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*Original article (Orijinal araştırma)*

# Landmark-based analysis of honey bee wing variation: findings from some regions of Van, Hakkari (Türkiye) and Iran [1]

## Balarısı kanat varyasyonunun landmark temelli analizi: Van, Hakkari (Türkiye) ile İran'ın bazı bölgelerden elde edilen bulgular

**Simanur AKÇAKAYA[2]** **Cengiz ERKAN[3]** **Cansu Özge TOZKAR[2*]**

## Abstract

This study investigates the geometric morphometric characteristics of honey bee colonies selected from certain regions of Eastern Anatolia and Iran. Wing samples from 1738 worker bees were collected from stationary colonies in the districts of Van and Hakkari (Türkiye), as well as Iran. Shape and size differences were examined using 20 landmark points on the right forewings of the samples. Procrustes ANOVA revealed significant differences between locations and apiaries ($p$<0.001). Canonical variate analysis (CVA) and principal component analysis (PCA) showed that Gevaş and Iran samples differentiated from other groups. Discriminant function analysis showed significant differences among all locations ($p$<0.0001). Substantial differences were observed between Iran and other locations, followed by differences between Gevaş and the other locations. The distribution pattern of Hakkari samples being closer to Iranian samples rather than Van samples emerged as an intriguing finding in the study. Deformation grid analysis highlighted specific landmark points contributing to these differences. The results indicate that the geometric morphometric differences in the region have been preserved, while also pointing to the potential hybridization effects caused by migratory beekeeping practices and queen bee trade. This study provides critical baseline data for understanding the morphological variation of honey bees in the region and highlights the importance of conserving locally adapted honey bee populations.

**Keywords:** Eastern Anatolia, geometric morphometrics, honey bee diversity, landmark analysis, wing variation

## Öz

Bu çalışma, Doğu Anadolu ve İran'ın belirli bölgelerinden seçilmiş bal arısı kolonilerinin geometrik morfometrik özelliklerini incelemektedir. Van ve Hakkari (Türkiye) illeri ile İran'daki sabit arıcılık kolonilerinden toplam 1738 işçi arının kanat örnekleri toplanmıştır. Örneklerin sağ ön kanatlarında 20 landmark noktası kullanılarak şekil ve boyut farkları incelenmiştir. Procrustes ANOVA, lokasyonlar ve arılıklar arasında önemli farklar olduğunu ortaya koymuştur ($p$<0.001). Kanonik değişken analizi (CVA) ve temel bileşen analizi (PCA), Gevaş ve İran örneklerinin diğer gruplardan farklılaştığını göstermiştir. Ayırıcı fonksiyon analizi, tüm lokasyonlar arasında önemli farklar olduğunu göstermiştir ($p$<0.0001). İran ve diğer lokasyonlar arasında belirgin farklar ortaya çıkarken bunu Gevaş ve diğer lokasyonlar arasındaki farklar izlemiştir. Hakkari örneklerinin Van örneklerinden ziyade İran örneklerine daha yakın bir dağılım paterni göstermesi araştırmada ilginç bir bulgu olarak ortaya çıkmıştır. Deformasyon ızgarası analizi, bu farklara katkıda bulunan belirli landmark noktalarını vurgulamıştır. Sonuçlar, bölgedeki geometrik morfometrik farklılıkların korunduğunu ortaya koymakla birlikte, göçer arıcılık faaliyetleri ve ana arı ticaretinin yol açabileceği melezleşme etkilerine de işaret etmektedir. Bu araştırma, bölgedeki bal arılarının morfolojik varyasyonunu anlamak için kritik temel veriler sağlamaktadır ve bölgeye uyum sağlamış yerel bal arısı popülasyonlarının korunmasının önemini vurgulamaktadır.

**Anahtar sözcükler:** Doğu Anadolu, geometrik morfometri, balarısı çeşitliliği, landmark analizi, kanat varyasyonu

[1] This study was part of the MSc thesis of the first author at the Institute of Natural and Applied Sciences, Van Yüzüncü Yıl University.

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## Introduction

The Western honey bee, *Apis mellifera* L., 1758 (Hymenoptera: Apidae) is vital for ensuring environmental, agricultural, and economic sustainability. They greatly benefit the ecosystem through their contribution to the pollination of flowering plants and agricultural crops. Additionally, they support plant diversity and preserve biodiversity by pollinating wild plants for wildlife. Honey bees contribute to the global economy by providing natural products such as pollen, royal jelly, propolis, honey, and beeswax to beekeeping, agriculture, and related industries.

As a result of the adaptation of *A. mellifera* to different regions worldwide, various geographic subspecies have emerged. Each subspecies possesses morphological and physiological characteristics specific to its geographical region and exhibits genetic variations. It is noted that the Anatolian geography, which encompasses various climate types, has been influential in the evolutionary process of honey bees in Türkiye (Kence, 2006). Thus, Anatolia is one of the important bee gene centers. Honey bees in Türkiye are categorized under the species *A. mellifera*. *Apis mellifera carnica* Pollmann, 1879 is found in the Thrace region*, Apis mellifera meda* Skorikov, 1929 in the Southeastern Anatolia region*, Apis mellifera syriaca* Skorikov, 1929 in a small area in Hatay-Antakya, *Apis mellifera caucasica* Gorbachev, 1916 from Samsun to Northeast Anatolia, and *Apis mellifera anatoliaca* Maa, 1953 in the Aegean, Central Anatolia, Mediterranean, and western and central parts of the Black Sea regions. Iranian bees were first described by Skorikov in 1929 as *A. mellifera meda*. Although the distribution of this subspecies was initially reported as the Caspian Sea and Northern Iran, subsequent studies have shown that it extends to Syria and Northeastern Türkiye. The *A. mellifera meda* subspecies is widely distributed, and the presence of six geographically distinct local *A. mellifera meda* populations has been reported (Ruttner, 1988).

Quantitative morphometric analyses are frequently used in the delineation of many species. The method, which is based on the direct measurement of individual characteristics, has generally yielded successful results (Schwarzfeld & Sperling, 2014). Many researchers have conducted studies using morphological traits such as the wing and body sizes of honey bees, as well as the lengths of legs, tongues, and hairs. Through these studies, groups can be formed, and comparisons can be made using quantitative traits (Taşkıran et al., 2017). The honey bee subspecies distributed in Türkiye and the Middle East exhibit distinct morphological characteristics. For instance, one of the most common subspecies in Türkiye, *A. mellifera anatoliaca*, is characterized by a broad abdomen, short wings, and yellow-colored bodies with orange-brown rings (Ruttner, 1988). Found in northeastern Anatolia, *A. mellifera caucasica* is notable for its long proboscis (up to 7.2 mm) and dark chitinous body covered with gray hairs (Ruttner, 1988; Kandemir et al., 2000). *A. mellifera syriaca*, distributed in southeastern Anatolia, particularly in Hatay, can be identified by its slender body, short hairs, yellow abdominal segments, and a bright yellow scutellum (Ruttner, 1988; Kandemir et al., 2000). These morphological differences influence the ecological adaptations, nectar collection capacities, and colony management traits of the subspecies, providing a critical foundation for genetic and biogeographic studies (Ruttner, 1988; Franck et al., 2000).

Wing shape is a key aspect of an insect's phenotype, closely linked to a critical feature as flight properties. Consequently, the study of geomorphometry or shape of wings, whether of honey bees or other winged insects, can have major impacts across different taxonomic levels for detecting geographical variations, population structures, shape differences, or taxonomic classification. Geomorphometry is a method that utilizes cartesian coordinates of landmark points instead of linear, angular, and proportional calculations in the quantitative interpretation of morphometric data. Geometric morphometry uses landmark points, which are adjusted through shifting, resizing, and orienting to remove size-related effects. Once aligned, the reference point arrangements vary only in shape and can be assessed reliably and cost-effectively using multivariate statistical methods for a large number of samples in a short time. With the advancement of geometric morphometric methods, researchers have begun to conduct classification studies of honey bee

subspecies using geomorphometric analyses of wing shapes (Kekeçoğlu et al., 2007; Tofilski, 2008; Francoy et al., 2009; Kandemir et al., 2011).

This study aimed to interpret honey bee samples collected from stationary colonies in Van, Hakkari, and Iran using geomorphometric wing analyses to reveal the similarities and differences between existing honey bee colonies. The results obtained from the parameters evaluated in this study serve as preliminary findings that will support future, more comprehensive phylogenetic studies representing the entire Eastern and Southeastern Anatolia region. Although our country is quite rich in honey bee genetic resources, unfortunately, these resources cannot be used effectively and protected. Another aim of this study was to contribute to making our honey bee genetic resources more accessible and conserved by providing geomorphometric diversity data.

## Materials and Methods

### Materials

The honey bee samples used in this study were obtained from stationary honey bee colonies in the regions of Van (Gevaş, Çatak, Özalp, Başkale), Hakkari (Otluca, Akçalı, Kanatlı), and Iran (Urmia-Bavan-Bardehzi-Gundikemele) between 2021 and 2022 (Table 1).

The locations from which honey bee samples were gathered, along with the number of samples collected and analyzed from each district, are shown in Table 1. A total of 1750 worker bee samples were collected from 24 apiaries, 69 hives.

The sampling process was conducted on worker bees inside the hive. Samples were individually collected using forceps and placed into sample beakers. Subsequently, the honey bees were anesthetized with ether, transferred to falcon tubes, and 96% ethyl alcohol was added. The falcon tubes were tightly sealed and stored at +4°C for geometric morphometric analyses.

From each hive, the right forewings of 30 worker bees were collected, resulting in an initial total of 2070 samples from 69 hives. During quality control, wings with deformities, damage, or inconsistent landmark identification were excluded to ensure reliable geometric morphometric measurements. Additional exclusions were made during analysis using MorphoJ software due to misaligned or statistically unsuitable TPS files. After these rigorous quality control steps, the final dataset comprised 1750 worker bee samples, of which 1738 high-quality samples were analyzed for geometric morphometric characteristics.

During the preparation stage for geometric morphometric analysis, only the right forewings of the bees were placed on microscope slides, while the other wings were separated but not used in the study. To prevent folding, alcohol was added to each wing. The right forewings were then fixed on microscope slides and labeled. During the digitization, processing, and editing of forewing images, wings fixed between slides for the landmark method were adjusted to the desired size using a Leica M165C stereomicroscope (Leica Microsystems, Wetzlar, Germany) at 20× magnification. High-resolution images were then captured using a Leica DFC450 digital camera (Leica Microsystems, Wetzlar, Germany), which has a resolution of 5 megapixels. The images were saved in JPEG format at 300 dpi. The wing photographs were labeled based on their locations and stored in separate folders on the computer.

Table 1. Study locations and beekeeping data across different altitudes in Eastern Türkiye and Iran

| Country | Province | District | Village | Number of Beekeepers | Number of Hives | Coordinates | Altitude (meters) | Field dates |
|---|---|---|---|---|---|---|---|---|
| Türkiye | Van | Gevaş (n=622) | Merkez | 1 | 3 | 38.298°N 43.106°E | 1.750 | July 2021 |
| | | | Göründü | 1 | 3 | 38.345°N 42.918°E | | |
| | | | Değirmitaş | 3 | 12 | 38.344°N 42.850°E | | |
| | | | Ortamahalle | 1 | 3 | 38.291°N 43.110°E | | |
| | | Çatak (n=188) | Dalbastı | 1 | 4 | 37.904°N 42.942°E | 1.500 | October 2021 |
| | | | Büyükağaç | 2 | 7 | 37.860°N 43.097°E | | |
| | | | Kaçit | 2 | 6 | 37.930°N 42.989°E | | |
| | | | Atlıhan | 1 | 3 | 37.919°N 42.821°E | | |
| | | Başkale (n=451) | Aşağı Darıca | 1 | 3 | 37.768°N 44.168°E | 2.320 | November 2022 |
| | | | Yukarı Darıca | 1 | 3 | 37.754°N 44.178°E | | |
| | | | Çaldıran | 1 | 3 | 37.790°N 44.125°E | | |
| | | Özalp (n=133) | Dönerdere | 1 | 3 | 38.737°N 44.123°E | 1.994 | November 2022 |
| | | | Yukarı Tulgalı | 1 | 3 | 38.772°N 44.260°E | | |
| Türkiye | Hakkari | Merkez (n=154) | Otluca | 1 | 3 | 37.601°N 43.693°E | 1.756 | November 2022 |
| | | | Akçalı | 1 | 3 | 37.711°N 43.992°E | | |
| | | | Kanatlı | 1 | 3 | 37.716°N 44.026°E | | |
| İran | Ürmiye | Merkez (n=190) | Merkez | 1 | 1 | 37.555°N 45.083°E | 1.330 | November 2022 |
| | | | Bavan | 1 | 1 | 37.546°N 44.762°E | | |
| | | | Gundikemele | 1 | 1 | 37.494°N 44.793°E | | |
| | | | Bardehzi | 1 | 1 | 37.494°N 44.793°E | | |

## Methods

### Marking the landmark points

The image set was transformed into a TPS format utilizing specialized morphometric software 'TPS utility'. During the landmark digitization procedure, TPS data structures were generated through the application of tpsUtil32 v. 1.78 (Rohlf, 2019) software (Figure 1).

The tpsDig2 v. 2.31 (Rohlf, 2018) software was utilized to obtain the wing coordinates. Following Bookstein's criteria for landmark identification, a set of twenty specific points on the right wings were precisely mapped and recorded digitally (Bookstein, 1990). This process was repeated for all samples, and TPS files were created for statistical analyses.

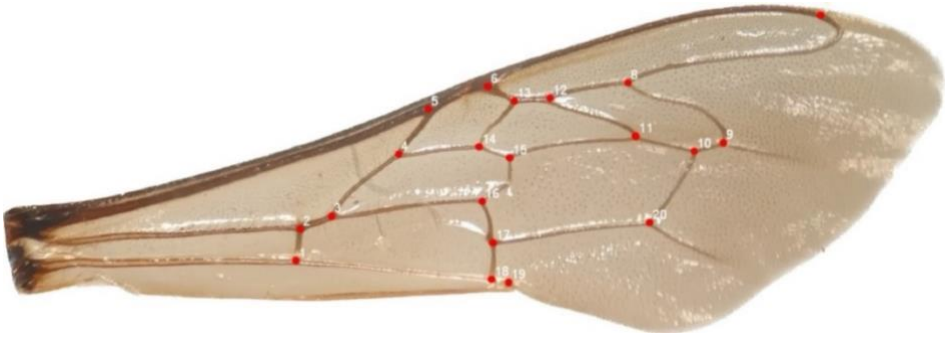

Figure 1. Landmark locations on the right forewing used in the study.

### MorphoJ analysis

The TPS files containing the raw coordinates of the landmarks were analyzed using MorphoJ software version 1.06 (Klingenberg, 2011) for the purpose of extracting 'x, y' coordinates and which subsequently facilitated the derivation of the size and shape parameters. The raw coordinates were aligned by removing scale, position, and orientation differences using the Procrustes Fit Function. Procrustes Fit Analysis, Procrustes ANOVA, Principal Component Analysis (PCA), Lollipop Graph, Discriminant Function Analysis (DFA) and Canonical Variate Analysis (CVA) were conducted with Morpho J software.

Procrustes ANOVA, used to determine variations between locations and apiaries in the study, is a technique applied to identify differences of the shape and size of centroids. Centroid size is a calculation method that determines the square root of the total of squared lengths from an object's landmarks to their centroid. Canonical variate analysis (CVA) is employed to assess and explain the differences among two or more groups within a given dataset. Mahalanobis distance (MD) is a powerful parameter for measuring multivariate distance, which calculates the separation between an individual observation and a statistical distribution. Procrustes distance, on the other hand, represents the square root of the total of squared deviations in landmark configurations between datasets. This measure is typically utilized to evaluate morphological similarities or differences between objects, reflecting the average shape variation between datasets. Discriminant Function Analysis (DFA) calculates the Mahalanobis distance relative to group centroids to classify an unknown entity. Principal Component Analysis (PCA) was utilized to illustrate variations in shape across the samples. The principal elements of the landmarks were displayed using a lollipop chart. Additionally, a PCA histogram showed the percentage of observations contributing to shape variability (Klingenberg, 2011).

## Results and Discussion

### Results

### Procrustes fit analysis (generalized Procrustes analysis)

The superimposition of the data was performed using Procrustes Fit Analysis. Out of the forewing samples, 1738 were included in the analyses by MorphoJ. Figure 2 displays a scatter diagram with the overlaid landmarks illustrating the overall morphological form of the right wing of the honey bee using twenty landmark points for the 1738 observations.

The Generalized Procrustes Analysis illustrating the scatter graph of the overlaid configuration set of 1738 right wing landmarks; blue dots indicate the average positions of the landmarks of all samples, while the small black dots denote the landmarks for individual specimens.

Figure 2. Superimposition of the landmarks on the forewing.

### Procrustes Anova (one-way analysis of variance)

Separate ANOVA tables present the outputs of the Procrustes ANOVA analysis for both size and shape of centroids. The Procrustes ANOVA test, a univariate analysis of variance applied to evaluate population differences, indicated significant size and shape differences among the populations based on their locations. The differences in centroid size were also found to be significant in statistical terms ($p$<0.001) (Table 2). Regarding centroid size, the variations among locations ($F$ = 149.47) showed a higher $F$ value than those among the apiaries ($F$ = 68.80). In terms of shape, greater differences were detected among locations ($F$ = 38.58) compared to those among the apiaries ($F$ = 17.16) based on the $F$ value (Table 2).

Table 2. Procrustes ANOVA results by location. Sum of squares (SS), mean squares (MS), degrees of freedom (df), Goodall's $F$ statistic ($F$), and $p$-value ($p$)

| Centroid size: | | | | | | | |
|---|---|---|---|---|---|---|---|
| Effect | SS | MS | df | $F$ | $P$ (param.) | | |
| Individual | 8713286.930017 | 1742657.386003 | 5 | 149.47 | <.0001 | | |
| Residual | 19902271.475878 | 11659.210003 | 1707 | | | | |
| Shape, Procrustes ANOVA: | | | | | | | |
| Effect | SS | MS | df | $F$ | $p$(param.) | Pillai tr. | $p$ |
| Individual | 0.13805243 | 0.0007669580 | 180 | 38,58 | <.0001 | 1.16 | <.0001 |
| Residual | 1.22165853 | 0.0000198799 | 61452 | | | | |

### Canonical variate analysis (CVA)

Canonical variate analysis was used to test whether there were differences in forewing shapes according to locations and apiaries. CVA generates canonical variables (CV) by rotating and adjusting the centroids and establishes Mahalanobis distance (MD) between categories calculated from the centroids of the observations. The distributions of populations were also demonstrated by canonical variate analysis. The variation among groups was scaled by the inverse of the within-group variation. The variance percentages among the CV (canonical variate) values of the locations (Van districts, Hakkari, and Iran) are provided in Table 3. Groups determined by location and apiaries showed diversity in the discriminant analysis based on Mahalanobis and Procrustes distances according to 10,000 permutation rounds ($p$<.0001).

Table 3. Eigenvalues, variance, and cumulative values among CV groups formed by location

| Groups | Eigen values | Variance % | Cumulative % |
|---|---|---|---|
| CV1 | 0.98187839 | 53.771 | 53.771 |
| CV2 | 0.38370883 | 21.013 | 74.784 |
| CV3 | 0.27139115 | 14.862 | 89.646 |
| CV4 | 0.12405223 | 6.794 | 96.440 |
| CV5 | 0.06501205 | 3.560 | 100.000 |

The scatter plot representing the canonical variate analysis was created to distinguish the similarities and differences in wing shapes of honey bees taken from different locations, considering the variables. According to the CVA graphs obtained from the canonical variate analysis, samples from L2 (Başkale), L3 (Çatak), L4 (Özalp) districts, and L5 (Hakkari) province were found to be intermingled, while comparing based on the Gevaş and Iran axes, the L3 (Çatak) ellipse showed more intersection with Gevaş samples, and the L2 (Başkale)-L5 (Hakkari) ellipses intersected more with L6 (Iran) samples. Although some overlaps were observed, certain samples from these four groups (L2-L3-L4-L5) clustered closely with the Gevaş (L1) and Iran (L6) groups. However, the Gevaş and Iran samples exhibited a noticeably different distribution compared to other groups (Figure 3).

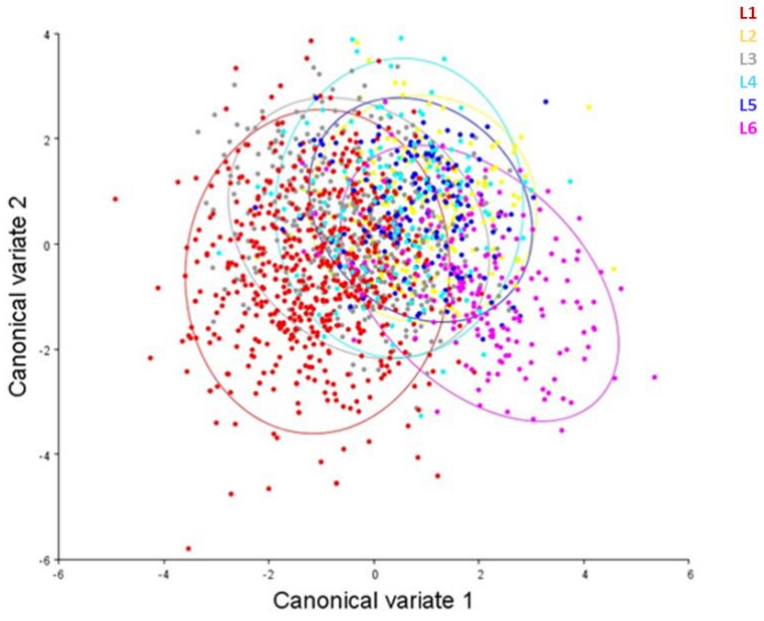


Figure 3. Scatter plot of form differences on the first two canonical variate axes, created by 20 landmarks on honey bee wing samples taken from six locations (L1-L6). L1-Gevaş is represented in red, L2-Başkale in yellow, L3-Çatak in gray, L4-Özalp in turquoise, L5-Hakkari in navy blue, and L6-Iran in pink.

According to another CVA graph created in the canonical variate analysis, the samples from the twenty-four apiaries, regardless of location, were found to be intermingled, indicating within-group variation.

**Principal component analysis (PCA) of geometric morphometric data**

As another method for variation analyses based on different locations in the landmark data (20 landmarks on the forewing), principal component analysis was applied. PCA was employed to illustrate shape changes in the observations. A PCA distribution chart also displayed the percents of samples contributing to shape deviations. Eigenvalues represent the separation of different directions. The extent to which each direction explains the overall phenotypic variation in the entire data set is shown with an orthogonal bar diagram. PCA revealed 36 principal components (PCs) accounting for the total variation.

According to the diagram, the first principal component (PC1) impacted the most to shape variation with 25%, followed by the second principal component (PC2) with 12%. The first five dimensions explained 60% of the overall variation, while the first ten dimensions explained 79% (Figure 4).

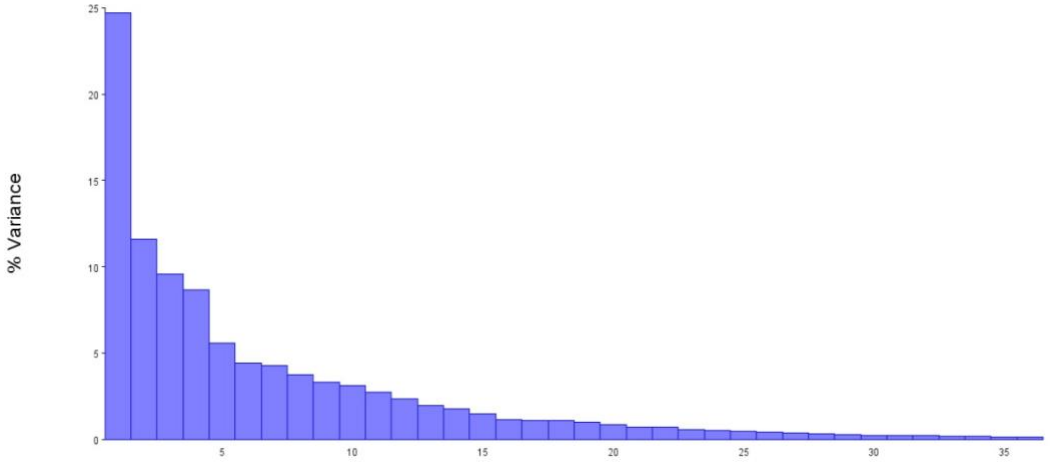


Figure 4. Variance distribution according to principal components as a result of principal component analysis.

The clusters formed in the PCA graph of the forewings of honey bee samples taken from six locations (Van districts, Hakkari, and Iran) exhibited a distribution similar to the CVA graph. Some samples from Gevaş (L1) and Iran (L6) showed a noticeable tendency to be distributed further from the center (Figure 5).

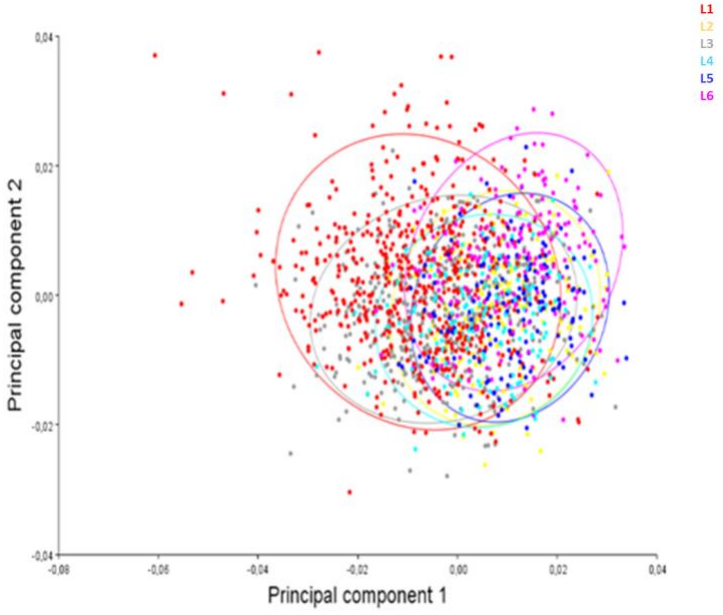


Figure 5. PCA graph of the forewings of honey bee samples taken from six locations. L1-Gevaş is represented in red, L2-Başkale in yellow, L3-Çatak in gray, L4-Özalp in turquoise, L5-Hakkari in navy blue, and L6-Iran in pink.

**Lollipop glut graph**

The shape changes in all forewings included in the analysis were shown in the 'lollipop glut' graph (Figure 6). The principal components of the landmarks were displayed in a lollipop diagram. The bars at the ends of the points represent the magnitude of shape change. The wing shape differences among honey

bee populations were visually analyzed using a deformation grid, which identified the landmarks where these differences were concentrated. The deformation grid defines the landmark regions that contribute most to the separation. The least change was observed at landmark points 9 and 10, while the greatest difference was seen at landmark point 7. This is followed by the magnitude of differences at landmark points 2 and 3. Variations among populations were also detected at the remaining landmark points.

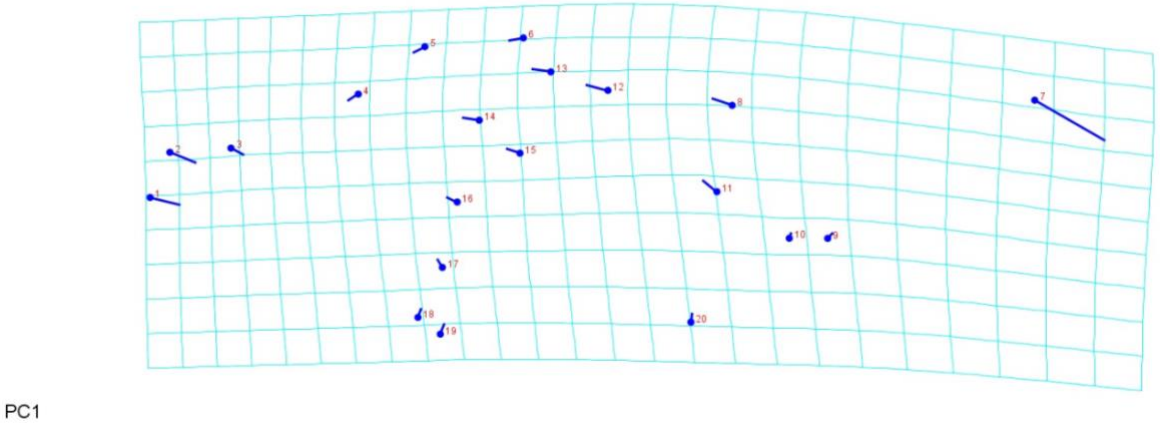


Figure 6. Landmark points where the wing shape differences were concentrated among honey bee populations. Blue points represent the average landmark values.

### Discriminant function analysis (DFA)

DFA selects the metrics that generates the most significant differences between data groups. An accurate classification test analyzes the mean values of these two groups in terms of Procrustes distances or Mahalanobis distances. Significant differences were found in pairwise comparisons among all locations (four districts of Van, Hakkari, and Iran) based on both Mahalanobis and Procrustes distances ($p$<.0001) (Table 4).

Table 4. Discriminant Function Analysis results for honey bee populations from different locations. (The permutation test using the T-square statistic is equivalent to a test using the Mahalanobis distance)

| Comparison | Procrustes Distance | Procrustes Distance (P) | T-Square (P) | Mahalanobis Distance | T-Square (value) | T-Square (P) |
|---|---|---|---|---|---|---|
| L1-L2 | 0.0170 | <.0001 | <.0001 | 2.4732 | 793.2917 | <.0001 |
| L1-L3 | 0.0081 | <.0001 | <.0001 | 1.5804 | 600.9674 | <.0001 |
| L1-L4 | 0.0157 | <.0001 | <.0001 | 2.3151 | 525.5498 | <.0001 |
| L1-L5 | 0.0195 | <.0001 | <.0001 | 2.3573 | 614.1914 | <.0001 |
| L1-L6 | 0.0216 | <.0001 | <.0001 | 3.0277 | 1231.4230 | <.0001 |
| L2-L3 | 0.0120 | <.0001 | <.0001 | 1.7549 | 459.6203 | <.0001 |
| L2-L4 | 0.0065 | <.0001 | <.0001 | 1.7482 | 282.9438 | <.0001 |
| L2-L5 | 0.0052 | <.0001 | <.0001 | 1.4762 | 270.1946 | <.0001 |
| L2-L6 | 0.0122 | <.0001 | <.0001 | 2.3100 | 678.3607 | <.0001 |
| L3-L4 | 0.0105 | <.0001 | <.0001 | 1.8260 | 390.9388 | <.0001 |
| L3-L5 | 0.0150 | <.0001 | <.0001 | 2.0070 | 529.3059 | <.0001 |
| L3-L6 | 0.0174 | <.0001 | <.0001 | 2.5553 | 1005.9193 | <.0001 |
| L4-L5 | 0.0077 | <.0001 | <.0001 | 1.4653 | 191.4848 | <.0001 |
| L4-L6 | 0.0145 | <.0001 | <.0001 | 2.6448 | 595.9205 | <.0001 |
| L5-L6 | 0.0135 | <.0001 | <.0001 | 2.2919 | 454.5249 | <.0001 |

According to Procrustes distances, shape differences were observed among all locations, with the greatest variation between Iran (L6) and other locations. Following Iran, the variation between Gevaş (L1) and other locations was also noteworthy. The most pronounced difference was between Iran (L6) and Gevaş (L1), followed by variations between Gevaş (L1)-Hakkari (L5), Iran (L6)-Çatak (L3), and Gevaş (L1)-Başkale (L2). Mahalanobis Distance (MD) quantifies how many standard deviations a point deviates from the average of a statistical distribution. As the distance increases, the variation between a sample and a distribution becomes broader. Accordingly, the widest variation was observed between Iran (L6) and Gevaş (L1) (Table 4).

The histogram data enabling pairwise comparisons based on discriminant scores confirmed the PCA and CVA results, showing variations between Gevaş and other locations (Figure 7 a-b-c-d-e), Iran and other locations, and overlaps between L2-L3-L4-L5 locations.

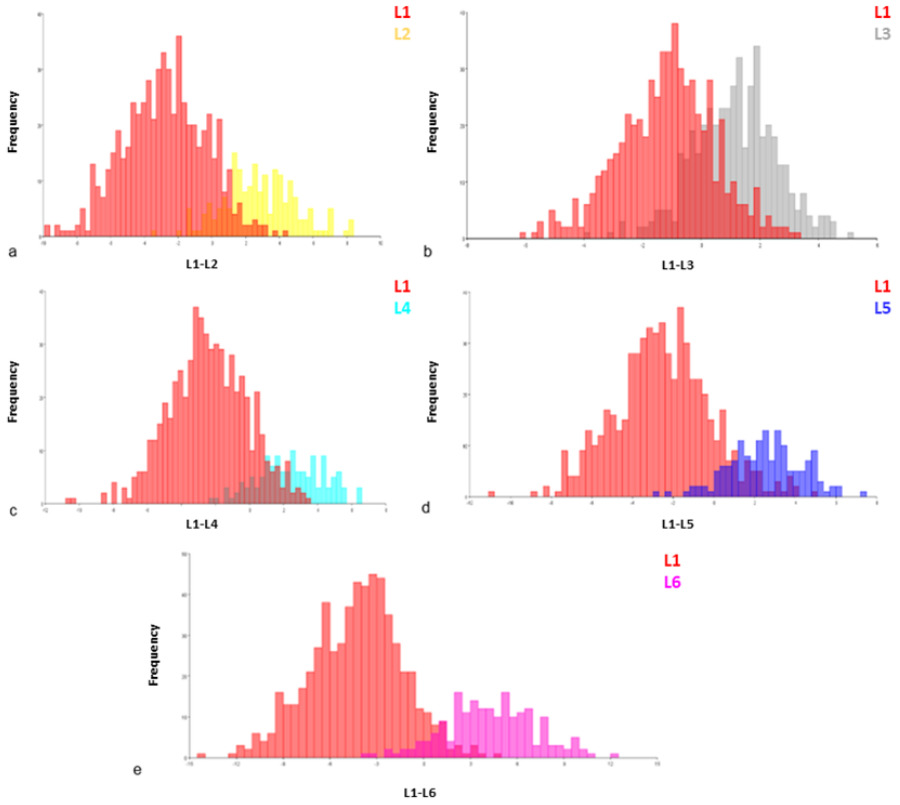


Figure 7. a) Histograms representing the measurement of discriminant scores for initial data variability among locations. a) Gevaş-L1 (red), Başkale-L2 (yellow) b) Gevaş-L1 (red), Çatak-L3 (gray) c) Gevaş-L1 (red), Özalp-L4 (turquoise) d) Gevaş-L1 (red), Hakkari-L5 (blue) e) Gevaş-L1 (red), Iran-L6 (pink).

Deformation grids were examined pairwise among locations, showing differences between landmarks 1 and 2 between L1 (Gevaş) and other locations (except L2-Çatak). Landmark 8 showed differences between L1 (Gevaş) and other locations; it was different between L6 (Iran) and other locations. The difference between L5 (Hakkari) and L1 (Gevaş) was minimal. The magnitude of the difference at landmark 8 was greatest between Gevaş and Iran. At landmark 7, samples from all locations showed differences, with the least difference between L1 (Gevaş) and L2 (Çatak), and the greatest difference between L1 (Gevaş) and L5 (Hakkari); L1 (Gevaş) and L6 (Iran). Samples from L1 (Gevaş) and L3 (Çatak) showed the most distinction from all other groups at landmark 7. Landmark 11 showed variation between L1 (Gevaş) and L2 (Başkale), L1 (Gevaş) and L6 (Iran), and between Iran and Çatak, Özalp, and Hakkari, with the largest change observed between Gevaş and Iran. Samples from L6 (Iran) showed distinct differences from other groups, particularly at landmark 8.

## Discussion and Conclusion

In this study, the clusters formed in the PCA graph of the forewing samples of honey bees collected from six locations (Van Districts, Hakkari, and Iran) showed a close distribution, and the intersection areas of the axes were dense. However, some samples from Gevaş and Iran exhibited a noticeable spread outside the center. It is thought that one of the main reasons for the most prominent differences emerging in the samples collected from the Iranian side of the border is the lower intensity of colony movements (migratory beekeeping and colony sales) and queen bee sales in this region compared to Türkiye. Although the research was conducted based on stationary beekeeping conditions, colony flow occurs in the regions during nectar flow periods. This disrupts the uniformity of local genotypes due to the mating behavior of queen bees. In this study, Hakkari samples showed a distribution pattern closer to Iranian samples rather than Van samples. In Badalı's (2010) study, which included samples from different locations including Iran, samples collected from Artvin, Iran, and Hakkari formed different clusters according to PCA analysis results, while samples from Iraq and Azerbaijan formed close clusters and showed similar characteristics. Honey bee populations in Southeastern Anatolia, including Hakkari, show distinct morphological traits that separate them from other regions in Türkiye, such as Van and Iran. This clustering is supported by geometric morphometric analyses, which highlight significant deviations in wing vein junctions among different populations (Kekeçoğlu & Soysal, 2010; Kekeçoğlu et al., 2020).

In our study, statistically significant differences were found in pairwise comparisons between all locations (4 districts of Van, Hakkari, and Iran) and apiaries, based on both Mahalanobis distances and Procrustes distances. In a study conducted by Özkan Koca (2012); according to Ruttner's (1988) classification, 3 populations in Iran (2nd Area Central and Western Iran, 3rd Area Northeastern Iran, 4th Area Subtropical areas of the Caspian Sea) clustered together using the DFA method. The differences between populations were also found to be significant according to pairwise test results. Colonies of all subspecies were distinctly separated from each other. While *A. m. meda* colonies in Eastern and Southeastern Anatolia were completely within their own groups, almost all (98.3%) of the colonies in Iran and Northern Iraq were within their own groups. A small portion (1.7%) of *A. m. meda* colonies in Iran and Northern Iraq was included in the Eastern and Southeastern Anatolia group. Morphometric analyses show distinct clustering of *A. m. meda* populations. Iranian populations form a separate cluster from Turkish populations, which include both *A. m. meda* and *A. m. caucasica* (Adl et al., 2007; Kence et al., 2009). Discriminant analysis and Mahalanobis distances reveal that Iranian, Central Anatolian, and Caucasian honey bee populations form distinct clusters, with geographical barriers likely contributing to these differences (Adl et al., 2007; Kekeçoğlu & Soysal, 2010).

According to the histogram data enabling pairwise comparisons based on discriminant scores, the greatest variation is observed between Gevaş district and other locations, and between Iran and other locations. In a morphometric analysis conducted by Özbakır & Fıratlı (2013), which is one of the studies on honey bee populations in different geographical regions using discriminant separation analysis for grouping, Syrian and Iranian honey bee samples formed different groups. Iranian honey bees were more similar to the samples from Hakkari, Van, and Şırnak, which are close to it; Syrian honey bees formed closer groups with samples from Mardin, Kilis, Hatay, and Şanlıurfa, which are neighboring it. According to Ftayeh et al. (1994), the bees in the region from Lake Van to the Mediterranean corner belong to one of the six ecotypes of Iranian honey bees (*A. m. meda*). Recent studies on the morphometric and genetic relationships of *Apis mellifera meda* populations in regions such as Eastern Anatolia, Southeastern Anatolia, Syria, and Iran reveal distinct clustering patterns influenced by geographic and ecological factors. (Adl et al., 2007; Bodur et al., 2007; Kence et al., 2009; Modaber et al., 2019). The genetic and morphometric variations among honey bee populations are significantly influenced by geographic proximity. For instance, bees from regions like Ardabil and Azarbaijan in Iran show genetic resemblance due to their geographical closeness (Rajabi-maham et al., 2018). Similarly, bees from Central Anatolia and Caucasian regions show closer genetic

relationships compared to those from Iran (Adl et al., 2007; Kekeçoğlu & Soysal, 2010). Ecological factors, such as local climate and flora, also contribute to the observed variations. The presence of distinct ecotypes in different regions of Türkiye, for example, is attributed to the diverse ecological conditions across these areas (Kandemir et al., 2000, Kekeçoğlu & Soysal, 2010).

In our study, according to Procrustes distances, it was observed that the shape differed among all locations. This difference was most pronounced between Iran and other locations. Following Iran, the variation between Gevaş and other locations was also noteworthy. According to the Mahalanobis distances, the widest variation is between Iran and Gevaş. In the study conducted by Badalı (2010), when the DFA distribution graph was examined, each group separated from each other with a high level of significance ($p$<0.001). The two groups consisting of Iranian and Artvin samples showed different clustering from other groups, but there were few signs of separation. While Azerbaijani and Hakkari samples were close to each other, Iraqi and Hakkari samples formed completely different groups. In our study, Hakkari samples showed similarity to Başkale, Özalp, and Iranian samples. Additionally, according to the DFA results using forewing data from Badalı (2010), it was determined that the first axis explained 47.3% of the total diversity, and the second axis explained 35.1%. In our study, according to the DFA results, it was evident that the first axis explained 77.5% of the total variation, and the second axis explained 22.5%.

