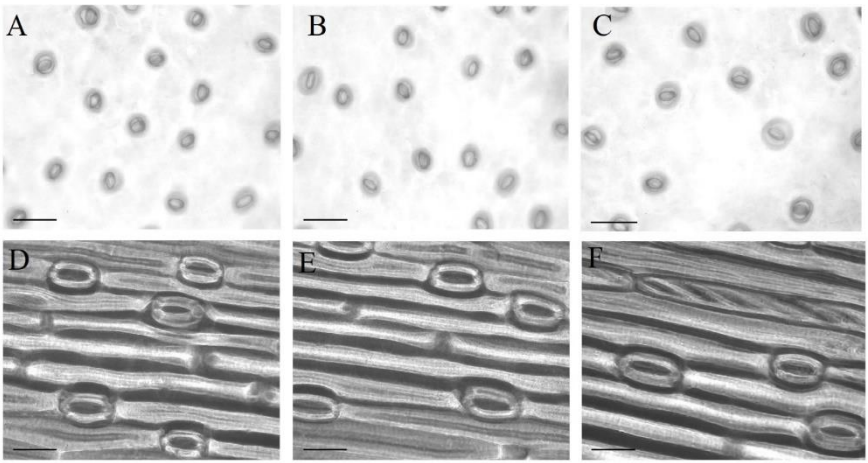
When the applications were evaluated separately according to leaf types, the highest droplet contact diameter on hairy ($\overline{X}$=1.53± 0.06 mm) and hairless ($\overline{X}$=1.57± 0.05 mm) leaves was determined in the 3rd application where spreader-adhesive with code number SA3 was used. The SA3 sample was followed by the applications in which SA9 ($\overline{X}$=1.50±0.01 mm) coded samples were used in hairy leaves and SA4 ($\overline{X}$=1.47±0.059 mm) coded samples were used in hairless leaves. The mean droplet contact diameter of the SA3 sample was found to be significantly larger compared to the SA9 and SA4 treatments (F=18.831 **, P <.001). The lowest droplet contact diameters were determined in the treatments using SA5 ($\overline{X}$=1.27±0.07 mm) for hairy leaves and SA11 ($\overline{X}$=1.27±0.06 mm)

for hairless leaves. In the applications using commercial spreader-adhesive, the same results were obtained for both leaf types. When the overall averages of the samples on hairy and hairless leaves were examined, the highest droplet contact diameter ($\overline{X}$=1.55±0.02 mm) was observed in the application where the spreader-adhesive coded as SA3 was used. This application was followed by the 4th application ($\overline{X}$=1.41±0.07 mm) and the applications ($\overline{X}$=1.40 mm) in which SA9, SA10, and SA11 coded samples were used. In the analysis, it was determined that the average droplet contact diameter of the SA3 sample was significantly larger compared to SA4, SA9 and other applications (F=2.909 **, P < .001). Among the sample spreader-adhesives, the lowest droplet contact diameter ($\overline{X}$=1.27±0.04 mm) was determined in the sample coded as SA7. The mean contact diameter of the commercial spreader-adhesive was observed to be 1.37±0.00 mm. Previous studies have shown that as the contact diameter of the spreader-adhesive increases, the contact angle decreases and the covered area increases (Bernacki et al., 1972:891; Gaskin, 2005:179). In addition, since the hairs disrupt the angle in hairy leaves, an accurate measurement cannot be made (Gaskin, 2005:179). Therefore, the evaluation in this study was made based on the droplet contact diameters rather than the contact angle of the droplets. Yu et al. (2009b:324) stated that the addition of a spreader-adhesive increased the coverage area of droplets, whose diameters ranged from 246 to 886 μm, on hairy (4.5-10.1 times) and solid-waxy (3.4 - 4.1 times) leaves. In their study, Xu et al. (2010:58) obtained droplets with diameters of 300 μm and 600 μm and compared these droplets in terms of various properties. The fact that droplet angles or diameters are different is due to the static electric charge of the surfaces, the electrical charge of the applied liquid, pH level, chemical structure and density of the applied material, humidity, temperature, and the structure of the leaf surface (Toraman & Bayat, 2019:1). On the other hand, the use of different amounts of spreader-adhesives, different tools (syringe, micropipette, special spray devices, etc.), and trials of them from different heights on various plants in studies can also affect the droplet diameters. In this study, a micropipette was used. Droplets in different sizes could be obtained by testing different sample spreader-adhesives from

different heights by using special nozzle sprays. The size of the droplets is important because the droplet diameter affects the evaporation time and the wetting area of the insecticide (Yu et al. 2009 a, 2009b:324). However, the evaporation time and wetting area are also affected by humidity, the chemical composition and density of the sample used, the leaf surface, and the location of the leaves where the droplets accumulate (Yu et al. 2009a, 2009b:324; Xu et al., 2010:58).