In this study, ANOVA showed greater variation in terms of $F$ value for centroid size between locations compared to apiaries belonging to beekeepers. Based on the $F$ value, more differences were detected in terms of shape between locations than between apiaries. In the study by Dolatti et al. (2013) centroid sizes of forewings in different geographical regions of Iran were compared. The results showed a significant difference in the centroid sizes of forewings ($F$ = 10.6, $p$= 0.000). In a study where honey bee samples from 1987-1988 were assessed with MorphoJ software, the Procrustes ANOVA test utilized to assess population disparities revealed statistically meaningful shape variations between sites ($p$< 0.0001), but not meaningful regarding size of centroids. In the same study, the Procrustes ANOVA test with recent (2017) honey bee samples showed statistically significant differences in shape between locations ($p$< 0.0001) (Kösoğlu et al., 2021).

According to the CVA graphs obtained from canonical variate analysis, it was observed that the samples from Başkale, Özalp districts, and Hakkari province overlapped. Although there were occasional overlaps, this quartet including Çatak district clustered closely with the groups formed by the Gevaş and Iranian samples. However, it was seen that Gevaş samples and Iran samples had different distributions compared to other groups. In addition, the Çatak ellipse intersected more with Gevaş samples, while Başkale and Hakkari ellipses intersected more with Iran samples. These results were confirmed by deformation grids. The close distribution of the samples from Hakkari to the Iranian samples is interpreted as an indication of natural or artificial bee entry from Iran into Türkiye across the border. The Gevaş region stands out from the other sampled regions in terms of both the number of colonies and production techniques. Accordingly, it is assumed that there is a gene flow of the same genotype into the region. Furthermore, the observed uniformity in the district colonies suggests the possibility of selection, even under beekeeping conditions. In the study by Kösoğlu et al. (2021), where wing samples from different geographical regions were analyzed using Morpho J software, the CVA from the populations indicated that old and recent groups formed distinct clusters. While historical and new sets were located in two clusters on the graph, differences represented by populations within their own groups (old or new) were also observed. The CVA graph of the samples taken from 24 apiaries also indicated intra-group variation in our study.

The rich flora of Van province, the change of climatic characteristics over short distances, its geographical location, and beekeeping culture provide an extremely favorable environment for production activities. Therefore, it is of great importance to determine the honey bee subspecies *Apis mellifera meda*, which is accepted to exist in part of the Eastern Anatolia region based on previous research (Özdil et et al., 2012), and to protect local genotypes adapted to the region. Scientific research conducted so far has

revealed that the genetic characterization of honey bee populations in Van province was related to the local race *Apis mellifera meda* (Bodur et al., 2007; Kence et al., 2009). However, there are also findings indicating heterogeneity in honey bee populations in Van province (Tunca & Kence, 2011). In this study, fixed honey bee populations from Van districts and Hakkari, along with samples from Iran, were evaluated for genetic similarity and difference levels and current population potential using geomorphometric methods. It is thought that due to migratory beekeeping, queen bee trade, and selective breeding activities, *Apis mellifera meda* has been hybridized with *Apis mellifera syriaca*, *Apis mellifera caucasica*, and *Apis mellifera anatoliaca* races from time to time in Van province and its surroundings. In the survey study conducted by Erkan & Aşkın (2001), the bee races used in migratory beekeeping activities in Van province were also evaluated. At the end of the study, 66% of the breeders used Caucasian bees, while 20% used Iranian bees. This situation confirms the theory that migratory beekeeping and queen bee sales affect the local bee gene pool in the long term. However, the use of different bee breeds may cause differentiation in the local gene pool. Nevertheless, the direction and extent of this effect have not yet been comprehensively investigated.

Another example of hybridization between subspecies is the analysis results of honey bee samples collected from 55 different locations in 7 different geographical regions of Türkiye, using morphometric observations as well as molecular methods. According to these results, a close relationship was found between *A. m. anatoliaca*, *A. m. meda*, and *A. m. caucasica* subspecies (Kekeçoğlu & Soysal, 2010). In the study conducted by Kence et al. (2009), samples from 7 populations of honey bees, including 5 *Apis mellifera meda* populations from Iran and 2 populations (Artvin, Hakkari) from Türkiye, were examined. Of the three groups that emerged from the morphometric analysis, the first included all Iranian populations, while the rest contained *A. m. meda* and Caucasian bees from Türkiye. Our study was conducted with honey bee samples from stationary beekeepers in Özalp, Başkale, Çatak, and Gevaş districts, as well as Hakkari and Iran, which were thought to be less affected by migratory beekeeping activities and did not purchase queen bees. The results revealed that there are still preserved geomorphometric differences as well as similarities between Van (Gevaş), Iran, and Hakkari populations. The overlapping distributions observed may result from the limited number of samples collected from certain regions (particularly Iran) and the influence of environmental and anthropogenic factors (e.g., migratory beekeeping, queen bee trade, and hybridization). Although the sampled regions are geographically distant, the distribution of honey bees and the mating behavior of queen bees reduce the effect of this distance. The data on the presence of *A. m. meda*, which shows local characteristics in honey bee populations in Van province, needs to be updated.

Morphometric methods provide great advantages when used to assess biodiversity and for taxonomic purposes. Classical and geometric morphometry are important tools for identifying and distinguishing subspecies in honey bees. While traditional morphometry is restricted to distance measurements and distance rotations, geomorphometric analyses not only encompass these measurements indirectly but also facilitate wing morphology analysis through the landmark-based technique, making it an accepted cheap, fast, and precise method for identifying honey bee races and populations. Kandemir et al. (2011) stated that the analysis of landmarks found in wing shapes was a powerful and reliable method for distinguishing honey bee subspecies. Oleksa & Tofilski (2015) showed that in particular research, morphometry was more effective than molecular markers in identifying subspecies, and morphological traits were better suited for differentiating ecotypes among honey bee races. In this study, geometric morphometric analysis method, which is more reliable and advantageous than classical morphometry for the classification and identification of *Apis mellifera* L. subspecies, was used; these analyses were performed to evaluate the effect of hybridization on fluctuating asymmetry. In some previous studies conducted with honey bee samples collected from various regions of Türkiye, the Van region was represented by a limited number of populations, and it was concluded that the samples showed similarities to *A. m. meda* based solely on classical morphometric data. In this study, honey bee samples from stationary colonies taken from Van, Hakkari, and Iran were measured and evaluated using the geometric morphometry method.

*Apis mellifera*, which is vital for agricultural economy, shows diversity in terms of morphological and genetic characteristics. Morphometric, geomorphometric, and molecular studies on honey bees are very useful methods for determining genetic and geographical differences. Scientific research reveals that Anatolia is a honey bee gene center and that there are five separate honey bee races and ecological forms in this region (Smith, 2002). Today, no country has such a diversity of honey bee races together. Factors such as honey bee diseases and pests, pesticides, commercial queen bee sales that have not been tested for suitability to regions, uncontrolled mating, and migratory beekeeping can reduce biodiversity in bees, and in some cases can cause serious yield losses or even colony losses. (Hristov et al., 2020). Especially the desire to increase yield causes breeders to turn to genotypes that can exhibit their characteristics in their own geographical conditions, which can lead to the deterioration of local genotypes on the one hand and the homogenization of the gene pool and the reduction of diversity on the other hand. Migratory beekeeping, which is conducted for the same purpose and gains a different dimension day by day, carries the same risks and can threaten the ecological adaptation and genetic uniqueness of local genotypes (Jara et al., 2020). For these reasons, the identification and protection of local honey bee races is extremely important. These determinations should be taken into account not only for the economic contributions of beekeeping but also for the cultural history, ecological structure, and scientific future of our country.

In this study, the similarities of the samples taken from Başkale, Çatak, and Özalp districts of Van province indicate the hybridization of honey bee colonies due to migratory beekeeping, queen bee trade, and selective breeding activities conducted in these regions. On the other hand, the analysis results can be interpreted as the honey bees in Gevaş district being less exposed to activities that would cause hybridization due to their more distant positioning between Gevaş and Iran, but it would be appropriate to examine the district samples with other studies. Expanding the sampling to cover broader areas will contribute to the identification of honey bee populations from Gevaş and other regions and aid in the conservation of local bees as genetic resources. For this purpose, the distance of these district bees from other genotypes of the country should also be evaluated. Right forewing size and shape revealed variation between different locations in this study.

It is crucial to determine population structures and evaluate regional differences, as anatomical differences influenced by agroecological factors and beekeeping activities may indicate different subspecies or ecotypes of the same species. Our current dataset includes few samples from Iran. Increasing the number of samples and expanding the geographical coverage in Iran in future studies would allow for a more comprehensive understanding of honey bee population structures. While wing morphometry is increasingly used to determine phenotypic variations between specific levels in insects, it is used for taxonomic purposes in honey bees. This study provides preliminary data for more comprehensive future research on honey bee populations in the Eastern Anatolia region. To ensure the reliable identification samples, it is recommended to establish a database that includes geometric morphometric profiles specific to this region, along with geographical and genetic markers. Environmental data can be collected to associate morphometric variations with ecological factors. Molecular analyses, such as mitochondrial DNA sequencing or microsatellite markers, can be applied to validate morphometric differentiation and assess genetic purity. Thus, it can be clearly determined to what extent the stationary honey bee gene pool in Van province is *Apis mellifera meda* and the impact of migratory beekeeping practices on stationary populations, thereby revealing the current status of honey bee biodiversity.

## References


Adl, M., H. Gençer, Ç. Firatli & R. Bahreini, 2007. Morphometric characterization of Iranian (*Apis mellifera meda*), Central Anatolian (*Apis mellifera anatoliaca*) and Caucasian (*Apis mellifera caucasica*) honey bee populations. Journal of Apicultural Research, 46 (3): 225-231.

Badalı, M. N., 2010. İran'ın Kuzeyinde Yayılış Gösteren Balarısı Popülasyonlarının Morfometrik ve Geomorfometrik Analizi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Ankara, Türkiye, 63 s (in Turkish with abstract in English).

Bodur, Ç., M. Yıldırım & A. Özkan, 2007. Morphometric and genetic analysis of honey bee populations in the East Anatolian region. Turkish Journal of Zoology, 31 (1): 41-51.

Bookstein, A., 1990. Informetric distributions. Part I: unified overview. Journal of the American Society for Information Science, 41 (5): 368-375.

Dolatti, L., J. N. Rafie & H. Khalesro, 2013. Landmark-based morphometric study in the fore and hind wings of an Iranian race of European honey bee (*Apis mellifera meda*). Journal of Apicultural Science, 57 (2): 187-197.

Erkan, C. & Y. Aşkın, 2001. Van ili Bahçesaray İlçesi'nde arıcılığın yapısı ve arıcılık faaliyetleri. Yuzuncu Yıl University Journal of Agricultural Sciences, 11 (1): 19-28 (in Turkish with abstract in English).

Franck, P., L. Garnery, M. Solignac & J. M. Cornuet, 2000. Molecular confirmation of a fourth lineage in honey bees from the Near East. Apidologie, 31 (2): 167-180.

Francoy, T. M., D. Wittmann, V. Steinhage, M. Drauschke, S. Müller, D. R. Cunha & L. S. Gonçalves, 2009. Morphometric and genetic changes in a population of *Apis mellifera* after 34 years of Africanization. Genetics and Molecular Research, 8 (2): 709-717.

Hristov, P., R. Shumkova, N. Palova & B. Neov, 2020. Factors associated with honey bee colony losses: A mini-review. Veterinary Sciences, 7 (4): 166 (1-17).

Jara, L., C. Ruiz, R. Martín-Hernández, I. Muñoz, M. Higes, J. Serrano & P. Rúa, 2020. The effect of migratory beekeeping on the infestation rate of parasites in honey bee (*Apis mellifera*) colonies and on their genetic variability. Microorganisms, 9 (1): 22 (1-18).

Kandemir, I., A. Özkan & S. Fuchs, 2011. Reevaluation of honey bee (*Apis mellifera*) microtaxonomy: A geometric morphometric approach. Apidologie, 42 (5): 618-627.

Kandemir, İ., M. Kence & A. Kence, 2000. Genetic and morphometric variation in honey bee (*Apis mellifera*) populations of Turkey. Apidologie, 31 (3): 343-356.

Kekeçoğlu, M. & M. Soysal, 2010. Genetic diversity of bee ecotypes in Turkey and evidence for geographical differences. Romanian Biotechnological Letters, 15 (6): 5646-5653.

Kekeçoğlu, M., M. Bouga, M. İ. Soysal & P. Harizanis, 2007. Morphometrics as a tool for the study of genetic variability of honey bees. Journal of Tekirdag Agricultural Faculty, 4 (1): 7-15.

Kekeçoğlu, M., M. Kambur, M. Uçak, T. Çaprazlı & S. Bir, 2020**.** Biodiversity of honey bees (*Apis mellifera* L.) in Turkey by geometric morphometric analysis. Biological Diversity and Conservation, 13 (3): 1-10.

Kence, A., 2006. Genetic diversity of honey bees in Turkey and the importance of its conservation. Uludag Bee Journal, 6 (3): 25-32.

Kence, M., H. J. Farhoud & R. I. Tunca, 2009. Morphometric and genetic variability of honey bee (*Apis mellifera* L.) populations from northern Iran. Journal of Apicultural Research, 48 (4): 247-255.

Klingenberg, C. P., 2011. MorphoJ: An integrated software package for geometric morphometrics. Molecular Ecology Resources, 11 (2): 353-357.

Kösoğlu, M., R. I. Tunca, N. Özsoy & Y.T. Tuna, 2021. Determination of the wing morphology differentiation of old and recent honey bee samples from western Turkey using geometric morphometrics. Turkish Journal of Entomology, 45 (4): 463-474.

Modaber, M., N. Rafie & H. Rajabi-Maham, 2019. Population genetic structure of native Iranian population of *Apis mellifera meda* based on intergenic region and COX2 gene of mtDNA. Insectes Sociaux, 66 (4): 413-424.

Oleksa, A. & A. Tofilski, 2015. Wing geometric morphometrics and microsatellite analysis provide similar discrimination of honey bee subspecies. Apidologie, 46 (1): 49-60.

Özbakır, G. O. & C. Fıratlı. 2013. Morphometric Classification of Honey bee Populations *(Apis mellifera* L.) Along the Southeast border of Turkey. Bulgarian Journal of Agricultural Science. 19 (6): 1396-1400.

Özdil, F., İ. Aytekin, F. İlhan & S. Boztepe, 2012. Genetic variation in Turkish honey bees *Apis mellifera anatoliaca, A. m. caucasica, A. m. meda* (Hymenoptera: Apidae) inferred from RFLP analysis of three mtDNA regions (16S rDNA-COI-ND5). European Journal of Endocrinology, 109 (2): 161-167.

Özkan Koca, A., 2012. Ortadoğu'da Yayılış Gösteren *Apis mellifera* L. (Hymenoptera Apidae) Alt Türlerinin Geometrik Morfometri Yöntemleriyle Analizi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, (Unpublished) Doktora Tezi, Ankara, Türkiye, 167 s (in Turkish with abstract in English).

Rajabi-Maham, H., T. Ghasemi & S. Pashaei-Rad, 2018. Genetic diversity evaluation of Persian honey bees (*Apis mellifera meda*) in North West of Iran, using microsatellite markers. Journal of Wildlife and Biodiversity, 2 (1): 37-46.

Rohlf, F. J., 2018. TpsDig Version 2.31 Ecology & Evolution. SUNY at Stone Brook, USA.

Rohlf, F. J., 2019. TpsUtil32 v.1.78 software: tpsUtil (Version 1.78). SUNY Stony Brook, Stony Brook Morphometrics.

Ruttner, F., 1988. "Morphometric Analysis and Classification, 66-78". In: Biogeography and Taxonomy of Honey bees (Ed. F. Ruttner). Springer Berlin, Heidelberg, Germany, 284 pp.

Schwarzfeld, M. D. & F. A. H. Sperling, 2014. Species delimitation using morphology, morphometrics, and molecules: definition of the *Ophion scutellaris* Thomson species group, with descriptions of six new species (Hymenoptera, Ichneumonidae). ZooKeys, 462: 59-114.

Smith, D. D. R., 2002. Genetic diversity in Turkish honey bees. Uludag Bee Journal, 2 (3): 10-17.

Taşkıran, Ö., N. M. Dayıoğlu & D. Kabakcı, 2017. Bal Arılarının (*Apis mellifera* L.) Sınıflandırılması ve Ekolojik Koşulların Morfolojisi Üzerine Etkisi. Arıcılık Araştırma Dergisi, 9 (2): 68-77.

Tofilski, A., 2008. Using geometric morphometrics and standard morphometry to discriminate three honey bee subspecies. Apidologie, 39 (5): 558-563.

Tunca, I. R. & M. Kence, 2011. Genetic diversity of honey bee (*Apis mellifera* L.: Hymenoptera: Apidae) populations in Turkey revealed by RAPD markers. African Journal of Agricultural Research, 6 (29): 6217-6225.

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*Original article (Orijinal araştırma)*

# The effect of some biopesticides on the root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) damaging tomato plants[1]

## Bazı biyopestisitlerin domateste zararlı kök-ur nematodu *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerine etkisi

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## Abstract

In recent years, biopesticides have been widely investigated for the control of plant parasitic nematodes (PPNs). In this study, the effects of *Bacillus*-based biopesticides on the root-knot nematode (*Meloidogyne incognita*) (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) were investigated. The study was conducted at the Nematology Laboratory and Growth Room of the Department of Plant Protection, Faculty of Agriculture, Bolu Abant İzzet Baysal University in 2023. The efficacy of *Bacillus subtilis* (Biopesticide-I), *Bacillus licheniformis* strain RTI184 (Biopesticide-II) and *Paecilomyces lilacinus* strain 251 (Biopesticide-III) was compared with chemical nematicides and an untreated control. Treatments were applied at different times: (A) 5-7 days before transplanting, (B) at transplant by drench, (C) just after transplanting, and (D) 14 days after transplanting (DAT). The lowest gal formation was observed in Biopesticide-III + Nematicide-II (CD) treatment (0.40±0.24), followed by Nematicide-I (CD) (3.40±0.24) and Biopesticide-I (BD) (4.20±0.37), while the highest was observed in Biopesticide-I (CD) (5.00±0.31). The number of second-stage juveniles was significantly reduced by Biopesticide-III + Nematicide-II (CD) (99.76%), Nematicide-I (CD) (70.29%) and Biopesticide-I (BD) (57.36%). The results indicate that *Bacillus*-based biopesticides are effective in reducing root-knot nematode damage and can be used to control *M. incognita* in tomato plants.

**Keywords:** *Bacillus licheniformis* strain RTI184, *Bacillus subtilis*, biopesticide, nematicide, root-knot nematode

## Öz

Son yıllarda, biyopestisitler bitki paraziti nematodların (BPN) kontrolü için yaygın olarak araştırılmaktadır. Bu çalışmada *Bacillus* bazlı biyopestisitlerin kök-ur nematodu *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerindeki etkileri incelenmiştir. Çalışma, 2023 yılında Bolu Abant İzzet Baysal Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nematoloji Laboratuvarı ve iklim odasında yürütülmüştür. Çalışmada *Bacillus subtilis* (Biyopestisit-I), *Bacillus licheniformis* suşu RTI184 (Biyopestisit-II) ve *Paecilomyces lilacinus* suşu 251'in (Biyopestisit-III) etkinliği, kimyasal nematisitler ve uygulama içermeyen bir kontrol ile karşılaştırılmıştır. Uygulamalar farklı zamanlarda gerçekleştirilmiştir: (A) dikimden 5-7 gün önce, (B) dikim sırasında daldırma yoluyla, (C) dikimden hemen sonra ve (D) dikimden 14 gün sonra (DAT). En düşük gal oluşumu Biyopestisit-III + Nematisit-II (CD) uygulamasında (0,40±0,24), ardından Nematisit-I (CD) (3,40±0,24) ve Biyopestisit-I (BD) (4,20±0,37) uygulamalarında gerçekleşmiş, en yüksek ise Biyopestisit-I (CD) (5,00±0,31) uygulamasında gözlenmiştir. İkinci dönem larva sayıları Biyopestisit-III + Nematisit-II (CD) (%99,76), Nematisit-I (CD) (%70,29) ve Biyopestisit-I (BD) (%57,36) ile önemli ölçüde azalmıştır. Sonuçlar, *Bacillus* bazlı biyopestisitlerin kök-ur nematodu zararını azaltmada etkili olduğunu ve domates bitkilerinde *M. incognita*'nın mücadelesinde kullanılabileceğini göstermektedir.

**Anahtar sözcükler:** *Bacillus licheniformis* RTI184 suşu, *Bacillus subtilis*, biyopestisit, nematisit, kök-ur nematodu

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## Introduction

The tomato is among the most lucrative vegetable crops, widely cultivated in tropical, subtropical, and temperate climates globally (Naika et al., 2005). This vital crop experiences substantial productivity losses attributable to a number of diseases, including bacterial, fungal, viral, and nematode infections (Netscher & Sikora, 1990). Plant-parasitic nematodes (PPNs) represent a significant threat to global agricultural output. The damage inflicted by PPNs is projected to lead to a significant 12.3% reduction in global yield, amounting to approximately $157 billion each year. The current taxonomy recognizes approximately 4,100 species of PPNs, with root-knot nematodes (RKNs) and cereal cyst nematodes (CCNs) being acknowledged as significant plant diseases, whereas other species exhibit a more restricted host range (Singh et al., 2015).

Among the PPNs, root-knot nematodes (*Meloidogyne* spp.) are especially infamous, causing enormous agricultural losses estimated at $100 billion annually (Elling, 2013). More than 100 species of RKNs have been found, with four species *Meloidogyne incognita* (Kofoid & White,1919) Chitwood,1949, *Meloidogyne javanica* (Treub,1885) Chitwood,1949, *Meloidogyne hapla* Chitwood, 1949 and *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 (Tylenchida: Meloidogynidae) predominating and causing up to 90% damage to infected plants (Hunt & Handoo, 2009; Lunt et al., 2014; Khan et al., 2023). These nematodes are obligate, sedentary parasites with a wide host range, able to infect more than 5,000 plant species, including vegetables, fruits, field crops, and ornamental plants (Blok et al., 2008). These organisms are very hard to manage as they have a short life cycle, high reproductive potential and they attack the roots of the plants continuously (Sikora et al., 1992).

Conventional control methods for RKNs include soil solarization, non-fumigant and fumigant nematicides, and planting RKN-resistant varieties (Giannakou & Anastasiadis, 2005; Jordan, 2018). The prevalence and regularity of nematicide use over the past decades have created substantial disadvantages, as these substances are highly harmful both to human health and the soil ecosystem (Rajasekharan et al., 2020). Consequently, there is an imperative for the formulation of effective, ecologically sustainable alternative strategies for the control of RKNs. Biological control of pests is one of the most promising ways to control PPNs (Hallmann et al., 2009; Collange et al., 2011).

Products containing antagonistic microorganisms, usually bacteria or fungi, are generally referred to as bioproducts or biopesticides and are commonly known as microbial pesticides. Biopesticides are considered an integral part of integrated pest management (IPM) strategies (Arora et al., 2000). Recent international research investigated the potential of antagonistic microorganisms to alleviate the adverse effects of PPNs (Zheng et al., 2016; Abd- Elgawad et al., 2021). Many microorganisms proved to be highly effective as biological control agents against RKNs (Kerry, 2000). Investigations have focused on bacterial strains of the genera *Bacillus*, *Pseudomonas*, and *Pasteuria* for the control of RKNs (Aballay et al., 2012; Mahesha et al., 2017).

This study aims to evaluate the nematicidal efficacy of locally derived commercial biopesticides against *M. incognita*, the predominant species of RKN affecting tomato plants in controlled environments.

## Materials and Methods

### Nematode population

The RKN species, *M. incognita*, was used in the experiment. The cultures of the nematodes were established from different egg masses and were then maintained on a tomato cultivar in a controlled growth room at the Faculty of Agriculture, Abant İzzet Baysal University. During the study, the environmental temperature was controlled at 25 ± 2°C, with relative humidity kept at %60±10. Infected roots were carefully cleaned to remove attached dirt. Egg masses from the infected plants were collected with care and

immersed in distilled water. These were then placed in a BOD incubator at 28 ± 2°C to obtain the second-stage juveniles (J2). The juvenile suspension was calibrated to a final concentration of 100 juveniles per milliliter of distilled water.

**Experimental design and set up**

The experimental setup followed a completely randomized design, featuring five treatments with five replications each. The experiments were conducted in 500 cc plastic pots. The soil mixture, composed of 75% sand and 25% peat, was sterilized in an autoclave at 121°C. Three-week-old sensitive tomato seedlings (variety Falcon) were planted in each pot for each treatment. The J2 inoculum, standardized at a rate of 500 J2 per pot, was carefully added into two wells near the plant roots using a 5 ml micropipette, immediately before the biopesticide and nematicide treatments, according to the experimental design. The biopesticides and nematicides listed in the table below have been used in the experiment (Table 1).

Table 1. Biopesticides and nematicides used in the experiment

| Code | Active substance | Trade name of product | Company (Türkiye) |
|---|---|---|---|
| Biopesticides-I | *Bacillus subtilis* | Basuka | Ecobio Agriculture Products Ind. Ltd. Co. |
| Biopesticides-II | *Bacillus licheniformis* (RTI184) | Accudo | FMC Türkiye Industrial Products Ind. Ltd. Co. |
| Biopesticides-III | *Paecilomyces lilacinus* strain 251 | Bioact DC 216 | Bayer Türk Chemistry Ind. Ltd. Co. |
| Nematicide- I | Abamectin 20 g/L | Tervigo 20 SC | Syngenta Agriculture Ind. Anon. Co. |
| Nematicide- II | Fluopyram 400 g/L | Velum Prime SC 400 | Bayer Türk Chemistry Ind. Ltd. Co. |

In the study investigating the effect of some biopesticides on the reproduction of the RKN species *M. incognita*, the treatments were applied at different times to assess their impact on various stages of the nematode's life cycle (Table 2).

Table 2. Application times of biopesticides and nematicides treatments

| Timing/Application code | A | B | C | D |
|---|---|---|---|---|
| | 5-7 days before transplantation to soil without plant | At transplantation by dipping | Just after transplantation | 14 days after transplantation (14DAT) |

Immediately after inoculation, the biopesticides and nematicides used in the trial were applied at recommended doses, with consideration that tomato seedlings must be planted at 1500 plants/da according to the (Table 3). After application, the pots were irrigated to enhance the effect.

Table 3. Used treatments in the experiment with used product formulation, application rates and and times

| No | Treatments | Form. | Rate (ml/hL or g/hL) | Time |
|---|---|---|---|---|
| 1 | Biopesticides-I | SG | 1 L/ha (0.05 L/1000 plant) | ABD |
| 2 | Biopesticides-I | SG | 1 L/ha | BD |
| 3 | Biopesticides-I | SG | 1 L/ ha | CD |
| 4 | Biopesticides-II | SC | 1 L/ha + 0.5L/ha | CD |
| 5 | Biopesticides-III + Nematicide-II | DC-SC | 0.75 L/ha+0.6L/ha | CD |
| 6 | Nematicide-I | SC | 4 L /ha | CD |
| 7 | Control (+) (nematode Applied) | NA | NA | NA |
| 8 | Control (-) | NA | NA | NA |

***** SG: water soluble granules, DC: dispersible concentrates: SC: suspension concentrate, NA: Non-application.

### Evaluation of the trial

Following a period of eight weeks from the commencement of the experiment, the tomato plants were ready for harvesting. The plants were harvested by cutting them at ground level, and their roots were extracted from the soil with great care. The roots were then washed gently under a stream of running water to remove any adhering soil particles. Subsequent to this process, the fresh and dry weights of the roots were meticulously measured and recorded. The severity of root galling, evaluated using a scale ranging from 0 to 10 as per Zeck (1971), provided insights into the extent of damage caused by nematode infestation. The improved Baermann funnel method (Hooper, 1986) was used to determine the population density of *M. incognita*. Finally, the collected second-stage juveniles (J2) were counted under an inverted microscope.

### Statistical analysis

The SPSS software (version 15.00; SPSS, Chicago, IL, USA) was used to statistically evaluate the experimental data. Analysis of variance (ANOVA) was employed to assess the significance of differences among the various parameters measured in the experiment. ANOVA enabled the comparison of means across many treatment and control groups. The Duncan test was used for post-hoc mean comparisons at a significance threshold of $p<0.05$ to identify homogeneous groupings.

## Results

### The impact of the treatments on the reproduction of the nematode

The results demonstrated that all treatments considerably diminished tomato root galling in comparison to the untreated control. The combination of Biopesticides-III with Nematicide-II exhibited the greatest efficacy, diminishing root galling by 93.81%, closely succeeded by Nematicide-I (CD), which achieved a reduction of 51.43%. Biopesticide-I (BD), Biopesticide-I (ABD), and Biopesticide-II (CD) shown significant decreases in root galling of 40.00%, 33.69%, and 33.33%, respectively (Table 4).

Moreover, all tested treatments significantly decreased the J2 population in the soil at recommended dosage rates compared to the untreated control. Biopesticides-III + Nematicide-II and Nematicide-I were particularly effective, reducing J2 in the soil by 99.76% and 70.29%, respectively. Biopesticides-I (BD), Biopesticides-I (CD), and Biopesticides-I (ABD) followed with reductions of 57.36%, 49.66%, and 42.31%, respectively (Table 4).

Table 4. The impact of biopesticides and nematicides on the root-gall index and second-stage juveniles (J2)

| Treatments | Time | Root-gall index (Mean ± SE)* | Mean J2 /250 g soil (Mean ± SE) | Decrease in galls over control (%) | Decrease in J2 over control (%) |
|---|---|---|---|---|---|
| Biopesticides-I | ABD | 4.60±0.24$^{c}$ | 954.00±117.32$^{cd}$ | 33.69 | 42.31 |
| Biopesticides-I | BD | 4.20±0.37$^{bc}$ | 712.00±133.01$^{bc}$ | 40.00 | 57.36 |
| Biopesticides-I | CD | 5.00±0.31$^{c}$ | 832.00±54.99$^{cd}$ | 27.14 | 49.66 |
| Biopesticides-II | CD | 4.60±0.24 $^{c}$ | 1250.00±96.85 $^{e}$ | 33.33 | 24.18 |
| Biopesticides-III + Nematicide-II | CD | 0.40±0.24$^{a}$ | 4.00±2.44$^{a}$ | 93.81 | 99.76 |
| Nematicide-I | CD | 3.40±0.24$^{b}$ | 490.00±53.85$^{b}$ | 51.43 | 70.29 |
| Control (+) applied nematod | NA | 7.00±0.31$^{d}$ | 1656.00±62.81$^{f}$ | 0.00 | 0.00 |
| Control (-) | NA | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00 | 0.00 |

***** Each value is the mean of five replicates. Values in each column labeled with the same letter(s) indicate no statistically significant differences within the acceptable significance range ($p<0.05$), according to Duncan's multiple-range analysis.

Furthermore, the combined treatments of Biopesticides-III + Nematicide-II demonstrated more significant efficacy compared to single treatments with Biopesticides-I and Biopesticides-II. Notably, transplant drench applications of *Bacillus*-based biopesticides proved more effective than other biopesticide treatments. These findings underscore the potential of integrated pest management strategies involving both biological products and nematicides for effective control of RKNs in tomato cultivation. Such integrated approaches hold promises for sustainable nematode management practices in agriculture.

**The impact of treatments on plant growth parameters**

According to the findings, the presence of nematode populations had a notable negative impact on plant growth indices compared to the nematode-free control. Significant increases in plant height were observed in treatments involving Biopesticides-III + Nematicide-II and Biopesticides-II. Conversely, plant height was significantly reduced at Biopesticides-I (ABD) and Biopesticides-I (BD) treatments compared to the nematode-free control. Other treatments did not differ significantly from the untreated nematode inoculated plants in terms of their effect on plant height (Table 5).

Regarding root weight, all treatments showed no significant difference from the control, except for Nematicide-I and Biopesticides-III + Nematicide-II, which significantly increased root weight compared to the control (+). The maximum biometric parameters were recorded in plants treated with Biopesticides-III + Nematicide-II (Table 5).

When compared to untreated inoculated plants, neither Biopesticides-I nor Biopesticides-II significantly impacted the growth indices of tomato plants. These results underscore the differential effects of various treatments on tomato plant growth under nematode-infested conditions, highlighting the potential benefits of integrated approaches such as Biopesticides-III + Nematicide-II in mitigating the negative impact of nematode infestations on plant development and productivity (Table 5).

Table 5. The impact biopesticides and nematicides on the growth parameters of tomato plants

| Treatments | Time | Plant height (Mean ± SE)* | Root fresh weight (Mean ± SE) | Root dry weight (Mean ± SE) |
|---|---|---|---|---|
| Biopesticides-I | ABD | 30.20±0.58$^{a}$ | 24.05±1.98$^{ab}$ | 2.34±0.16$^{ab}$ |
| Biopesticides-I | BD | 29.40±2.06$^{a}$ | 21.55±1.29$^{ab}$ | 2.09±0.20$^{a}$ |
| Biopesticides-I | CD | 39.80±2.05$^{bc}$ | 20.39±0.99$^{a}$ | 1.90±0.23$^{a}$ |
| Biopesticides-II | CD | 42.40±1.43$^{cd}$ | 22.45±2.07$^{ab}$ | 2.15±0.35$^{a}$ |
| Biopesticides-III + Nematicide-II | CD | 45.40±0.92$^{d}$ | 43.99±2.80$^{c}$ | 4.69±0.29$^{c}$ |
| Nematicide-I | CD | 37.80±1.62$^{b}$ | 44.24±4.56$^{c}$ | 4.95±0.48$^{c}$ |
| Control (+) applied nematod | NA | 26.60±0.50$^{a}$ | 18.58±1.94$^{a}$ | 1.89±0.23$^{a}$ |
| Control (-) | NA | 50.20±0.86$^{e}$ | 49.06±1.10$^{c}$ | 5.24±0.13$^{c}$ |

***** Each value is the mean of five replicates. Values in each column labeled with the same letter(s) indicate no statistically significant differences within the acceptable significance range ($p$<0.05), according to Duncan's multiple-range analysis.

## Discussion

Globally, agricultural practices have traditionally relied on chemical nematicides to control RKNs because of their rapid and often effective control (Burkett-Cadena et al., 2008). However, concerns over their environmental impacts, including soil toxicity and effects on non-target organisms, have caused the development of stringent regulatory controls and total bans on several compounds over the past two decades (Mukhtar et al., 2013). The situation has been marked by a dire need for sustainable options that enhance the current Integrated Pest Management (IPM) systems.

Research has demonstrated that rhizobacteria belonging to the *Bacillus* genus function efficiently as biological control agents for RKNs. These bacteria address PPNs through several mechanisms, such as the synthesis of volatile organic compounds (VOCs), which impede nematode growth and attract beneficial microorganisms. The fact that these organisms can induce systemic resistance in plants makes them a very interesting environmentally friendly substitute for chemical treatments (Siddiqui & Mahmood, 1999).

The current study was conducted to assess the efficiency of various *Bacillus*-based bioproducts in managing *Meloidogyne incognita* (Kofoid and White) Chitwood, 1949 (Tylenchida: Meloidogynidae) under controlled growth room conditions. The utilization of biopesticides, specifically Biopesticides-I (BD), Biopesticides-I (ABD), and Biopesticides-II (CD), has demonstrated a significant decrease in root galling, with observed reductions between 33.33% and 40.00%. Our findings are consistent with prior research demonstrating various RKN control experiments conducted by rhizobacteria related to the *Bacillus* genus. For example, Freitas et al. (2005) reported that *B. cereus* isolates to control *M. javanica* observed reduce the intensity of galls approximately 55% when applied via tomato seeds. Colagiero et al. (2018) reported that *in vitro* tests using *B. cereus* and *B. licheniformis* against *M. incognita* showed that *B. licheniformis* significantly reduced the ability of *M. incognita* second-stage juveniles to infect tomato roots. Ramalakshmi et al. (2020) reported that the biocontrol potential of 2 native *B. thurungiensis* was evaluated for their effective control of *M. incognita* under greenhouse conditions. The final nematode population in the soil was 52.4% and 59.5%, and gall index values were reduced by 46.7% and 66.7%. Xiao et al. (2018) reported that *Bacillus cereus* strain Jdm1 was tested for activity as a biocontrol agent against *M. incognita*. The root galling severity decreased 43%, with improved growth compared to control plants. Hussain et al. (2020) reported that tomato roots in combined application of culture filtrate of *B. subtilis* was effective in reducing the root galling by 58.79%. Huang et al. (2020) reported that *Bacillus* genus bacteria reduced the second-stage juveniles of RKN rates to 64.58%. Yanyan et al. (2023) reported that treatments with cultures of *Bacillus firmus* strain YB-1503 reduced root gall index by 65.8% and increased plant growth, a significant improvement over the control. Niazi (2024) reported that the combination of *B. subtilis* and *Pseudomonas putida* cultures reduced the effects of *M. incognita* on tomato plants, reducing the nematode population by 50% and gall formation by 60%.

Although the results under controlled conditions are promising, the extrapolation of biocontrol efficacy from the laboratory to the field is problematic (Meyer, 2003; Tian et al., 2007). The differences between pot trials and field results can be explained by the soil texture, climatic conditions, and microbial competition. Meyer (2003) and Tian et al. (2007) highlighted the importance of understanding these complex interactions in order to improve the applicability and efficacy of *Bacillus*-based biopesticides in real agricultural scenarios.

Furthermore, comparisons with chemical nematicides show that chemical treatments gave higher levels of suppression than biopesticides under the same conditions. In addition, the combination of the fungal biopesticide *P. lilacinus* strain 251 and the nematicide fluopyram also achieved very high levels of control. This suggests that biologicals alone may not provide sufficient efficacy and that their use in combination with chemical nematicides is important to ensure higher yields and effective pest management. Therefore, the use of biopesticides and chemical nematicides in an integrated sustainable pest management system should be considered.

In conclusion, the study showed that *Bacillus*-based biopesticides, including *B. subtilis* and *B. licheniformis*, can effectively reduce *M. incognita* population densities and can be used in insecticide-based management strategies. Although chemical nematicides provide better control, these biopesticides are safe for plants and the environment. Further research is needed to improve their efficacy under field conditions, considering factors such as soil composition, climate and microbial interactions.

## References


Aballay, E., S. Prodan, A. Mårtensson & P. Persson, 2012. Assessment of rhizobacteria from grapevine for their suppressive effect on the parasitic nematode *Xiphinema index*. Crop Protection, 42: 36-41.

Abd-Elgawad, M. M., 2021. Biological control of nematodes infecting eggplant in Egypt. Bulletin of the National Research Centre, 45 (1): 1-9.

Arora, R., G. S. Battu & D. S. Bath, 2000. Management of insect pests of cauliflower with biopesticides. Indian Journal of Ecology, 27 (2): 156-162.

Blok, V. C., J. T. Jones, M. S. Phillips & D. L. Trudgill, 2008. Parasitism genes and host range disparities in biotrophic nematodes: the conundrum of polyphagy versus specialisation. BioEssays, 30 (3): 249-259.

Burkett-Cadena, M., N. Kokalis-Burelle, K. S. Lawrence, E. Van Santen & J. W. Kloepper, 2008. Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biological Control, 47 (1): 55-59.

Colagiero, M., L. C. Rosso & A. Ciancio, 2018. Diversity and biocontrol potential of bacterial consortia associated to root-knot nematodes. Biological Control, 120: 11-16.

Collange, B., M. Navarrete, G. Peyre, T. Mateille & M. Tchamitchian, 2011. Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. Crop Protection, 30 (10): 1251-1262.

Elling, A. A., 2013. Major emerging problems with minor *Meloidogyne* species. Phytopathology, 103 (11): 1092-1102.

Freitas, L. G., W. S. Neves, C. F. S. Fabry, B. M. Marra, M. M. Coutinho, R. S. Romeiro & S. Ferraz, 2005. Isolation and selection of rhizobacteria for the control of root-knot nematodes, *Meloidogyne* spp. on tomato. Nematologia Brasileira, 29 (2): 215-220.

Giannakou, I. O. & I. Anastasiadis, 2005. Evaluation of chemical strategies as alternatives to methyl bromide for the control of root-knot nematodes in greenhouse cultivated crops. Crop Protection, 24 (6): 499-506.