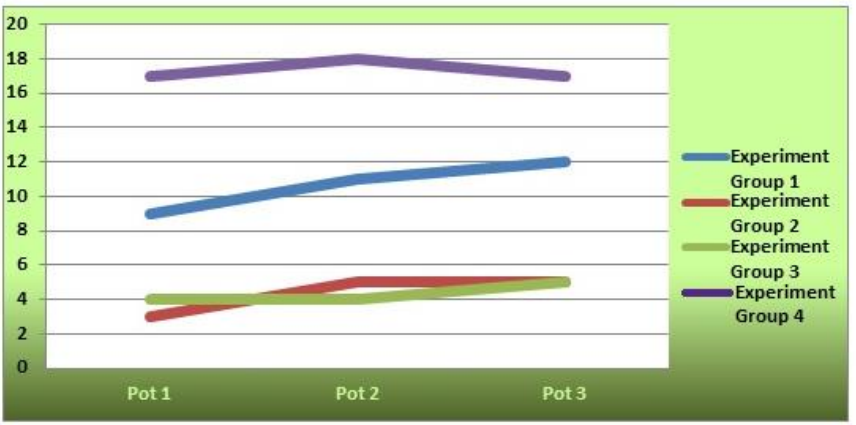
The effect of sample spreaders-adhesives on stomata was investigated using *Citrus limon* L. and *Triticum aestivum* L. leaves. Three separate leaves taken from the plants were used for each sample. According to the method applied in the study, microscope examination began approximately 2 hours after sample spreaders-adhesives were applied to the leaves. As a result of the examinations made under the microscope, it was seen that stomata were open in the preparations prepared for all samples and photographs were taken from the samples with good images (Figure 2). The leaves were examined again after waiting for 6 hours and the stomata were seen to be open. In previous studies were reported that resinous spreader-adhesives close stomata. Morever it has been determined that when commonly used monodisperse (tetraethylene glycol monododecyl ether) and polydisperse alcohol ethoxylate (BrijL4) are used at a 10% level, they completely cover the leaf surface but also close the stomata, prevent the leaf from performing photosynthesis and transpiration, and cause leaf death within 2-6 hours (Baales et al., 2022:1). The stomata are vital for plants. For these reasons, the spreader-adhesives used should not close the stomata, but should keep them open to increase the diffusion of the applied pesticide or fertilizer into the leaf. It can be said that the samples prepared in the study have these features because they do not close the stomata.

Figure 2. Stomata obtained from the lower surface of Citrus limon L. leaves A: 2 h after application of SA3 sample, B: 6 h after application of SA9 sample, C: 2 h after application of SA13 (+control) (for comparison) and stomata obtained from the upper surface of Triticum aestivum L. leaves D: 2 h after application of SA3 sample, E: 6 h after application of SA9 sample, F: 2 h after application of SA13 (+control) (for comparison). The scale bar is 100 µm



Bean plants, aphids and insecticides (Y) were used to investigate the effect of the spreader-adhesive sample SA3 in combination with currently used drugs. At this stage of the study, four different experimental groups (group 1: water + Y, group 2: commercial spreading adhesive + Y, group 3: SA3 spreading adhesive sample + Y, group 4: water) were formed. Three pots were prepared for each group (one plant per pot) and 20 aphids were added to the bean plants in the pots. After the samples prepared for the experimental groups were applied twice at 3-day intervals, the number of aphids remaining alive in the pots was determined (Figure 3). When the results were analyzed, it was determined that the SA3 coded sample spreader-adhesive (group 3) showed the same efficiency on the mechanism of action of Y insecticide as the commercial spreader-adhesive (group 2) which is widely used today.

Figure 3. Number of bean aphid survivors in 4 different experimental groups (1st Group: water + Y, 2nd Group: commercial spreader - adhesive + Y, 3rd Group: SA3 spreader - adhesive sample + Y, 4th Group: water) applied to bean plants 2 times at 3-day intervals (At the beginning of the application there are 20 aphids on each plant).



When the results of the study were evaluated, it was observed that the spreader-adhesive coded as SA3, which contains *A. hippocastanum* + Water + Sunflower oil + Saponin (100 µl 100 ml^{-1}), could be an alternative to synthetic spreader-adhesives, in particular. There are various spreader-adhesives on the market. It is important to know the properties and effects of these substances. It has been determined that when some spreader-adhesives, especially those derived from cellulose, are prepared by dilution with pure water as recommended on their labels, they increase the surface tension of the solution. Even, it has been revealed that the surface tension values of H and C spreader-adhesives are at the level of the surface tension value of pure water, and they do not improve the application liquid's droplet characteristics such as adhesion and contact angle on the applied surfaces (Camacho et al., 2001:602; Temeldaş, 2007; Toraman et al., 2018:68). Spreader-adhesives that are widely sold on the pesticide market and include contents such as carboxymethylcellulose and sodium carboxymethylcellulose which do not provide effective results have these properties (Toraman et al.,

2018:68). It has been stated that some spreader-adhesives change the performance of pesticides in applications, their effects can be positive or negative, and in this case, their use also has economic effects (Toraman & Bayat, 2016:308). It has been determined that spreader-adhesives containing surfactants such as trisiloxane + allyloxy polyethylene glycol, alkyl ethoxylate, and Alkylphenol ethoxylate create the largest contact diameter by spreading rapidly and increase adhesion (Toraman & Bayat 2019:1). However, it has been stated that some alkylphenol ethoxylates used as surfactants are toxic to animals such as fish, birds, and mammals (Hoffman, 2004). Moreover, surfactants used in a wide variety of fields such as spreader-adhesive, detergent, and drug production are mostly synthetic. These substances are not biodegradable and negatively affect the ecosystem by mixing with groundwater or other water sources in the environment (Gürkok & Özdal, 2021:7). During our research, we determined that some farmers continue to use substances such as sugar to ensure the pesticide or fertilizer sticks and to prevent it from falling off the leaf, as well as dish detergent to spread it on the surface immediately. Some chemicals in dish detergents are harmful to human health, and sugar causes damage to the plant and increases the use of pesticides due to the fact that it attracts some harmful insects or other living things to the plant.

Because the general goal of current studies is sustainability, it is necessary to develop natural spreaders-adhesives by using plant or animal-based materials that can be produced by living organisms, biodegrade, and do not harm the ecosystem. Therefore, this preliminary study, which aims to produce a natural spreader-adhesive, is scientifically significant. In the study, it was determined that the SA3-coded product (*A. hippocastanum* + water + sunflower oil + saponin), which underwent certain processes, promoted maximum spreading and created resistance to flow by adhering to the surface. These are the desired properties in spreader-adhesives. The produced product can be an alternative to synthetic or organic spreader-adhesives that do not fully possess the desired properties. The effectiveness of spreader-adhesives depends on many factors such as surface wettability, product type, product concentration, pH, plant structure, and the temperature of the application environment

(Gaskin et al., 2005:179). Therefore, studies using different methods can also be conducted to improve the product. In this study, 2 different adhesives, 6 different spreaders, 2 different densities of spreader-adhesives from selected samples, one type of commercial spreader-adhesive and insecticide were used, and the experiments were carried out in 3 replications. In future studies, the proposed product can be improved or new products can be created by using different plants and natural waste products, various natural spreaders, various fertilizers and pesticides applied to plants, leaves of different plants, performing many repetitions, using advanced technological devices. Additionally, the production of *A. hippocastanum* and *S. officinalis* can be an option for farmers.

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Integrated Pest Management:A sustainable Approach to Pest Control

BÖLÜM 9

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Integrated Pest Management

According to the Food and Agriculture Organization of the United Nations (FAO), "Integrated Pest Management (IPM) means the careful consideration of all available pest control techniques and

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subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM promotes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms" (FAO 2020). This definition highlights IPM as a framework that not only addresses pest management but also places equal importance on environmental sustainability and human well-being. In today's agriculture, Integrated Pest Management (IPM) has become a key approach for managing pesticide use. Unlike traditional methods that frequently rely on scheduled spraying, IPM aims to maximize the effectiveness of pesticide applications while minimizing their overall use. This is done through practices like frequent field inspections, making decisions based on economic thresholds, and taking into account factors such as heat accumulation and past pest trends. It is an agroecological approach to pest and disease management (Pretty *et al*.,2010) By using pesticides only when truly necessary and in a focused way, IPM promotes both efficacy and safety. Integrated Pest Management (IPM) is a comprehensive, ecological approach that uses cultural, biological, and chemical methods to maintain pest populations below economically harmful levels. IPM prioritizes prevention and monitoring over reactive measures. Core strategies include crop rotation, sanitation, resistant varieties, and habitat management. Intervention is based on pest

surveillance data and defined thresholds principles rely on an integrated process: preventing pest outbreaks, monitoring pests and their natural enemies, using a combination of biological, physical, and chemical methods, and regularly assessing management effectiveness. This multidisciplinary approach enhances crop productivity and preserves ecological balance, supporting long-term agricultural sustainability. The shift toward IPM is essential due to the limitations of conventional pest control. Today, intensive farming has been shown to have reached its limits. In pest management, questions relating to the questions of sustainability have often been raised (van Lenteren 1998), in particular, the many harmful consequences of the massive use of pesticides: farmers, consumers and society in general face more socio-economic difficulties (Bourguet and Guillemaud 2016; Sheahan *et al.* 2017); there is mounting pollution of water, soil and the atmosphere (Aubertot *et al*. 2005;Burdon *et al*.,2019) biodiversity is being eroded, particularly that of insects (Foucart 2019;Hallmann *et al*.2017; Sánchez-Bayo and Wyckhuys 2019) and birds Integrated Pest Management (IPM) tackles not just the immediate effects of pests on agricultural yields, but it also supports the overarching goals of sustainable development. This includes safeguarding natural resources, ensuring public health protection, and fostering social and economic prosperity helps to conserve biodiversity and safeguard ecosystem services by lowering reliance on chemical pesticides. Additionally, it makes farming systems more resilient to

environmental shocks and pest outbreaks. By lowering input costs, increasing agricultural yields, and guaranteeing safer, better-quality produce for customers, IPM also directly helps farmers. Thus, IPM represents a holistic and sustainable approach to pest management, aligning agricultural productivity with environmental stewardship and public health protection.