Hallmann, J., K. G. Davies & R. Sikora, 2009. “Biological Control Using Microbial Pathogens, Endophytes and Antagonists, 380-411” In: Root-Knot Nematodes (Eds. R. N. Perry, M. Moens & J. L. Starr). CABI International, Wallingford, UK, 488 pp.

Hooper, D. J., 1986. “Extraction of Nematodes from Plant Material, 59-80”. In: Laboratory Methods for Work with Plants and Soil Nematodes (Eds. J. F. Southey). Ministry of Agriculture, Fisheries and Food, London, UK, 202 pp.

Huang, K., Q. Jiang, L. Liu, S. Zhang, C. Liu, H. Chen & Y. Zhang, 2020. Exploring the key microbial changes in the rhizosphere that affect the occurrence of tobacco root-knot nematodes. AMB Express, 10: 72 (1-11).

Hunt, D. J. & Z. A. Handoo, 2009. “Taxonomy, Identification and Principal Species, 55-97” In: Root-Knot Nematodes (Eds. R. N. Perry, M. Moens & J. L. Starr). CABI International, Wallingford, UK, 488 pp.

Jordan, S., 2018. Yield to the resistance: The impact of nematode resistant varieties on alfalfa yield. Natural Resource Modeling, 31 (2): 1-11.

Kerry, B. R., 2000. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. Annual Review of Phytopathology, 38 (1): 423-441.

Khan, A., A. Khan, A. Ali, S. Fatima & M. A. Siddiqui, 2023. Root-knot nematodes (*Meloidogyne* spp.): Biology, plant-nematode interactions and their environmentally benign management strategies. Gesunde Pflanzen, 75 (6): 1-19.

Lunt, D. H., S. Kumar, G. Koutsovoulos & M. L. Blaxter, 2014. The complex hybrid origins of the root knot nematodes revealed through comparative genomics. PeerJ - Life & Environment, 2: e356 (1-25).

Mahesha, H. S., N. G. Ravichandra, M. S. Rao, N. C. Narasegowda, S. Shreeshail & H. Shivalingappa, 2017. Bio-efficacy of different strains of *Bacillus* spp. against *Meloidogyne incognita* under in vitro. International Journal of Current Microbiology and Applied Sciences, 6 (11): 2511-2517.

Meyer, S. L., 2003. United States Department of Agriculture-Agricultural Research Service research programs on microbes for management of plant-parasitic nematodes. Pest Management Science: Formerly Pesticide Science, 59 (6-7): 665-670.

Mukhtar, T., M. Z. Kayani & M. A. Hussain, 2013. Nematicidal activities of *Cannabis sativa* L. and *Zanthoxylum alatum* Roxb. against *Meloidogyne incognita*. Industrial Crops and Products, 42: 447-453.

Naika, S., J. V. L. De Jeude, M. De Goffau, M. Hilmi & B. Van Dam, 2005. Cultivation of Tomato. Digigrafi, Wageningen, Netherlands, 92 pp.

Netscher, C. & R. A. Sikora, 1990. "Nematode Parasites of Vegetables, 237-283". In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, R. Sikora & J. Bridge). CABI International, Wallingford, UK, 629 pp.

Niazi, P., 2024. Isolation and Characterization of a (Surfactin-Like Molecule) Produced by *Bacillus subtilis*: Antagonistic Impact on Root-Knot Nematodes. Scientific Research Communications, 4 (2): 132-149.

Rajasekharan, S. K., S. Kim, J. C. Kim & J. Lee, 2020. Nematicidal activity of 5-iodoindole against root-knot nematodes. Pesticide Biochemistry and Physiology, 163: 76-83.

Ramalakshmi, A., R. Sharmila, M. Iniyakumar & V. Gomathi, 2020. Nematicidal activity of native *Bacillus thuringiensis* against the root knot nematode, *Meloidogyne incognita* (Kofoid and White). Egyptian Journal of Biological Pest Control, 30 (1): 1-9.

Siddiqui, Z. A. & I. Mahmood, 1999. Role of bacteria in the management of plant parasitic nematodes: A review. Bioresource Technology, 69 (2): 167-179.

Sikora, R. A., 1992. Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. Annual Review of Phytopathology, 30 (1): 245-270.

Singh, S., B. Singh & A. P. Singh, 2015. Nematodes: A threat to sustainability of agriculture. Procedia Environmental Sciences, 29: 215-216.

Tian, B., J. Yang & K. Q. Zhang, 2007. Bacteria used in the biological control of plant-parasitic nematodes: Populations, mechanisms of action, and future prospects. FEMS Microbiology Ecology, 61 (2): 197-213.

Xiao, L., J. W. Wan, J. H. Yao, H. Feng & L. H. Wei, 2018. Effects of *Bacillus cereus* strain Jdm1 on *Meloidogyne incognita* and the bacterial community in tomato rhizosphere soil. 3 Biotech, 8 (8): 319 (1-8).

Yanyan, F. U., Z. H. A. N. G. Jie, Z. H. U. Wenqian, X. I. A. Mingcong, S. U. N. Runhong, X. U. Wen & L. I. Dongmei, 2023. Identification and mechanism of strain YB-1503 for biological control of *Meloidogyne incognita*. Chinese Journal of Biological Control, 39 (2): 429-437.

Zeck, W. M., 1971. A Rating scheme for field evaluation of root-knot nematode infestation. Pflanzenschutz Nachrichten, 10: 141-144.

Zheng, Y. K., X. G. Qiao, C. P. Miao, K. Liu, Y. W. Chen, L. H. Xu & L. X. Zhao, 2016. Diversity, distribution and biotechnological potential of endophytic fungi. Annals of Microbiology, 66 (2): 529-542.

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# Effect of pepper variety on the degradation behaviors of pirimicarb[1]

Biber çeşitlerinin pirimicarb'ın bozunma davranışları üzerindeki etkisi

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## Abstract

In pesticide residue trials, selecting crop varieties that accurately represent agricultural practices and morphological diversity is essential to obtaining reliable and applicable results. Generally, widely grown varieties are given priority, but differences in pesticide residues may occur due to the morphological and physiological characteristics of plant varieties. This study investigated the degradation behaviors of pirimicarb in five pepper varieties in Tokat, Türkiye, in 2023. Pirimicarb, an insecticide registered against the peach aphid *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), is widely used in peppers, tomatoes, sugar beets, and citrus fruits. While effective in pest control, pirimicarb inhibits acetylcholinesterase, posing neurotoxic risks to target and non-target organisms. Prolonged exposure may cause endocrine disruption and oxidative stress, making residue monitoring essential for food safety. Initially, a rapid and sensitive QuEChERS-LC-MS/MS method was verified to analyze pirimicarb in peppers. Analysis results show that pirimicarb in all varieties decreased below EU-MRL (0.5 mg $kg^{-1}$) 24 hours after application. Significant variations in degradation rates and half-lives were observed among the varieties, attributed to their morphological and physiological differences. This research fills a critical gap by revealing the impact of varietal differences on the fate of pesticides, providing valuable data to optimize application strategies and ensure consumer safety.

**Keywords:** Acute risk, chronic risk, dissipation kinetics, method verification, pesticide residue

## Öz

Pestisit kalıntısı denemelerinde, tipik tarımsal uygulamaları ve morfolojik çeşitliliği doğru bir şekilde temsil eden ürün çeşitlerinin seçilmesi, güvenilir ve uygulanabilir sonuçlar elde etmek için önemlidir. Genellikle yaygın olarak yetiştirilen çeşitlere öncelik verilmekle birlikte, bitki çeşitlerinin morfolojik ve fizyolojik özellikleri nedeniyle pestisit kalıntılarında farklılıklar meydana gelebilir. Türkiye'nin Tokat ilinde 2023 yılında yürütülen bu çalışmada beş biber çeşidinde pirimikarb'ın bozunma davranışları araştırılmıştır. Şeftali yaprak bitine, *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) karşı tescilli bir insektisit olan pirimicarb, biber, domates, şeker pancarı ve turunçgillerde yaygın olarak kullanılmaktadır. Pirimicarb zararlı kontrolünde etkili olmasına rağmen asetilkolinesterazı inhibe ederek hedef ve hedef olmayan organizmalar için nörotoksik riskler oluşturur. Uzun süreli maruziyet endokrin bozulmasına ve oksidatif strese yol açabilir, bu da gıda güvenliği için kalıntı izlemeyi zorunlu hale getirir. Başlangıçta, biber örneklerinde pirimicarb'ı analiz etmek için hızlı ve hassas bir QuEChERS-LC-MS/MS yöntemi doğrulanmıştır. Analiz sonuçları, tüm çeşitlerde pirimicarb'ın uygulamadan 24 saat sonra AB-MRL (0,5 mg $kg^{-1}$) altına indiğini göstermektedir. Farklı biber çeşitleri arasında bozunma oranları ve yarı ömürlerde önemli farklılıklar gözlemlenmiştir. Bu farklılıkların, çeşitlerin morfolojik ve fizyolojik özelliklerinden kaynaklandığı düşünülmektedir. Bu araştırma, çeşit farklılıklarının pestisitlerin akıbeti üzerindeki etkisini ortaya koyarak, pestisit uygulama stratejilerini optimize etmek ve tüketici güvenliğini sağlamak için değerli veriler sağlayarak kritik bir boşluğu doldurmaktadır.

**Anahtar sözcükler:** Akut risk, kronik risk, parçalanma kinetiği, metot doğrulama, pestisit kalıntısı

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## Introduction

Peppers are one of the most widely consumed vegetables globally and in Türkiye, either fresh or processed. Pepper cultivation is practiced in almost every region of Türkiye, both in open fields and greenhouses (Altuntaş et al., 2021). Beyond domestic markets, pepper production contributes significantly to the national economy, with Türkiye exporting 312.213 tons of pepper valued at approximately $89 million in 2021. The primary export destinations included Germany (57%), the Netherlands (13%), and the United Kingdom (6%) (TUIK, 2024a). However, various biotic stress factors, including insect pests and plant diseases, pose serious threats to pepper production. Pests such as two-spotted spider mite [*Tetranychus urticae* Koch, 1836 (Acarina: Tetranychidae)], aphids [*Aphis gossypii* Glover, 1877; *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)], and whiteflies [*Bemisia tabaci* (Gennadius, 1889); *Trialeurodes vaporariorum* (Westwood, 1856). (Hemiptera: Aleyrodidae)], as well as diseases like bacterial canker (Cmm), *Clavibacter michiganensis* subsp. *michiganensis* (Smith) (Actinobacteriota: Microbacteriaceae), and gray mold, *Botrytis cinerea* Pers. (Ascomycota: Sclerotiniaceae), leading to significant economic losses if not effectively managed (Anonymous, 2022; Can & Ulusoy, 2022).

Chemical control remains one of the most employed strategies for pest management due to its effectiveness, rapid results, and cost-efficiency, especially in large-scale production systems. Compared to biological and cultural control methods, chemical pesticides provide immediate and broad-spectrum protection against a wide range of pests, making them indispensable in intensive agricultural practices where high yields and economic sustainability are prioritized. However, the excessive or improper use of pesticides can result in the accumulation of harmful residues in food products, potentially exceeding the maximum residue limits (MRLs) established by regulatory bodies. Consuming such contaminated foods can result in acute or chronic poisoning in humans, with symptoms ranging from mild irritation to severe health issues, including death (Solomon, 2000). These effects may manifest as mild headaches, nausea, flu-like symptoms, skin rashes, and blurred vision (WHO, 2010). In more severe cases, pesticides pose serious threats to human health, leading to neurological disorders, paralysis, blindness, and even death (Damalas & Eleftherohorinos, 2011). Additionally, studies have linked pesticide exposure to cancer, reproductive damage, and endocrine disruption (Whyatt et al., 2007; Thongprakaisang et al., 2013; Donkor et al., 2016). Epidemiological studies highlight the increased risk of leukemia and lymphoma among agricultural workers exposed to pesticides (Alavanja et al., 2004; Jurewicz & Hanke, 2008). Furthermore, prenatal exposure to certain pesticides has been associated with developmental delays and behavioral issues in children (Eskenazi et al., 2007; Rauh et al., 2006). Consequently, monitoring pesticide degradation in agricultural products is essential for ensuring food safety and compliance with legal residue limits.

Pirimicarb is a systemic carbamate insecticide widely used for controlling aphids, particularly the peach aphid (*M. persicae),* in various crops, including peppers, tomatoes, sugar beets, and citrus fruits (Anonymous, 2024). Its mode of action involves the inhibition of acetylcholinesterase (AChE), leading to an accumulation of acetylcholine at synaptic junctions, which results in neurotoxicity and paralysis in target pests (Riva et al., 2018). Pirimicarb can enter the human body through inhalation, dermal contact, or oral intake (Archibald et al., 1994; Zhou et al., 1996). Evidence also suggests its carcinogenic and mutagenic potential (Piel et al., 2019). Recent studies indicate that pirimicarb exposure may disrupt the endocrine system and contribute to developmental and reproductive issues (Gupta et al., 2020). Moreover, chronic exposure to pirimicarb has been associated with liver and kidney damage in animal studies. Its potential to induce oxidative stress and DNA damage further highlights the importance of monitoring its residue levels in food products (Zhang et al., 2022). Despite these concerns, pirimicarb continues to be approved for use in the European Union, as outlined in the latest European Food Safety Authority (EFSA) pesticide assessment reports (EFSA, 2024). Given its systemic nature, pirimicarb can penetrate plant tissues and persist in different plant parts, necessitating detailed investigations into its degradation behavior and residue dissipation patterns. The dissipation of pirimicarb in crops is influenced by numerous factors, including environmental conditions, application method, and crop morphology (Jacobsen et al., 2015; Alister et al., 2017).

Accurate pesticide residue analysis is essential for understanding dissipation behavior and ensuring food safety. This study analyzed pirimicarb residue levels using Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS), a highly sensitive and selective analytical technique. LC-MS/MS enables detecting and quantifying pesticide residues at trace levels, ensuring precise and reliable results. Its high specificity and ability to separate complex sample matrices make it an indispensable tool in pesticide residue studies. Furthermore, LC-MS/MS offers high-throughput capabilities, which are particularly advantageous for evaluating pesticide degradation under varying environmental conditions (Chen et al., 2020).

The degradation behaviors of pesticides in plants depend heavily on factors such as the cultivated species (Lu et al., 2014), plant variety (İş, 2019), growth rate and metabolism (Jacobsen et al., 2015), application frequency (Şarkaya-Ahat, 2015), application timing, formulation type (Alister et al., 2017), volatilization (Jacobsen et al., 2015), and abiotic factors like rainfall, temperature, sunlight, and humidity (Liu et al., 2014; Sharma et al., 2014; Malhat, 2017; Balkan & Kara, 2023). Numerous studies worldwide have examined the dissipation kinetics of pesticides in peppers (Antonious, 2004; Feng-Shou et al., 2008; Hem et al., 2011; Lu et al., 2014; Feng et al., 2021). However, research on this topic in Türkiye is limited (Cönger et al., 2012; Şarkaya-Ahat, 2015; Balkan et al., 2024). Most existing studies have been conducted on a single plant variety. Studies investigating the degradation behaviors of pesticides based on plant variety were scarce globally and in Türkiye.

Selecting crop varieties accurately representing agricultural practices is critical in pesticide residue trials. Test Guideline No. 509 Crop Field Trial emphasizes the importance of prioritizing commonly cultivated crop varieties to ensure the relevance and applicability of the results (OECD, 2021). However, pepper fruits vary significantly in shape, color, and size depending on their type (e.g., bell, long, or capia peppers), so solely focusing on commonly cultivated varieties may not fully capture the variability in residue degradation patterns. Therefore, while prioritizing commonly cultivated varieties is essential for generating relevant data, it is equally important to consider the potential variability introduced by less common varieties. Residue dissipation patterns may differ significantly due to each variety's morphological and physiological characteristics, such as flesh thickness, surface texture, and fruit size. To the best of our knowledge, no studies have been conducted on the degradation behaviors of pirimicarb in different pepper varieties. This study aims to investigate the degradation behaviors of pirimicarb in five different pepper varieties under field conditions. The findings will provide valuable insights into the role of varietal differences in pesticide dissipation and contribute to developing more effective pesticide residue management strategies.

## Materials and Methods

### Chemicals and reagents

The pesticide reference material of pirimicarb and its metabolites of pirimicarb-desmethyl and pirimicarb-desmethyl-formamido (with 99.17%, 99.30%, and 98.52% purity, respectively) was procured from Dr. Ehrenstorfer GmbH in Augsburg, Germany. The commercial wettable powder formulation of pirimicarb containing 50%, was obtained from Doğal Kimya, Türkiye. Chemicals such as acetonitrile, methanol, anhydrous magnesium sulfate, anhydrous sodium acetate, ammonium formate with purity over 99.0%, and acetic acid were supplied by Merck in Darmstadt, Germany. Additionally, PSA (Primary Secondary Amine) with a particle size of 40 µm was provided by Supelco Analytical in Bellefonte, PA, USA.

### Field trials

The field studies were conducted in 2023 at the Agricultural Application and Research Center of Tokat Gaziosmanpaşa University, in Tokat, Türkiye. The study utilized five different pepper varieties. The selection of pepper varieties was based on their commercial importance and widespread cultivation in Türkiye. The varieties Köylüm f1 (three-lobed), Tufan f1 (charleston), İstek f1 (bell pepper), Forte f1 (long

green), and Nefer f1 (capia) were chosen as they represent different morphological and physiological characteristics such as fruit shape, size, flesh thickness, and surface texture. The experimental plots were designed with a length of 5 m and a width of 2.8 m, with plants spaced 25 cm apart within rows and 140 cm apart between rows. A randomized block design with three replications was used, with each plot containing 20 pepper plants. Pepper plants were grown without pesticide applications, following recommended agronomic practices. Drip irrigation was used to cultivate experimental plants. Pesticides were applied according to SANTE/2019/12752 guideline (SANTE, 2019). The pesticide was applied using a battery-powered knapsack sprayer equipped with a conical spray nozzle at a pressure of 0.4 MPa. The pesticide was applied at the recommended dose of 50 g 100 $L^{-1}$. A randomized block design with three replications was used, with each plot containing 20 pepper plants. Pepper samples were harvested and analyzed 24 hours before applying the pesticide, confirming the absence of residues. Pepper samples were collected and analyzed 24 hours before pesticide application to confirm the absence of residues. This step was conducted to establish a baseline residue level, ensuring that any detected residues post-application could be attributed exclusively to the applied treatment, thereby eliminating potential confounding factors such as prior contamination or environmental deposition. Spraying occurred at the early fruit ripening stage, one week before the expected harvest. During the study, Tokat recorded an average relative humidity of 55.6% (ranging from 48.3% to 61.3%) and an average temperature of 22.5°C (ranging from 20.4°C to 24.5°C). There was no precipitation during the study period.

**Sample collection and storage**

Pepper samples were collected according to the Commission's 2002/63/EC regulation, which outlines the protocols for the formal sampling of pesticide residues in plant and animal products (EC, 2002). Pepper samples (12-24 pieces, approximately 2 kg) for dissipation kinetics were collected at zero time (2 hours post-spraying), $1^{st}$, $3^{rd}$, $5^{th}$, $7^{th}$,$10^{th}$, and $14^{th}$ after pesticide application (OECD, 2021). Latex gloves and polyethylene bags were used to minimize the risk of contamination during the harvesting process. After being collected, the samples were swiftly delivered to the laboratory for immediate analysis.

**Sample preparation, extraction, and clean-up**

The QuEChERS AOAC Method 2007.01 was applied to the extraction and clean-up procedures (Lehotay, 2007). A 4-blade blender homogenized the pepper samples (2 kg). A 15 g portion of the homogenized pepper sample was accurately weighed into a 50 mL Falcon tube. Subsequently, 15 mL of acetonitrile containing 1% acetic acid was added to facilitate extraction. During the QuEChERS extraction, 0.4 g of anhydrous magnesium sulfate and 0.1 g of anhydrous sodium acetate per gram of sample enhanced phase separation and improved analyte recovery. The mixture was vigorously shaken for 60 seconds to ensure thorough interaction between the sample and the solvent. The tube was centrifuged at 5000 rpm for 5 minutes to separate the organic phase. An 8 mL aliquot of the supernatant was transferred into a new 15 mL Falcon tube for further purification. 150 mg of magnesium sulfate and 50 mg of PSA per milliliter sample were added to eliminate co-extractive matrix components and potential interferences. The tube was then shaken for approximately 60 seconds to ensure effective adsorption of unwanted compounds. Subsequently, the sample was centrifuged again at 5000 rpm for 5 minutes. A 1 mL portion of the purified extract was filtered and transferred into glass vials for analysis. The final pesticide residue was determined using LC-MS/MS, ensuring high sensitivity and selectivity in quantifying pirimicarb and its metabolites.

**Analytical instruments and conditions**

The analyses were conducted using a Shimadzu® LC-MS 8050 system, renowned for its advanced UPLC and MS/MS capabilities. Chromatographic separation was executed on a Raptor Biphenyl (2.1 mm x 100 mm, 2.7 µm particle size) from Restek Pure Chromatography (USA). The mobile phase comprised 10 mmol/L ammonium formate in distilled water (A) and methanol (B). The mobile phase gradient initiated

at 50% B, ramped up to 95% B over 3.2 minutes, returned to 50% B at 3.21 minutes, and was maintained at 50% B from 3.21 to 4.75 minutes. Each sample injection volume was precisely 5 µL. The mobile phase flow rate was consistently maintained at 0.5 mL $min^{-1}$, with the column temperature regulated at 50°C. LabSolution® software (Version 5.118) was used to precisely manage all instrument parameters.

**Method verification**

The analytical method was subject to rigorous in-house verification following European SANTE parameters, which cover a variety of critical metrics such as linearity, mean recovery, limits of detection (LOD) and quantification (LOQ), accuracy, precision and measurement uncertainty (SANTE, 2021). Linearity was evaluated using matrix-matched calibration, with concentrations ranging from 5 to 200 µg $kg^{-1}$. The recovery of pirimicarb and its metabolites from the matrix was assessed by analyzing blank samples that were fortified at three concentration levels (10, 50, and 100 µg $kg^{-1}$). Chromatograms of pirimicarb and its metabolites obtained through LC-MS/MS analysis are provided in Figure 1, demonstrating the separation and detection efficiency of the analytical method.

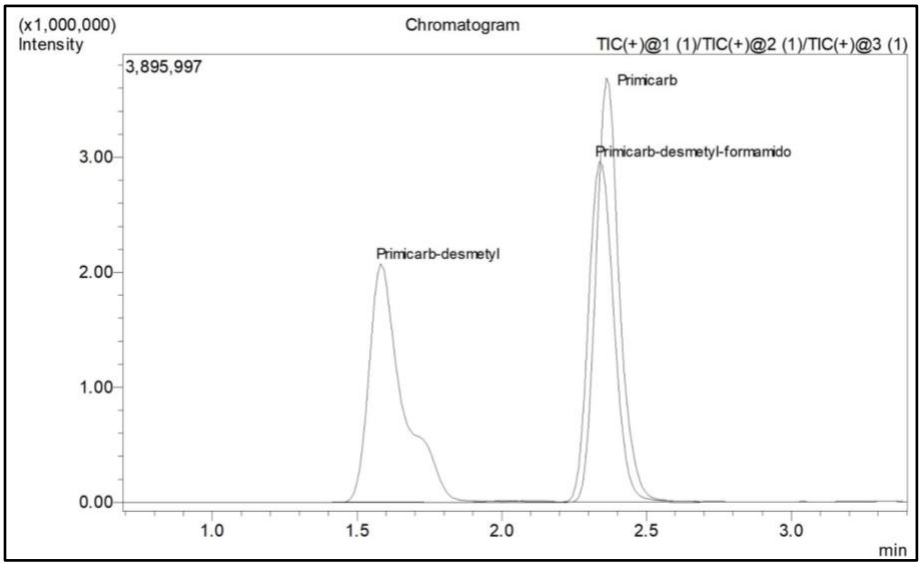


Figure. 1. Chromatogram of pirimicarb and its metabolites.

**Statistical analysis**

The dissipation kinetics of pesticides in pepper over a period were characterized by a single first-order kinetic model. Determining half-life ($t_{1/2}$) has been executed according to the following Eq 1 and Eq 2 (EPA, 2015).

$$C_t = C_0 \times e^{(-kt)} \quad (1)$$

$$t_{1/2} = \ln 2/k \quad (2)$$

where $C_0$ is the initial (zero-time) concentration of pesticide residues obtained from field experiments, while $C_t$ is the residue concentration at a given time, k is the dissipation coefficient, $t_{1/2}$ is the time interval required for the residue concentration to decline to half of its initial value ($C_0$) after application. An one-way analysis of variance (ANOVA) was conducted on the data using the SPSS 20.0 package software. The Tukey multiple comparison test was employed to ascertain whether the means differed at the 5% level.

In assessing the acute and chronic risks, the estimated dietary exposure was compared to ARfD, expressed in mg $kg^{-1}$ bw $day^{-1}$ and ADI, expressed in mg $kg^{-1}$ bw $day^{-1}$. The ADI was established at 0.035 mg $kg^{-1}$ body weight per day, while the ARfD was determined to be 0.1 mg $kg^{-1}$ body weight per day

(IUPAC, 2025). The acute hazard quotient (HQa), representing the risk to consumer health from short-term or acute exposure, is calculated by dividing the estimated short-term intake (ESTI, mg $kg^{-1}$ $day^{-1}$) by the acute reference dose (ARfD). In contrast, chronic hazard quotients (HQc), which assess the risk associated with long-term or chronic exposure, are derived by dividing the estimated daily intake (EDI, mg $kg^{-1}$ $day^{-1}$) by the acceptable daily intake (ADI) (EFSA, 2015). For the Turkish population, an average adult body weight of 73.7 kg was assumed (TUIK, 2024b; Yelaldı et al., 2024), along with an average daily pepper consumption (FC) of 0.077 kg per person (TUIK, 2022). Furthermore, median pesticide residue (MR, mg $kg^{-1}$) and high pesticide residue (HR, mg $kg^{-1}$) values observed at 7, 10, and 14 days were included in the analysis. The calculations were performed using the following equations.

$$ESTI=HR\times FC/body\ weight \quad (3)$$

$$HQ_a=ESTI/ARfD \quad (4)$$

$$EDI=MR\times FC/body\ weight \quad (5)$$

$$HQ_c=EDI/ADI \quad (6)$$

$HQ_a$ and $HQ_c$ values exceeding 1 were categorized as indicative of unacceptable risk. Higher values were associated with elevated levels of risk.

## Results and Discussion

### Method verification

Matrix-matched calibration solutions at concentrations of 5, 10, 25, 50, 100, and 200 µg/L were meticulously prepared and analyzed in triplicate using LC-MS/MS. The calibration curve demonstrated excellent linearity, with a correlation coefficient ($R^2$) greater than 0.998. The LODs and LOQs were found to be below the MRLs established by the EU for peppers (0.5 mg $kg^{-1}$) (EU-MRL, 2025). The mean recovery ranged from 89.30% to 109.83%, with a maximum relative standard deviation (RSD) of 10.82% (Table 1).

Table 1. Method optimization and verification data of pirimicarb, pirimicarb-desmethyl, and pirimicarb-desmethyl-formamido

| Analyte | Precursor ion, m/z | Product ion, m/z (CE, eV) | RT (min) | Linear regression equation Y = aX + b | Correlation coefficient ($r^2$) | LOD (µg $kg^{-1}$) | LOQ (µg $kg^{-1}$) | Spike level (µg $kg^{-1}$) | Repeatability Recovery, % (RSD, %) | Reproducibility Recovery, % (RSD, %) | U'% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pirimicarb | 239.2 | 72.1 (-22) | 2.407 | Y=184.492X - 5604.52 | 0.99977 | 1.90 | 6.34 | 10 | 98.52 (8.09) | 99.95 (7.51) | 18.14 |
| | | 182.2 (-15) | | | | | | 50 | 103.53 (7.42) | 104.10 (8.27) | |
| | | | | | | | | 100 | 109.83 (7.77) | 105.61 (5.08) | |
| Pirimicarb-desmethyl | 225.1 | 72.1 (-23) | 1.689 | Y=139.231X-234.789 | 0.99899 | 1.84 | 6.13 | 10 | 90.66 (9.35) | 93.67 (8.83) | 14.01 |
| | 224.9 | 168.1(-15) | | | | | | 50 | 105.33 (7.75) | 103.40 (6.13) | |
| | | | | | | | | 100 | 104.94 (6.87) | 104.11 (4.39) | |
| Pirimicarb-desmethyl-formamido | 253.1 | 72.1 (-22) | 2.395 | Y=114.347X+88366.2 | 0.99848 | 2.01 | 6.69 | 10 | 94.22 (10.82) | 89.30 (8.19) | 23.79 |
| | | 225.1 (-10) | | | | | | 50 | 99.49 (5.82) | 108.76 (6.64) | |
| | | | | | | | | 100 | 105.25 (6.75) | 103.35 (4.78) | |

CE, Collision energy; RT, Retention time; LOD, limit of detection; LOQ, limit of quantification; U', expanded measurement uncertainty. Y represents the instrument response, X is the analyte concentration, 'a' denotes the slope of the calibration curve, and 'b' is the intercept.

The expanded measurement uncertainty (U') remained below the default threshold of 50% (SANTE, 2021). Recovery results at all spiking levels confirmed that the method performance criteria for pesticide residue analysis, underscoring its accuracy and robustness.

**Degradation behaviors of pirimicarb in different pepper varieties**

In Commission Regulation (EU) 2016/71, pirimicarb residues are expressed as the sum of pirimicarb and pirimicarb-desmethyl (European Union, 2016). In this study, the total of pirimicarb, pirimicarb-desmethyl, and pirimicarb-desmethyl-formamido was represented as pirimicarb. The required waiting period before harvest, known as the pre-harvest interval (PHI), was set at 7 days for pirimicarb applied to peppers. This means that after the pesticide application, a minimum of 7 days must pass before the peppers can be harvested to ensure residue levels have declined sufficiently. The European Union has also established MRL for pirimicarb in peppers, which is 0.5 mg $kg^{-1}$. This limit represents the highest legally permissible concentration of pesticide residue allowed in the final product to ensure food safety (EU-MRL, 2025). The residues, half-lives, and dissipation rates of pirimicarb in different pepper varieties are presented in Table 2. The dissipation kinetics of pirimicarb residues in different pepper varieties over time are shown in Figure 2.

Table 2. Residues, rate of degradation, and half-life of pirimicarb in different pepper varieties

| Time after application, day | Three-lobed Residue, µg $kg^{-1}$ (Loss, %) | Bell pepper Residue, µg $kg^{-1}$ (Loss, %) | Long green Residue, µg $kg^{-1}$ (Loss, %) | Charleston Residue, µg $kg^{-1}$ (Loss, %) | Capia Residue, µg $kg^{-1}$ (Loss, %) |
|---|---|---|---|---|---|
| Zero time* | 829.55 | 870.57 | 947.37 | 247.68 | 581.69 |
| 1 | 376.75 (54.58) | 456.38 (44.99) | 353.07 (57.44) | 113.55 (86.31) | 183.31 (77.90) |
| 3 | 201.15 (75.75) | 283.23 (65.86) | 127.05 (84.69) | 51.23 (93.82) | 93.20 (88.77) |
| 5 | 128.79 (84.47) | 95.95 (88.43) | 75.90 (90.85) | 26.96 (96.75) | 72.29 (91.29) |
| 7** | 78.25 (90.57) | 87.88 (89.41) | 49.88 (93.99) | 23.09 (97.22) | 62.66 (92.45) |
| 10 | 39.13 (95.28) | 54.43 (93.44) | 28.42 (96.57) | 12.11 (98.54) | 40.30 (95.14) |
| 14 | 3.43 (99.59) | 6.38 (99.23) | 8.73 (98.95) | 3.48 (99.58) | 12.61 (98.48) |
| Kinetics equation | $C_t = 713.55e^{-0.348x}$ | $C_t = 722.64e^{-0.316x}$ | $C_t = 492.76e^{-0.302x}$ | $C_t = 157e^{-0.276x}$ | $C_t = 293.83e^{-0.226x}$ |
| k ($day^{-1}$) | 0.348 | 0.316 | 0.302 | 0.276 | 0.226 |
| $R^2$ | 0.936 | 0.957 | 0.854 | 0.895 | 0.844 |
| $t_{1/2}$ (day) | 1.99a | 2.19a | 2.30a | 2.51ab | 3.07b |

*: Samples were taken after two hours of spraying, **PHI, $R^2$: Determination coefficient;
a-c: means with the same letter are not significantly different from each other ($p$>0.05 ANOVA followed by Tukey test).

The dissipation kinetics of pirimicarb residues in different pepper varieties over time are depicted in Figure 2. As observed, the initial residue levels varied across the pepper varieties, with bell pepper exhibiting the highest and charleston pepper the lowest concentration. The residues declined rapidly within the first 24 hours, and by the seventh day, all varieties had residue levels below the MRL of 500 µg $kg^{-1}$. The differences in degradation rates are thought to be influenced primarily by the morphological (e.g., fruit color, size, shape, flesh thickness, and surface structure), physiological, and biochemical characteristics of the pepper varieties. These inherent traits likely account for the variation in residue levels observed among the different pepper types.

The initial concentrations of pirimicarb residues for three-lobed, bell pepper, long green, charleston, and capia pepper varieties were determined as 829.55, 870.57, 947.37, 247.68, and 581.69 µg $kg^{-1}$, respectively. The initial residue concentrations of pirimicarb varied among the five pepper varieties, which can be attributed to their distinct morphological and physiological characteristics. Factors such as surface texture, cuticle composition, wax content, fruit size, and differences in surface-area-to-volume ratio may play a role in pesticide deposition. The half-lives of these varieties were calculated as 1.99, 2.19, 2.30, 2.51, and 3.07 days. Except for charleston pepper, the initial concentrations of pirimicarb residues in the other pepper varieties exceeded the MRL. Within 24 hours, the residues in all varieties decrease below the MRL, indicating that peppers could be safely consumed one day after application, considering the MRL for pirimicarb (500 µg $kg^{-1}$) (EU-MRL, 2025). However, the residue level of bell pepper on the first day (456.38 µg $kg^{-1}$) was close to the MRL, emphasizing the importance of monitoring residue dissipation in this variety. The degradation rates of pirimicarb residues varied across pepper varieties, with capia pepper exhibiting the longest half-life.

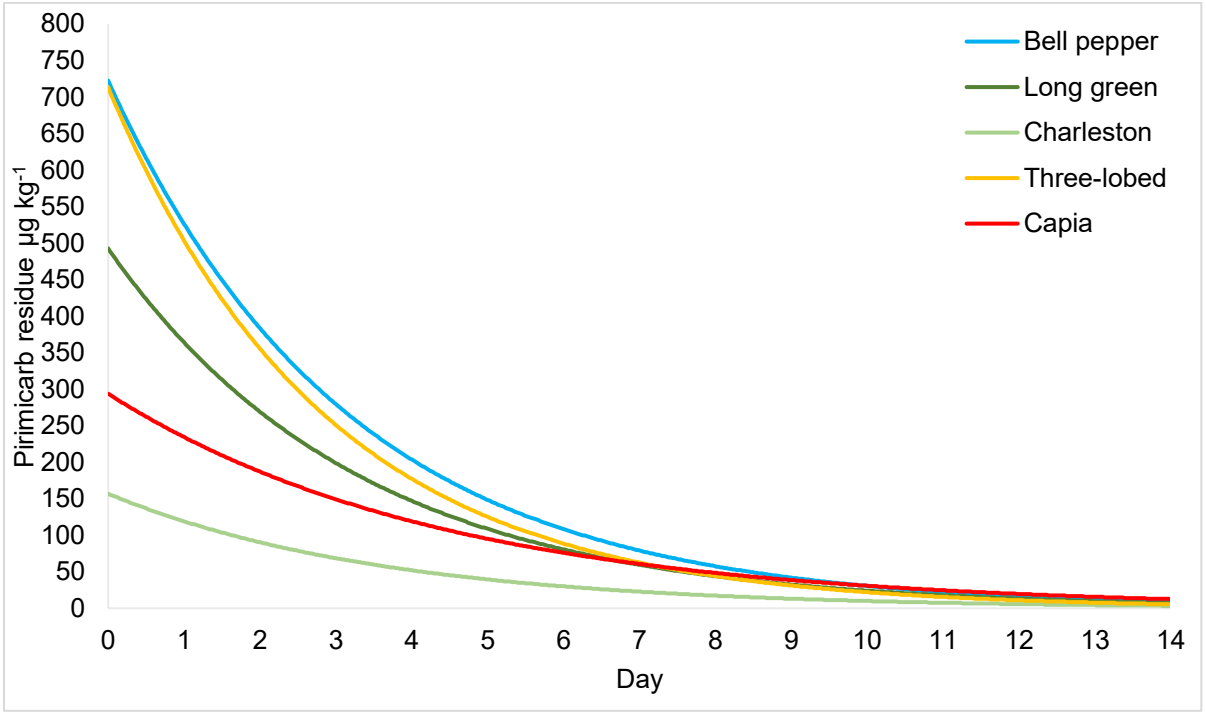


Figure. 2. Dissipation kinetic curves of pirimicarb in different pepper varieties.

To the best of our knowledge, no studies have specifically examined the degradation behaviors and residue levels of pirimicarb in different pepper varieties. By focusing on multiple pepper types, this research fills a significant gap and provides new insights into how varietal differences influence pesticide behavior. Although no directly comparable studies exist, İş (2019) demonstrated the impact of peach variety on pesticide degradation, highlighting the role of morphological traits in degradation rates. Similarly, Alister et al. (2017) reported that grape berry size influenced pesticide residue distribution, emphasizing the importance of morphological factors in residue degradation. These findings underscore the significance of physical and structural characteristics, such as surface texture, fruit size, and flesh thickness, in determining degradation behaviors. For instance, the surface texture of peppers may affect the adherence and penetration of pirimicarb, thereby influencing its degradation rates. Furthermore, differences in fruit size and flesh thickness likely impact the internal distribution and retention of the pesticide.

This study emphasizes the need to understand better how specific morphological and physiological traits of pepper varieties interact with pesticide behavior. Future research should systematically investigate these factors under controlled conditions to clarify the relationship between varietal characteristics and degradation dynamics. Such studies would facilitate the development of more tailored pesticide application strategies, improving both efficacy and safety in vegetable production. Given the scarcity of studies in this area, further research is needed to validate these findings under varying environmental and agronomic conditions and to assess their implications for consumer safety and sustainable agricultural practices. Additionally, the results could inform region-specific guidelines for safe pesticide use, particularly in areas where pepper cultivation is economically significant.

**Health risk assessment**

In recent years, the evaluation of pesticide hazards has garnered significant attention from consumers, particularly in Türkiye. This growing concern is reflected in a range of studies, which highlight the potential risks associated with pesticide residues in agricultural products (Çatak & Tiryaki, 2020; Balkan & Yılmaz, 2022; Serbes & Tiryaki, 2023; Balkan et al., 2024; Polat & Tiryaki, 2024; Yelaldı et al., 2024; Isci

et al., 2025; Keklik et al., 2025a, b). Increased awareness has prompted more rigorous pesticide safety assessments, emphasizing the need for effective risk management strategies to ensure public health and food safety. In this study, the health risk assessment of pirimicarb residues in different pepper varieties was conducted, and the results are presented in Table 3.

Table 3. The results of long-term risk assessments of lufenuron

| Pepper variety | MR (mg $kg^{-1}$) | HR (mg $kg^{-1}$) | EDI (mg $kg^{-1}day^{-1}$) | ESTI (mg $kg^{-1}day^{-1}$) | $HQ_c$ | $HQ_a$ |
|---|---|---|---|---|---|---|
| Three-lobed | 0.040 | 0.078 | 4.19E-05 | 8.14E-05 | 0.120 | 0.081 |
| Bell pepper | 0.050 | 0.088 | 5.16E-05 | 1.04E-04 | 0.147 | 0.104 |
| Long green | 0.029 | 0.050 | 2.90E-05 | 4.99E-05 | 0.083 | 0.050 |
| Charleston | 0.013 | 0.023 | 1.29E-05 | 2.31E-05 | 0.037 | 0.023 |
| Capia | 0.039 | 0.063 | 3.85E-05 | 6.27E-05 | 0.110 | 0.063 |

MR, Median pesticide residue; HR, High pesticide residue; EDI, estimated daily intake; ESTI, estimated short-term intake; $HQ_a$; acute hazard quotient, $HQ_c$; chronic hazard quotient.

All varieties' HQc values were below 1, indicating no significant health risk. Similarly, the HQa values were within acceptable limits, confirming the safety of pirimicarb residues in the tested pepper varieties under the current usage conditions. However, it was observed that bell pepper and three-lobed pepper had slightly higher HQc and HQa values compared to the other varieties, suggesting the importance of monitoring residue levels, particularly in these varieties.

## Conclusion

This study provides a comprehensive analysis of the degradation behaviors and residue levels of pirimicarb in different pepper varieties, offering valuable insights into the influence of varietal differences on pesticide behavior. The findings demonstrated that pirimicarb residues degrade rapidly across nearly all pepper varieties, falling below the EU-MRL of 500 µg $kg^{-1}$ within 24 hours of application. The significant variation in half-lives among the pepper types highlights the role of intrinsic morphological and physiological traits, such as surface texture, fruit size, and flesh thickness, in influencing degradation rates. By addressing a critical gap in the literature, this research emphasizes the importance of understanding how varietal differences impact pesticide dissipation. The results substantially affect consumer safety and sustainable agricultural practices, particularly optimizing pesticide application strategies.

The health risk assessment confirmed that chronic (HQc) and acute (HQa) health risk values for all pepper varieties were within acceptable limits, indicating no significant health risks under current usage conditions.

Future studies should focus on validating these findings under diverse environmental and agronomic conditions. Additionally, controlled experiments are necessary to systematically examine the effects of varietal differences on pesticide behavior, which could guide the development of region-specific guidelines for safe pesticide use in pepper production.

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## References

Alavanja, M. C., J. A. Hoppin & F. Kamel, 2004. Health effects of chronic pesticide exposure: Cancer and neurotoxicity. Annual Review of Public Health, 25 (2004): 155-197.

Alister, C., M. Araya, K. Becerra, J. Saavedra & M. Kogan, 2017. Pre-harvest interval periods and their relation to fruit growth stages and pesticide formulations. Food Chemistry. 221 (2017): 548-554.

Altuntaş, Ö., R. Küçük & M. Değirmenci, 2021. Investigation of promising genotypes selected from Arapgir bell pepper population in terms of their plant characteristics. Yuzuncu Yıl University Journal of Agricultural Sciences, 31 (1): 1-10 (in Turkish with abstract in English).

Anonymous, 2022. Biber hastalık ve zararlılar ile mücadele. (Web page: https://www.tarimorman.gov.tr/GKGM/Belgeler/Uretici_Bilgi_Kosesi/Dokumanlar/biber_hastalik_ve_zararlilari_ile_mucadele.pdf) (Date accessed: December 2024) (in Turkish).

Anonymous, 2024. Plant protection products database. (Web page: https://bku.tarimorman.gov.tr/Arama/Index?csrt=11430343323884281979) (Date accessed: December 2024) (in Turkish).

Antonious, G. F., 2004. Residues and Half-Lives of Pyrethrins on Field Grown Pepper and Tomato. Journal of Environmental Science and Health Part B, 39 (4): 491-503.

Archibald, B. A., K. R. Solomon & G. R. Stephenson, 1994. Estimating pirimicarb exposure to greenhouse workers using video imaging. Archives of Environmental Contamination and Toxicology, 27 (2): 126-129.

Balkan, T. & K. Kara, 2023. Dissipation Kinetics of Some Pesticides Applied Singly or in Mixtures in/on Grape Leaf. Pest Management Science, 79 (3): 1234-1242.

Balkan, T. & Ö. Yılmaz, 2022. Investigation of insecticide residues in potato grown in Türkiye by LC-MS/MS and GC-MS and health risk assessment. Turkish Journal of Entomology, 46 (4): 481-500.

Balkan, T., K. Kara & M. Kızılarslan, 2024. Investigation of the dissipation kinetics of lufenuron in pepper grown under field conditions. Turkish Journal of Entomology, 48 (4): 439-448.

Can, E. & M. R. Ulusoy, 2022. Adana ili açık alan biber yetiştiriciliğinde sorun olan Arthropoda Şubesi'ne bağlı zararlı ve yararlı türlerin saptanması. Çukurova Tarım ve Gıda Bilimleri Dergisi, 37 (1): 79-87 (in Turkish with abstract in English).

Çatak, H. & O. Tiryaki, 2020. Insecticide residue analyses in cucumbers sampled from Çanakkale open markets. Turkish Journal of Entomology, 44 (4): 449-460.

Chen, M., J. Zhang, H. Yang, Y. Ma & H. Cui, 2020. Advances in pesticide residue analysis: Recent analytical methods and their applications. Food Chemistry, 315 (2020): 126158.

Cönger, E., P. Aksu, N. Yigit, S. Dokumacı, Z. Baloğlu & A. A. Burçak, 2012. Studies on residue behaviour of certain pesticides used in vegetables. Plant Protection Bulletin, 52 (3): 273-288 87 (in Turkish with abstract in English).

Damalas, C. A. & I. G. Eleftherohorinos, 2011. Pesticide exposure, safety issues, and risk assessment indicators. International Journal of Environmental Research and Public Health, 8 (5): 1402-1419.

Donkor, A., P. Osei-Fosu, B. Dubey, R. Kingsford-Adaboh, C. Ziwu & I. Asante, 2016. Pesticide residues in fruits and vegetables: Risk assessment and monitoring. Environmental Science and Pollution Research, 23 (18): 18966-18987.

EC, 2002. European Commission: Commission Directive 2002/63/EC of 11 July 2002 establishing Community methods of sampling for the official control of pesticide residues in and on products of plant and animal origin and repealing Directive 79/700/EEC. Official Journal of the European Communities, L 187 (45): 30-43.

EFSA, 2015. European Food Safety Authority: Revisiting the International Estimate of Short-Term Intake (IESTI equations) used to estimate the acute exposure to pesticide residues via food. EFSA Supporting Publication, 12 (12): 1-81.

EFSA, 2024. European Food Safety Authority: Peer review of the pesticide risk assessment of the active substance pirimicarb. EFSA Journal, 22 (10): 1-31.

EPA, 2015. Standard operating procedure for using the nafta guidance to calculate representative half-life values and characterizing pesticide degradation. (Web page: https://www.epa.gov/sites/default/files/2015-08/documents/ftt_sop_using_nafta_guidance_version2.pdf) (Date accessed: September 2024).

Eskenazi, B., A. R. Marks, A. Bradman, K. Harley, D. B. Barr, C. Johnson, N. Morga & N. P. Jewell, 2007. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environmental Health Perspectives, 115 (5): 792-798.

EU-MRL, 2025. European Union (EU-MRL) Pesticides Database: Pesticide Residues MRLs. Directorate General for Health & Consumers. (Web page: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/mrls) (Date accessed: January 2025)

European Union, 2016. Commission Regulation (EU) 2016/71 of 26 January 2016 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for pirimicarb in or on certain products. Official Journal of the European Union, L 20 (59): 8-27.

Feng-Shou, D., Y. Shuang, L. Xin-Gang, J. Jim-Peng, Z. Yong-Quan, L. Chong-Jiu & Y. Jim-Ren, 2008. Fate of fluazinam in pepper and soil after application. Agricultural Sciences in China, 7 (2): 193-199.

Feng, Y., A. Zhang, Y. Bian, L. Liang & B. Zuo, 2021. Determination, residue analysis, dietary risk assessment, and processing of flupyradifurone and its metabolites in pepper under field conditions using LC-MS/MS. Biomedical Chromatography, 36 (4): e5312.

Gupta, R. C., D. Chang, K. Nam & A. Bafila, 2020. ''Toxicological Profile of Carbamate Pesticides, 469-479''. Handbook of Toxicology of Chemical Warfare Agents (Ed. Gupta R.C). Academic Press, 1198 pp.

Hem, L., J. Choi, J. Park, M. Mamun, S. Cho, A. M. Abd El-Aty & J. Shim, 2011. Residual pattern of fenhexamid on pepper fruits grown under greenhouse conditions using HPLC and confirmation via tandem mass spectrometry. Food Chemistry, 126 (2011): 1533-1538.

İş, M., 2019. Determination of residual amounts of kresoxim methyl, boscalid and tetraconazole in some peach varieties according to the waiting periods. Çanakkale Onsekiz Mart Üniversitesi, MSc Thesis, Çanakkale, 38 pp (in Turkish with abstract in English).

Isci, G., O. Golge & B. Kabak, 2025. Infant and toddler health risks associated with pesticide residue exposure through fruit-and vegetable-based baby food. Journal of Food Composition and Analysis, 137: 106870.

IUPAC, 2025. The PPDB-Pesticide properties database, international union of pure and applied chemistry. (Web page: http://sitem.herts.ac.uk/aeru/iupac/Reports/420.htm) (Date accessed: January 2025)

Jacobsen, R. E., P. Fantke & S. Trapp, 2015. Analysing half-lives for pesticide dissipation in plants. SAR and QSAR in Environmental Research, 26 (4): 325-342.

Jurewicz, J. & W. Hanke, 2008. Prenatal and childhood exposure to pesticides and neurobehavioral development: Review of epidemiological studies. International Journal of Occupational Medicine and Environmental Health, 21 (2): 121-132.

Keklik, M., E. Odabas, O. Golge & B. Kabak, 2025a. Pesticide residue levels in strawberries and human health risk assessment. Journal of Food Composition and Analysis, 137 (2025): 106943.

Keklik, M., O. Golge, M. Á. González-Curbelo & B. Kabak, 2025b. Pesticide residues in peaches and nectarines: Three-year monitoring data and risk assessment. Food Control, 172 (2025): 111141.

Lehotay, S., 2007. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: Collaborative study. Residues and Trace Elements, 90 (2): 485-520.

Liu, X., Y. Yang, Y. Cui, H. Zhu, X. Li, Z. Li, K. Zhang & D. Hu, 2014. Dissipation and residue of metalaxyl and cymoxanil in pepper and soil. Environmental Monitoring and Assessment, 186 (2014): 5307-5313.

Lu, M., W. W. Jiang, J. Wang, Q. Jian, Y. Shen, X. Liu & X. Yu, 2014. Persistence and dissipation of chlorpyrifos in *Brassica chinensis,* lettuce, celery, asparagus lettuce, eggplant, and pepper in a greenhouse. PLOS One, 9 (6): e101290.

Malhat, F. M., 2017. Persistence of metalaxyl residues on tomato fruit using high performance liquid chromatography and QuEChERS methodology. Arabian Journal of Chemistry, 10 (S1): 765-768.

OECD, 2021. Test No. 509: Crop Field Trial, OECD Guidelines for the Testing of Chemicals, Section 5, OECD Publishing, Paris. (Web page: https://www.oecd.org/content/dam/oecd/en/publications/reports/2021/06/test-no-509-crop-field-trial_g1ghbba1/9789264076457-en.pdf) (Date accessed: February 2024)

Piel, C., C, Pouchieu, L. Migault, B. Béziat, M. Boulanger, M. Bureau, C. Carles, A. Grüber, Y. Lecluse, V. Rondeau, X. Schwall, S. Tual, P. Lebailly & I. Baldi, 2019. Increased risk of central nervous system tumours with carbamate insecticide use in the prospective cohort AGRICAN. International Journal of Epidemiology, 48 (2): 512–526.

Polat, B. & O. Tiryaki, 2024. Herbicide contamination of Batak plain agricultural soils and risk assessment. Journal of Environmental Science and Health Part B, 59 (5): 203-208.

Rauh, V. A., R. Garfinkel, F. P. Perera, H. F. Andrews, L. Hoepner, D. B. Barr, R. Whitehead, D. Tang & R. W. Whyatt, 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics, 118 (6): e1845-e1859.

Riva, C., M.B. Sokolowski, J. Normand, J.S.D.O. Santos & M.P. Halm Lemeille, 2018. Effect of Oral Exposure to the Acaricide Pirimicarb, a New Varroacide Candidate, on Apis Mellifera Feeding Rate. Pest Management Science, 74 (8): 1790-1797.

SANTE, 2019. SANTE/2019/12752, On data requirements for setting maximum residue levels, comparability of residue trials and extrapolation of residue data on products from plant and animal origin, 2-55. (Web page: https://food.ec.europa.eu/document/download/d0729db4-fe2f-4750-b3d4-f7aa913c51d1_en?filename=pesticides_mrl_guidelines_app-d.pdf) (Date accessed: September 2024).

SANTE, 2021. SANTE/11312/2021, Analytical quality control and method validation procedures for pesticide residues analysis in food and feed, 1-55. (Web page: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.pdf) (Date accessed: April 2022).

Şarkaya Ahat, C., 2015. Domates ve Biberde Ardışık Pestisit Uygulamasının Pestisitlerin Parçalanma Kinetiğine Olan Etkisi. Adnan Menderes Üniversitesi, (Unpublished) MSc Thesis, Aydın, 64 pp (in Turkish with abstract in English).

Serbes, E. B. & O. Tiryaki, 2023. Determination of insecticide residues in "Bayramiç Beyazı" nectarines and their risk analysis for consumers. Turkish Journal of Entomology, 47 (1): 73-85.

Sharma, K. K., I. Mukherjee, B. Singh, S. K. Sahoo, N. S. Parihar, B. N. Sharma, V. D. Kale, R. V. Nakat, A. R. Walunj, S. Mohapatra, A. K. Ahuja, D. Sharma, G. Singh, R. Noniwal & S. Devi, 2014. Residual behavior and risk assessment of flubendiamide on tomato at different agro-climatic conditions in India. Environmental Monitoring and Assessment, 186 (11): 7673-7682.

Solomon, G., 2000. Pesticides and human health: A resource for health care professionals. University of California, San Francisco.

Thongprakaisang, S., K. Thiantanaviboon, N. Rangkadilok, T. Suriyo & J. Satayavivad, 2013. Glyphosate induces human breast cancer cells growth via estrogen receptors. Food and Chemical Toxicology, 59 (2013): 129-136.

TUIK, 2022. Turkish Statistical Institute. (Web page: https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr) (Date accessed: January 2025) (in Turkish).

TUIK, 2024a. Foreign Trade Statistics. (Web page: https://biruni.tuik.gov.tr/disticaretapp/disticaret.zul?param1=25&param2=0&sitcrev=0&isicrev=0&sayac=5802) (Date accessed: December 2024) (in Turkish).

TUIK, 2024b. Türkiye Health Interview Survey. (Web page: https://data.tuik.gov.tr/Bulten/DownloadIstatistikselTablo?p=WEBW229PP/91tMV2m71fU6pRWq2F1ZD/lzOFFk0bNDi2rjAC8QDCRN62nr2M3n1K) (Date accessed: September 2024) (in Turkish).

WHO, 2010. Public health impact of pesticides used in agriculture. World Health Organization. (Web page: https://apps.who.int/iris/handle/10665/39772) (Date accessed: September 2024).

Whyatt, R. M., R. Garfinkel, L. A. Hoepner, D. Holmes, M. Borjas & M. K. Williams, 2007. Within- and between-home variability in indoor air insecticide levels during pregnancy among an inner-city cohort from New York City. Environmental Health Perspectives, 115 (3): 383-390.

Yelaldı, A., K. Kara & T. Balkan, 2024. Investigation of insecticide residues in fig and health risk assessment. Turkish Journal of Entomology, 48 (3): 319-326.

Zhang, W., H. Song, Y. Liu & T. Wang, 2022. Oxidative stress and genotoxicity of carbamate pesticides in mammalian cells: A review. Chemosphere, 287 (2022): 132216

Zhou, Q., J. Yang & X. Liu, 1996. Loss of pirimicarb residues from contaminated fabrics. Bulletin of Environmental Contamination and Toxicology, 57 (1): 29-33.

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*Original article (Orijinal araştırma)*

# Isolation of *Bacillus zhangzhouensis* OBB Liu et al. (Caryophanales: Bacillaceae) from native *Polyphylla fullo* (L., 1758) (Coleoptera: Scarabaeidae) larvae, PCR-based detection of *cry1* gene and evaluation of its biological control potential[1]

Yerel *Polyphylla fullo* (L., 1758) (Coleoptera: Scarabaeidae) larvalarından *Bacillus zhangzhouensis* OBB Liu et al. (Caryophanales: Bacillaceae)'nin izolasyonu, *cry1* geninin PCR tabanlı tespiti ve biyolojik kontrol potansiyelinin değerlendirilmesi

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## Abstract

*Bacillus zhangzhouensis* OBB Liu et al. (Caryophanales: Bacillaceae) isolate was obtained from *Polyphylla fullo* (L., 1758) (Coleoptera: Scarabaeidae) larvae in the Microbiology Laboratory of the Biology Department of the Faculty of Science of Atatürk University in 2024. Additionally, the presence of the *cry1* gene was identified. After endospore staining of the isolate, the presence of crystal protein was detected by phase contrast microscopy and SEM analysis. As a result of PCR, only the presence of the cry1 gene was detected and confirmed. The total protein contents were compared with those of *Bacillus thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) by performing SDS-PAGE analysis using a crystal protein and spore mixture. *B. zhangzhouensis* OBB showed bands ~ 250 kDa and ~ 80 kDa, while *B. thuringiensis* showed bands corresponding to ~70 kDa and ~45 kDa. Probit analysis was used to determine the $LC_{50}$ value of the isolates, and the Abbott method was used to determine the mortality percentages of the larvae. Spore-crystal mixtures of *B. thuringiensis* and *B. zhangzhouensis* OBB isolates were tested against *P. fullo* larvae at doses of 1000, 2000, and 4000 ppm. The highest mortality rate was determined in the spore-crystal mixture of *B. zhangzhouensis* OBB isolate at 4000 ppm dose.

**Keywords:** *Bacillus zhangzhouensis* OBB, biopesticides, *cry* genes, *Polyphylla fullo*, SDS-PAGE

## Öz

*Bacillus zhangzhouensis* OBB Liu et al. (Caryophanales: Bacillaceae) izolatı, 2024 yılında Atatürk Üniversitesi Fen Fakültesi Biyoloji Bölümü Mikrobiyoloji Laboratuvarında *Polyphylla fullo* (L., 1758) (Coleoptera: Scarabaeidae) larvalarından elde edilmiştir ve *cry1* geninin varlığı tanımlanmıştır. İzolatın endospor boyaması yapıldıktan sonra faz kontrast mikroskobu ve SEM analizi ile kristal protein varlığı tespit edilmiştir. PCR sonucunda sadece cry1 geninin varlığı tespit edilmiştir ve doğrulanmıştır. Kristal protein ve spor karışımı kullanılarak SDS-PAGE analizi ile *Bacillus thuringiensis* (Berliner, 1915) (Bacteria: Bacillaceae) ile arasındaki total protein içerikleri karşılaştırılmıştır. *Bacillus zhangzhouensis* OBB ~250 kDa ile ~80 kDa arasında bantlar gösterirken, *B. thuringiensis,* 70 kDa ve ~45 kDa'ya karşılık gelen bantlar göstermiştir.İzolatların $LC_{50}$ değerini belirlemek için probit analizi, larvaların ölüm yüzdelerini belirlemek için ise Abbott yöntemi kullanılmıştır. *B.thuringiensis* ve *B. zhangzhouensis* OBB izolatlarının spor-kristal karışımları (1000, 2000 ve 4000 ppm) dozlarında *P. fullo* larvalarına karşı test edilmiştir. En yüksek ölüm oranı *B. zhangzhouensis* OBB izolatının 4000 ppm dozunda spor-kristal karışımında belirlenmiştir.

**Anahtar sözcükler:** *Bacillus zhangzhouensis* OBB, biyopestisitler, *cry* genleri, *Polyphylla fullo*, SDS-PAGE

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## Introduction

With the urbanization brought about by rapid population growth, agricultural areas are decreasing day by day and the amount of agricultural products per capita is declining. Türkiye, which used to be a self-sufficient country in terms of agricultural products, now imports agricultural products from many countries. One of the most important reasons for this is that pest control in economically important plants cannot be done consciously and completely (Bülbüloğlu, 2000). There are around 30,000 beetle species in the Scarabaeidae family (Insecta: Coleoptera) worldwide. *Polyphylla fullo* (L., 1758) (Coleoptera: Scarabaeidae) is one of the most significant members of this family. For *P. fullo* larvae, the term "Manas bug" is often used. *P. fullo* is one of the most damaging pests of orchards, vineyards, ornamental plants, grass, potatoes, peppers, tomatoes, and a number of other crops in Türkiye. It also drastically lowers the production of plants and forests (Anonymous, 2008). April and May saw the emergence of *P. fullo* adults from the soil as the temperature warmed. After nightfall, adults fly to the trees, mate, and deposit their eggs in the dirt of overgrown and uncultivated gardens. After hatching from the eggs, the larvae dwell in groups, feed on the grass roots, and shed their skins to become second-stage larvae after two months. Larvae in their second stage consume a substantial amount of food. In the fall, they hibernate deep in the ground. A feeding that can cause serious harm occurs from March or April until the first part of June. After that, they undergo a skin change and develop into third-stage larvae. During their one-year lifespan, the third-stage larvae do considerable harm. They pupate in a dirt nest that is 15-35 cm below the earth's surface in July. The pupae remain in the nest until the next spring, maturing into adults in the following autumn, in September. After hatching in this manner, an individual matures into an adult three years later and gives birth to offspring once every three years. Adult insects ruin things by eating flowers and foliage. They can leave behind leafless fruit and forest trees in heavily populated areas (Anonymous, 2008, 2011a). They target the root systems of several crops, such as ornamental plants, grass, grape vines, and young fruit trees. While older larvae nibble at tree roots, causing the plants to dry up and die, younger larvae feed on the roots of herbaceous plants (Borror et al., 1981). In Türkiye, the damage rate of these insects ranges from 50% to 80%, depending on the crop and soil type (Erler & Ates, 2015). Considering the damage they cause, controlling pests is very important for optimizing product yield. In addition, chemical pesticides registered worldwide are used against these insects. Due to the negative effects of chemical pesticides, including their non-biodegradability, persistence, and toxicity, an environmentally friendly approach is being adopted for crop pests (Buss, 2006). As an alternative to the traditional synthetic pesticides currently used for pre-harvest and post-harvest management of crop pests and diseases, biopesticides have garnered significant attention in the field of pest control in recent years (Kour et al., 2020; Yadav et al., 2020). Since microbial pesticides are an ecologically benign way to reduce pest populations in the agricultural business, they are very useful for both farmers and researchers (Kour et al., 2019a, b, c). Compared to readily accessible chemicals, the use of biopesticides to control insect pests is more cost-effective, ecologically friendly, and efficient (Thakur et al., 2020). According to Koul & Dhaliwal (2003), biopesticides protect crops over their whole growth phase, do not harm plants, and manage a significant number of insect pests. Biopesticides do not damage people or the environment; they only affect the pests they are intended to kill (Lengai & Muthomi, 2018). These days, biopesticides are widely employed in organic agriculture (Seiber et al., 2014) and play a significant role in the agricultural market (Nawaz et al., 2016).

The most important of these methods is biological control. Bacteria, viruses, fungi, nematodes, organisms belonging to the protozoa group and agents developed with recombinant techniques constitute the elements used in biological control. Among these, soil group bacteria are the most promising biological control agents (Bülbüloğlu, 2000). Today, many microorganisms are used in the biological control of insects. Although more than 100 bacterial species have been defined as insect pathogens so far, only the *Bacillus* species are commercially preferred as control agents. Bacteria of the genus *Bacillus* are microorganisms that attract attention due to their metabolic properties such as antibiotic, enzyme, and toxin

production, their industrial importance, and their easy production (Rosovitz et al., 1998). In addition, their sporulation abilities and diversity of metabolic activities provide significant advantages that facilitate their spread across diverse environments (Wipat & Harwood, 1999). *Bacillus* species produce peptide antibiotics used in the pharmaceutical industry, such as tyrocidine (*Bacillus brevis* Burmeister), subtilin, and bacilicin (*Bacillus subtilis* G.). *Bacillus thuringiensis*, *Bacillus sphaericus* Meyer & Neide and *Bacillus popilliae* Dutky species are pathogenic to various insect larvae and are used as biological control agents (Rosovitz et al., 1998). *Bacillus thuringiensis* (Bt) Berliner (Bacteria: Bacillaceae) is a bacterial species naturally found in soil and is an agricultural agent of economic importance. Bt is one of the most successful microbial insecticide agents and has been used and researched for years, especially in agriculture and forestry, in the biological control of pests such as mosquitoes, which are effective virus carriers, especially in agriculture and forestry, thanks to the proteins they produce in vegetative form and during sporulation. Bt has great commercial importance, especially because it is effective against many agricultural pests. Insecticidal proteins are insecticidal crystal proteins (Cry), vegetative insecticidal proteins (Vip) and secreted insecticidal proteins (Sip). Of these, extensive research has been done on Cry proteins, and various plant or crop varieties have been developed. Genes encoding insecticidal proteins are usually carried on plasmids. Plasmid transfer among these isolates provides gene and toxin diversity in Bt isolates (de Maagd et al., 2001). In addition, the collection of Bt samples from different geographical regions is important in finding new insecticidal genes and proteins. Studies in the literature focus on Bt isolation, characterization and bioactivity from different geographies of the world. New Bt collections are obtained to investigate the insecticidal activities of these isolates. Effective strains need to be found for the production of new commercial products (Baranek et al., 2015; Boukedi et al., 2016).

When it comes to controlling certain insect species from the Lepidoptera, Diptera, and Coleoptera orders, Bt is a safe organism that targets specific species. However, this study is the first to discover the cry gene in *Bacillus zhangzhouensis* OBB Liu et al. (Caryophanales: Bacillaceae), is the first. While there are entomopathogenic nematode studies on *P. fullo* insects in the literature, no entomopathogenic bacteria studies have been reported. In addition, the application of Cry proteins to *P. fullo* larvae and adults has unique value in terms of using a locally sourced strain and investigating the potential of this local strain to be used as a bioinsecticide. Furthermore, the collection of local samples is important for finding new insecticidal genes and proteins. The discovery of strains that can form the active ingredient of new commercial products also increases the unique value. Since the study is naturally of biological origin, it will not harm the environment or humans. This study aimed to screen *cry* genes in *B. zhangzhouensis* OBB isolate from *P. fullo using* PCR method, to detect cry proteins by SEM analysis, to determine the protein profile by SDS-PAGE, and examine its insecticidal activity. In this way, the potential used on Coleoptera will be determined. The effective *B. zhangzhouensis* OBB isolate we found will be an important alternative in breaking the resistance mechanism developed against both chemical pesticides and existing Bt preparations.

## Materials and Methods

### Sample collection

*Polyphylla fullo* larvae from the study were gathered in high concentrations from a range of urban and peri-urban locations in Türkiye, such as fields, gardens, and vineyards. Tokat, Türkiye; latitude: 40°18'50"N; longitude: 36°33'15"E. The field-collected larvae were stored in plastic containers filled with dirt. The larvae were kept in six 11-liter plastic containers and were regularly supplemented with food in the form of fruits. In September 2023, the larvae samples were gathered (Figure 1).

Figure 1. *Polyphylla fullo* larvae collected from dried tree roots in Tokat/Türkiye.

**Isolation of bacteria from *Polyphylla fullo***

The dead larvae of *P. fullo* were used to isolate bacteria. 70% ethyl alcohol was used to surface sterilize *Polyphylla fullo* larvae. After removing the insects' guts, the samples were cleaned two or three times with sterile distilled water to remove any residual alcohol. The intestines were crushed using 10 milliliters of phosphate buffer (PB, 50 mM, pH 7.4). 10 grams of Mueller Hinton, 10 grams of yeast extract, 3 grams of $K_2HP0_4$, 2 grams of glucose, and 0.5 grams of trehalose were added to one liter of MYPGP medium (Sharpe et al., 1970). 30 milliliters of MYPGP medium were mixed with 500 microliters of crushed intestinal sample, and the mixture was cultured for 48 hours at 30°C. Subsequently, 50 µl of MYPGP agar medium was added to the bacterial culture, which had been diluted to a concentration of $10^{-9}$. The colony was selected and inoculated into new MYPGP agar medium following an overnight incubation period at 30°C (Kang et al., 2012).

**Gram and endospore staining of isolates of *Bacillus zhangzhouensis* OBB**

Gram staining (Claus, 1992) and spore staining (Reynolds et al., 2009) characteristics of the isolate were determined.

**Scanning Electron Microscopy**

Isolates of *B. zhangzhouensis* OBB were incubated for seven days at 30°C while being shaken at 250 rpm in T3 medium. For ten minutes, the cell cultures were centrifuged at 4000 rpm. Three times, the pellets were reconstituted in sterile distilled water. After being fixed for 12 hours at 4°C in 2.5% glutaraldehyde, the cells were rinsed with sterile distilled water. Sterilized distilled water was used to dissolve them. SEM images were taken at Bilecik Seyh Edebali University Central Research Laboratory (Suludere et al., 1992).

**Molecular identification of isolate**

Genomic DNA Miniprep (BioBasic) was used to isolate the genomic DNA of the bacteria. In accordance with William et al., 1991, PCR was used to analyze the 16S rRNA sequences of the bacterial isolates from which genomic DNA was extracted. Universal primers were used to sequence the 16S rRNA gene. Applied Biological Materials' Taq 2X PCR Master Mix (G888) was utilized. The following ingredients were used to create a total of 25 µl of reaction mixture: One microliter of a 10 µM forward primer, one microliter of a 10 µM reverse primer, one microliter of a 5 ng/µl DNA solution, twenty-five microliters of PCR Taq 2X master mix, and twenty-two microliters of clean water. After that, the samples were put through a reaction cycle in a Sensoquest thermocycler. PCR stages were performed according to Mehtap et al., 2022. A 1.2% agarose gel was used to visualize the results of the polymerase chain reaction (PCR). The band in the gel was sent to MACROGEN in the Netherlands for sequencing. Confirmation was made by comparing with similar species registered in the NCBI database.

***Bacillus zhangzhouensis* OBB phylogeny**

BioEdit software was used to assemble the sequence (Hall et al., 2011). The isolate's sequencing was submitted into the GenBank database. To compare the sequences with the RefSeq database, the NCBI GenBank nBLAST search engine (http: //www.ncbi.nlm.nih.gov) was utilized. Phylogenetic analysis was performed using the isolate's sequence and those of closely related species. Phylogenetic tree of isolates was created with Neighbor-Joining analysis using Mega 11.0 program and performed with 1000 replicates with Bootstrap method (Figure 3).

**PCR analysis of *Bacillus zhangzhouensis* OBB isolates *cry* genes**

To find *cry* genes (Ben-Dov et al., 1995), PCR was conducted using the primers specified in Table 1.

Table 1. Primers used to screen for the presence of *cry* genes

| Gene | Primer sequences (5' -> 3') | Tm (°C) |
|---|---|---|
| *cry1* (277 bp) | CATGATTCATGCGGCAGATAAAC (f)<br>TTGTGACACTTCTGCTTCCCATT (r) | 59 |
| *cry2* (701 bp) | GTTATTCTTAATGCAGATGAATGGG (f)<br>CGGATAAAATAATCTGGGAAATAGT (r) | 55 |
| *cry3* (604bp) | CGTTATCGCAGAGAGATGACATTAAC (f)<br>CATCTGTTGTTTCTGGAGGCAAT (r) | 59 |
| *cry4* (439 bp) | GCATATGATGTAGCGAAACAAGCC (f)<br>GCGTGACATACCCATTTCCAGGTCC (r) | 59 |

**SDS-PAGE Analysis**

*Bacillus zhangzhouensis* OBB and *B. thuringiensis* isolates were used. *Bacillus thuringiensis* isolates were used as controls. The isolates were cultured in T3 medium for ten days and centrifuged at 14000 rpm, and +4°C. Following centrifugation, the larger proteins (Cry) stay in the pellet. The pellet is used directly in SDS after being repeatedly cleaned with pure water. Protein concentrations were measured using the methods Bradford (1976) described. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (10% PAGE) was used to test for purified cry proteins (Mehtap, 2022).

**Preparation of Spore-Crystal Proteins**

100 milliliters of liquid sporulation medium T3 (3 g $L^{-1}$ tryptone, 2 g $L^{-1}$ tryptose, 1.5 g $L^{-1}$ yeast extract, 0.005 g $L^{-1}$ $MnCl_2$, 6 g $L^{-1}$ $NaH_2PO_4$, and 7.1 g $L^{-1}$ $Na_2HPO_4$) were used to cultivate the activated isolate. It was incubated for seven days at 200 rpm and 30 °C in an incubator that was constantly shaking. Following the incubation period, the samples were centrifuged for 20 minutes at 15000 rpm. The pellet that resulted

from this process (spore-crystal protein) was then rinsed twice in 20 milliliters of sterile distilled water and centrifuged for 10 minutes at 15000 rpm (Travers et al., 1987). The spore-crystal protein mixture was precipitated by centrifugation, and then it was dried for 24 hours at 60°C to create a powder.

**Bioassays**

Spore-crystal suspensions of *B. zhangzhouensis* OBB and *B. thuringiensis* isolates were applied at 1000 ppm, 2000 ppm, and 4000 ppm rates. Ten larvae were tested for the bacterial isolate. The experiments were performed in triplicate. The experiments were performed on tomatoes grown in 30 cm diameter pots (Figure 2). The setups were arranged with three repetitions for six different applications. Tomato seedlings were planted in nine pots to form the groups and were prepared for the experiment after growing for 8-10 days. Only water was used in the control group. Effectiveness tests of different concentrations were conducted. Mortality data were calculated using the Abbott formula (Abbott, 1925). Lethal concentrations ($LC_{50}$) were calculated for the bacterial isolate (Finney, 1952).

Figure 2. Application of spore-crystal mixture to tomato seedlings.

**Statistical Analysis**

SPSS 22.0 was used for statistical analysis. It was analyzed with the Probit Analysis-MSChart 2.0 program to calculate the $LC_{50}$ value. All data were shown as mean±standard deviation (Mean±SD). Data of other tests were subjected to one-way analysis of variance (ANOVA) and evaluated using the LSD test ($p<0.05$).

## Result and Discussion

16S rRNA, and BLAST data indicated that *B. zhangzhouensis* OBB bacterium had identified. *B. zhangzhouensis* is a Gram-positive, rod-shaped, aerobic bacterium. Its colonies have an oval endospore in the middle and are spherical, creamy white, and translucent. The bacterium has catalase-positive and oxidase-positive results. Based on the findings of the BLAST analysis, a phylogenetic tree was created using the NCBI database (Figure 3).

The isolates' motility traits, endospore structure, cell shape, and Gram staining were all investigated. Gram staining produced purple bacilli, which were identified as Gram-positive under a light microscope. To diagnose the presence of spores, endospore staining was performed. Under a microscope, spores stained with malachite green were identified. Coomassie Brilliant Blue was used to stain the parasporal crystal proteins of the isolates, which were then observed under an Olympus CKX41 phase contrast microscope (Figure 4). Electron microscopy also revealed the existence of crystal protein (Figure 5).

To ascertain whether crystal proteins were present, light and electron microscopy were used to analyze the spore-crystal combination produced from the *B. zhangzhouensis* OBB strain (Figure 5). The crystals were found to be square, cubic, and bipyramidal in shape based on electron microscope examinations (Figure 5). Multiple crystal proteins can be synthesized by subspecies. All three of these isolates from the reference strain *Btk* HD-1 produced bipyramidal crystals. While *Cry2* creates cuboidal crystals that exhibit lepidopteran toxicity, *Cry1* toxins are linked to the formation of bipyramidal crystals and toxicity against lepidopteran insects, including *S. frugiperda* (Monnerat et al., 2007). Bipyramidal, cubic, flat rhomboid, spherical, and composite forms are all possible for crystal proteins (Yu et al., 2015). The Cry proteins varied among the isolates, according to a microscopic examination. Bipyramidal and other crystal forms were found in the samples during examination. The diversity of *cry* genes is further supported by these studies.

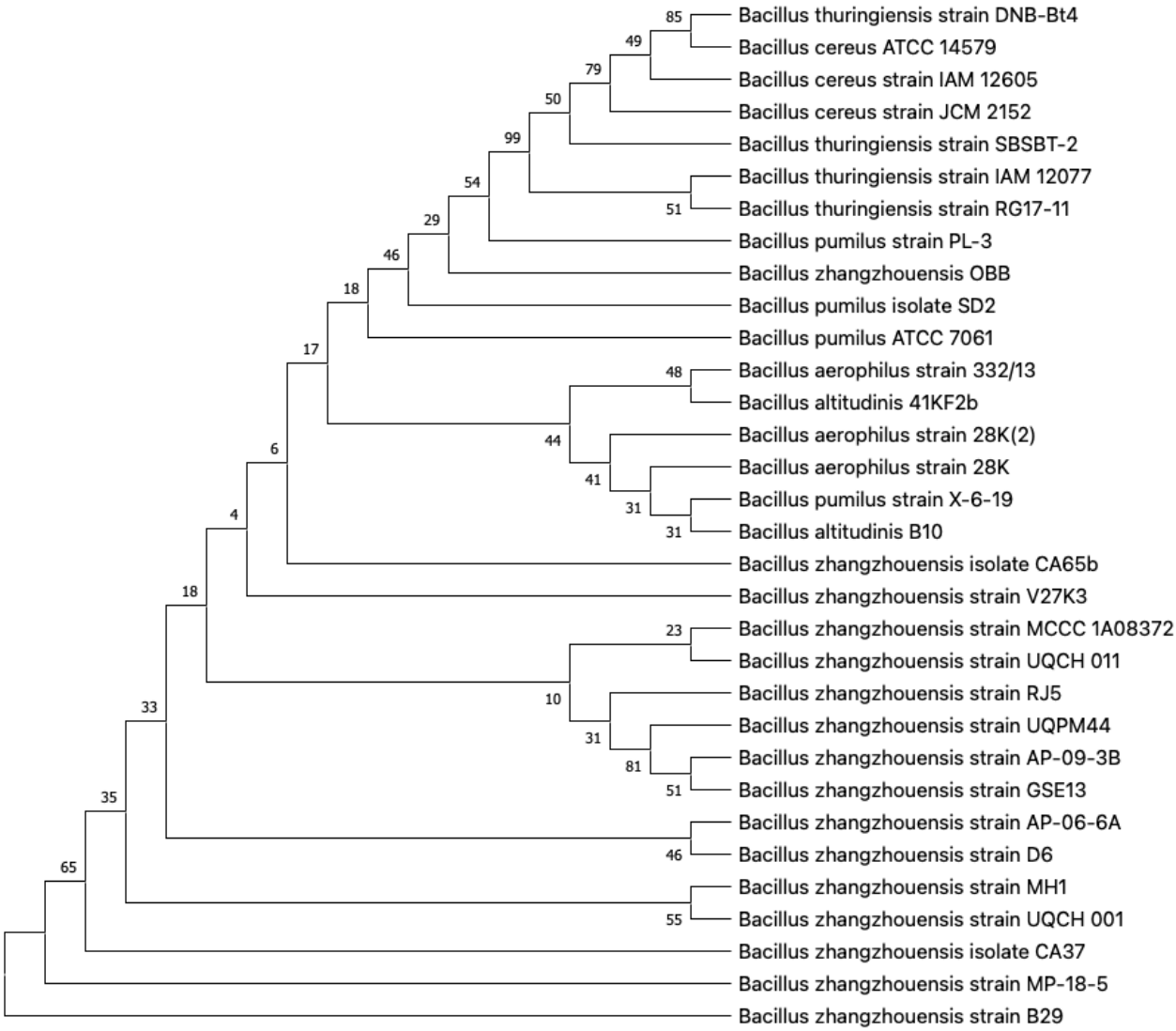


Figure 3. Phylogenetic tree of the 16S rRNA gene region of the isolate.

Figure 4. Endospore structure of *Bacillus zhangzhouensis* OBB bacteria under phase contrast microscope (100x).

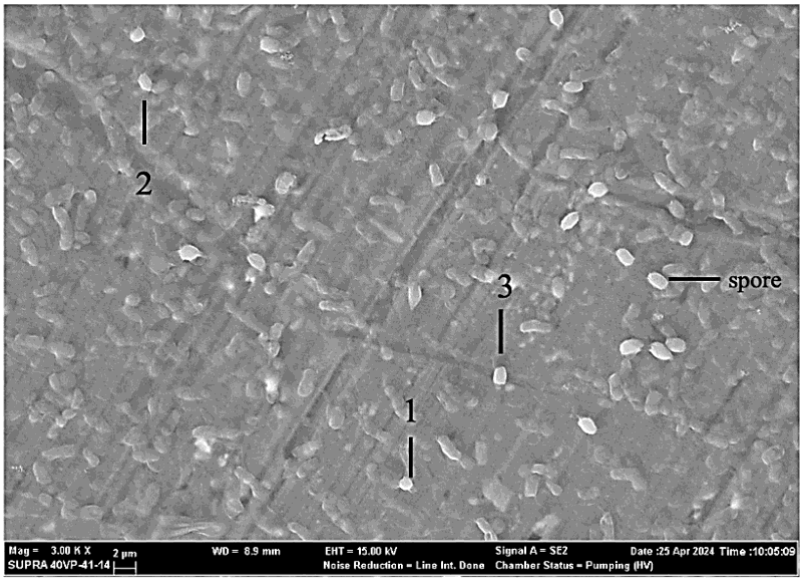


Figure 5. Using scanning electron microscopy (SEM) investigation of several crystal morphologies, the *Bacillus zhangzhouensis* OBB isolate was observed: (1) spherical crystal, (2) bipyramidal crystal and (3) square crystal.