Components of IPM

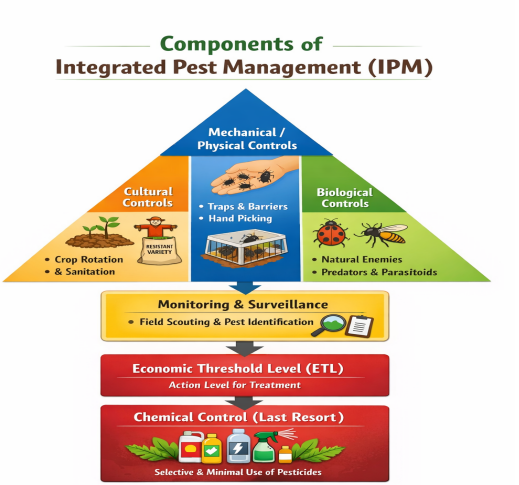

Fig.1

Figure 1 illustrates the key components of an IPM program, Integrated Pest Management (IPM) takes a comprehensive approach to managing pests in a way that's sustainable for both the environment and the economy. As illustrated in the accompanying figure, IPM incorporates a variety of strategie

These include prevention and cultural practices, monitoring and decision-making tools, biological control methods, and chemical control as a last resort. Prevention and cultural practices involve techniques like sanitation, crop rotation, intercropping, and the use of resistant plant varieties to create conditions that deter pest populations from establishing. Monitoring and decision-making tools, such as economic injury levels, action thresholds, scouting, and sampling techniques, empower farmers to evaluate pest populations and identify the right time to take action. Biological control methods leverage natural enemies, enhance and conserve beneficial insects, and incorporate genetic and classical biological control (CBC) strategies to maintain pest populations at manageable levels. Additionally, judicious use of chemical inactivation techniques, including biopesticides, targeted pesticide applications, and advancements in nanotechnology, plays a crucial role when other methods fall short. By combining these various approaches, Integrated Pest Management (IPM) effectively tackles pest issues while minimizing risks to both public health and the environment.

Prevention and Cultural Control Methods: The goal of preventative measures is to lessen the likelihood of pest outbreaks before they happen. Crop rotation, intercropping, weed and crop residue management, and the use of pest-resistant cultivars are among the practices.

The effectiveness of crop rotation in controlling pest populations is due to various factors, such as the spatial and temporal separation of host crops, the inclusion of nonhost crops that serve as barriers or trap crops, and the promotion of beneficial organisms through increased biodiversity.The mechanisms underlying the pest-suppressive effects of intercropping are intricate, involving elements such as resource competition, physical obstacles, allelopathy, and habitat modification.Sanitation procedures are cultural management methods that entail the elimination and destruction of pest-infested plant material, crop leftovers, and other sources of pest inoculum from agricultural fields and adjacent areas.The goal of using resistant cultivars in IPM programs is to reduce reliance on pesticides, reduce production losses, and improve the general resilience and sustainability of crops. Antixenosis, antibiosis, and tolerance are the three primary categories into which the many mechanisms of resistance in crop plants can be categorized.

Monitoring and Decision Making: To accurately identify the species and identify pests early on, fields must be regularly scouted. Visual inspection, the use of sweep nets, sticky traps, pheromone traps, and remote sensing technologies are some of the instruments and approaches used in addition to these sampling designs to monitor pest populations and their detrimental impacts on agricultural plants. The use of remote sensing methods, including satellite images, aerial photography, and unmanned aerial vehicles (UAVs), to track crop health and identify insect outbreaks on a wide geographic scale is

growing. Appropriate sample designs and the integration of various monitoring instruments and methodologies enable IPM practitioners to make data-driven judgments about the need for and timing of pest control treatments. Artificial intelligence (AI) has recently been applied to IPM for identification and decision-making.

Biological Control: Utilizing natural enemies such as parasitoids, pathogens, and predators to control pest populations. By regulating pest populations through a variety of strategies, including as direct predation, parasitism, and infection, these advantageous organisms may often keep pest concentrations below thresholds that can be economically detrimental. A thorough understanding of the biology and ecology of natural enemies with regard to target pests and the agricultural environment is necessary for their widespread incorporation into IPM.