The PCR study of the toxin genes of the *B. zhangzhouensis* OBB strain's toxin genes revealed that the strain carries the *cry1* gene. The *cry1* gene in *B. zhangzhouensis* OBB was shown to produce a 277 bp band on 1% agarose gel (Figure 6). In the literature, it is seen that many researchers have investigated the *cry* gene content and distribution in Bt collections in different parts of the world. In the general results obtained, *cry1* is the most abundant gene group, followed by the *cry2* genes (Baig &Mehnaz, 2010). Jain et al. (2017) found that the *cry1* gene (100%) was the most abundant among the Bt isolates they obtained from India. Bozlağan et al. (2010) obtained 60 Bt isolates from soil samples collected from different regions of Kayseri and showed that 28% of them were *cry1* positive. In the study conducted by Alper et al. (2014) in which the *cry* gene contents of 288 Bt isolates from fig orchards were screened, *cry1* (36%) was found to be the most common, while the rates of *cry2 (*3%) and *cry3* (1%) were lower. In another study, 24 of 44 soil isolates were *cry1*, and 14 were *cry2*. It was determined that these isolates contain these genes (Lone et al., 2017).

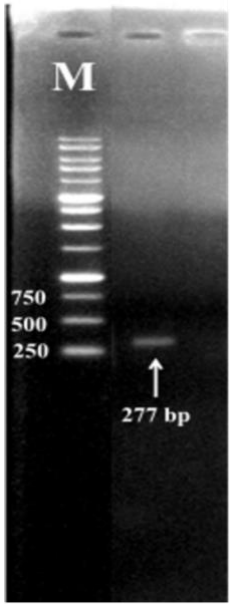


Figure 6. Visualization of *cry1* gene of *Bacillus zhangzhouensis* OBB in 1.2% agarose gel as a result of PCR *(*M: Marker (Ecotech-1 kb DNA Ladder).

Spore-crystal mixtures of *B. thuringiensis* and *B. zhangzhouensis* OBB bacteria were applied at doses of 1000, 2000, and 4000 ppm. The highest mortality rate on *P. fullo* larvae was determined to be 90% effective at 4000 ppm spore-crystal concentration of *B. zhangzhouensis* OBB. The lowest mortality rate was calculated as 55% at 1000 ppm spore crystal concentration of *B. thuringiensis* isolate (Figure 7). The effect of bacterial isolates on the larvae of the pest was determined by probit analysis for 4000 ppm concentration and it was determined that *B. zhangzhouensis* OBB had the highest $LC_{50}$ value of 6,11. The lowest $LC_{50}$ value of bacterial isolates on the pest was *B. thuringiensis* with 4.87 for 4000 ppm concentration (Table 2).

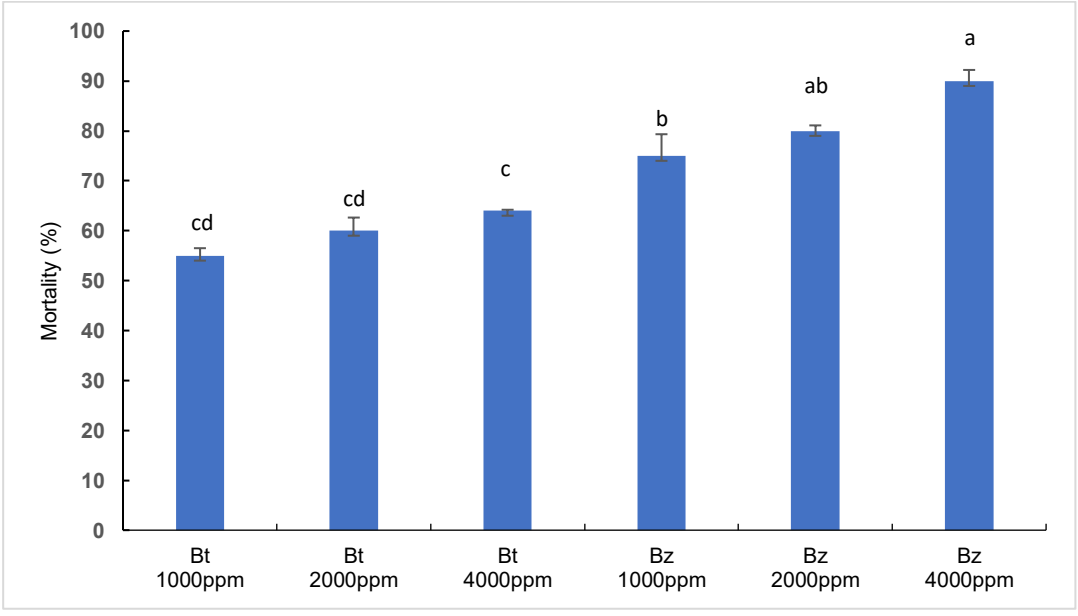


Figure 7. Lethal effect of spore-crystal mixture of *Bacillus thuringiensis* (Bt) and *Bacillus zhangzhouensis* OBB (Bz) isolates at doses of 1000, 2000, 4000 ppm on *Polyphylla fullo* larvae.
* Means followed by different letters within a column are statistically different ($p<0.05$) from each other.

Table 2. Probit analysis results of isolates

| Isolates | N | Slope±SD | $LC_{50}$(Confidence interval, 95%) | $X_2$ | df | p |
|---|---|---|---|---|---|---|
| *Bacillus thuringiensis* | 30 | 0,80 ± 0,055 | 4,87 (4,52-5,43) | 6,62 | 3 | ,0001 |
| *Bacillus zhangzhouensis* OBB | 30 | 0,90 ± 0,117 | 6,11 (5,73-6,75) | 0,50 | 3 | ,0001 |

Figure 8 illustrates the distinction between control and applications. Given that the *cry1* gene was the most commonly found gene in this collection and that the Cry1 protein has particular insecticidal properties against Lepidoptera (Tarekegn & Teferra, 2023), one could wonder if the presence of the cry protein gene in the related *B. zhangzhouensis* OBB was caused by a different insect ecology brought about by different geographic and climatic conditions. Saadoun et al. (2001) reported that the $LC_{50}$ values of Bt isolates isolated from soil ranged between 4.60 to 8.65 spore-crystal conc./ml for *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) larvae and 5.30 to 6.74 spore-crystal conc./ml for *C. pulex*. In the study conducted by Wang et al., it was determined that spore toxin mixtures of Bt isolates carrying *cry1* genes were 71-83% effective against *Helicoverpa virescens* (Fabricus, 1777) and *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae) larvae (Wang et al., 2003). The highest mortality rate was determined in the spore-crystal mixture at the dose of 4000 ppm. It shows that the *B. zhangzhouensis* OBB isolate is more lethal than other isolates. According to the results, the *B. zhangzhouensis* OBB isolates that were acquired for this investigation can be employed as biological control agents.

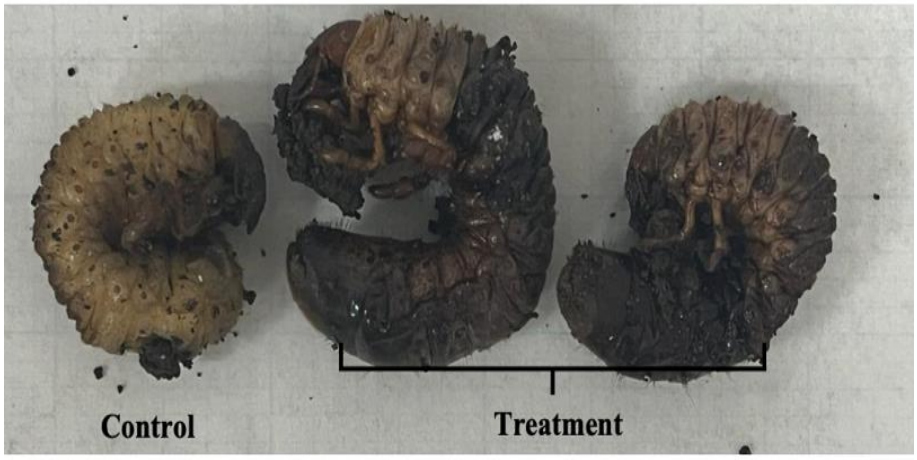


Figure 8. Difference between water only (control) and 10 days after application of *Bacillus zhangzhouensis* 4000 ppm.

SDS-PAGE was used to compare the spore-crystal mixture in Bt and *B. zhangzhouensis* OBB isolates. *B. zhangzhouensis* OBB displayed bands between 250 kDa and 80 kDa, whereas Bt displayed bands corresponding to 70 kDa and 45 kDa (Figure 9). Similarly, the Bt strain isolated from *Balaninus nucum* showed the presence of *cry1* and *cry2* genes and their corresponding Cry1 and Cry2 proteins with ~130 kDa and ~65 kDa in SDS-PAGE analysis (Kati et al., 2007). The spore crystal mixture collected from 80 Bt species isolated from the *Sichuan basin* showed protein bands ranging from 40 to 130 kDa belonging to the major Cry protein family in SDS-PAGE analysis (Zhu et al., 2009). Boonmee et al. (2019) characterized 511 Bt isolates in Thailand and detected the presence of lepidopteran toxic genes using PCR and found that the molecular mass of the proteins was between ~65 and ~130 kDa in SDS-PAGE analysis. The spore-crystal suspension of the isolates was analyzed using SDS-PAGE, and we found that the electrophoretic bands were highly varied. Several proteins were expressed by *B. zhangzhouensis* OBB. This is thought to be because Bt usually produces more than one parasporal crystal and therefore protein profiles may differ among strains. In addition, the profile of the same Cry protein in different studies may differ due to environmental conditions. This is because the expression of certain cry genes is affected by certain environmental conditions and its turned on or off (Sevim et al., 2012).

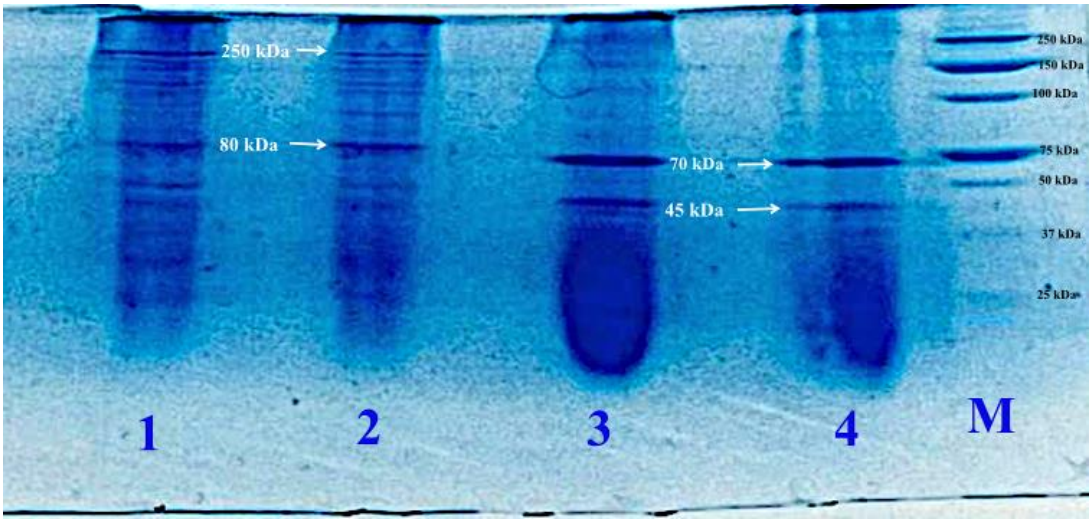


Figure 9. Comparison of spore-crystal mixture with SDS-PAGE analysis; 1-2: *Bacillus zhangzhouensis* OBB, 3-4: *Bacillus thuringiensis.*

## Conclusion

According to current knowledge, the cry gene is only found in *B. thuringiensis* and some other bacteria. We were able to identify the cry protein in *B. zhangzhouensis* OBB strain. Since the cry1 gene is the most common gene in this collection and the cry1 protein has specific insecticidal activities against Lepidoptera, different geographical and climatic environments may have created a different ecology in insects, which in turn led to the presence of the cry protein gene in *B. zhangzhouensis.* It was determined that the mortality rate of *B. zhangzhouensis* OBB bacteria was much higher than *B. thuringiensis.* Considering the effects of cry proteins and the harmful effects of the insect reported in literature, these proteins have the potential to be developed as biopesticides and used against pests. In addition, the use of harmful pesticides and chemicals causes various health and environmental problems; we think that it will be an alternative to existing *Bacillus* isolates to overcome this problem.

## Acknowledgment

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## References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18 (2): 265-267.

Alper, M., H. Güneş, A. Tatlıpınar, B. Çöl, S. Civelek, C. Özkan & B. Poyraz, 2014. Distribution, occurrence of cry genes, and lepidopteran toxicity of native *Bacillus thuringiensis* isolated from fig tree environments in Aydin Province. Turkish Journal of Agriculture and Forestry, 38 (6): 898-907.

Anonymous, 2008. Zirai Mücadele Teknik Talimatları. Tarımsal Araştırmalar Genel Müdürlüğü. (Web page: http: //www. tarimorman.gov.tr) (Date Accessed: 7 February 2024) (in Turkish).

Baig, D. N. & S. Mehnaz, 2010. Determination and distribution of cry-type genes in halophilc *Bacillus thuringiensis* isolates of Arabian Sea sedimentary rocks. Microbiological Research, 165 (5): 376-383.

Baranek, J., A. Kaznowski, E. Konecka & S. Naimov, 2015. Activity of vegetative insecticidal proteins Vip3Aa58 and Vip3Aa59 of *Bacillus thuringiensis* against lepidopteran pests. Journal of Invertebrate Pathology, 130 (1): 72-81.

Ben-Dov, E., S. Boussiba & A. Zaritsky, 1995. Mosquito larvicidal activity of *Escherichia coli* with combinations of genes from *Bacillus thuringiensis* subsp. *israelensis*. Journal of Bacteriology, 177 (10): 2581-2587.

Boonmee, K., S. N. R, Thammasittirong & A. Thammasittirong, 2019. Molecular characterization of lepidopteran-specific toxin genes in *Bacillus thuringiensis* strains from Thailand. 3 Biotech, 9 (4): 117 (1-11).

Borror, D. J., D. M. De Long & C. A. Triplehorn, 1981. An Introduction to the Study of Insects. Sounder College Publishers, New York, 838 pp.

Boukedi, H., S. Sellami, S. Ktari & N. Belguith-Ben Hassan, S. Tounsi & L. Abdelkefi Mesrati, 2016. Isolation and characterization of a new *Bacillus thuringiensis* strain with a promising toxicity against Lepidopteran pests. Microbiological Research,186 (187): 9-15.

Bozlağan, L., A. Ayvaz, F. Oztürk, L. Açik, M. Akbulut & S. Yilmaz, 2010. Detection of the cry1 gene in *Bacillus thuringinsis* isolates from agricultural fields and their bioactivity against two stored product moth larvae. Turkish Journal of Agriculture and Forestry, 34 (2): 145-154.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72 (1-2): 248-254.

Bülbüloğlu, Ö., 2000. Çeşitli toprak örneklerinden izole edilen *Bacillus thuringiensis*' lerin izolasyonu, karakterizasyonu ve insektisidal etkilerinin belirlenmesi. Karadeniz Teknik Üniversitesi, Fen Bilimleri Enstitüsü, (Unpublished) Master Thesis, Trabzon, 117 s (in Turkish with abstract in English).

Buss, E. A., 2006. White grub biology and management. (Web page: http: //edis.ifas.ufl.edu/LH037) (Date accessed: 7 February 2024).

Claus, D., 1992. A standardized gram staining procedure. World Journal of Microbiology and Biotechnology, 8 (4): 451-452.

De Maagd, R. A., A. Bravo & N. Crickmore, 2001. How *Bacillus thuringiensis* has evolved specific toxins to colonize the insect world. Trends in Genetics, 17 (4): 193-199.

Erler, F. & A. O. Ates, 2015. Potential of two entomopathogenic fungi, *Beauveria bas*siana and *Metarhizium anis*opliae (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. Journal of Insect Science, 15 (1): 44 (1-6).

Finney, D. J., 1952. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. Cambridge University Press, Cambridge, England, 256 pp.

Hall, T., I. Biosciences & C. J. G. B. B. Carlsbad, 2011. BioEdit: an important software for molecular biology. GERF Bull Bioscience, 2 (1): 60-61.

Jain, D., D. Sita Sunda, S. Sanadhya, J. Dhruba Nath & S. K. Khandelwal, 2017. Molecular characterization and PCR-based screening of cry genes from *Bacillus thuringiensis* strains. 3 Biotech, 7 (4): 1-8.

Kang, T. H., S. H. Han, H. Y. Weon, Y. B. Lee, N. Kim & S. H. H. C. Nam, 2012. Purification and identification of *Paenibacillus* sp., isolated from diseased larvae of *Allomyrina dichotoma* (Linnaeus, 1771) (Coleoptera: Scarabaeidae) in Insect Farms. International Journal of Industrial Entomology, 25 (2): 195-203.

Kati, H., K. Sezen & Z. Demirbag, 2007. Characterization of a highly pathogenic *Bacillus thurinigensis* strain isolated from common cockchafer, *Melolontha melontha*. Folia Microbiologica, 52 (2): 146-152.

Koul, O. & G. S. Dhaliwal, 2003. "Predators and Parasitoids: An Introduction, 1-16". In: Predators and Parasitoids (Eds. O. Koul & G. S. Dhaliwal). CRC Press, London and New York, 208 pp.

Kour, D., K. L. Rana, A. N. Yadav, N. Yadav, M. Kumar, V. Kumar, P. Vyas, H. S. Dhaliwal & A. K. Saxena, 2020. Microbial biofertilizers: bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatalysis and Agricultural Biotechnology, 23 (1): 101487 (1-11).

Kour, D., K. L. Rana, T. Kaur, B. Singh, V.S. Chauhan, A. Kumar & V. K. Gupta, 2019a. "Extremophiles for Hydrolytic Enzymes Productions: Biodiversity and Potential Biotechnological Applications, 321-372". In: Bioprocessing for Biomolecules Production (Eds. G. Molina, V. Gupta, B. Singh & N. Gathergood). Wiley Blackwell, 506 pp.

Kour, D., K. L. Rana, N. Yadav, A. N. Yadav, A. Kumar, V. S. Meena & A. K. Saxena, 2019b. Rhizospheric Microbiomes: Biodiversity, Mechanisms of Plant Growth Promotion, and Biotechnological Applications for Sustainable Agriculture, 19-65". In: Plant Growth Promoting Rhizobacteria for Agricultural Sustainability (Eds. A. Kumar & V.S. Meena). Springer, Singapore, 314 pp.

Kour, D., K. L. Rana, N. Yadav, A.N. Yadav, J. Singh, A. A. Rastegari & A. K. Saxena, 2019c. Agriculturally and Industrially Important Fungi: Current Developments and Potential Biotechnological Applications, 1-64". In: Recent Advancement in White Biotechnology Through Fungi: Volume 2: Perspective for Value-Added Products and Environments (Eds. A. N. Yadav, S. Singh, S. Mishra & A. Gupta). Springer, Cham, 504 pp.

Lengai, G. M. & J. W. Muthomi, 2018. Biopesticides and their role in sustainable agricultural production. Journal of Biosciences and Medicines, 6 (6): 7-41.

Lone, A. L., A. Malik & J. V. Padaria, 2017. Characterization of lepidopteran spesific cry1 and cry2 gene harbouring native *Bacillus thuringiensis* izolates toxic aganist *Helicoverpa armigera*. Biotechnology Reports, 15 (1): 27-32.

Mehtap, U., 2022. Local isolate of *Bacillus thuri*ngiensis (Berliner, 1915) (Bacteria: Bacillaceae) from *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae: Spilomelinae) includes cry1, cry3 and cry4 genes and their insecticidal activities. Turkish Journal of Entomology, 46 (2): 227-237.

Monnerat, R. G., A. C. Batista, P. T. de Medeiros, E. S. Martins, V. M. Melatti, L. B. Praça & C. Berry, 2007. Screening of Brazilian *Bacillus thuringiensis* isolates active against *Spodoptera frugiperda*, *Plutella xylostella* and *Anticarsia gemmatalis*. Biological Control, 41 (3): 291-295.

Nawaz, M., J. I. Mabubu & H. Hua, 2016. Current status and advancement of biopesticides: microbial and botanical pesticides. Journal of Entomology and Zoology Studies, 4 (2): 241-246.

Reynolds, J., R. Moyes & D. P. Breakwell, 2009. Differential staining of bacteria: endospore stain. Current Protocols in Microbiology, 15 (1): A.3J.1-A.3J.5.

Rosovitz, M. J., M. I. Voskuil & G. H. Chambliss, 1998. Bacillus, Topley and Wilson's Microbiology and Microbial Infections, Systematic, Bacteriology. Oxford University Press, New York, 730 pp.

Saadoun, I., F. Al-Momani, M. Obeidat, M. Meqdam & A. Elbetieha, 2001. Assessment of toxic potential of local Jordanian *Bacillus thuringiensis* strains on *Drosophila melanogaster* and *Culex* sp. (Diptera). Journal of Applied Microbiology, 90 (6): 866-872.

Seiber, J. N., J. Coats, S. O. Duke & A. D. Gross, 2014. Biopesticides: state of the art and future opportunities. Journal of Agricultural and Food Chemistry, 62 (48): 11613-11619.

Sevim, A., E. Eryüzlü, Z. Demirbağ & S. Demir, 2012. A novel cry2Ab gene from indigenous isolate *Bacillus thuringiensis* subs *kurstaki*. Journal of Microbiology and Biotechnology, 22 (1): 133-140.

Sharpe, E. S., G. S. Julian & C. Crowell, 1970. Characteristics of a new strain of *Bacillus popilliae* sporogenic in vitro, Applied Microbiology, 19 (4): 681-688.

Suludere, Z., Y. Kalender, L. Çakmakçı, B. Alten, C. Ayvalı & G. Çetinkaya, 1992. Türkiye'nin çeşitli yörelerinden izole edilen bazı *Bacillus sphaericus* ve *Bacillus thuringiensis* suşlarının spor ve parasporal kristallerinin elektron mikroskobuyla incelenmesi. Journal of Agricultural Forestry ,16 (1): 1-14 (in Turkish with abstract in English).

Tarekegn, M. M. & M. Teferra, 2023. Isolation and molecular characterization of *Bacillus thuringiensis* strains obtained from different habitats in Northwest Ethiopia. Food Science and Applied Biotechnology, 6 (1): 134-142.

Thakur, N., S. Kaur, P. Tomar, S. Thakur & A. N. Yadav, 2020. "Microbial Biopesticides: Current Status and Advancement for Sustainable Agriculture and Environment, 243-282". In: New and Future Developments in Microbial Biotechnology and Bioengineering (Eds. A. A. Rastegari, A. N. Yadav & N. Yadav). Elsevier, 351 pp.

Travers, R. S., P. A. W. Martin & C. F. Reichelderfer, 1987. Selective process for efficient isolation of soil *Bacillus* spp. Applied and Environmental Microbiology, 53 (6): 1263-1266.

Wang, J., A. Boets, J. V. Rie & G. Ren, 2003. Characterization of cry1, cry2, and cry9 genes in *Bacillus thuringiensis* isolates from China. Journal of Invertebrate Pathology, 82 (1): 63-71.

William, G. W., M. B. Susan, A. P. Dale & J. L. David, 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173 (2): 697-703.

Yadav, A. N., A. A. Rastegari & N. Yadav, 2020. Microbiomes of Extreme Environments: Biodiversity and Biotechnological Applications. CRC Press, 292 pp.

Yu, Z., L. Gong, Q. Li, G. Huang, L. He, P. Li & A. Zheng, 2015. Diversity of insecticidal crystal protein genes of *Bacillus thuringiensis* isolated from soil and cloning of novel haplotypes of cry genes. Annals of Microbiology, 65 (1): 2179-2186.

Zhu, J., F. Tan, J. Tang, Y. Li, A. Zheng & P. Li, 2009. Characterization of insecticidal crystal protein cry gene of *Bacillus thuringiensis* from soil of Sichuan Basin, China and cloning of novel haplotypes cry gene. Annals of Microbiology, 59 (1): 1-8.

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*Original article (Orijinal araştırma)*

# The effect of weed control strategies on *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) and *Helicotylenchus* spp. Steiner, 1945 (Tylenchida: Hoplolaimidae) in banana under water stress[1]

Yabancı ot kontrol stratejilerinin su stresi altındaki muzda *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) ve *Helicotylenchus* spp. Steiner, 1945 (Tylenchida: Hoplolaimidae) üzerine etkisi

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## Abstract

This study was conducted to evaluate the effects of different weed control practices on the nematode populations in the banana greenhouse of Alata Horticultural Research Institute, Mersin, Southern Türkiye, during the years 2022-2024. The greenhouse experiment was conducted under two irrigation regimes: Full (100%) and Deficit (50%) irrigation, with a split-plot design and three replications for treatments per irrigation. Weed control treatments, including herbicide combinations pre-planting (indaziflam, oxyfluorfen, pendimethalin) and post-planting (glyphosate, diquat), were applied to subplots. Additionally, geotextile mulching and mowing were compared to control plot (weedy) with herbicide combinations. Nematode densities were measured in relation to weed coverage for *Helicotylenchus*, *Meloidogyne* and total nematodes. Irrigation regimes had no significant effect on weed control but did affect nematode populations, particularly in the second year. Based on weed coverage, herbicide combinations (indaziflam, oxyfluorfen+glyphosate and pendimethalin+glyphosate) were effective in suppressing nematodes during the first 6 months, with diquat herbicide also helping before harvest. Geotextile mulching suppressed weeds and affected nematode populations. The results showed that weed control was more effective in reducing nematode densities in the first year, while in the second year nematode populations differed as a function of weed coverage. This highlights the importance of managing weed hosts to control nematodes in banana production.

**Keywords:** Density, host weeds, indirect control, irrigation, nematodes

## Öz

Bu çalışma, farklı yabancı ot mücadele uygulamalarının 2022-2024 yıllarında Alata Bahçe Kültürleri Araştırma Enstitüsü, Türkiye'nin güneyi, Mersin'deki muz serasında nematod popülasyonları üzerindeki etkilerinin değerlendirilmesi amacıyla yürütülmüştür. Sera denemesi iki sulama rejimi altında: Tam (%100) ve Kısıtlı (%50) sulama, bölünmüş parsel deneme tasarımında ve her sulamada uygulamalar üç tekerrür olacak şekilde kurulmuştur. Dikim öncesi (indaziflam, oxyfluorfen, pendimethalin) ve dikim sonrası (glyphosate, diquat) herbisit kombinasyonlarını içeren yabancı ot kontrol uygulamaları alt parsellere uygulanmıştır. Ayrıca, jeotekstil malçlama ve biçme, herbisit kombinasyonları ile kontrol parseliyle (yabancı otlu) karşılaştırılmıştır. Nematod yoğunlukları, *Helicotylenchus*, *Meloidogyne* ve toplam nematodlar yabancı ot kaplama alanları ile ilişkili olarak ölçülmüştür. Sulama rejimlerinin yabancı ot kontrolü üzerinde önemli bir etkisi olmamıştır, ancak özellikle ikinci yılda nematod popülasyonlarını etkilemiştir. Yabancı ot kaplama alanlarına bağlı olarak, herbisit kombinasyonları (indaziflam, oxyfluorfen+glyphosate ve pendimethalin+glyphosate) ilk 6 ay boyunca nematodları baskılamada etkili olmuş, diquat herbisiti de hasattan önce baskılamayı sürdürmüştür. Jeotekstil malçlama yabancı otları baskılamış ve nematod popülasyonlarını etkilemiştir. Sonuçlar, yabancı ot kontrolünün ilk yılda nematod yoğunluklarını azaltmada daha etkili olduğunu, ikinci yılda ise nematod popülasyonlarının yabancı ot kaplama alanına göre farklılık gösterdiğini ortaya koymuştur. Bu durum, muz üretiminde nematodları kontrol etmek için yabancı ot konukçularını yönetmenin önemini vurgulamaktadır.

**Anahtar sözcükler:** Yoğunluk, konukçu yabancı otlar, dolaylı mücadele, sulama, nematodlar

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## Introduction

Banana production is a key player in the global economy, providing essential food and serving as a major export product, particularly in Latin America, Asia, and Africa (Uddin et al., 2002; Dassou et al., 2024). The industry highlights its broad economic and social impact, supporting millions of jobs in sectors ranging from agriculture to distribution (Dassou et al., 2024). Furthermore, sustainable banana farming practices can improve soil health and biodiversity, contributing to environmental conservation (Subba et al., 2023; Swafo & Dlamini, 2023). The sensitivity of banana plants to climate change highlights the need for adaptive and sustainable practices to ensure the long-term viability of this important crop.

The interaction between biotic factors (like plants and pests) and abiotic factors (such as soil and climate) is essential in influencing crop-weed dynamics, ultimately affecting productivity, sustainability, and pest population control within agroecosystems (Yeates et al., 1993; Koenning et al.,1999). In systems like long-term monoculture farming, variations in plant biodiversity and population density can trigger shifts in pest populations (Goodey et al., 1965; Quénéhervé et al., 2006). These interactions are heavily influenced by crop management practices, which affect both plant-pest dynamics and overall biodiversity (Govaerts et al., 2007a). In such environments, weeds often become key hosts for pests, particularly when cultivated crops are not present (Goodey et al., 1965; Siddiqui et al., 1973). Weeds in many cases act as alternative hosts for plant-parasitic nematodes, thereby intensifying pest-related challenges (Bélair & Benoit, 1996; Castillo et al., 2008). This highlights the importance of integrated pest management strategies that consider both weed control and the broader ecological context.

Plant-parasitic nematodes are obligate parasites that rely on host plants to fulfill their entire life cycle (Yeates et al., 1993; Koenning et al.,1999). Weeds play a critical role in supporting nematode populations by serving as alternative hosts, allowing nematodes to thrive and maintain their viability, even in the absence of cultivated crops (Egunjobi & Bolaji, 1979). This perpetuates nematode populations and increases the damage to cultivated plants (Goodey et al., 1965; Siddiqui et al., 1973). Effective weed control has been demonstrated to lower nematode populations and reduce yield losses in crops (Quénéhervé et al., 2006; Rich et al., 2008; Singh et al., 2010; Kokalis-Burelle & Rosskopf, 2012; Mendes et al., 2020).

Studies, conducted in Türkiye, have reported the relationship between root-knot nematodes, *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) and weed species in banana production areas, particularly in the Mediterranean region of Türkiye. A survey covering 2% of banana cultivation areas identified several weed species frequently contaminated by root-knot nematodes, including *Amaranthus retroflexus* L. (Caryophyllales: Amaranthaceae) (46.34%), *Portulaca oleracea* L. (Caryophyllales: Portulacaceae) (40.63%), and *Solanum nigrum* L. (Solanales: Solanaceae) (37.84%). Molecular analyses further identified numerous weed species-such as *Abutilon theophrasti* Medic. (Malvales: Malvaceae), *Amaranthus* spp., *Cucumis melo* var. *agrestis* Naudin. (Cucurbitales: Cucurbitaceae), *Erodium cicutarium* (L.) L'Hér. (Geraniales: Geraniaceae), *Kickxia commutata* (Bernh. ex Rchb.) Fritsch (Lamiales: Plantaginaceae), *Malva* spp. (Malvales: Malvaceae), *Mercurialis annua* L. (Malpighiales: Euphorbiaceae), *P. oleracea*, *S.nigrum*, and *Sonchus oleraceus* L. (Asterales: Asteraceae) as suitable hosts for root-knot nematodes (*Meloidogyne javanica* and *M. incognita*) (Dinçer et al., 2024). It indicates that certain weeds in banana fields can sustain nematode populations, which could damage subsequent crops. The presence of weeds in banana cultivation areas has been demonstrated to be a contributing factor to the problem of nematodes, which may result in substantial yield and quality losses (Özarslandan & Elekcioğlu, 2010; Özarslandan & Dinçer, 2015). In addition, numerous plant species are known to host the major parasitic nematodes affecting bananas (Isaac et al., 2007; Fongod et al., 2010; Dinçer et al., 2024). Studies like these highlight the importance of adopting effective weed management strategies to control nematode populations and reduce their negative impact on banana production.

The goal of this study was to evaluate the effects of chemical and alternative weed control methods on nematode populations under two irrigation types (Full 100% and Deficit 50%) in a banana greenhouse. To achieve this aim, we evaluated the effectiveness of various herbicide combinations, including Indaziflam, Oxyfluorfen, Pendimethalin, Glyphosate, and Diquat, as well as alternative methods such as Geotextile mulching and mowing in controlling weeds that serve as hosts for nematodes. Specifically, the effects of these weed management practices on the densities of *Helicotylenchus* spp. Steiner, 1945 (Tylenchida: Hoplolaimidae), *Meloidogyne* spp. and total nematode populations were observed. Potential relationships between weed management strategies and weed-nematode dynamics were also investigated. The aim is to determine how different weed control approaches affect nematode populations in banana, *Musa* spp. (Zingiberales: Musaceae), so that pest management strategies can be proposed to improve banana production systems.

## Materials and Methods

### Study area, treatments and irrigation

This experimental study was conducted between 2022 and 2024 in a newly established banana greenhouse (cv. Alata Azman) at the Alata Research Directorate of Agricultural Experiment Management in Mersin, Türkiye. The experiment used a split-plot design with two main factors: irrigation levels (main plot factor) and weed control treatments (subplot factor). The two irrigation levels, full irrigation (100%) and deficit irrigation (50%), were assigned to the main plots with three replications per level in a randomised per block design. Seven weed control treatments were tested, consisting of four chemical herbicide combinations (indaziflam, oxyfluorfen, pendimethalin, glyphosate and diquat), two alternative methods (geotextile mulching and mowing) and weedy control plot. These treatments were randomly assigned to subplots, except for the Geotextile mulch, resulting in six randomly assigned plots per block (three total blocks at one irrigation). Each main plot was divided into seven subplots where different weed control treatments were applied. The linear mixed-effects model employed for the analysis was μ: Overall average, αi: Irrigation level effect (fixed), βj: Effect of weed control method (fixed), (αβ)ij: Irrigation×Weed control interaction (fixed), ρk: Block effect (random), and ϵijkl: Error term (random) (Equation 1).

$$Yijkl=\mu+\alpha i+\beta j+(\alpha\beta)ij+\rho k+\epsilon ijkl \quad \text{(Equation 1)}$$

The objective of the experiment was to evaluate two irrigation methods: full (IR100) and deficit (IR50) irrigation using a drip irrigation system consisting of a fertilizer tank, header, water distribution pipes, and 16 mm drippers with a 4 L $h^{-1}$ flow rate. Initially, all plants received equal water for the first 120 days. After this period, irrigation levels were adjusted according to the data from a Class A evaporation pond, regulated by a coefficient Kcp (0.45-1.2) (Liu et al., 2008). Irrigation was performed every two days with full irrigation (IR100) providing adequate water for banana cultivation as recommended by previous studies (Carr, 2009; Arantes et al., 2018). From April 14, 2022 to March 12, 2024, the total water applied was 3301 mm for IR100 and 1727 mm for IR50, across 178 irrigation events. Daily evaporation rates were tracked using a Class A pan evaporimeter, following US Weather Service standards to accurately calculate evaporation (Epan) (Eid & Maklad, 2019). Key variables included irrigation (IR in mm), plant spacing (Sp in m), row spacing (Sl in m), cumulative evaporation (Epan), crop coefficient (Kc: 1.0 for IR100, 0.5 for IR50), plant cover (P), and irrigation efficiency (Ea, %) (Equation 2).

$$IR=(SpxSlxEpanxKcxP)/Ea \quad \text{(Equation 2)}$$

Each subplot (6m²) contained three banana plants. The percentage (%) of weed coverage was recorded to assess the impact of treatments on weed growth. Weed species were identified using the Flora of Turkey (Davis, 1965-1988). Soil samples for nematode analysis were collected from around the base of three banana trunks per subplot using an auger (100 g per sample). The samples were processed using the 'Improved Baermann Funnel' method (Barker, 1985), and nematode populations were counted under a

microscope. The reproductive rate (R0) of the nematodes was calculated by comparing the final population (Pf) at the end of the year with the initial population (Pi) at the start, using Equation 3. This experimental setup aimed to assess the impact of various weed control methods on nematode populations in banana cultivation under different irrigation conditions. For nematode identification, morphological characterization was conducted on the second stage juveniles recovered from the soil. The nematodes were vermiform, slender and annulated. The body of *Helicotylenchus* was short or medium in length, spiral as a result of fixation, the stylet was strong, and the median bulb was developed. The ovary was paired in females, and the female's tail was short, hemispherical, convex or conical. The tail of the male was short and conical. In *Meloidoyne*, the stylet was delicate, narrow, and sharply pointed, with small knobs. The excretory pore was distinct. The tail was conoid with a hyaline terminus distinctive in most species (Kepenekci & Ökten, 1996; Evlice & Bayram, 2016).

*Nematode density: R0=Pf/Pi* (Equation 3)

For chemical weed control in banana cultivation, the following herbicides were used: 500 g $L^{-1}$ Indaziflam (100 ml $ha^{-1}$), 480 g $L^{-1}$ Oxyfluorfen (400 ml $ha^{-1}$) and 455 g $L^{-1}$ Pendimethalin (3000 ml $ha^{-1}$) were applied during pre-planting, and 441 g $L^{-1}$ Glyphosate (3000 ml $ha^{-1}$) and 200 g $L^{-1}$ Diquat (8000 ml $ha^{-1}$) was applied during post-planting. The purpose of the pre-planting applications in the first year was to avoid the damaging on young banana seedlings. In the second year, as the banana plants matured, herbicide applications were adjusted to avoid direct contact with plants, and they were tailored based on weed density. Additionally, Geotextile mulching combined with mowing was used as an alternative weed control method applied once. Weed population measurements, based on weed coverage percentage (10-15%), were taken by comparing herbicide-treated plots and alternative control plots with untreated weedy control plots (TAGEM, 2019). The timing of both chemical and mowing applications was recorded according to target weed densities. The experimental setup was established in April, in the light of the first 6-month observations in September and pre-harvest observations in March. During the first 6-months, under two irrigation regimes, the following combinations were applied: (1) Indaziflam, (2) Oxyfluorfen+Glyphosate, (3) Pendimethalin+Glyphosate, (4) Glyphosate+Glyphosate, and (5) Mowing (applied twice). An additional application was made during the pre-harvest period based on weed populations, involving such combinations as (1) Indaziflam+Diquat, (2) Oxyfluorfen+Glyphosate+Diquat, (3) Pendimethalin+Glyphosate+Diquat, (4) Glyphosate+Glyphosate+Diquat, and (5) Mowing (applied three times).

### Statistical analysis

Analysis of variance-ANOVA was used to assess the significance of the main effects and their interactions. The fixed effects included irrigation, weed control treatments, and their interaction, while blocks were considered a random effect. Linear regression analysis was also used to show the interaction between weed coverage - *Helicotylenchus*, *Meloidogyne* and total nematode densities and graphs were presented. All statistical analyses were performed using JMP software (version 5.0.1, SAS Institute Inc., Cary, NC, USA), with residual diagnostics verifying the assumptions of normality and homogeneity of variances. Least Significant Difference (LSD) test was employed for pairwise comparisons to detect significant differences among treatment means at 5% and 1% significance levels.

## Results

In the 2022-2024 banana greenhouse experiment, 25 weed species belonging to a total of 15 families were identified in the weed species observations made in accordance with the relevant practices to determine the effect of chemical and alternative weed control practices on nematode densities. These weed species were found to be hosts of *Helicotylenchus* and *Meloidogyne* nematodes, which are the other important pests (Table 1).

Table 1. Weed species in the experimental area of the banana greenhouse (2022-2024)

| Plant family | Weed species | Greenhouse | | Examples of hosts for nematode species* (*Helicotylenchus* spp. and *Meloidogyne* spp.) |
|---|---|---|---|---|
| | | First year (2022-2023) | Second year (2023-2024) | |
| Amaranthaceae | *Amaranthus* spp. | ✓ | ✓ | *Helicotylenchus* sp., *Meloidogyne javanica* Treub,1885 |
| Asteraceae | *Capsella bursa-pastoris (*L.) Medik. | ✓ | ✓ | *Meloidogyne* sp. |
| | *Sonchus* spp. | ✓ | ✓ | *M. incognita* Kofoid&White,1919 |
| Boraginaceae | *Heliotropium europaeum* L. | ✓ | ✓ | *Meloidogyne* sp. |
| Brassicaceae | *Cardamine occulta* Hornem. | ✓ | ✓ | *Meloidogyne* sp. |
| | *Malva* spp. | ✓ | | *Helicotylenchus multicinctus* (Cobb,1893) Golden,1956, *M. incognita* |
| Caryophyllaceae | *Stelleria media* (L.) Vill. | ✓ | ✓ | *M. incognita* |
| Convolvulaceae | *Convolvulus arvensis* L. | ✓ | ✓ | *M. javanica* |
| Cyperaceae | *Cyperus rotundus* L. | ✓ | ✓ | *H. dihystera* (Cobb,1893) Sher,1961, *M. javanica* |
| Euphorbiaceae | *Chrozophora tinctoria (*L.) A.Juss. | ✓ | | *Meloidogyne* sp. |
| | *Euphorbia* spp. | ✓ | ✓ | *M. arenaria* Neal,1889 |
| | *Mercurialis annua* L. | ✓ | ✓ | *Meloidogyne* sp. |
| Lamiaceae | *Lamium amplexicaule* L. | ✓ | ✓ | *M. hapla* Chitwood,1949, *M. incognita* |
| Musaceae | *Musa* spp. (Banana) | Crop | Crop | *H. dihystera, H. multicinctus, M. arenaria, M. enterolobii* Yang&Eisenback,1983, *M. hapla, M. incognita, M. javanica* |
| Oxalidaceae | *Oxalis* spp. | ✓ | ✓ | *M. arenaria*, *M. hapla*, *M incognita, M. javanica* |
| Plantaginaceae | *Veronica* spp. | ✓ | | *M. incognita*, *M. javanica* |
| Poaceae | *Digitaria sanguinalis (*L.) Scop. | ✓ | ✓ | *H. dihystera*, *M. arenaria*, *M incognita, M javanica*, *M. naasi* Franklin,1965 |
| | *Eleusine indica (*L.) Gaertn. | ✓ | ✓ | *Helicotylenchus* sp., *M. arenaria*, *M. incognita*, *M. javanica* |
| | *Setaria* spp. | ✓ | ✓ | *H. dihystera*, *M. arenaria*, *M. hapla*, *M. incognita*, *M javanica* |
| | *Sorghum halepense* (L.) Pers. | ✓ | ✓ | *H. dihystera, M. arenaria*, *M. enterolobii, M. incognita*, *M. javanica* |
| Portulacaceae | *Portulaca oleracea* L. | ✓ | ✓ | *H. multicinctus, M. arenaria*, *M. enterolobii*, *M. hapla*, *M. incognita*, *M. javanica* |
| Solanaceae | *Physalis* spp. | ✓ | | *M. arenaria, M. hapla, M incognita, M javanica* |
| | *Solanum nigrum* L. | ✓ | | *M. arenaria, Meloidogyne chitwoodi* Golden et al.,1980, *M. hapla, M incognita, M. javanica* |
| Urticaceae | *Parietaria judaica* L. | | ✓ | - |
| | *Pilea microphylla* (L.) Liebm. | | ✓ | *M. incognita* |
| | *Urtica* spp. | ✓ | ✓ | *M. hapla, M. incognita* |

* The reference data used as example host were from Goodey et al. (1965), Caveness (1967), Davidson & Townshend (1967), Stoyanov (1967), Siddiqui et al. (1973), Dabaj & Jenser (1990), McKenry (1992), Powers & Pitty (1993), Queneherve et al. (1995), Levin et al. (2005), Kaur et al. (2007), Rich et al. (2008), Singh et al. (2010), Kokalis-Burelle & Rosskopf (2012), Mendes et al. (2020).

Nematode densities of *Helicotylenchus* and *Meloidogyne* populations were assessed in soil samples which were taken from the banana trial plots of the two key observation points: after 6-months (1st observation) and pre-harvest (2nd observation). The results revealed that the weed control applications at the 6-month mark led to higher nematode densities compared to those observed just before harvest. This change was likely due to the increasing number of weed control treatments, higher water application, and the absence of a sufficient developmental period for nematodes to build populations (Table 2). Nematode densities, on the other hand, were generally lower under full irrigation across all weed control treatments. Herbicide treatments, particularly Indaziflam and the combination of Oxyfluorfen+Glyphosate, effectively reduced nematode populations when compared to the weedy control plots, which had the highest nematode densities. This reduction pattern persisted across both the 6-month and pre-harvest observation periods. However, under deficit irrigation, nematode populations increased, particularly in the weedy control plots emphasizing the detrimental effects of water stress in the absence of weed management. Even under deficit irrigation, herbicide treatments still managed to reduce nematode populations compared to untreated plots. The differences observed between irrigation regimes may have resulted from the interactions between soil moisture, weed management, and nematode ecology. In addition, soil moisture plays a critical role in nematode survival, mobility, and reproduction. While excessive moisture can limit nematode activity due to oxygen depletion, moderate moisture levels can enhance nematode movement and infectivity.

Table 2. Nematode density (number 100 g soil$^{-1}$) of weed control treated plots in greenhouse banana (2022-2024)

| Irrigation | Treatments | Total | | *Helicotylenchus* | | *Meloidogyne* | |
|---|---|---|---|---|---|---|---|
| | | 2022-2023 | 2023-2024 | 2022-2023 | 2023-2024 | 2022-2023 | 2023-2024 |
| 1st Observation (6-months) | | | | | | | |
| Full irrigation (100%) | Indaziflam | 6.18 | 0.64 | 5.70 | 11.00 | 6.86 | 0.10 |
| | Oxyfluorfen+Glyphosate | 4.16 | 1.41 | 3.27 | 10.67 | 10.67 | 0.25 |
| | Phendimethalin+Glyphosate | 1.76 | 1.14 | 1.71 | 6.00 | 1.91 | 0.58 |
| | Glyphosate+Glyphosate | 2.83 | 4.27 | 3.43 | 19.33 | 1.25 | 0.50 |
| | Geotextile mulching | - | - | - | - | - | - |
| | Mowing (2 times) | 2.00 | 2.33 | 3.08 | 7.33 | 0.38 | 1.08 |
| | Weedy control | 11.69 | 2.07 | 7.58 | 19.17 | 22.86 | 0.56 |
| Deficit irrigation (50%) | Indaziflam | 3.07 | 2.18 | 2.70 | 18.33 | 6.33 | 0.24 |
| | Oxyfluorfen+Glyphosate | 3.68 | 3.81 | 3.00 | 24.33 | 9.33 | 1.25 |
| | Phendimethalin+Glyphosate | 2.71 | 4.64 | 1.64 | 34.67 | 11.67 | 0.55 |
| | Glyphosate+Glyphosate | 10.32 | 1.47 | 7.00 | 15.67 | 28.00 | 0.18 |
| | Geotextile mulching | - | - | - | - | - | - |
| | Mowing (2 times) | 2.39 | 2.44 | 1.42 | 25.67 | 7.40 | 0.19 |
| | Weedy control | 16.25 | 6.64 | 2.62 | 24.67 | 75.33 | 1.73 |
| | Average | 5.59 | 2.75 | 3.60 | 18.07 | 15.17 | 0.60 |
| 2nd Observation (Pre-harvest) | | | | | | | |
| Full irrigation (100%) | Indaziflam+Diquat | 5.06 | 0.21 | 8.20 | 4.33 | 0.57 | 0.00 |
| | Oxyfluorfen+Glyphosate+Diquat | 4.44 | 0.74 | 4.50 | 6.67 | 4.00 | 0.00 |
| | Phendimethalin+Glyphosate+Diquat | 2.23 | 0.76 | 2.24 | 6.67 | 2.18 | 0.08 |
| | Glyphosate+Glyphosate+Diquat | 5.00 | 0.87 | 4.24 | 4.33 | 7.00 | 0.00 |
| | Geotextile mulching | 1.62 | 1.18 | 1.35 | 8.67 | 4.33 | 0.00 |
| | Mowing (3 times) | 3.05 | 1.60 | 3.25 | 7.67 | 2.75 | 0.08 |
| | Weedy control | 3.81 | 0.46 | 3.26 | 4.67 | 5.29 | 0.09 |
| Deficit irrigation (50%) | Indaziflam+Diquat | 1.60 | 3.29 | 1.26 | 26.33 | 4.67 | 0.52 |
| | Oxyfluorfen+Glyphosate+Diquat | 0.59 | 1.89 | 0.46 | 13.00 | 1.67 | 0.50 |
| | Phendimethalin+Glyphosate+Diquat | 1.71 | 2.56 | 1.60 | 21.33 | 2.67 | 0.00 |
| | Glyphosate+Glyphosate+Diquat | 2.42 | 1.22 | 2.63 | 14.33 | 1.33 | 0.03 |
| | Geotextile mulching | 5.42 | 1.03 | 5.43 | 19.33 | 5.33 | 0.05 |
| | Mowing (3 times) | 1.32 | 1.62 | 1.27 | 17.33 | 1.60 | 0.10 |
| | Weedy control | 4.31 | 5.21 | 3.15 | 21.00 | 9.33 | 0.91 |
| | Average | 3.04 | 1.62 | 3.06 | 12.55 | 3.77 | 0.17 |

The study evaluated the impact of weed control methods on nematode densities and weed coverage in a banana greenhouse, specifically after the first 6-months of treatment implementation. While no statistically significant effects were observed in the interaction between irrigation and treatment factors for total nematode density, some emerging differences were observed for *Helicotylenchus* spp., *Meloidogyne* spp., or weed coverage during the first 6-months of both study periods (2022-2023 and 2023-2024). In the first year, there was a statistically significant difference in the total nematode density and *Meloidogyne* density. In the second year, irrigation had a significant effect on total nematode and *Helicotylenchus* densities. Weed control treatments were very effective on weed coverage in both years. In addition, there was a parallel in both nematode densities and weed coverage, especially in the weedy control plots under different irrigation levels during in the first year. In contrast, weed control treatments resulted in a reduction in nematode densities in the second year (Table 3). The analysis of the average total nematode and *Meloidogyne* densities in the second year revealed that all of the treatments, except for the weedy control, significantly reduced nematode densities in the soil. This reduction was correlated with the weed population management, suggesting that effective weed control may indirectly influence nematode dynamics. Although no statistically significant difference was observed in the average density of *Helicotylenchus*, a significant effect on weed coverage was observed. The herbicide treatments (Indaziflam, Oxyfluorfen+Glyphosate, Pendimethalin+Glyphosate, and Glyphosate+Glyphosate) were the most effective ones in reducing weed coverage over the two years, while double mowing treatment had relatively little effect on weed control. In summary, the findings indicated that weed management practices significantly reduced both weed coverage and nematode densities within the first 6-months of application (Table 3).

Table 3. Initial observations (6-months) of total nematode, *Helicotylenchus*, *Meloidogyne*, and weed coverage in banana plants under two different irrigation levels (2022-2023)

| Treatments | Irrigation | Total nematod (number) | | *Helicotylenchus* (number) | | *Meloidogyne* (number) | | Weed coverage (%) | |
|---|---|---|---|---|---|---|---|---|---|
| 1st Observation (6-months) | Year | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| IrrigationxTreatments factor | | | | | | | | | |
| Indaziflam | Ir100 | 700.00 | 260.00 | 380.00 | 220.00 | 320.00 | 40.00 | 5.00 | 1.00 |
| | Ir50 | 613.00 | 407.00 | 487.00 | 367.00 | 127.00 | 40.00 | 11.70 | 6.00 |
| Oxyfluorfen+Glyphosate | Ir100 | 693.00 | 253.00 | 480.00 | 213.00 | 213.00 | 40.00 | 8.30 | 3.70 |
| | Ir50 | 687.00 | 687.00 | 500.00 | 487.00 | 187.00 | 200.00 | 4.30 | 2.70 |
| Phendimethalin+Glyphosate | Ir100 | 493.00 | 220.00 | 353.00 | 120.00 | 140.00 | 100.00 | 12.70 | 2.00 |
| | Ir50 | 507.00 | 773.00 | 273.00 | 693.00 | 233.00 | 80.00 | 6.00 | 5.70 |
| Glyphosate+Glyphosate | Ir100 | 547.00 | 427.00 | 480.00 | 387.00 | 67.00 | 40.00 | 10.00 | 2.00 |
| | Ir50 | 1307.00 | 353.00 | 747.00 | 313.00 | 560.00 | 40.00 | 10.00 | 2.70 |
| Geotextile mulching | | | | | | | | | |
| Mowing (2times) | Ir100 | 267.00 | 233.00 | 247.00 | 147.00 | 20.00 | 87.00 | 18.30 | 55.00 |
| | Ir50 | 493.00 | 553.00 | 247.00 | 513.00 | 247.00 | 40.00 | 15.00 | 43.30 |
| Weedy control | Ir100 | 2027.00 | 510.00 | 960.00 | 383.00 | 1067.00 | 127.00 | 23.30 | 70.00 |
| | Ir50 | 1733.00 | 620.00 | 227.00 | 493.00 | 1507.00 | 127.00 | 21.70 | 68.30 |
| LSDirrigation | | N.S | 133** | N.S | 117** | N.S | N.S | N.S | N.S |
| LSDtreatmens | | 729** | N.S | N.S | N.S | 499** | N.S | 7.2** | 2.5** |
| LSDirrigationxtreatments | | N.S | N.S | N.S | N.S | N.S | N.S | N.S | N.S |
| 1st Observation (6-months) | | Averages (2 year) | | | | | | | |
| Treatments factor | | | | | | | | | |
| Indaziflam | | 495.00a | | 363.34 | | 131.67a | | 5.92a | |
| Oxyfluorfen+Glyphosate | | 580.00a | | 420.00 | | 160.00a | | 4.75a | |
| Phendimethalin+Glyphosate | | 498.34a | | 360.00 | | 138.34a | | 6.58a | |
| Glyphosate+Glyphosate | | 658.34a | | 481.67 | | 176.67a | | 6.16a | |
| Geotextile mulching | | | | | | | | | |
| Mowing (2times) | | 386.67a | | 288.33 | | 98.34a | | 32.92b | |
| Weedy control | | 1222.50b | | 515.83 | | 706.67b | | 45.83c | |
| LSDtreatmens | | 421* | | N.S. | | 256** | | 14.23** | |

1) Separate letters indicate the differences between the averages. 2) N.S.:Not Significant,*p≤0.05,**P≤0.01.

The findings also revealed that the irrigation and weed control treatments had a significant effect on nematode densities and weed coverage, especially in the pre-harvest periods of both years. In the first year, a significant interaction between irrigation and treatments affected total nematode and *Helicotylenchus* densities. Specifically, under deficit irrigation (Ir50), herbicide combinations such as Oxyfluorfen+Glyphosate+ Diquat and mowing treatments (applied three times) were the most effective ones in reducing nematode densities. However, no significant effects were found in the second year. In addition, treatments such as Indaziflam+Diquat and Geotextile Mulching significantly reduced weed coverage, especially under both irrigation levels in the second year, while mowing and weedy treatments showed the highest weed coverage. In two years, full irrigation resulted in lower nematode densities and better weed control compared to deficit irrigation. The combination of higher soil moisture and herbicide treatments, such as Indaziflam+Diquat and Oxyfluorfen+Glyphosate induced the significantly reduced nematode populations, as moisture supports better herbicide efficacy and minimized nematode habitat by suppressing weed hosts (Table 4).

Table 4. Second observations (Pre-harvest) of total nematode, *Helicotylenchus*, *Meloidogyne*, and weed coverage in banana plants under two different irrigation levels (2023-2024)

| Treatments | Irrigation | Total nematod (number) | | *Helicotylenchus* (number) | | *Meloodogyne* (number) | | Weed coverage (%) | |
|---|---|---|---|---|---|---|---|---|---|
| 2nd Observation (Pre-harvest) | Year | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| IrrigationxTreatments factor | | | | | | | | | |
| Indaziflam+Diquat | Ir100 | 573.00b-e | 87.00 | 547.00bcd | 87.00 | 26.70 | 0.00 | 2.00 | 4.30ab |
| | Ir50 | 320.00abc | 613.00 | 227.00ab | 527.00 | 93.30 | 86.70 | 1.00 | 2.30a |
| Oxyfluorfen+Glyphosate+Diquat | Ir100 | 740.00cde | 133.00 | 660.00d | 133.00 | 80.00 | 0.00 | 35.00 | 56.70d |
| | Ir50 | 110.00a | 340.00 | 77.00a | 260.00 | 33.30 | 80.00 | 17.00 | 18.30bcd |
| Phendimethalin+Glyphosate+Diquat | Ir100 | 623.00bcde | 147.00 | 463.00bcd | 133.00 | 160.00 | 13.30 | 25.00 | 48.30d |
| | Ir50 | 320.00abc | 427.00 | 267.00abc | 427.00 | 53.30 | 0.00 | 15.00 | 21.70c |
| Glyphosate+Glyphosate+Diquat | Ir100 | 967.00e | 87.00 | 660.00d | 87.00 | 37.30 | 0.00 | 21.00 | 30.00c |
| | Ir50 | 307.00abc | 293.00 | 280.00abc | 287.00 | 26.70 | 6.70 | 16.00 | 21.70c |
| Geotextile mulching | Ir100 | 367.00abc | 173.00 | 280.00abc | 173.00 | 86.70 | 0.00 | 0.00 | 0.00a |
| | Ir50 | 867.00de | 407.00 | 760.00d | 387.00 | 106.70 | 20.00 | 0.00 | 0.00a |
| Mowing (3times) | Ir100 | 407.00abc | 160.00 | 260.00abc | 153.00 | 160.00 | 6.70 | 63.30 | 90.00e |
| | Ir50 | 273.00ab | 367.00 | 220.00ab | 347.00 | 53.30 | 20.00 | 71.70 | 80.00e |
| Weedy control | Ir100 | 660.00bcde | 113.00 | 413.00abcd | 93.00 | 247 | 20.00 | 71.70 | 93.30e |
| | Ir50 | 460.00abcd | 487.00 | 273.00abc | 420.00 | 187.00 | 66.70 | 65.00 | 80.00e |
| LSDirrigation | | 167** | 122** | 132* | 102** | N.S | 30** | N.S | 6.0** |
| LSDtreatmens | | N.S | N.S | N.S | N.S | N.S | N.S | 13** | 11** |
| LSDirrigationxtreatments | | 441* | N.S | 348* | N.S | N.S | N.S | N.S | 16* |
| 2nd Observation (Pre-harvest) | | Averages (2 year) | | | | | | | |
| Treatments factor | | | | | | | | | |
| Indaziflam+Diquat | | 398.34 | | 346.67 | | 51.67 | | 2.42a | |
| Oxyfluorfen+Glyphosate+Diquat | | 330.83 | | 282.50 | | 48.34 | | 31.75b | |
| Phendimethalin+Glyphosate+Diquat | | 379.16 | | 322.50 | | 56.67 | | 27.50b | |
| Glyphosate+Glyphosate+Diquat | | 413.34 | | 311.67 | | 101.67 | | 22.16b | |
| Geotextile mulching | | 453.34 | | 400.00 | | 53.34 | | 0.00a | |
| Mowing (3times) | | 301.67 | | 245.00 | | 56.67 | | 76.25c | |
| Weedy control | | 430.00 | | 300.00 | | 130.00 | | 77.50c | |
| LSDtreatmens | | N.S. | | N.S. | | N.S. | | 9.59** | |

1) Separate letters indicate the differences between the averages. 2) N.S.:Not Significant,*p≤0.05,**P≤0.01.

Analysis of mean regression curves by year revealed a positive correlation between total nematode densities (including *Meloidogyne*) and weed coverage at the end of the 6 months in both irrigation regimes. A remarkably similar positive correlation was observed between *Helicotylenchus* densities and weed coverage under full irrigation while a negative correlation was found under deficit irrigation (Figure 1). We hypothesized that weed coverage may have played a critical role in nematode population dynamics, with increased weed density correlating with higher nematode populations, particularly under water-limited conditions. Under full irrigation, nematode densities and weed coverage were significantly lower compared to those observed under deficit irrigation. This trend was most pronounced for *Meloidogyne*, which exhibited higher densities under water stress. The findings supported the hypothesis that full irrigation could enhance herbicide efficacy by improving crop health and reducing weed hosts, ultimately limiting nematode populations. Adequate moisture is known to improve better herbicide uptake, resulting in more uniform weed control and reduced nematode habitat.

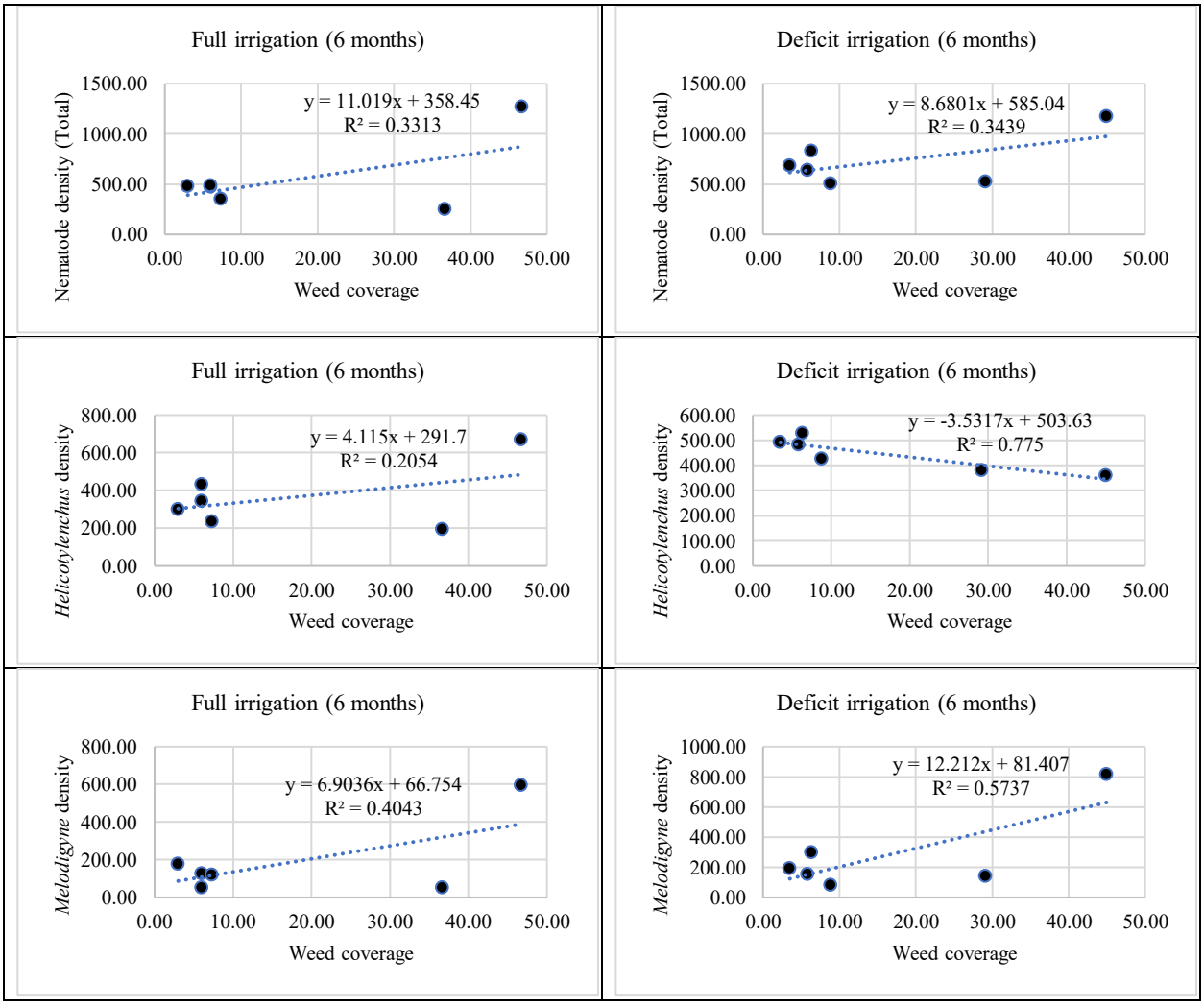


Figure 1. Weed treatment effects of full and deficit irrigation on weed coverage (%) - nematode density (number 100 g soil$^{-1}$) after 6-months observation (average 2022-2024).

The regression curve analysis of nematode densities and weed coverage during the pre-harvest period, based on the two-year average, revealed distinct patterns depending on the irrigation levels. A negative correlation was observed between total nematode and *Helicotylenchus* densities and weed coverage, as lower densities of both were associated with higher weed coverage. Conversely, a positive correlation was found between *Meloidogyne* densities and weed coverage, with nematode populations increasing as weed coverage increased (Figure 2). These trends may be related to the interaction between soil moisture, weed control, and nematode dynamics. Under full irrigation, the relationship between total nematode density and weed coverage was relatively balanced, suggesting that optimum moisture levels could stabilize both weed control and nematode management. This indicates that full irrigation provides a balanced environment, where both weed, and nematode populations are effectively controlled. In contrast, a slight negative correlation between weed coverage and nematode density was observed under deficit irrigation, particularly for *Helicotylenchus*. This implies that water scarcity limits the effectiveness of both weed and nematode management, as water-stressed plants and weeds become more resilient hosts for nematodes.

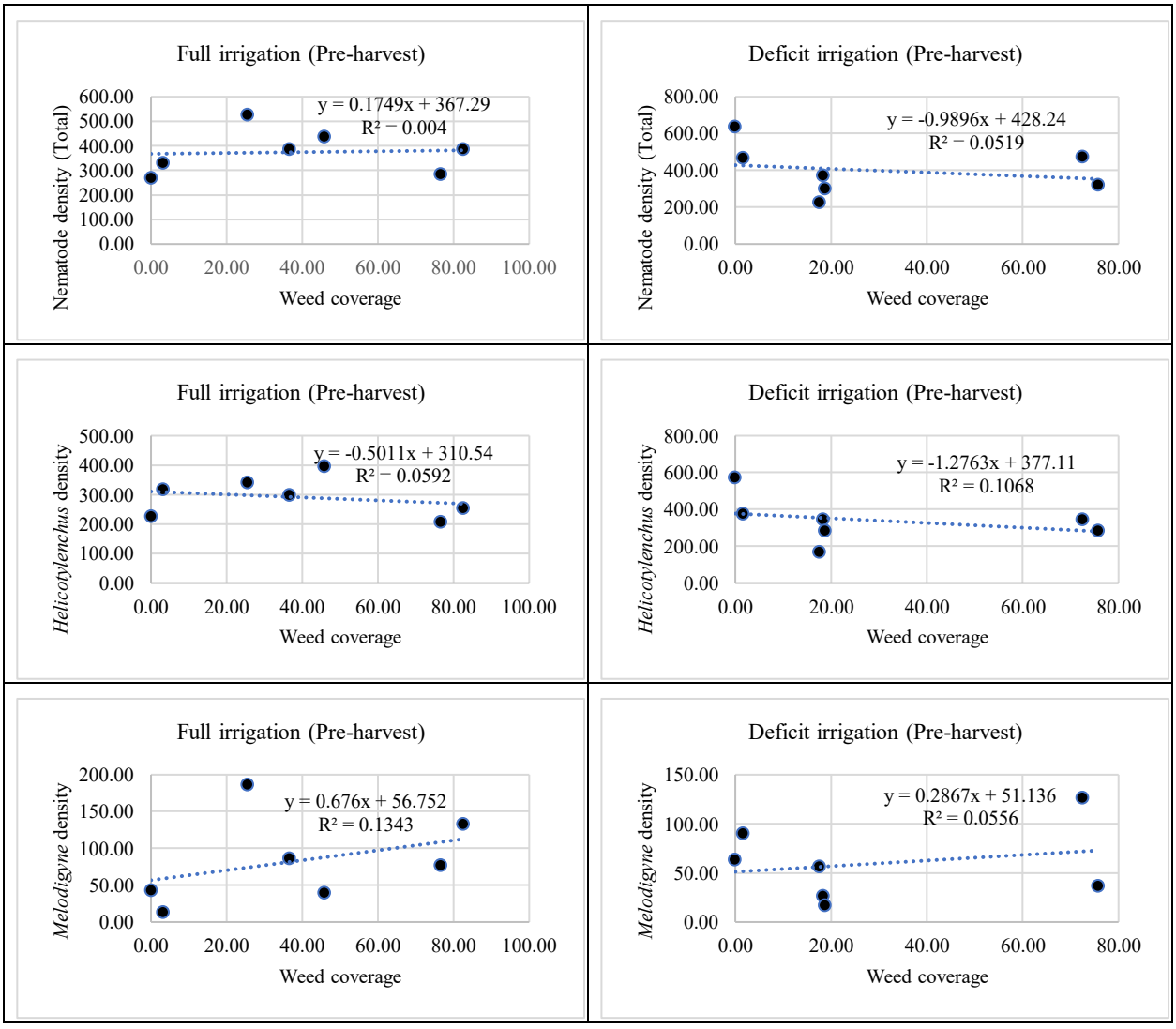


Figure 2. Weed treatment effects of full and deficit irrigation on weed coverage (%) - nematode density (number 100 g soil$^{-1}$) pre-harvest observation (average 2022-2024).

## Discussion

Weed species in banana production areas of Colombia and Brazil pose significant challenges to control efforts (Moura Filho et al., 2015; Quintero-Pertúz et al., 2020). Weeds inhibit seedling growth and serve as hosts for nematodes (Isaac et al., 2007; Fongod et al., 2010). Our results showed that weed control significantly affected nematode densities and weed coverage, particularly in the first six months. Higher nematode densities were observed in irrigated areas, likely due to favorable conditions for nematode development, including irrigation and dense weeds. Nematode densities were highest in the weedy and mowing plots, suggesting early weed density supported nematode populations. Reduced densities later were due to increased weed control, reduced irrigation, and insufficient time for nematodes to recover (Tables 2, 3 & 4). Full irrigation reduced nematode activity, as Wallace (1964) noted, while drought stress increased plant vulnerability to nematodes (Barker & Olthof, 1976). Herbicides reduced nematode populations by controlling weed hosts (Stirling et al., 1992). Geotextile mulching reduced nematode populations by altering soil temperature and moisture, making conditions less favorable for nematodes, particularly under deficit irrigation. These findings align with Govaerts et al. (2007b) and Klose et al. (2008), who emphasized

that consistent soil moisture improves herbicide penetration and reduces nematode survival. In contrast, deficit irrigation increased nematode densities, as water stress heightened plant susceptibility to nematodes, reducing herbicide effectiveness and promoting weed survival, which in turn provided more habitat for nematodes. These results highlight the importance of optimal irrigation and weed control strategies in integrated pest management for banana cultivation. Weed control during the first six months, particularly under full irrigation, resulted in higher nematode densities than in the pre-harvest period. The use of herbicide combinations combined with irrigation contributed to a gradual reduction in nematode populations over time. These results are consistent with the findings of Özarslan & Dinçer (2015), who suggested that weeds can increase nematode populations, leading to reduced yield and quality in bananas. Although the interaction between irrigation and treatment did not significantly affect total nematode, *Helicotylenchus*, or *Meloidogyne* densities, differences in nematode densities were observed in the first year. In the second year, a significant reduction in total nematode and *Helicotylenchus* densities was observed, probably due to the increased effectiveness of the weed management strategies. Weed coverage was a significant factor influencing nematode densities in our study, with higher weed coverage in weedy plots correlating with higher nematode populations. This observation correlates with findings reported in previous studies, which suggest that dense weed populations create favorable habitats for nematodes, particularly around banana roots (Araya et al., 1998; Araya & Blanco, 2001). Additionally, nematodes continue to thrive on host plants from common families such as Euphorbiaceae, Poaceae, and Solanaceae (Araya & De Waele, 2005; Quénéhervé et al., 2006; Duyck et al., 2009; Dinçer et al., 2024).

Treatments with (1) Indaziflam, (2) Oxyfluorfen+Glyphosate, and (3) Phendimethalin+Glyphosate were applied during the first 6 months, followed by the addition of Diquat in the pre-harvest period (Indaziflam+Diquat, Oxyfluorfen+Glyphosate+Diquat, and Phendimethalin+Glyphosate+Diquat). Geotextile mulching was also effective in reducing nematode densities. The reduction in weed coverage resulted in lower nematode populations, suggesting that effective weed control supports nematode management. While no significant changes in *Helicotylenchus* densities were observed, the statistical significance of weed coverage highlights its role in nematode dynamics. This suggests that *Helicotylenchus* densities may be less responsive to weed management than *Meloidogyne*, indicating different ecological niches for these nematodes (Tables 3 & 4). Furthermore, Robinson et al. (1991) and Bhattacharyya & Madhava (1992) emphasized that mowing weeds, rather than using herbicides, is essential for maintaining soil moisture and supporting nematode retention in banana fields. A study in Costa Rica showed that while weed management had no significant effect on nematode numbers or banana root damage, differences in root thickness were observed for *Radopholus similis* (Cobb,1893) (Thorne,1949) and *Helicotylenchus* spp., and weed control increased banana panicle weight, contributing to more sustainable practices (Araya & De Waele, 2005). Additionally, uncontrolled low population levels of *Meloidogyne* species on weeds like *Amaranthus* sp., *S. nigrum*, *Crassocephalum crepidioides* (Benth.) S.Moore (Asterales: Asteraceae), *Commelina benghalensis* L. (Commelinales: Commelinaceae), and *Eleusine indica* (L.) Gaertn. (Poales: Poaceae) may lead to significant yield losses in the future (Jonathan & Rajendran, 2000). It provides insight into the critical role of effective weed management in reducing nematode populations and improving banana yields.

Regression analyses revealed complex relationships between nematode densities and weed coverage. Both full and deficit irrigation showed a positive correlation between total nematode and *Meloidogyne* densities and weed coverage after six months, but *Helicotylenchus* densities were positively correlated only under full irrigation, with a negative correlation under deficit irrigation. Over two years, total nematode and *Helicotylenchus* densities decreased with increased weed coverage, especially under deficit irrigation, while *Meloidogyne* densities were positively correlated with weed coverage, indicating species-specific responses to irrigation (Figures 1 & 2). These results emphasize the importance of managing irrigation to influence nematode dynamics and suggest that tailored weed management can help mitigate nematode pressures under varying irrigation regimes. Soil moisture significantly affects herbicide effectiveness by

improving penetration and weed suppression, thus reducing nematode host availability (Ozores-Hampton et al., 2012). In contrast, deficit irrigation increases nematode and weed densities due to water stress, weakening plant defenses and reducing herbicide efficacy, allowing more weeds to serve as nematode reservoirs (Wang et al., 2006). Adequate moisture under full irrigation enhances herbicide absorption, leading to better weed control and fewer nematode habitats, while deficit irrigation promotes weed survival and nematode invasion, particularly for *Helicotylenchus*, which relies on weakened roots. These findings highlight the critical role of optimal irrigation in managing both weeds and nematodes and underscore the need for integrated management strategies that consider moisture levels and pest dynamics.

In conclusion, this study highlights the importance of integrated weed management strategies for nematode control in banana production. Future research should explore the underlying mechanisms driving these relationships and the potential for developing targeted management practices that optimize both weed and nematode control. A better understanding of these dynamics will ultimately contribute to more sustainable agricultural practices and improved crop health (Swafo & Dlamini, 2023; Dassou et al., 2024).

## Conclusion

This study aimed to highlight the interaction between weed control practices in banana cultivation and changes in host weed populations and nematode densities. The results showed that effective weed management can significantly reduce the presence of both harmful nematodes and weeds in banana fields. The use of different irrigation regimes showed that both the number of chemical applications and alternative weed control methods reduced weed populations over time, leading to a reduction in nematode populations due to the lack of suitable host plants. It was also found that effective and appropriate weed control strategies influenced nematode densities. The study also showed that, in addition to the favourable growth conditions provided by weeds, the amount of irrigation may influence nematode populations. Further research is therefore needed to gain a better understanding of the relationship between weed control practices and nematodes and their long-term effects.

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## References

Arantes, A. M., S. L. R. Donato, D. L. De Siqueira & E. F. Coelho, 2018. Gas exchange in 'pome' banana plants grown under different irrigation systems. Engenharia Agrícola, 38 (2): 197-207.

Araya, M. & D. De Waele, 2005. Effect of weed management on nematode numbers and their damage in different root thickness and its relation to yield of banana (*Musa* AAA cv. Grande Naine). Crop Protection, 24 (7): 667-676.

Araya, M. & F. Blanco, 2001. Changes in the stratification and spatial distribution of the banana (*Musa* AAA cv. Grande Naine) root system of poor, regular, and good developed plants. Journal of Plant Nutrition, 24 (11): 1679-1693.

Araya, M., A. Vargas & A. Cheves, 1998. Changes in distribution of roots of banana (*Musa* AAA cv. Valery) with plant height, distance from the pseudostem, and soildepth. The Journal of Horticultural Science and Biotechnology, 73 (4): 437-440.

Barker, K. R. & T. H. A. Olthof, 1976. Relationships between nematode population densities and crop responses. Annual Review of Phytopathology, 14: 327-353.

Barker, K. R., 1985. “Nematode Extraction and Bioassays, 19-35”. In: Advanced Treatise on *Meloidogyne* (Vol. 2) Methodology, (Eds. K. R.Barker, C. C. Carter & J. N. Sasser). North Carolina: Raleigh, North Carolina State University Graphics, USA, 223 pp.

Bélair, G. & D. L. Benoit, 1996. Host suitability of 32 common weeds to *Meloidogyne hapla* in organic soils of South Western Quebe. The Journal of Nematology, 28 (4S): 643-647.

Bhattacharyya, R. K. & R. V. N. Madhava, 1992. Root penetration in depth of cv Robusta Banana (AAA) as influenced by soil covers and soil moisture regimes. Banana Newslett. Australia, 15: 18-19.

Carr, M. K. V., 2009. The water relations and irrigation requirements of banana (*Musa* spp.). Experimental Agriculture, 45 (3): 333-371.

Castillo, P., H. F. Rapoport, J. E. Palomares Rius & R. M. Jiménez Diaz, 2008. Suitability of weed species prevailing in Spanish vineyards as hosts for root-knot nematodes. European Journal of Plant Pathology, 120 (1): 43-51.

Caveness, F. E., 1967. Shade house host ranges of some Nigerian nematodes. Plant Disease Reporter, 51 (1): 33-37.

Dabaj, K. H. & G. Jenser, 1990. Some weed host-plants of the northern root-knot nematode *Meloidogyne hapla* in Hungary. Nematologia Mediterranea, 18 (2): 139-140.

Dassou, A. G., S. Tovignan, F. Vodouhè & S. D. Vodouhè, 2024. Meta-analysis of agroecological technologies and practices in the sustainable management of banana pests and diseases. Environment, Development and Sustainability, 26 (9): 21937-21954.

Davidson, T. R. & J. L. Townshend, 1967. Some weed hosts of the southern root-knot nematode, *Meloidogyne incognita*. Nematologica, 13 (3): 452-458.

Davis, P. H., 1965-1988. Flora of Turkey and the East Aegean Islands (Vol. 1-10 Series). Edinburgh University Press, Edinburgh, Great Britain, 7041 pp.

Dinçer, D., M. Özkil, H. Torun & A. Özarslandan, 2024. The importance of host weed species for root-knot nematodes, *Meloidogyne* spp. Göldi, 1897 (Tylenchida: Heteroderidae) in banana plantations. Turkish Journal of Entomology, 48 (2): 183-194.

Duyck, P. F., S. Pavoine, P. Tixier, C. Chabrier & P. Quénéhervé, 2009. Host range as an axis of niche partitioning in the plant-feeding nematode community of banana agroecosystems. Soil Biology and Biochemistry, 41 (6): 1139-1145.

Egunjobi, O.A. & E. I. Bolaji, 1979. Dry season survival of *Pratylenchus* spp. in maize fields in Western Nigeria. Nematologia Mediterranea, 7 (2): 129-135.

Evlice, E. & Ş. Bayram, 2016. Identification of root-knot nematode species (*Meloidogyne* spp.) (Nemata: Meloidogynidae) in the potato fields of Central Anatolia (Turkey) using molecular and morphological methods Türkiye Entomoloji Bülteni, 6 (4): 339-347.

Fongod, A. G. N., D. A. Focho, A. M. Mih, B. A. Fonge & P. S. Lang, 2010. Weed management in banana production: The use of *Nelsonia canescens* (Lam.) Spreng as a nonleguminous cover crop. African Journal of Environmental Science and Technology, 4 (3): 167-173.