Chemical Control: Chemical management is only used as a last option when non-chemical and preventative measures are unable to keep pest populations below economically destructive levels. To reduce hazards to people, beneficial creatures, and the environment, pesticides should be used sparingly, selectively, and in conjunction with other suitable techniques. Instead of normal calendar-based spraying, IPM bases the choice to apply pesticides on meticulous field observation and the creation of economic thresholds. This makes sure that only when insect populations are high enough to result in substantial financial loss are pesticides utilized. Choosing

the appropriate pesticide is essential when chemical control is required. Target-specific, short-lived, and ecologically safe products such botanicals, bio-pesticides, or selective insecticides are preferred since they have little effect on non-target creatures like pollinators and natural enemies. In order to guarantee efficient management and avoid contaminating soil, water, and food items, proper dose, timing, and application technique are also crucial.

Advantages of IPM

Benefits to the environment: Environmental benefits: Excessive pesticide usage is frequently linked to environmental deterioration, which causes a number of other issues like soil and water contamination as well as biodiversity loss. An environmentally acceptable substitute is Integrated Pest Management (IPM), in which any control techniques are implemented after carefully weighing the possible environmental implications. IPM reduces the risks associated with traditional pesticide usage by emphasizing the adoption of sustainable and ecologically safe pest management techniques. In addition to ensuring efficient pest management, IPM supports cultural practices, biological control, and other sustainable methods that support ecosystems' long-term resilience and health.

Minimizes residue hazards of pesticides: The use of chemical pesticides is significantly decreased in an Integrated Pest Management (IPM) program, which significantly lowers the risks associated with pesticide residues. The efficient management of pest issues without sacrificing crop safety is ensured by the planned

application of biological, cultural, and mechanical control techniques. Consequently, IPM encourages food that is devoid of toxic residues, enhancing customer confidence and promoting more sustainable, healthier farming practices.

A Balancing Act for Non-target Species: Traditional pest management techniques frequently damage non-target species and upset the natural equilibrium. On the other hand, by reducing the use of toxic pesticides and safeguarding beneficial species, Integrated Pest Management (IPM) encourages accuracy and sustainability. IPM promotes soil health, water quality, and biodiversity by using cultural practices and biological control agents. This environmentally friendly method creates more robust and sustainable agricultural systems by promoting better ecosystems that can naturally control pests.

Preserving Natural Predators: The preservation of natural predators that control insect populations is a fundamental component of Integrated insect Management (IPM). IPM safeguards birds, beneficial insects, and other species that are essential to preserving ecological balance by reducing the usage of broad-spectrum pesticides. By using natural biological regulation, this method supports sustainable pest management, increases biodiversity, and lessens reliance on chemical control. In the end, maintaining natural enemies within an IPM framework promotes ecosystems that are stronger and healthier.

Protecting human health: By reducing the use of dangerous chemical pesticides that can cause acute poisoning and long-term health problems including cancer and neurological diseases, Integrated Pest Management (IPM) protects human health. IPM lessens direct exposure for both agricultural workers and customers by focusing on safer, more focused, and need-based pest management techniques. In addition to guaranteeing food safety, this strategy shields susceptible groups from the negative consequences of pesticide residues, promoting healthier communities and more environmentally friendly farming methods.

CONCLUSION

As a possible substitute for the overuse and careless application of chemical pesticides, integrated pest management (IPM) has emerged as a promising and sustainable paradigm for crop protection. IPM aims to reduce threats to the environment and public health while maintaining pest populations below economically detrimental levels by combining a variety of preventive, biological, cultural, and chemical control techniques in a synergistic manner. Adopting IPM techniques has been shown to have several advantages, such as reduced pesticide usage and related dangers, greater crop yields and quality, improved biodiversity, and increased agricultural systems' resilience and profitability. As a possible substitute for the overuse and careless application of chemical pesticides, integrated pest management (IPM) has emerged as a promising and sustainable paradigm for crop protection. IPM aims to reduce threats to the

environment and public health while maintaining pest populations below economically detrimental levels by combining a variety of preventive, biological, cultural, and chemical control techniques in a synergistic manner. Adopting IPM techniques has been shown to have several advantages, such as reduced pesticide usage and related dangers, greater crop yields and quality, improved biodiversity, and increased agricultural systems' resilience and profitability.

Future Perspectives

Future perspectives in Integrated Pest Management (IPM) emphasize the need for innovative, adaptive, and sustainable approaches to address emerging challenges in modern agriculture. Rapid changes in climate, intensified cropping systems, global trade, and evolving pest resistance patterns are reshaping pest dynamics, necessitating continuous refinement of IPM strategies. Climate change, in particular, is expected to alter pest phenology, geographic distribution, and population growth rates, leading to the emergence of new pest complexes and increased pressure from existing ones. Therefore, predictive modeling using climate-based forecasting tools and degree-day models will play an increasingly critical role in proactive pest management. Technological advancements are expected to significantly enhance IPM implementation. The integration of precision agriculture tools such as remote sensing, geographic information systems (GIS), unmanned aerial vehicles (UAVs), and automated pest detection systems will enable real-time