Goodey, J. B., M. T. Franklin & D. J. Hooper, 1965. T.Goodey's: The Nematode Parasites of Plants Catalogued Under Their Hosts (Third Edition). Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, 214 pp.

Govaerts, B., M. Fuentes, M. Mezzalama, J. M. Nicol, J. Deckers, J. D. Etchevers, B. Figueroa-Sandoval & K. D. Sayre, 2007a. Infiltration, soil moisture, root rot and nematode populations after 12 years of different tillage, residue and crop rotation managements. Soil and Tillage Research, 94 (1): 209-219.

Govaerts, B., M. Mezzalama, Y. Unno, K. D. Sayre, M. Luna-Guido, K. Vanherck, L. Dendooven & J. Deckers, 2007b. Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. Applied Soil Ecology, 37 (1-2): 18-30.

Isaac, W. P., R. A. I. Brathwaite, J. E. Cohen & I. Bekele, 2007. Effects of alternative weed management strategies on *Commelina diffusa* Burm. infestations in Fairtrade banana (*Musa* spp.) in St. Vincent and the Grenadines. Crop Protection, 26 (8): 1219-1225.

Jonathan, K. I. & G. Rajendran, 2000. Pathogenic effect of root-knot nematode, *Meloidogyne incognita* on banana, *Musa* sp. Indian Journal of Nematology, 30 (1): 13-15.

Kaur, R., J. A. Brito & J. R. Rich, 2007. Host suitability of selected weed species to five *Meloidogyne* species. Nematropica, 37 (1): 107-120.

Kepenekci, İ. & M. E. Ökten, 1996. Beypazarı (Ankara) ilçesi'nde havuç ile münavebeye giren domates ekiliş alanlarında saptanan *Helicotylenchus* (Tylenchida, Haplolaimidae) cinsine bağlı türler. Turkish Journal of Entomology, 20 (2): 137-148.

Klose, S., H. A. Ajwa, G. T. Browne, K. V. Subbarao, F. N. Martin, S.A. Fennimore & B.B. Westerdahl, 2008. Dose response of weed seeds, plant-parasitic nematodes, and pathogens to twelve rates of metam sodium in a California soil. Plant disease, 92 (11): 1537-1546.

Koenning, S. R., C. Overstreet, J. W. Noling, P. A. Donald, J. O. Becker & B. A. Fortnum, 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. Journal of Nematology, 31 (4S): 587-618.

Kokalis-Burelle, N. & E. N. Rosskopf, 2012. Susceptibility of several common subtropical weeds to *Meloidogyne arenaria*, *M.incognita*, and *M.javanica*. Journal of Nematology, 44 (2): 142-147.

Levin, R., J. A. Brito, W. T. Crow & R. K. Schoellhorn, 2005. Host status of several perennial ornamental plants to four root-knot nematode species in growth room and greenhouse experiments. 44th annual meeting fort lauderdale. Journal of Nematology, 37 (3): 379.

Liu, H. J., S. Cohen, J. Tanny, J. H. Lemcoff & G. Huang, 2008. Estimation of banana (*Musa* sp.) plant transpiration using a standard 20 cm pan in a greenhouse. Irrigation and Drainage Systems, 22 (3-4): 311-323.

McKenry, M. V., 1992. Cover crops and nematode species. Plant Protection Quarterly, 2 (4): 4-8.

Mendes, M. L., D. W. Dickson & W. T. Crow, 2020. Yellow and purple nutsedge and coffee senna as hosts of common plant nematodes in Florida. Journal of Nematology, 52 (1): 1-9.

Moura Filho, E. R., L. P. M. Macedo & A. R. S. Silva, 2015. Phytosociological survey of weeds in banana. Holos, 31 (2): 92-97.

Özarslandan, A. & D. Dinçer, 2015. Plant parasitic nematodes in banana fields in Türkiye. Plant Protection Bulletin, 55 (4): 361-372.

Özarslandan, A. & İ. H. Elekcioğlu, 2010. Identification of the root-knot nematode species (*Meloidogyne* spp.) (Nemata: Meloidogynidae) collected from different parts of Turkey by molecular and morphological methods. Turkish Journal of Entomology, 34 (3): 323-335.

Ozores-Hampton, M., R. McSorley & P.A. Stansly, 2012. Effects of long-term organic amendments and soil sanitation on weed and nematode populations in pepper and watermelon crops in Florida. Crop Protection, 35: 89-95.

Powers, L. E. & A. Pitty, 1993. Research notes: Resistance of common weeds in Honduras to *Meloidogyne incognita*. Nematropica, 23 (2): 209-211.

Quénéhervé, P., C. Chabrier, A. Auwerkerken, P. Topart, B. Martiny & S. Marie-Luce, 2006. Status of weeds as reservoirs of plant-parasitic nematodes in banana fields in Martinique. Crop Protection, 25 (8): 860-867.

Queneherve, P., F. Drob & P. Topart, 1995. Host status of some weeds to *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp. and *Rotylenchulus reniformis* associated with vegetables cultivated in polytunnels in Martinique. Nematropica, 25 (2): 149-157.

Quintero-Pertúz, I., E. Carbonó-Delahoz & A. Jarma-Orozco, 2020. Weeds associated with banana crops in Magdalena department, Colombia. Planta Daninha, 38: e020217466 (1-13).

Rich, J. R., J. A. Brito, R. Kaur & J. A. Ferrell, 2008. Weed species as hosts of *Meloidogyne*: A review. Nematropica, 39 (2): 157-185.

Robinson, J. C., A. J. Alberts & R. E. Reynolds, 1991. Water relations. C/N ratio in leaves of 'Robusta' banana as influenced by soil covers and soil moisture regimes. Banana Newsletter Australia, 14: 15-16.

Siddiqui, I. A., S. A. Sher & A. M. French, 1973. Distribution of Plant Parasitic Nematodes in California. State of California Department of Food and Agriculture, Division of Plant Industry, USA, 324 pp.

Singh, S. K., U. R. Khurma & P. J. Lockhart, 2010. Weed hosts of root-knot nematodes and their distribution in Fiji. Weed Technology, 24 (4): 607-612.

Stirling, G. R., J. M. Stanton & J. W. Marshall, 1992. The importance of plant-parasitic nematodes to Australian and New Zealand agriculture. Australasian Plant Pathology, 21 (3): 104-107.

Stoyanov, D., 1967. Additions to host records of *Meloidogyne* sp., *Helicotylenchus multicinctus*, and *Rotylenchulus reniformis*. Nematologica, 13 (1): 173.

Subba, S., S. Chowdhury, S. Chhetri, H. Meena & S. Debnath, 2023. Floor management of banana orchard using banana biomat mulch and leguminous cover crop for sustainable production. The Pharma Innovation Journal, 12 (1): 680-684.

Swafo, S. M. & P. E. Dlamini, 2023. Unlocking the land capability and soil suitability of Makuleke farm for sustainable banana production. Sustainability, 15 (1): 453 (1-15).

TAGEM, 2024. Yabancı Ot Standart İlaç Deneme Metodları. General Directorate of Agricultural Research and Policies, Republic of Türkiye Ministry of Agriculture and Forestry, Türkiye, 158 pp (Web page: https://www.tarimorman.gov.tr/TAGEM/Belgeler/yayin/Yabancı%20Ot%20Standart%20İlaç%20Deneme%20Metotları.pdf) (Date accessed: January 2025).

Uddin, M. S., M. J. Rahman, M. A. Mannan, S. A. Begum, A. F. M. F. Rahman & M. R. Uddin, 2002. Plant biodiversity in the homesteads of saline area of Southeastern Bangladesh. Pakistan Journal of Biological Sciences, 5 (6): 710-714.

Wallace, H. R., 1964. The Biology of Plant Parasitic Nematodes. New York, St. Martin's Press, USA, 280 pp.

Wang, K. H., R. McSorley & N. Kokalis-Burelle, 2006. Effects of cover cropping, solarization, and soil fumigation on nematode communities. Plant and Soil, 286 (1-2): 229-243.

Yeates, G. W., D. A. Wardle & R. N. Watson, 1993. Relationships between nematodes, soil microbial biomass and weed-management strategies in maize and asparagus cropping systems. Soil Biology and Biochemistry, 25 (7): 869-876.

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# A taxonomic study on nematode diversity of some terrestrial trees in Türkiye: a case study in Tekirdağ

## Türkiye'deki bazı odunsu ağaç türlerinin nematod çeşitliliği üzerine taksonomik bir çalışma: Tekirdağ ili örneği

**Lerzan ÖZTÜRK**[1*]

## Abstract

The current study was conducted in 2024 in Süleymanpaşa, Tekirdağ to determine the nematode biodiversity in the black poplar [*Populus nigra* L. (Malpighiales: Salicaceae)], Himalayan cedar [*Cedrus deodara (*Lamb.) G.Don (Pinales: Pinaceae)], cypress [*Cupressus sempervirens* L. (Pinales: Cupressaceae)], oriental plane [*Platanus orientalis* L. (Proteales: Platanaceae)], common ash [*Fraxinus excelsior* L. (Lamiales: Oleaceae)], stone pine [*Pinus pinea* L. (Pinales: Pinaceae)], and black locust [*Robinia pseudoacacia* L. (Fabales: Fabaceae)] growing areas. Soil samples were collected from the 0-60 cm soil depth of each tree rhizosphere. Nematodes were extracted using the centrifuge flotation method, and identifications were made using polytomous keys. In the study, 38 genera were identified in the rhizosphere soil of stone pine, 32 in Himalayan cedar, 36 in cypress, 34 in oriental plane, 31 in black locust, 27 in common ash, and 27 in black poplar. *Cephalobus* Bastian, 1865 (Rhabditida: Cephalobidae), *Aphelenchus* Bastian, 1865 (Aphelenchida: Aphelenchidae), *Filenchus* Andrassy, 1954 (Tylenchida: Tylenchidae), and *Geocenamus* Thorne & Malek, 1968 (Tylenchida: Merliniidae) were common genera across all tree species. Taxonomic diversity indices were calculated to compare nematode diversities at different taxonomic levels. The Shannon diversity index (H′) ranged from 2.99 to 3.41, Evenness (J′) from 0.90 to 0.95, Maturity index (MI) from 2.29 to 2.48, and Plant-parasitic index (PPI) from 2.84 to 3.02. Analysis by trophic groups revealed that plant parasitic and bacterial feeder nematodes were more prevalent in all tree species.

**Keywords:** Nematode fauna, soil, taxonomy, woody trees, Türkiye

## Öz

Kara kavak [*Populus nigra* L. (Malpighiales: Salicaceae)], Himalaya sediri [*Cedrus deodara* (Lamb.) G.Don (Pinales: Pinaceae)], servi [*Cupressus sempervirens* L. (Malpighiales: Salicaceae)], Doğu çınarı [*Platanus orientalis* L. (Proteales: Platanaceae)], Adi dişbudak [*Fraxinus excelsior* L. (Lamiales: Oleaceae)], Karaçam [*Pinus pinea* L. (Pinales: Pinaceae)] ve Yalancı akasya [*Robinia pseudoacacia* L. (Fabales: Fabaceae)] ağaçlarının yetişme alanlarındaki nematod biyoçeşitliliğini belirlemek için 2024 yılında Süleymanpaşa Tekirdağ'da bir çalışma yapılmıştır. Her ağacın rizosferinde 0-60 cm derinlikten toprak örnekleri alınmıştır. Nematodlar, santrifüj yöntemi kullanılarak ekstrakte edilmiş ve teşhisleri polytomous anahtarlar kullanılarak yapılmıştır. Çalışmada, Karaçam rizosfer topraklarında 38 cins, Himalaya sedirinde 32 cins, servide 36 cins, doğu çınarında 34 cins, yalancı akasyada 31 cins, adi dişbudakta 27 cins ve kara kavakta 27 cins tespit edilmiştir. *Cephalobus* Bastian, 1865 *(*Rhabditida: Cephalobidae*), Aphelenchus* Bastian, 1865 *(*Aphelenchida: Aphelenchidae), *Filenchus* Andrassy, 1954 *(*Tylenchida: Tylenchidae) ve *Geocenamus* Thorne & Malek, 1968 (Tylenchida: Merliniidae) tüm ağaç türlerinde bulunmuştur. Nematod çeşitliliklerini farklı taksonik düzeyde karşılaştırmak için birçok taksonomik çeşitlilik indeksi hesaplanmıştır. Shannon diversity index (H′) 2.99 ile 3.41 arasında, Evenness index (J′) 0.90 ile 0.95 arasında, Maturity (MI) 2.29 ile 2.48 arasında ve Plant-parasitic index (PPI) 2.84 ile 3.02 arasında değişmiştir. Trofik gruplara göre yapılan analizde, bitki paraziti ve bakteri ile beslenen nematodların tüm ağaç türlerinde yaygın olduğu belirlenmiştir.

**Anahtar sözcükler:** Nematod faunası, toprak, taksonomi, odunsu ağaçlar, Türkiye

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## Introduction

Terrestrial trees are critical components of ecosystems worldwide, and forests established with these trees provide habitat for countless species and essential ecological services that sustain life on Earth. Covering 31% of the world's land area, forest ecosystems are crucial, containing elements vital for human life such as water, soil, and energy (Stanturf & Mansourian, 2020). Forests, housing approximately 60.000 tree species, offer habitats for plants, animals, and people, particularly in underdeveloped countries (Anonymous, 2020). They also yield resources like wood, fibre, oil, and fruit, supporting industries in timber, food, medicine, and cosmetics (Anonymous, 1993). Located at the crossroads of Europe and Asia in the northern hemisphere, Türkiye features several climate types including continental, Mediterranean, and Black Sea, each characterized by distinct precipitation patterns. Türkiye hosts 800 taxa of terrestrial tree species, with oak covering 29.4%, red pine 22.85%, and Scots pine 17.54% of the total forest area as of 2022. The reported area for the cultivation of these tree species in Tekirdağ province is 1.098.12 hectares (Anonymous, 2023). Many of these forest tree species are now being cultivated in parks, gardens, and other areas (Anonymous, 2022).

Trees harbour a diverse community of organisms within their ecosystems, both above and below ground. Some of these organisms can be harmful to trees, while others can coexist without causing harm.Hundreds of nematode species inhabit agricultural fields, pastures, soil, water, plant roots, and animals. Among these, approximately 4.100 species are known as plant parasites, capable of feeding on or inside roots of plants, and 27.000 nematodes with different feeding habitats were identified in soils (Decraemer & Hunt, 2006; Háněl & Čerevková, 2010). Damage by plant parasitic nematodes can be particularly significant in young seedlings and saplings, often drastically reducing their survival rates soon after planting. On the other hand, other free-living nematode species exhibit feeding behaviours such as omnivore, predator, fungivore and bacterivore, posing no threat to plants. These species play crucial roles in decomposing animal and plant organic matter in the soil and are essential components in the cycling of mineral nutrients in the soil. The population density of these free-living nematodes in soil provides insights into nutrient cycling, mineral content, productivity, and soil acidity, distinct from those influenced by plant-feeder nematodes (Yadav et al., 2018).

In the global context, extensive research has identified diverse nematode communities, often focusing on identifying *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1981 one of the most destructive nematodes (Karmezi et al., 2022). For instance, Lawton et al. (1998) documented 374 nematode species in Cameroon's tropical forests, highlighting the richness of nematode biodiversity in such environments. Similarly, Skwiercz (2012) reported 119 nematode species across various forest types in Poland, showcasing global variations in nematode fauna. In a study conducted on Mount Ararat in Türkiye, nematodes belonging to 62 genera were identified across various habitats, including mountain grasslands, wildflower meadows, riverbeds, marshes, and chalk grasslands (Çakmak et al., 2021). *Rotylenchus conicaudatus* Atighi et al., 2011 (Nematoda: Hoplolaimidae), *Heterodera trifolii* Goffart, 1932 and *Tylenchorhynchus mangiferae* Luqman & Khan, 1986 were nematodes identified for the first time at species level (Çakmak et al., 2019). In the other studies in Türkiye, *Bursaphelenchus* species have been identified in the provinces that located in Anatolian part of the country (Akbulut et al., 2006, 2008; Öztürk, 2019; Taşdemir et al., 2020).

In Türkiye, particularly in the Northwestern European part, there is limited data on nematode diversity associated with forest trees, with only two species identified so far: *Xiphinema pachtaicum* Tulaganov, 1938 in cypress, poplar, pine, acacia, and spruce, and *Xiphinema index* Thorne & Allen, 1950 in cypress (Öztürk et al., 2023). This underscores the necessity for comprehensive surveys to understand the nematode communities associated with forest trees. The present study aims to fill this gap by investigating nematode presence in habitats where terrestrial trees grow. The study categorize nematodes into different functional groups based on their feeding habits: fungivores (feeding on fungi), bacterivores (feeding on bacteria),

predators (feeding on other nematodes or microorganisms), omnivores (feeding on various organic matter), and plant parasitics nematodes. By determining the diversity and functional roles of nematodes across 7 forest tree species, this study aims to provide valuable insights into soil ecology, and forest tree health management.

## Materials and Methods

### Nematological survey

The nematological survey was conducted in May 2024 in several locations in Tekirdağ province (Figure 1). As part of the study, soil samples were collected from woodlands, parks, and gardens in the Süleymanpaşa district, and surrounding villages. The survey focused on areas where various tree species are cultivated and 140 soil samples were collected. For each tree species, 20 soil samples were collected from 20 distinct locations (Table 1).


Figure 1. A map illustrating the survey locations in Süleymanpaşa district, its geographical position within the province, and its location in Turkey.

Table 1. The number of samples per tree species

| Common name | Scientific name | Number of samples |
|---|---|---|
| Oriental plane | *Platanus orientalis* L. | 20 |
| Black poplar | *Populus nigra* L. | 20 |
| Black locust | *Robinia pseudo-acacia* L. | 20 |
| Cypress | *Cupressus sempervirens* L. | 20 |
| Common ash | *Fraxinus excelsior* L. | 20 |
| Himalayan cedar | *Cedrus deodara* (Lamb.) G.Don | 20 |
| Stone pine | *Pinus pinea* L. | 20 |

Attention was paid to ensuring that there was at least a 1 km distance between the areas being sampled. According to the data obtained from the meteorological station at the Tekirdağ Viticulture Research Institute during the study year, the average air temperature in January was 6°C, the soil temperature was 8.2°C, and the precipitation was 56.4 mm. In February, the average air temperature was 9.4°C, the soil temperature was 9.6°C, and the precipitation was 34.8 mm. In March, the air temperature

was recorded as 9.82°C, the soil temperature as 11.5°C, and the precipitation as 83 mm. In April, the average air temperature was 15.72°C, the soil temperature was 15.9°C, and the precipitation was 82.8 mm. In May, the air temperature increased to 16.25°C, while the soil temperature was 17.3°C, and the precipitation dropped to its lowest level at 27.8 mm. These data reflect the climatic conditions during the study period and played a crucial role in determining the timing of sampling.

In the sampled areas, trees of the same genus were found clustered together, with no other tree species present; only weeds were observed. The sampled trees were identified as follows: species identification was conducted using published diagnostic keys. Subsequent examinations focused on parameters such as leaf arrangement on the branch, leaf morphology (needle-like, compound, etc.), leaf margin characteristics (serrated or smooth), trunk color, trunk structure, bark morphology, tree height, and seed structure.

For species identification, the dichotomous keys of Schulz et al. (2005), Lavin et al. (2012), Lange (2014), Atha & Boom (2017), and Anonymous (2024a, b) were utilized. Additionally, expert engineers within Tekirdağ Metropolitan Municipality and Tekirdağ Viticulture Research Institute, possessing extensive knowledge and experience in species identification, was consulted. Furthermore, some sampled trees had species identification labels affixed by the Tekirdağ Metropolitan Municipality.

During the surveys, soil samples were collected from a depth of 0-60 cm within the canopy projection of the trees. At this stage, soil samples were collected from multiple points and combined into a single bag to create a composite sample. The sampled areas were non commercial areas. In poplar trees (Populus spp.), a spacing of 2.40-3 meters was observed between individual trees, whereas other tree species exhibited a spacing of 4.80 to 5 meters. Nematodes in the soil samples were extracted using the centrifuge flotation method described by Jenkins (1964). Initially, 200 grams of soil were placed in a bowl with water. The mixture was thoroughly mixed and passed through a 200-mesh sieve, followed by a 400-mesh sieve. The nematodes remaining at the bottom of the 400-mesh sieve were collected into a tube. The collected nematode suspension was first centrifuged at 1750 rpm for five minutes. Next, a solution containing 475 g/L of sugar was added to the tubes instead of water, and the suspension was centrifuged again for one minute. Finally, the suspension was passed through a 400-mesh sieve, and the remaining nematodes were collected. Female and if present male nematodes were then killed by heating to 55°C for slide preparation. The dead nematodes were fixed with solutions TAF (40% formaldehyde + triethanolamine + distilled water), Seinhorst I (glycerol + distilled water), and Seinhorst II (glycerol + ethanol), according to the method described by Seinhorst (1959), before being mounted on slides.

Nematodes were examined under a microscope at magnifications ranging from 10X to 100X. To identify at genus and species level polytomous keys from several sources, including Loof & Jairajpuri (1968), Choudhary & Jairajpuri (1984), Geraert & Raski (1987), Anderson (1979), Abolafia & Santiago (1996), Handoo & Golden (1989), Loof & Luc (1990), Brzeski (1991), Handoo et al. (2007), Scholze & Sudhaus (2011), Wu et al., (2016), and Tran et al. (2024), were used. Leica DM 1000 microscope was used in the examination and morphometric measurement of nematodes. The morphometric measurements were conducted using Leica Application Suite. The identified nematodes were then classified based on order, family, colonizer-persister (c-p) value, and feeding habitat. In some genera, such as *Laimidorus* and *Dorylaimus*, female individuals were not captured; therefore, identifications were limited to the genus level. The nematodes were classified based on Siddiqi (2000).

The number of samples in which the species or genus was recorded was divided by the total number of samples to calculate the frequency of occurrence (F%) of each species. A heat map of genera abundance was generated for each forest tree using XLSTAT software. Diversity indices, including Shannon-Wiener, Simpson's Diversity, Richness, and Evenness, Maturity index (MI), Plant-parasitic index (PPI) were calculated for each terrestrial tree. MI stands for free-living nematodes that are omnivorous, predatory, or feed on bacteria and fungi, while PPI stands for plant-feeding nematodes (Bongers, 1990). The indices were calculated as follows (Pielou, 1966; Bongers, 1990; Neher & Darby, 2009; Öztürk & Avcı, 2023). Other indices were generated with Phyton 3.12, and Sieriebriennikov et al. (2014).

Shannon-Weiner $H' = \sum[(pi) \times log\ (pi)]$

Evenness $J' = \frac{\mathrm{H}\prime}{\ln(\mathrm{S})}$ Richness $R = \frac{S-1}{ln\ N}$

Simpson's Diversity index $1 - D = 1 - [\sum n\ (n-1)/N(N-1)]$

Pi: the abundance of species in the genera; S: total genera number; $n_i$: The number of nematodes in a genur; N: Total number of nematodes in sample belonging to all genera

Maturity index (only bacterial feeder, fungal feeder, omnivore and predator nematodes) $MI = \sum c(i) \times pi$

Plant-parasitic index (only Plant-parasitic nematodes) $PPI = \sum c(i) \times pi$

pi represents the ratio of free-living nematodes in group i to all collected individuals in the area

c (i) represents the colonizer-persister values of nematodes in the area

## Results and Discussion

### Nematode fauna composition in terrestrial trees

Forty-four nematode genera belonging to eight orders, 10 suborders, 16 superfamilies, 23 families, and 31 subfamilies were identified in terrestrial tree-growing areas in Tekirdağ. Order Tylenchida exhibited the highest overall values across all tree species, indicating its prevalence in these environments. In contrast, Triplonchida was less prominent, absent from the Oriental plane, and Common ash, suggesting a limited presence. Tylenchida dominated each tree species with values ranging from 34.2 to 44.1, comprising a significant portion of the nematode community. Following Tylenchida were Dorylaimida and Rhabditida, contributing nine and seven genera, respectively (Figure 2).

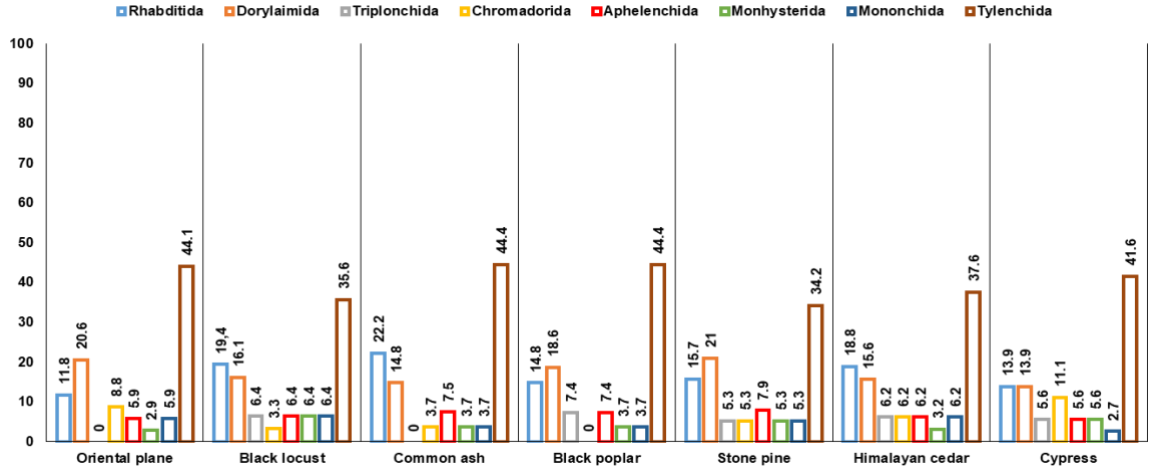


Figure 2. The proportion (%) of eight nematode orders among tree species.

The taxonomic classification of identified genera was represented in Table 2.

Table 2. Taxonomic classification of 44 nematode genera associated with terrestrial trees surveyed in Tekirdağ

| Genera | Order | Suborder | Superfamily | Families | Subfamily |
|---|---|---|---|---|---|
| *Acrobeles* | Rhabditida | Cephalobina | Cephaloboidea | Cephalobidae | Cephalobinae |
| *Alaimus* | Dorylaimida | Dorylaimina | Alaimoidea | Alaimidae | Alaiminae |
| *Achromadora* | Chromadorida | Chromadorina | Chromadoroidea | Achromadoridae | Achromadorinae |
| *Acrobeloides* | Rhabditida | Cephalobina | Cephaloboidea | Cephalobidae | Cephalobinae |
| *Aphelenchus* | Aphelenchida | Aphelenchina | Aphelenchoidea | Aphelenchidae | Aphelenchinae |
| *Aphelenchoides* | Aphelenchida | Aphelenchina | Aphelenchoidea | Aphelenchioididae | Aphelenchoidinae |
| *Aporcelaimellus* | Dorylaimida | Dorylaimina | Dorylaimoidea | Aporcelaimidae | Aporcelaiminae |
| *Basiria* | Tylenchida | Tylenchina | Tylenchoidea | Tylenchidae | Boleodorinae |
| *Boleodorus* | Tylenchida | Tylenchina | Tylenchoidea | Tylenchidae | Boleodorinae |
| *Cervidellus* | Rhabditida | Cephalobina | Cephaloboidea | Cephalobidae | Cephalobinae |
| *Cephalobus* | Rhabditida | Cephalobina | Cephaloboidea | Cephalobidae | Cephalobinae |
| *Clarkus* | Mononchida | Mononchina | Mononchoidea | Mononchidae | Prionchulinae |
| *Coslenchus* | Tylenchida | Tylenchina | Tylenchoidea | Tylenchidae | Tylenchinae |
| *Mesocriconema* | Tylenchida | Tylenchina | Criconematoidea | Criconematidae | Criconematinae |
| *Discolaimus* | Dorylaimida | Dorylaimina | Dorylaimoidea | Qudsianematidae | Discolaiminae |
| *Ditylenchus* | Tylenchida | Tylenchina | Sphaerularioidea | Anguinidae | Anguininae |
| *Dorylaimus* | Dorylaimida | Dorylaimina | Dorylaimoidea | Dorylaimidae | Dorylaiminae |
| *Filenchus* | Tylenchida | Tylenchina | Tylenchoidea | Tylenchidae | Tylenchinae |
| *Geomonhystera* | Monhysterida | Monhysterina | Monhysteroidea | Monhysteridae | Monhysterinae |
| *Geocenamus* | Tylenchida | Tylenchina | Tylenchoidea | Merliinidae | Merliniinae |
| *Helicotylenchus* | Tylenchida | Tylenchina | Tylenchoidea | Hoplolaimidae | Hoplolaiminae |
| *Laimydorus* | Dorylaimida | Dorylaimina | Dorylaimoidea | Dorylaimidae | Laimydorinae |
| *Mesodorylaimus* | Dorylaimida | Dorylaimina | Dorylaimoidea | Dorylaimidae | Laimydorinae |
| *Mesorhabditis* | Rhabditida | Rhabditina | Rhabditoidea | Rhabditidae | Rhabditinae |
| *Monhystera* | Monhysterida | Monhysterina | Monhysteroidea | Monhysteridae | Monhysterinae |
| *Mylonchulus* | Mononchida | Mononchina | Mononchoidea | Mylonchulidae | Mylonchulinae |
| *Plectus* | Chromadorida | Chromadorina | Plectoidea | Plectidae | Plectinae |
| *Panagrolaimus* | Rhabditida | Rhabditina | Rhabditoidea | Rhabditidae | Panagrolaiminae |
| *Prismatolaimus* | Triplonchida | Tobrilina | Prismatoloidea | Prismatolaimidae | Prismatolaiminae |
| *Prodorylaimus* | Dorylaimida | Dorylaimina | Dorylaimoidea | Dorylaimidae | Laimidorinae |
| *Psilenchus* | Tylenchida | Tylenchina | Tylenchoidea | Tylenchidae | Psilenchinae |
| *Pratylenchoides* | Tylenchida | Tylenchina | Tylenchoidea | Merliniidae | Pratylenchoidinae |
| *Pratylenchus* | Tylenchida | Tylenchina | Tylenchoidea | Pratylenchidae | Pratylenchinae |
| *Paratylenchus* | Tylenchida | Tylenchina | Tylenchoidea | Tylenchulidae | Paratylenchinae |
| *Rhabditis* | Rhabditida | Rhabditina | Rhabditoidea | Rhabditidae | Rhabditinae |
| *Rotylenchus* | Tylenchida | Tylenchina | Tylenchoidea | Hoplolaimidae | Rotylenchulinae |
| *Tripyla* | Triplonchida | Tripylina | Tripyloidea | Tripylidae | Tripylinae |
| *Tylenchus* | Tylenchida | Tylenchina | Tylenchoidea | Tylenchidae | Tylenchinae |
| *Tylenchorhynchus* | Tylenchida | Tylenchina | Tylenchoidea | Telotylenchidae | Telotylenchinae |
| *Tylencholaimus* | Dorylaimida | Dorylaimina | Tylencholaimoidea | Tylencholaimidae | Tylencholaiminae |
| *Tylocephalus* | Chromadorida | Chromadorina | Plectoidea | Plectidae | Wilsonematinae |
| *Xiphinema* | Dorylaimida | Dorylaimina | Longidoroidea | Longidoridae | Xiphinematinae |
| *Wilsonema* | Chromadorida | Chromadorina | Plectoidea | Plectidae | Wilsonematinae |
| *Zygotylenchus* | Tylenchida | Tylenchina | Tylenchoidea | Pratylenchidae | Pratylenchinae |

The nematode genera varied by tree species, with 38 genera found in Stone pine, 32 in Himalayan cedar, 36 in Cypress, 34 in Oriental plane, 31 in Black locust, and 27 each in Common ash and Black poplar. A diverse community of nematodes was identified, comprising 15 genera of bacterial feeder nematodes, such as *Tylocephalus* Crossman, 1933 and *Geomonhystera* Andrassy, 1981, four genera of fungal feeders, including *Ditylenchus* Filipjev, 1936 and *Tylencholaimus* De Man, 1876, five genera of omnivores like *Prodorylaimus* Andrassy, 1959 and *Laimydorus* Siddiqi, 1969, and five genera of predators, notably *Clarkus* Jairajpuri, 1970 and *Mylonchulus* Cobb, 1916. In the bacterial feeder group, species-level identification was achieved for 10 species, highlighting the richness of this group. In the plant-parasitic nematode genera found in the areas, 21 nematodes were identified at the species level. Of these, nine species, including *Boleodorus thylactus* Thorne, 1941, could feed on fungal cultures and therefore grouped as root-fungal feeders. The distribution of plant-parasitic nematodes varied significantly across tree species: 17 species were found in Stone pine, 17 in Himalayan cedar, 16 in Cypress, 19 in Oriental plane, 12 in Black locust, 15 in Common ash, and 13 in Black poplar . Among the plant-parasitic species, endoparasitic (13.6%), ectoparasitic (81.8%), and semi-endoparasitic (4.5%) nematodes were identified, with ectoparasitic nematodes being the most dominant across all tree species (Table 3, Figure 3).

Table 3. The nematodes recorded on a per-terrestrial tree (Sp: stone pine, Hc: Himalayan cedar, C: Cypress, As: Oriental plane, Bl: Black locust, Ca: Common ash, Bp: Black poplar)

| Genera/species | | c-p | Feeding habitat | Sp | Hc | C | Op | Bl | Ca | Bp |
|---|---|---|---|---|---|---|---|---|---|---|
| *Acrobeles* | *Acrobeles ciliatus* | 2 | Bacterial feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| | *Acrobeles complexus* | 2 | Bacterial feeder | ■ | | ■ | ■ | ■ | | |
| | *Acrobeles cylindricus* | 2 | Bacterial feeder | ■ | | | | | | |
| *Alaimus* | *Alaimus primitivus* | 4 | Bacterial feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Achromadora* sp. | | 3 | Bacterial feeder | ■ | | ■ | ■ | ■ | | |
| *Acrobeloides* | *Acrobeloides nanus* | 2 | Bacterial feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Cervidellus* | *Cervidellus vexilliger* | 2 | Bacterial feeder | ■ | ■ | | | ■ | ■ | |
| *Cephalobus* | *Cephalobus persegnis* | 2 | Bacterial feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Geomonhystera* | *Geomonhystera villosa* | 1 | Bacterial feeder | ■ | ■ | ■ | | ■ | ■ | ■ |
| *Mesorhabditis* sp. | | 1 | Bacterial feeder | | | ■ | | ■ | | |
| *Monhystera* sp. | | 2 | Bacterial feeder | ■ | | ■ | ■ | ■ | | |
| *Panagrolaimus* | *Panagrolaimus rigidus* | 1 | Bacterial feeder | ■ | ■ | | | | ■ | |
| *Plectus* sp. | | 2 | Bacterial feeder | | ■ | ■ | | | | |
| *Prismatolaimus* | *Prismatolaimus intermedius* | 3 | Bacterial feeder | ■ | ■ | ■ | | ■ | | ■ |
| *Rhabditis* | | 1 | Bacterial feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Tylocephalus* | *Tylocephalus auriculatus* | 2 | Bacterial feeder | ■ | | ■ | ■ | | | |
| *Wilsonema* | *Wilsonema schuurmanstekhoven* | 2 | Bacterial feeder | ■ | ■ | ■ | ■ | | ■ | |
| *Aphelenchus* | *Aphelenchus avenae* | 2 | Fungal feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Aphelenchoides* | *Aphelenchoides sacchari* | 2 | Fungal feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Ditylenchus* | *Ditylenchus myceliophagus* | 2 | Fungal feeder | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Tylencholaimus* | *Tylencholaimus proximus* | 4 | Fungal feeder | ■ | | | ■ | | | |
| *Aporcelaimellus* | *Aporcelaimellus obscuroides* | 5 | Omnivore | | ■ | | | ■ | ■ | ■ |
| *Dorylaimus* sp. | | 4 | Omnivore | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Laimydorus* sp. | | 4 | Omnivore | ■ | | ■ | ■ | | | |
| *Mesodorylaimus* sp. | | 5 | Omnivore | ■ | | ■ | ■ | ■ | | |
| *Prodorylaimus* sp. | | 5 | Omnivore | ■ | | ■ | | | | ■ |
| *Discolaimus* sp. | | 4 | Predator | ■ | ■ | | ■ | | | |

Table 3. continued

| Genera/species | | c-p | Feeding habitat | Sp | Hc | C | Op | Bl | Ca | Bp |
|---|---|---|---|---|---|---|---|---|---|---|
| *Clarkus* | *Clarkus papilatus* | 4 | Predator | ■ | ■ | ■ | ■ | ■ | | |
| *Mylonchulus* sp. | | 4 | Predator | ■ | ■ | | ■ | ■ | ■ | ■ |
| *Tripyla* sp. | | 3 | Predator | ■ | ■ | ■ | | ■ | | ■ |
| *Basiria* | *Basiria graminophila* | 2 | Root-fungal feeder [Ek] | ■ | | ■ | ■ | | ■ | |
| *Boleodorus* | *Boleodorus thylactus* | 2 | Root-fungal feeder [Ek] | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Coslenchus* | *Coslenchus costatus* | 2 | Root-fungal feeder [Ek] | | | ■ | | ■ | | |
| | *Coslenchus turkeyensis* | 2 | Root-fungal feeder [Ek] | | ■ | | ■ | | | |
| *Filenchus* | *Filenchus thornei* | 2 | Root-fungal feeder [Ek] | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| | *Filenchus cylindricus* | 2 | Root-fungal feeder [Ek] | ■ | ■ | | ■ | | ■ | |
| | *Filenchus sheri* | 2 | Root-fungal feeder [Ek] | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Geocenamus* | *Geocenamus brevidens* | 3 | Plant-parasitic [Ek] | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| *Mesocriconema* | *Mesocriconema xenoplax* | 3 | Plant-parasitic [Ek] | ■ | ■ | ■ | ■ | | ■ | ■ |
| *Helicotylenchus* | *Helicotylenchus digonicus* | 3 | Plant-parasitic [Ek] | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| | *Helicotylenchus pseudorbustus* | 3 | Plant-parasitic [Ek] | ■ | ■ | | ■ | | ■ | |
| *Psilenchus* | *Psilenchus hilarulus* | 2 | Root-fungal feeder [Ek] | ■ | ■ | | ■ | ■ | ■ | ■ |
| *Pratylenchoides* | *Pratylenchoides alkani* | 3 | Plant-parasitic [Sen] | ■ | ■ | | | | ■ | ■ |
| *Pratylenchus* | *Pratylenchus thornei* | 3 | Plant-parasitic [En] | ■ | ■ | ■ | ■ | | | ■ |
| | *Pratylenchus neglectus* | 3 | Plant-parasitic [En] | ■ | ■ | ■ | ■ | ■ | | |
| *Paratylenchus* | *Paratylenchus nainianus* | 2 | Plant-parasitic [Ek] | ■ | ■ | ■ | ■ | ■ | ■ | |
| *Rotylenchus* | *Rotylenchus cypriensis* | 3 | Plant-parasitic [Ek] | ■ | ■ | ■ | ■ | ■ | | ■ |
| *Tylenchus* | *Tylenchus davainei* | 2 | Root-fungal feeder [Ek] | ■ | | ■ | ■ | | | ■ |
| *Tylenchorhynchus* | *Tylenchorhynchus annulatus* | 3 | Plant-parasitic [Ek] | | | ■ | ■ | | ■ | |
| *Xiphinema* | *Xiphinema pachtaicum* | 5 | Plant-parasitic [Ek] | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| | *Xiphinema index* | 5 | Plant-parasitic [Ek] | | ■ | | | | | |
| *Zygotylenchus* | *Zygotylenchus guaverei* | 3 | Plant-parasitic [En] | | | ■ | ■ | ■ | ■ | ■ |

* Ek: Ectoparasite En: Endoparasite Sen: Semi-endoparasitFigure 3. Comparative graph showing the % proportion of trophic groups in each terrestrial tree.