monitoring and early detection of pest outbreaks at field and landscape scales. Artificial intelligence (AI) and machine learning algorithms can further support accurate pest identification, population forecasting, and decision-making, reducing dependency on calendar-based pesticide applications.Biological control will remain a cornerstone of future IPM programs. Strengthening conservation biological control through habitat diversification, ecological engineering, and reduced pesticide disturbance will enhance the effectiveness of natural enemies. Advances in molecular biology, genomics, and microbiome research may facilitate the development of improved biocontrol agents, including entomopathogenic microbes and endophytes with pest-suppressive properties. Emerging technologies such as RNA interference (RNAi), sterile insect techniques (SIT), and enhanced semiochemical-based mating disruption offer promising alternatives to conventional insecticides and warrant further field-level validation.Host plant resistance will also gain renewed importance. The use of resistant cultivars and rootstocks, supported by molecular breeding, marker-assisted selection, and gene-editing technologies, can provide durable and environmentally benign pest control solutions. Combining host resistance with biological and cultural practices will strengthen system-level resilience and reduce reliance on chemical inputs.Socio-economic and policy dimensions will be equally important in shaping the future of IPM. Farmer education, participatory research, extension services, and decision-support

platforms must be strengthened to encourage widespread adoption of IPM practices. Economic incentives, regulatory frameworks promoting reduced-risk pesticides, and harmonization of food safety standards will further support sustainable pest management transitions.In conclusion, the future of IPM lies in its evolution as a knowledge-intensive, technology-driven, and ecologically grounded approach. Continued interdisciplinary research, innovation, and stakeholder engagement will be essential to ensure that IPM remains effective, economically viable, and environmentally responsible in safeguarding global food security.

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BÖLÜM 10

Insect Pest Complex of Apple and Their Integrated Management Strategies

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Introduction

Pests represent one of the most critical challenges in global agriculture, inflicting substantial economic losses by damaging crops through direct feeding, oviposition, transmission of pathogens, and production of honeydew that fosters sooty molds (Angela, 2018). In fruit production, particularly apples a crop valued at billions annually worldwide these threats can reduce yields by 20-50% (Anucha, 2024) or more if unmanaged, compromising fruit quality, size, and marketability while increasing production costs. Integrated Pest Management (IPM) has emerged as the cornerstone strategy, emphasizing scouting, economic thresholds, biological controls, cultural practices, and judicious pesticide use to sustain productivity while minimizing environmental impacts and resistance development. Apple orchards face a diverse array of pests with complex life cycles, often overlapping generations that demand precise timing for interventions. Key threats include codling moth *(Cydia pomonella),* whose larvae tunnel into fruit cores, rendering apples unmarketable; woolly apple aphid (*Eriosoma lanigerum*), which forms protective waxy colonies on roots and branches, stunting tree growth; and San Jose scale (*Quadraspidiotus perniciosus*), an armored scale causing bark cracking and fruit blemishes via sap-feeding. Additional pests like leafminers (e.g., *Lyonetia clerkella*) create serpentine mines that impair photosynthesis, spider and rust mites induce leaf stippling and russeting, and leafrollers (e.g., obliquebanded) web foliage and scar fruit surfaces. Effective IPM begins with dormant oils and delayed-dormant sprays to target overwintering stages of scales and mites, progresses to pheromone traps for timing codling moth and leafroller controls at petal fall through cover sprays, and incorporates predators like parasitic wasps and predatory mites to suppress aphids and scales naturally. Sanitation, resistant rootstocks, and degree-day

models further optimize outcomes, ensuring resilient orchards amid evolving pest (Zhou *et al.*, 2024)

Important pests of Apple

1.Woolly apple aphid (*Eriosoma lanigerum*) is a reddish-brown aphid covered with white, wool-like wax secretions that give it a characteristic fluffy appearance (Fig 1a,1b). It infests roots, trunks, limbs, shoots, and occasionally fruit of apple trees, forming colonies protected by the waxy material. (Baker, 1915).

Figure 1a

Fig. 1b

Symptoms

Woolly apple aphid causes stunting in young and mature trees through the formation of root galls or aerial galls on branches and trunks. Heavily infested leaves curl into rosettes. High populations

lead to honeydew accumulation, which promotes sooty mold growth. Aphids may also infest fruit, especially in the calyx area. (UCIPM, 2014).

Life Cycle

The aphid overwinters as "naked" nymphs sheltered in cracks or under loose bark on apple trees. In spring, nymphs become active and produce large white, woolly colonies mainly on spurs and branches. Winged aphids may be produced which migrate to roots or other host plants; multiple generations occur during summer. Colonies can persist on roots underground, sometimes causing root damage and galls (Elizabeth *et al.,* 2019).

Management

Management includes use of resistant rootstocks (such as Malling 106 and 111) and varieties, biological control using the parasitic wasp *Aphelinus mali,* and careful pesticide use to avoid disrupting natural enemies. Delayed dormant applications and summer sprays targeting crawlers are effective. Organically acceptable methods emphasize resistant varieties and biological controls. Chemical options include insect growth regulators and selective insecticides applied while considering environmental impacts and resistance management (Walker, 1985).

2. Codling moth (*Cydia pomonella*) is a major pest of apples and pears worldwide, recognized by its small grayish moths with a 0.5- to 0.75-inch wingspan and coppery-brown banded forewings (Fig.2) Larvae are creamy-white to pinkish caterpillars with brown heads that bore into fruit, often called "apple worms." It primarily attacks

apples but also infests pears, quince, and other fruits like walnuts in some regions. (UCIPM, 2014).

Figure 2

Symptoms

Larvae cause two main damage types: shallow “stings” where they penetrate the skin but die shortly after, leaving red-ringed entry holes with frass, and deep entries tunneling to the core, filling fruit with frass and leading to rot or premature drop. Infested fruit becomes unmarketable, with brown frass-extruding holes near the calyx or stem end. High populations can destroy up to 95% of a crop if unmanaged.

Life Cycle

Codling moth overwinters as mature larvae in silken cocoons under bark, in soil, or litter. Adults emerge in spring near bloom, females lay up to 100 eggs on leaves or fruit, and larvae hatch to bore into

developing apples, feeding for 3-4 weeks before exiting to pupate. Typically, two generations occur per year in temperate regions, with activity peaking at dusk and dawn.

Management

Integrated strategies include sanitation (remove fallen fruit and debris), mating disruption with pheromones, and monitoring via traps to time sprays. Biological controls like predators and parasites help, while chemical options target eggs or young larvae using degree-day models. Home orchards benefit from trunk bands to trap larvae and resistant varieties where available.

San Jose scale (***Quadraspidiotus perniciosus*****)** is a small, armored scale insect with a hard, gray to black, cone-shaped waxy covering about 1-2 mm in diameter, featuring a central white knob and radiating grooves Females are wingless, yellow, and legless, while males are tiny winged insects resembling gnats. It attacks apples, pears, stone fruits, and ornamentals by sucking sap from bark, branches, leaves, and fruit. (Stanley, 1993).

Figure 3

Symptoms

Feeding causes red to purple halos or spots around punctures on fruit, leading to depressions, misshapen, dull-colored apples, and reduced market value. On branches and trunks, heavy infestations result in twig dieback, bark cracking, defoliation, and tree decline or death. Honeydew production promotes sooty mold, further impairing photosynthesis (Figure 3).

Life Cycle

Overwinters as immature scales (nymphs) on bark. In spring, males emerge as tiny flies, mate with sessile females, and crawlers (first-instar nymphs) hatch, dispersing by wind or crawling before settling to form scales. Multiple overlapping generations (2-5 per year) occur, with peak crawler activity in late spring and summer.

Management

Dormant or delayed-dormant oil sprays smother overwintering scales, while summer insecticide applications target crawlers using degree-day timing and monitoring with traps or double-sided tape. Promote natural enemies like parasitic wasps; avoid broad-spectrum pesticides that disrupt them. Sanitation, resistant rootstocks, and thorough coverage in older trees enhance control.

4.Apple leaf miner (*Lyonetia clerkella*) is a small moth whose larvae mine inside apple leaves, causing visible serpentine tunnels starting near the main veins. The mines start as narrow, white tracks and widen as the larva grows, turning brownish and often branching into several directions. This pest can have two to three generations

per year, with larvae active mainly from June to September, feeding inside leaves and spinning silken cocoons on the leaf underside after feeding. (European Forest Institute., 2025), (Figure 4).

Figure 4

Symptoms

Damaged leaves show winding, whitish or brownish trails (mines) that can cause parts of the leaf to turn brown and drop out, leading to defoliation in severe cases. Multiple larvae can infest a single leaf, impacting photosynthesis and overall tree vigor. The damage is visible as discolored, dead leaf tissue where the larvae have fed internally.

Life Cycle

Adults emerge in early summer, lay eggs on leaf surfaces, and larvae hatch to mine leaves. After feeding internally, larvae exit to pupate on the leaf or nearby. The species overwinters as pupae or adults in sheltered locations, with new generations emerging the following season.

Management

Management focuses on monitoring for leaf miner activity and timing insecticide applications to target early larval stages before they enter leaf tissue. Cultural practices such as removing fallen leaves and maintaining tree health reduce infestation. Insecticides, including specific systemic or contact options, are used based on regional recommendations and timing.

5.Apple mites: Particularly European red mite (*Panonychus ulmi*), two spotted spider mite, and apple rust mite, are tiny arachnids (less than 0.5 mm) that infest apple leaves, with spider mites showing webbing and rust mites appearing as elongated, annulate forms visible only under magnification. (University of Massachusetts.,2024) (Figure 5a, 5b).