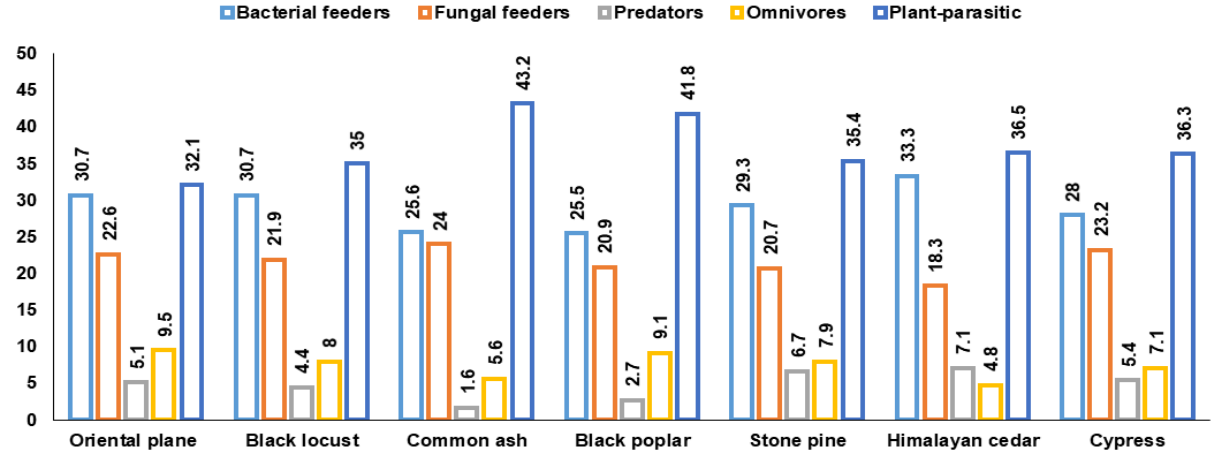


Figure 3. Comparative graph showing the proportion (%) of trophic groups in each terrestrial tree.

The identified nematodes were classified into five c-p classes (1-5) and five feeding groups. Species with c-p 2 values (22.2-29.6 %) and c-p 4 values (11.1-18.4%) were particularly prominent in the free-living nematode community across all tree species (Figure 4). The c-p 2 group primarily comprised nematodes from the families Cephalobidae and Plectidae. In contrast, the c-p 4 group included predatory and omnivore nematodes from the families Mononchidae and Dorylaimidae. Among the plant-parasitic nematode species, those with p-p 3 values (14.8-20.8%) were notably prevalent, belonging to the families Merliniidae, Pratylenchidae, and Hoplolaimidae.

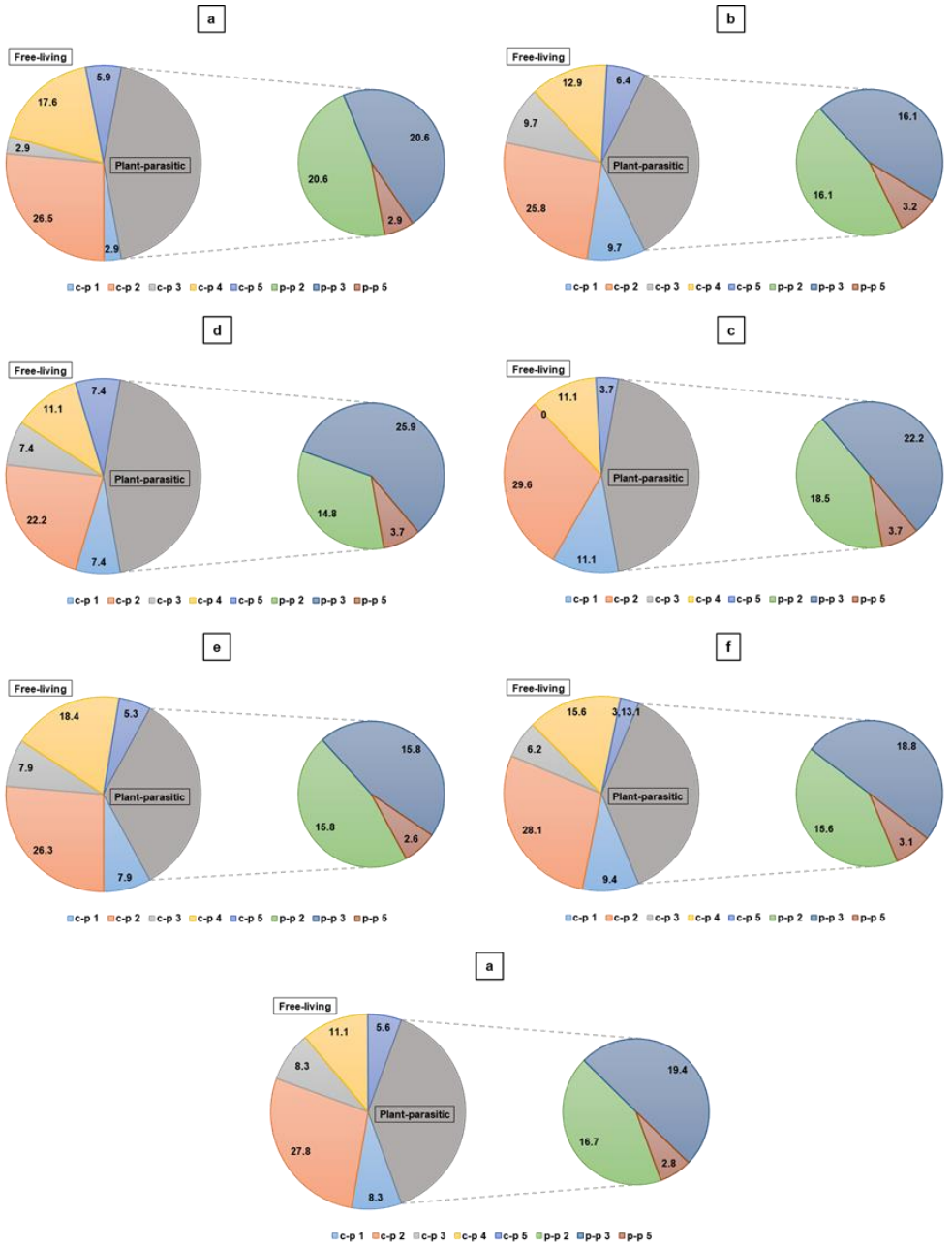


Figure 4. Comparative graph showing the proportion (%) of c-p groups in each terrestrial tree: a) Oriental plane; b) Black locust; c) Common ash; d) Black poplar; e) Stone pine; f) Himalayan cedar; g) Cypress.

Nematode genera such as *Acrobeles* von Linstow, 1877*, Alaimus* De Man, 1880*, Acrobeloides* Cobb, 1928*, Cephalobus, Aphelenchus, Aphelenchoides* Fischer, 1894*, Ditylenchus, Boleodorus* Thorne, 1941*, Helicotylenchus* Steiner*,* 1945, *Geocenamus,* and *Xiphinema* have been detected across all tree species (Figure 5). *Acrobeles ciliatus* Linstow, 1877*, Cephalobus persegnis* Bastian*,* 1865*,* and *Acrobeloides nanus* De Man, 1880 emerged as the most prevalent bacterial feeder nematodes in the study. Notably, *A. nanus* was identified as the most common species across all soil samples, with a remarkable occurrence rate of 94.2%, followed closely by *C. persignis* at 91.4% and *A. ciliatus* at 54%. These three species were consistently found in the soil of all tree species. Among fungal feeder species, *Aphelenchoides sacchari* Hooper, 1958 (80%), and *Aphelenchus avenae* Bastian, 1865 *(*96.4%) were the most frequently detected. Regarding plant-parasitic nematodes*, Geocenamus brevidens* stood out as the most abundant species, occurring in an impressive 98.2% of all soil samples. Additionally, six species *Boleodorus tylactus, Helicotylenchus digonicus* Perry, 1959*, Filenchus thornei (*Andrássy, 1954) Andrássy, 1963*, Filenchus sheri* Khan & Khan, 1978*,* and *Xiphinema pachtaicum* were consistently found across all tree species. In contrast, the *X. index* was found only in Himalayan cedar.

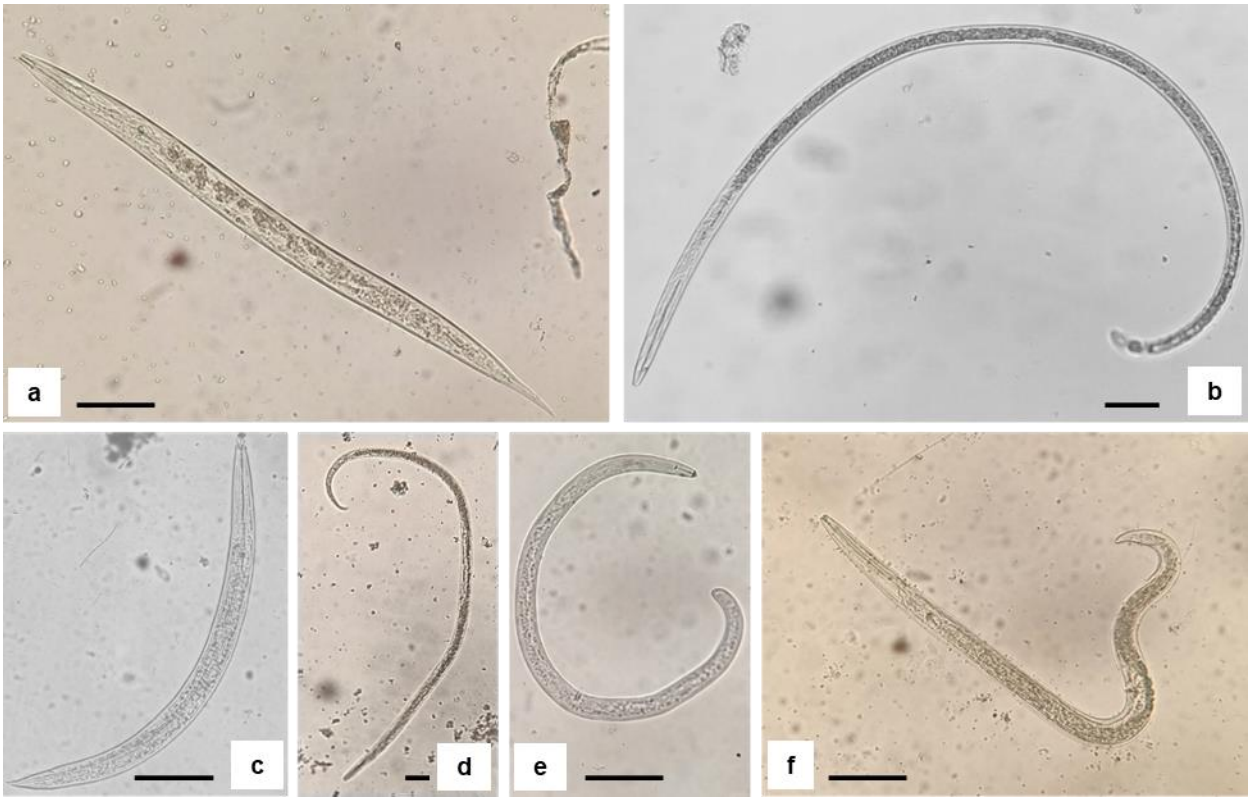


Figure 5. Nematode in this study from genera: a) *Rhabditis*; b) *Xiphinema*; c) *Acrobeles*; d) *Alaimus*; e) *Rotylenchus*; f) *Cephalobus* (a, c, e, f 20 µm; b 100 µm; d 200).

In terms of nematode density per 100 grams of soil samples, the average proportion of free-living nematodes in the total soil community ranged from 42% to 56.2%. In contrast, the proportion of plant-parasitic nematodes varied between 43.8% and 58%. Figure 6 illustrates the average nematode abundance found in 100 grams of soil, represented in a heat map generated in XLSTAT using Z scores. The dendrogram in the left axis represents terrestrial trees, and the axis at the bottom indicates identified nematode genera. A dendrogram at the top shows the clustering of these genera based on their abundance patterns. In the heat map, the colors green, red, and black convey varying levels of nematode abundance. A higher nematode populations were indicated with green color, suggesting a robust presence within the soil sample. In the heat map, the shades of red indicate that the nematode populations in the soil samples

for certain genera are low. According to heatmap some nematodes exhibit a clear preference for particular tree species. *Psilenchus* is more abundant in Cypress and Black locust, while *Xiphinema* and Helicotylenchus are more prevalent in Oriental plane. Ditylenchus shows relatively higher abundance in Common ash and Black poplar. In contrast, some nematodes are more evenly distributed. Figure 6 shows that the least number of individuals were found in the black poplar, while the most were found in the species that were present. Genera clustered in the same clade in a heat map share quite close or similar abundance patterns. Results on this map were consistent with the previous data in Table 3.

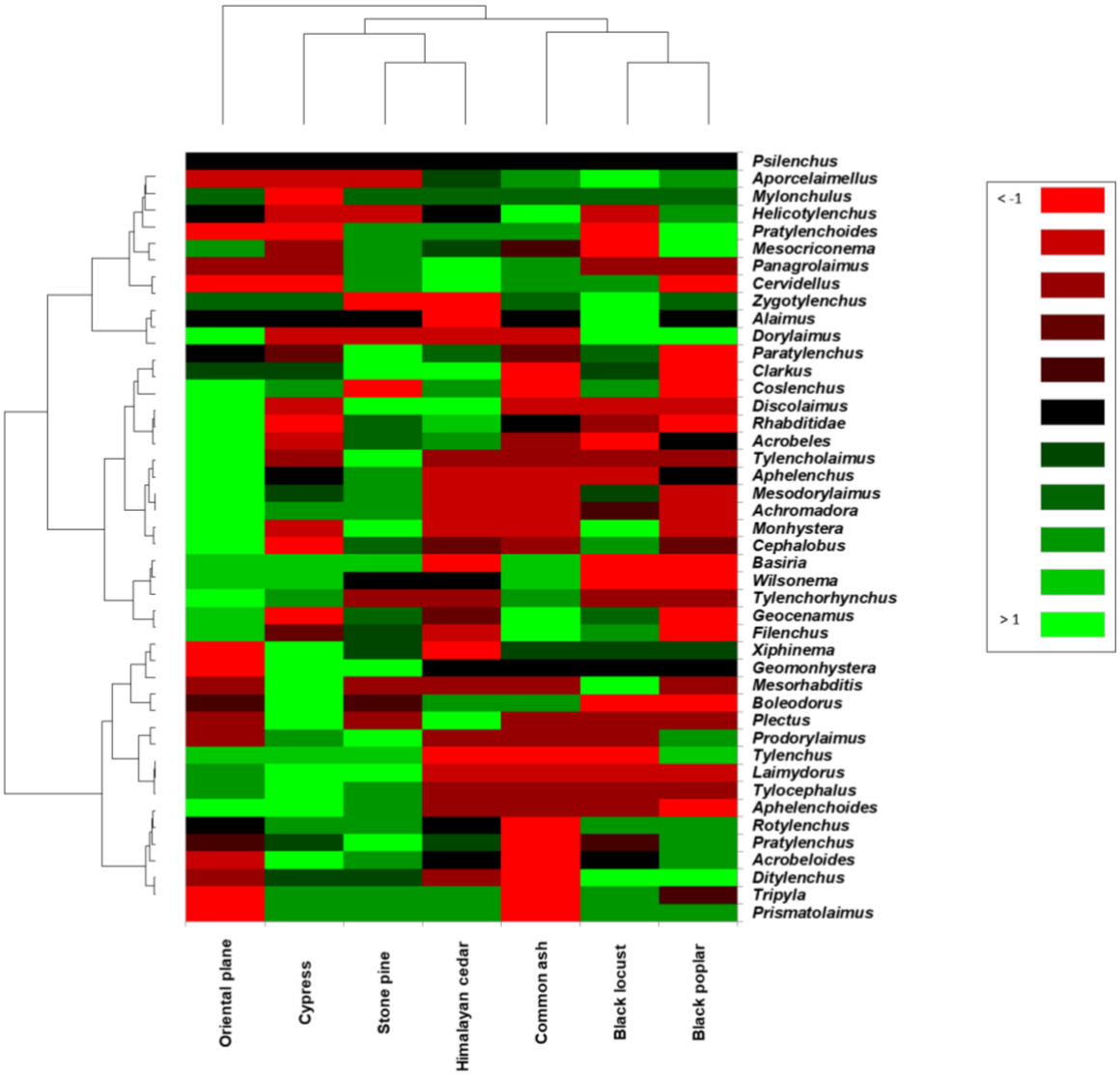


Figure 6. A heatmap illustrating the abundance of nematode genera found in sampled terrestrial trees. The names of the tree species are listed along the right axis, while the identified nematode genera are displayed along the bottom axis. A color key indicates the average nematode abundances per 100 grams of soil.

**Nematode biodiversity indices in terrestrial tree growing areas**

Table 4 summarises the biodiversity indices calculated using average nematode populations. There was significant variation in diversity, dominance, richness, and ecological indices among tree species, reflecting differences in soil biodiversity and ecosystem function.

Table 4. Average nematode diversity indices of terrestrial trees

| Diversity indices | Oriental plane | Black locust | Common ash | Black poplar | Stone pine | Himalayan cedar | Cypress |
|---|---|---|---|---|---|---|---|
| Shannon-Weiner (H') | 3.17 | 3.15 | 2.99 | 3.14 | 3.37 | 3.25 | 3.41 |
| Evenness (J') | 0.090 | 0.091 | 0.090 | 0.095 | 0.092 | 0.093 | 0.095 |
| Simpson's Diversity index (1-D) | 0.059 | 0.056 | 0.072 | 0.047 | 0.040 | 0.043 | 0.036 |
| Reciprocal Simpson index (1/D) | 16.72 | 17.58 | 14.49 | 21.25 | 24.40 | 22.74 | 25.67 |
| Maturity index (MI) | 2.45 | 2.29 | 2.24 | 2.48 | 2.41 | 2.28 | 2.33 |
| Maturity index 2-5 (MI) | 2.61 | 2.46 | 2.42 | 2.58 | 2.57 | 2.52 | 2.49 |
| Sigma maturity index (ΣMI) | 2.61 | 2.50 | 2.54 | 2.71 | 2.57 | 2.48 | 2.51 |
| Plant-parasitic index (PPI) | 2.93 | 2.88 | 2.94 | 3.02 | 2.86 | 2.85 | 2.84 |
| Berger-parker dominance index | 0.12 | 0.13 | 0.16 | 0.09 | 0.10 | 0.09 | 0.10 |
| Menhinick index | 2.64 | 2.71 | 2.47 | 2.63 | 2.93 | 2.64 | 3.17 |
| Margaleff richness index | 6.46 | 6.16 | 5.44 | 5.58 | 7.22 | 5.96 | 7.20 |
| Dominance (1-D) | 0.94 | 0.94 | 0.92 | 0.95 | 0.95 | 0.95 | 0.96 |
| Equitability index (J) | 0.86 | 0.89 | 0.87 | 0.92 | 0.91 | 0.93 | 0.92 |
| Channel index (CI) | 46.27 | 42.86 | 45.45 | 58.97 | 42.11 | 30.67 | 43.53 |
| Basal index (BI) | 25.57 | 28.78 | 30.00 | 27.97 | 25.95 | 25.03 | 28.03 |
| Enrichment index (EI) | 53.17 | 54.69 | 56.41 | 47.56 | 53.90 | 60.48 | 54.84 |
| Structure index (SI) | 63.97 | 55.89 | 50.96 | 62.53 | 62.75 | 59.42 | 57.51 |
| Bacterivore footprint | 14.35 | 13.47 | 14.30 | 10.80 | 20.06 | 20.40 | 22.92 |
| Fungivore footprint | 2.53 | 2.46 | 2.12 | 2.13 | 2.90 | 1.87 | 3.15 |
| Omnivore footprint | 33.25 | 23.80 | 23.32 | 31.24 | 25.30 | 21.72 | 27.06 |
| Predator footprint | 4.84 | 4.06 | 0.91 | 1.96 | 7.09 | 6.84 | 4.26 |
| Herbivore footprint | 10.17 | 11.01 | 11.68 | 11.05 | 12.38 | 9.45 | 11.70 |

The Shannon-Weiner index (H'), which measures species diversity, was highest for cypress (3.41) and stone pine (3.37), demonstrating that these trees supported the most diverse communities. In contrast, common ash (2.99) had the lowest diversity, suggesting a more limited range of species. Evenness (J'), which assesses how evenly species were distributed, was highest in black poplar and cypress (0.095), showing a more balanced species composition. In contrast, oriental plane and common ash (0.090) had slightly lower evenness, meaning that some species were more dominant than others.

Simpson's Diversity Index (1-D), which quantifies dominance, showed that cypress (0.036) and stone pine (0.040) had the lowest dominance values, meaning that no single species prevailed in these communities. Conversely, common ash (0.072) had the highest dominance, signifying that a few species were more abundant. The Reciprocal Simpson Index (1/D) followed the same trend, with cypress (25.67) and stone pine (24.40) having the highest values, revealing diverse communities, while common ash (14.49) was the least diverse.

Maturity indices (MI, MI 2-5, and ΣMI) provided insights into ecosystem stability. Black poplar (2.48) had the highest maturity index, emphasizing a more stable environment, whereas common ash (2.24) had the lowest, reflecting a younger or more disturbed ecosystem. The Plant-Parasitic Index (PPI) was highest in black poplar (3.02), confirming that plant-parasitic nematodes were more prevalent in its soil ecosystem, while cypress (2.84) had the lowest PPI, suggesting fewer plant-parasitic nematodes.

The Berger-Parker Dominance Index, which measures the dominance of the most abundant species, was highest for common ash (0.16), implying that its soil community was strongly influenced by a few species. In contrast, black poplar (0.09) had the lowest dominance, reflecting a more even species distribution. Species richness indices, such as the Menhinick and Margaleff indices, measure the number of species present. Cypress exhibited the highest richness values (Menhinick: 3.17, Margaleff: 7.20), closely followed by stone pine (Margaleff: 7.22), revealing that these trees hosted the most species. In contrast, common ash had the lowest richness (Menhinick: 2.47, Margaleff: 5.44), showing fewer species in its soil environment. The Equitability Index (J'), which evaluates species distribution fairness, was highest in Himalayan cedar (0.93) and black poplar (0.92), representing a more even species distribution. Oriental plane (0.86) had the lowest value, confirming that certain species were more dominant.

Ecological indices provided further insights into ecosystem functioning. The Channel Index (CI) was highest in black poplar (58.97), illustrating a well-structured food web, while Himalayan cedar (30.67) had the lowest, implying a less developed trophic structure. The Enrichment Index (EI), which measures nutrient cycling efficiency, was highest in Himalayan cedar (60.48), revealing a well-functioning microbial and nematode community, and lowest in black poplar (47.56). The Structure Index (SI), which reflects ecosystem complexity and resilience, was highest in oriental plane (63.97), highlighting a strong ecological framework, whereas common ash (50.96) had the lowest, suggesting a less stable community.

Trophic footprints, representing nematode community composition, varied significantly among trees. Cypress had the highest bacterivore footprint (22.92), proving high microbial activity in the soil, while black poplar had the lowest (10.80). The fungivore footprint was highest in cypress (3.15), showing strong fungal activity, and lowest in Himalayan cedar (1.87). The omnivore footprint, representing generalist feeders, was greatest in oriental plane (33.25) and lowest in Himalayan cedar (21.72). The predator footprint, indicating predatory nematode abundance, was highest in stone pine (7.09) and lowest in common ash (0.91), signifying limited predator presence in ash soils. Lastly, the herbivore footprint, representing plant-feeding nematodes, was highest in stone pine (12.38) and lowest in Himalayan cedar (9.45), confirming different levels of plant-nematode interactions.

From these results, cypress and stone pine emerged as the most diverse and ecologically stable tree species, with high species richness and low dominance, fostering a balanced and resilient soil ecosystem.

## Discussions

At the end of the study, 44 nematode genera were identified, indicating rich biodiversity in Tekirdağ terrestrial forests. The presence of nematodes from different functional groups, including fungal feeders, bacterial feeders, predators and omnivores, indicates a complex food web. The differences in the number of genera associated with different tree species (e.g., 38 in pine vs. 27 in ash) may be due to factors such as plant root structure and nutrient availability, as well as soil conditions, soil moisture, and soil microorganisms. Research by Bongers & Ferris (1999) highlighted how different plant species can influence soil nematode communities. Their findings support the observation that pine maintains the most diverse nematode fauna with 38 genera, suggesting that tree species with specific root structures or nutrient profiles can significantly influence nematode diversity. Similarly, several published researches demonstrated that specific tree species can support different nematode communities, resembling similarity with the findings from Tekirdağ. In a study conducted in Greece, several nematode genera, including *Bursaphelenchus xylophilus, Clarkus,* and *Tylencholaimus,* were found in pine areas (Karmezi et al., 2022). In Bulgaria, 48 plant-parasitic nematode species were identified in the same tree species, with *Geocenamus brevidens, Helicotylenchus digonicus,* and *Geocenamus brevidens* being commonly found, similar to the results in Tekirdağ (Peneva & Choleva, 1994). In Brazil, 35 nematode genera from 5 trophic groups have been identified in forest areas. Similar to the fauna in Tekirdağ, genera such as Aphelenchoides and Rhabditis were found to be the most common and abundant. In another research in Slovakia, 51 nematode genera

have been identified in forest areas consisting of various tree species, including *Fraxinus excelsior*. When nematodes were identified, it was observed that the number of free-living bacterivorous nematodes was greater (Cerevkova et al., 2021).,

The different nematode assemblages found in different tree species likely reflect differences in root exudates and soil nutrient profiles, which may influence nematode diversity and abundance. The lower number of nematode genera in some trees and locations in Tekirdağ may be due to predatory genera, such as Mylonchulus, that play a functional role in the suppression of the populations of other nematodes, including potential plant parasites. For instance, predators *Mylonchulus, Clarkus, Tripyla, Discolaimus,* and omnivores such as *Aporcelaimellus,* and *Dorylaimus* may feed on nematode genera *Aphelenchus, Aphelenchoides, Helicotylenchus, Plectus, Cephalobus, Acrobeloides, Rotylenchus,* and *Tylenchorrhynchus (*Small, 1987).

The higher prevalence of Tylenchida in all tree species revealed its importance in the province. Its significant values (ranging from 34.2 to 44.1) indicate its adaptability and ability to survive in various soil and plant conditions in Tekirdağ. The dominance of Tylenchida in the Tekirdağ study was consistent with the findings of Yeates, 1979, who noted that Tylenchida was common in various terrestrial ecosystems. Tylenchid species, such as *Mesocriconema xenoplax, Pratylenchus neglectus*, and *Pratylenchus thornei* are significant threats to plants due to their ability to cause substantial damage. These nematodes feed on roots, and cause deformation and lesions, leading to stunted growth and impaired nutrient uptake, which can severely affect plant health and agricultural productivity (Thompson & Clewett, 2021). Their presence in the soil not only disrupts root function but also makes plants more vulnerable to environmental stresses and diseases, ultimately resulting in reduced crop yields. Effective management strategies are essential to mitigate their impact and protect agricultural outputs.

The identification of 15 genera of bacterial feeders, four genera of fungal feeders, five genera of omnivores, and five genera of predators illustrates a well-structured nematode community in survey areas in Tekirdağ. Moreover, the bacterivore footprint is highest in Cypress (22.92), while the fungivore footprint is notably higher in Cypress (3.15). Predator footprints are significant in Stone Pine (7.09), reflecting a robust predator presence. Achieving species-level identification for 10 female species within the bacterial feeders emphasizes the richness and ecological significance of this group. Bacterial feeders play a crucial role in decomposing organic matter and regulating bacterial populations, thereby enhancing nutrient availability for plants. (Bardgett & van der Putten, 2014). Research has shown that the presence of diverse nematode communities is linked to improved soil structure and fertility, which is essential for sustainable agriculture (Pires et al., 2023). Their diversity is high in a healthy soil environment that can support a wide range of microbial activity. The presence of four genera of fungal feeders highlights their importance in the decomposition of organic materials, particularly in breaking down fungal biomass (Zhang et al., 2020). The identification of five genera of omnivores and five genera of predators indicates a biologically more community structure and soil food web (Ferris et al., 2012; Pires et al., 2023). Omnivorous nematodes can feed on both bacteria and other nematodes, facilitating energy transfer within the food web. Predators, on the other hand, play a critical role in controlling populations of bacterial and fungal feeders, helping to maintain balance within the ecosystem (Khan & Kim, 2007).

Seventeen diversity indices were calculated for each terrestrial tree. The higher Shannon-Weiner index values in Cypress (3.41) and Stone Pine (3.37) is the sign of a richer and more diverse nematode community (Krebs, 1985). This diversity can enhance ecosystem resilience, as varied species fulfill different ecological roles. Conversely, in Tekirdağ Common Ash (2.99) exhibits a lower Shannon-Weiner index. The Evenness values reveal a more balanced distribution of nematode species in Cypress and Black Poplar, which is critical for maintaining ecosystem stability. In a higher Evenness, no single species dominates the community (Pielou, 1966). The low Simpson's index values in Cypress and Stone Pine indicate a diverse community with lower dominance by any single species. This is advantageous for ecosystem functioning,

as it allows for a more balanced interaction among species. In contrast, the higher dominance observed in Black Locust could imply that a few species may dominate (Díaz et al., 2007). All calculated indices highlight the ecological roles and interactions of nematodes associated with different tree species. The indices data in Tekirdağ suggest that trees like Cypress and Stone Pine support more diverse and balanced nematode communities, which contribute positively to ecosystem resilience. In contrast, species like Common Ash and Black Locust may face challenges due to lower diversity and higher dominance, respectively. Understanding these dynamics is crucial for managing forest ecosystems and enhancing soil health.

## References


Abolafia, J. & R. Peña-Santiago, 1996. Nematodes of the order Dorylaimida from Andalucía Oriental, Spain. The genus Mesodorylaimus Andrássy, 1959. I. Two short-tailed new species. Russian Journal of Nematology, 4 (2): 173-180.

Akbulut, S., Elekçioğlu, İ. H. & A. Keten, 2008. First Record of *Bursaphelenchus vallesianus* Braasch, Schönfeld, Polomski, and Burgermeister in Turkey, Turkish Journal of Agriculture and Forestry, 32 (4): 5 (273-279).

Akbulut, S., P. Vieira, A. Ryss, B. Yuksel, A. Keten, M. Mota & V. Valadas, 2006, Preliminary survey of the pinewood nematode in Turkey. EPPO Bulletin, 36: 538-542.

Anderson, R. V., 1979. A supplemental key to species of *Helicotylenchus* Steiner, 1945 (Nematoda: Hoplolaimidae) described since 1972 and a description of *H. oscephalus* n.sp.. Canadian Journal of Zoology, 57 (2): 337-342.

Anonymous, 1993. More than wood. Special options on multiple use of forests. FAO report. (Web page: https://www.fao.org/sustainable-forest-management/toolbox/tools/tool-detail/en/c/224700/) (Date accessed: October, 2024).

Anonymous, 2020. The state of World's forest. FAO Report. (Web page: https://www.fao.org/state-of-forests/en/) (Date accessed: October, 2024).

Anonymous, 2022. Ormanlarımızda yayılış gösteren asli ağaç türleri. (Web page: https://www.ogm.gov.tr/tr/e-kutuphane-sitesi/Yayinlar/Asli%20A%C4%9Fa%C3%A7%20T%C3%BCrleri.pdf) (Date accessed: October, 2024) (in Turkish).

Anonymous, 2023. Tekirdağ İl Tarım Orman Müdürlüğü, Tarım Raporu. (Web page: https://tekirdag.tarimorman.gov.tr/Belgeler/TarimRaporlari/2023%20TARIM%20RAPORU.pdf) (Date accessed: October, 2024) (in Turkish).

Anonymous, 2024a. Dichotomous key to common trees of the Pacific Northwest. (Web page: https://treespnw.forestry.oregonstate.edu/) (Date accessed: May 2024).

Anonymous, 2024b. Key to selected conifer species on campus. (Web page: https://trees.stanford.edu/PDF/ParConiferKey.pdf) (Date accessed: May 2024).

Atha, D. & B. Boom, 2017. Field Guide to the Ash Trees of Northeastern United States. Center for Conservation Strategy, The New York Botanical Garden, Bronx, NY, 26 pp.

Atighi, M. R., E. Pourjam, M. Pedram, C. Cantalapiedra-Navarrete, E. J. Palomares-Rius & P. Castillo, 2011. Molecular and morphological characterisations of two new species of *Rotylenchus* (Nematoda: Hoplolaimidae) from Iran. Nematology, 13 (8): 951-964.

Bardgett, R. D. & W. H. van der Putten, 2014. Belowground biodiversity and ecosystem functioning. Nature, 515 (7528): 505-511.

Bongers, R., 1990. The maturity index: An ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83: 14-19.

Bongers, T. & H. Ferris, 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology and Evolution, 14 (6): 224-228.

Brzeski, M. W., 1991. Review of the genus *Ditylenchus* Filipjev, 1936 (Nematoda: Anguinidae). Revue Nematology, 14 (1): 9-59.

Cerevková, A., M. Renco, D. Miklisová & E. Gömöryová, 2021. Soil Nematode communities in managed and natural temperate forest. Diversity, 13 (7): 327 (1-13).

Choudhary, M. & S. M. Jairajpuri, 1984. Six new species of the genus *Alaimus* de Man, 1880 (Nernatoda : Alaimidae) frorn India. Revue Nématologie, 7 (2): 287-300.

Çakmak, T., Ç. Gözel, M. B. Kaydan & U. Gözel, 2019. First record of three plant parasitic nematode species from Mount Ararat (Ağrı) in Turkey. Turkish Journal of Entomology, 43 (2): 113-130.

Çakmak, T., Ç. Gözel, U. Gözel, T. D. Achiri & M. B. Kaydan, 2021. Biodiversity and distribution of soil nematodes in Mount Ararat, Turkey. Russian Journal of Nematology, 29 **(**1): 31-48.

Decraemer, W. & D. Hunt, 2006. "Structure and Classification, 3-32". In: Plant Nematology (Eds. R. N. Perry & M. Moens). CABI: Cambridge, MA, USA, 447 pp.

Díaz, S., S. Lavorel, F. de Bello, F. Quétier, K. Grigulis & T. M. Robson, 2007. Incorporating plant functional diversity effects in ecosystem service assessments. Proceedings of the National Academy of Sciences of the United States of America, 104 (52): 20684-20689.

Ferris, H., L. E. Pocasangre, E. Serrano, J. Muñoz, S. Garcia, G. Perichi & G. Martinez, 2012. Diversity and complexity complement apparent competition: Nematode assemblages in banana plantations. Acta Oecologia, 40: 11-18.

Geraert, E. & D. J. Raski, 1987. A reappraisal of Tylenchina (Nemata). Revue Nematologia, 10 (2): 143-161.

Handoo, Z. A., A. Khan & S. Islam, 2007. A key and diagnostic compendium to the species of the genus *Merlinius* Siddiqi, 1970 (Nematoda: Tylenchida) with description of *Merlinius khuzdarensis* n. sp. associated with date palm. Nematology, 9 (2): 251-260.

Háněl, L. & A. Čerevková, 2010. Species and genera of soil nematodes in forest ecosystems of the Vihorlat Protected Landscape Area, Slovakia. Helminthologia, 47 (2010): 123-135.

Jenkins, W. R., 1964. Rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disese Reports, 48 (9): 692.

Karmezi, M., A. Bataka, D. Papachristos & D. N. Avtzis, 2022. Nematodes in the pine forests of Northern and Central Greece. Insects, 13 (2):194 (1-15).

Khan, Z. & H. Y. Kim, 2007. A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes. Applied Soil Ecology, 35 (2): 370-379.

Krebs, C. J., 1985. Ecology: The Experimental Analysis of Distribution and Abundance, Third Edition. Harper & Row Publisher, New York, 800 pp.

Lange, D. J., 2014. *Pinus* L. (Pinaceae): Vegetative Key to Species in Cultivation. Ghent University Botanical Garden 14 pp.

Lavin, M., D. Isely & E. McClintock, 2012. *Robinia pseudoacacia*, in Jepson Flora Project (Eds.) Jepson eFlora. (Web page: https://ucjeps.berkeley.edu/eflora/eflora_display.php?tid=41521) (Date accessed: May 2024).

Lawton, J. H., D. E. Bignell, B. Bolton, G. F. Bloemers, P. Eggleton, P. M. Hammond, M. Hodda, R. D. Holt, T. B. Larsen, N. A. Mawdsley, N. E. Stork, D. S. Srivastava & A. D. Wyatt, 1998. Biodiversity inventories, indicator taxa, and effects of habitat modification in tropical forest. Nature, 391 (6662): 72-76.

Loof, P. A. A. & M. Luc, 1990. A revised polytomous key for the identification of species of the genus *Xiphinema* Cobb,1913 (Nematoda: Longidoridae) with exclusion of the *X. americanum*-group. Systematic Parasitology, 16 (1): 35-66.

Loof, P. A. A. & M. S. Jairajpuri, 1968. Taxonomic studies on the genus *Tylencholaimus* De Man, 1876 (Dorylaimoidea) with a key to the species. Nematologica, 14 (3): 317-350.

Neher, D. A. & B. J. Darby, 2009. "General Community İndices that can be Used for Analysis of Nematode Assemblages, 107-123". In: Nematodes as Environmental Indicators (Eds. M. Wilson & T. Kakouli-Duarte) CABI, Wallingford, UK, 338 pp.

Öztürk, L. & G. G. Avcı, 2023. A survey in sunflower fields in Tekirdağ (Türkiye) to determine soil health with nematode-based diversity indices. Turkish Journal of Entomology, 47 (4): 401-414.

Öztürk, L., T. Behmand, A. Öcal, G. G. Avcı & İ. H. Elekcioğlu, 2023. New data on plant hosts of Longidoridae and Trichodoridae nematodes in Türkiye. Plant Protection Bulletin, 63 (3): 5-16.

Öztürk, N., S. Akbulut & I. Baysal, 2019. Determination of pathogenicity of *Bursaphelenchus* species on different pine species under natural conditions in Düzce. Phytoparasitica, 47 (1): 89-97.

Peneva, V. & B. Choleva, 1994. Plant-parasitic nematodes associated with pine trees in Bulgaria. Eppo Bulletin, 24 (2): 459-466.

Pielou, E. C., 1966. The measurement of diversity in different types of biological collections. Journal of Theoretical Biology, 13: 131-144.

Pires, D., V. Orlando, L. R. Collett, D. Moreira, R. S. Costa & M. L. Inácio, 2023. Linking nematode communities and soil health under climate change. Sustainability, 15 (15): 1-23.

Scholze, V. S. & W. Sudhaus, 2011. A pictorial key to current genus groups of “Rhabditidae”. Journal of Nematode Morphology and Systematics, 14 (2): 105-112.

Schulz, C., P. Knopf & T. Stützel, 2005, Identification key to the Cypress family (Cupressaceae). Feddes Repert, 116 (1-2): 96-146.

Seinhorst, J. W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica, 4 (1): 67-69.

Sieriebriennikov, B., H. Ferris & R. G. M. de Goede, 2014. NINJA: An automated calculation system for nematode-based biological monitoring. European Journal of Soil Biology, 61: 90-93.

Skwiercz, A., 2012. Nematodes ( Nematoda ) in Polish forests. I. Species inhabiting soils in nurseries. Journal of Plant Protection Research, 52 (1): 169-179.

Small, R. W., 1987. A review of the prey of predatory soil nematodes. Pedobiologia, 30 (3): 179-206.

Stanturf, J. & S. Mansourian, 2020. Forest landscape restoration: state of play. Royal Society Open Science, 7 (12): 201218 (1-47).

Taşdemir, S., S. Akbulut, N. Kanzaki & N. Öztürk, 2020. Preliminary survey of nematodes associated with broadleaved trees in İzmit Forest Management Directorate, Turkey. Forest Pathology, 50 (6): e12642 (1-9).

Thompson, J. P. & T. G. Clewett, 2021. Impacts of Root-lesion nematode (*Pratylenchus thornei*) on plant nutrition, biomass, grain yield and yield components of susceptible/ıntolerant wheat cultivars determined by nematicide applications. Agronomy, 11 (2): 296 (1-25).

Tran, van D., van L. Vu, H. T. Nguyen & Q. P. Trinh, 2024. An updated species list of the genus *Rotylenchus* (Nematoda: Hoplolaimidae) and a browser-based interactive key for species identification. Australasian Plant Pathology, 53 (1): 79-88.

Wu, Wen-Jia, L. Yan, C. Xu, K. Wang, Y. S. Jin & J. Xie, 2016. A new species of the genus *Discolaimus* Cobb, 1913 (Nematoda: Dorylaimida: Qudsianematidae) from Qinghai, China. Zootaxa, 4088 (1): 129-138.

Yadav, S., J. Patil & R. S. Kanwar, 2018. The role of free living nematode population in the organic matter recycling. International Journal of Current Microbiology and Applied Sciences, 7 (6): 2726-2734.

Yeates, G. W., 1979. Soil nematodes in terrestrial ecosystems. Journal of Nematology, 11 (3): 213-229.

Zhang, Y., S. Li, H. Li, R. Wang, Q. K. Zhang & J. Xu, 2020. Fungi-Nematode Interactions: Diversity, Ecology, and Biocontrol Prospects in Agriculture. Journal of fungi (Basel, Switzerland), 6 (4): 206 (1-24).

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