Figure 5a

Figure 5b

Symptoms

Spider mites cause stippling (yellow-white spots) on leaves from cell puncturing, leading to bronzing, yellowing, or browning, with heavy webbing in advanced infestations. Rust mites produce a silvery or russeted leaf cast, fruit russeting, or blisters (in gall-forming types),

reducing photosynthesis and tree vigor. Severe damage results in leaf drop and weakened yields.

Life Cycle

Mites overwinter as eggs or adults under bud scales; spring activation leads to rapid reproduction with multiple generations (up to 10-20) per season, peaking in hot, dry conditions. Immatures resemble smaller adults; all stages feed actively, with rust mites active until late summer.

Management

Monitor with hand lenses or beat sheets; apply miticides targeting motile stages or eggs, timed via degree-days, while conserving predators like predatory mites. Dormant oils smother overwintering stages; avoid broad-spectrum insecticides that disrupt biological control. Cultural practices include irrigation to reduce dust and promote tree health.

6.Apple leaf rollers primarily obliquebanded (*Choristoneura rosaceana*) and redbanded (Argyrotaenia velutinana) leaf rollers, are small moths (0.5-1-inch wingspan) with tan to brown wings featuring distinctive bands or mottling. Larvae are green caterpillars (up to 1 inch long) with brown to black heads that roll leaves with silk for feeding shelters (Figure 6). They infest apples, causing damage to foliage and fruit. (Giving Grove, 2022).

Figure 6

Symptoms

Larvae feed on buds, leaves, and fruit, creating rolled or webbed leaves with frass and holes; severe defoliation occurs in spring. On fruit, they cause shallow surface scars or bites without deep tunneling or much frass, leading to irregular brown patches and reduced quality. Damage is often more evident in upper canopy and spur varieties.

Life Cycle

Overwinter as partially grown larvae under bark; they resume feeding in spring on buds and shoots before pupating in rolled leaves. Adults emerge late spring to lay eggs; second generation hatches in summer, with larvae dropping on silken threads when disturbed. Two main generations per year, peaking with new growth.

Management

Monitor buds and shoots early spring for larvae, webbing, or frass; use pheromone traps for timing. Apply insecticides targeting young larvae at egg hatch; conserve predators and parasitoids by avoiding

broad-spectrum sprays. Sanitation like removing debris and pruning infested parts aids control.

Conclusion

Apple production worldwide faces persistent challenges from a diverse complex of insect pests that threaten both yield and fruit quality. Key pests such as woolly apple aphid, codling moth, San Jose scale, leaf miners, mites, and leaf rollers exhibit multiple generations and concealed feeding habits, making their management highly demanding. Their damage ranges from direct feeding on fruit and foliage to physiological stress, premature fruit drop, and increased susceptibility to secondary infections. Effective and sustainable protection of apple orchards therefore relies on Integrated Pest Management (IPM) strategies that prioritize regular monitoring, accurate identification, and timely intervention based on economic thresholds. Incorporating biological control agents like *Aphelinus mali*, preserving natural predators, utilizing resistant cultivars and rootstocks, and applying selective pesticides only when necessary helps maintain ecological balance while reducing chemical dependency and resistance development. Cultural operations such as orchard sanitation, proper pruning, and removal of infested plant parts further strengthen pest suppression. As climate change and intensified cultivation alter pest dynamics, continuous research, grower training, and adoption of predictive tools like pheromone traps and degree-day models will remain critical to ensure economically viable and environmentally responsible apple production.

Future Research and Strategies

Future efforts in apple pest management must focus on developing more resilient and environmentally sustainable approaches as pest behavior and distribution continue to evolve. Climate change, globalization of trade, and shifts in orchard design will likely

influence pest population dynamics, promote invasive species, and increase the risk of resistance to pesticides. Therefore, research directions should include the development of advanced forecasting tools using remote sensing, artificial intelligence, and improved degree-day models to enhance early detection and optimize spray timing. Expanding the use of resistant cultivars and rootstocks, supported by molecular breeding and genomic tools, will help provide long-term suppression of pests such as woolly apple aphid and San Jose scale.Strengthening biological control through conservation of natural enemies, habitat diversification, and augmentation of parasitoids and predators remains essential. Innovative technologies like sterile insect techniques, RNA interference (RNAi), and semiochemicals for enhanced mating disruption offer promising alternatives to conventional insecticides. Additionally, studies focusing on soil–plant–pest interactions, particularly for root-feeding pests, will support the development of holistic orchard systems.

Improving grower education, field-level pest surveillance networks, and decision support systems will ensure that strategies are implemented effectively and consistently. Integration of sustainable practices such as organic amendments, botanical insecticides, and precision application methods will minimize environmental impacts and support ecological resilience. Ultimately, continued interdisciplinary research and adaptive management practices will be crucial for safeguarding apple industry productivity and profitability in the years ahead.

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GEÇİCİ KAPAK

Kapak tasarımı
devam ediyor.