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Occurrence of *Fusarium* spp. in *Humulus lupulus* L. and Cultivated Soils of Pazaryeri/Bilecik, Türkiye

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Abstract
Purpose: This study aimed to investigate the occurrence, diversity, distribution, and seasonal dynamics of *Fusarium* species associated with *Humulus lupulus L.*) (hop) plants and hop-cultivated soils in Pazaryeri (Bilecik, Türkiye), the only commercial hop-growing region in the country. Morphological and multilocus molecular identification methods were employed to characterize the isolates and to evaluate biodiversity patterns in relation to environmental conditions.
Method: Soil and hop samples were collected from production fields in Pazaryeri during September 2022 and August 2023. *Fusarium* isolates were recovered using selective media (SNA and SFA supplemented with Rose Bengal). Species identification was based on morphological characteristics and multilocus sequence analyses of the ITS and TEF1 gene regions. Phylogenetic analyses were conducted using MEGA 11.0, while community structure and diversity were assessed using Bray–Curtis similarity, Shannon diversity, and Simpson diversity indices.
Findings: A total of 98 *Fusarium* isolates were obtained, including 71 from soil samples and 27 from hop plant tissues. Morphological and multilocus molecular analyses identified eight species: *Fusarium oxysporum, F. venenatum, Neocosmospora solani (*synonym *F. solani), F. acuminatum, F. equiseti, F. camptoceras, F. coeruleum,* and *F. redolens. F. oxysporum* was the most prevalent species (25.51%), followed by *F. venenatum* (17.35%) and *N. solani* (15.31%). Biodiversity analyses revealed high species diversity within the study area and demonstrated that seasonal variation significantly influenced species dominance and distribution patterns. Furthermore*, F. venenatum* and *F. camptoceras* were recorded for the first time in Türkiye.
Conclusion: This study provides the first comprehensive assessment of *Fusarium* communities associated with hop cultivation in Türkiye. The findings indicate that hop-cultivated soils serve as important reservoirs for diverse *Fusarium* species, which can subsequently colonize plant tissues during growth. Environmental factors, particularly seasonal conditions and wind patterns, influence the distribution and dispersal of these fungi. The detection of two previously unreported species highlights the ecological significance of the region and contributes valuable information to the Turkish mycobiota. These results provide a foundation for future disease management and monitoring programs in hop production systems.

Keywords: *Humulus lupulus, Fusarium sp.,* Pazaryeri, biodiversity

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Pazaryeri/Bilecik (Türkiye) Bölgesinde Humulus lupulus L. ve Kültür Topraklarında Fusarium Türlerinin Varlığı

Özet
Amaç: Bu çalışmanın amacı, Türkiye'nin tek ticari şerbetçiotu yetiştirme bölgesi olan Pazaryeri (Bilecik)'nde, şerbetçiotu *(Humulus lupulus L.*) (hop) bitkileri ve yetiştirme topraklarında bulunan *Fusarium* türlerinin çeşitliliğini, yaygınlığını, dağılımını ve mevsimsel değişimlerini belirlemektir. Ayrıca, elde edilen izolatların morfolojik ve moleküler yöntemlerle karakterizasyonu yapılarak çevresel faktörlerle ilişkili biyoçeşitlilik desenleri değerlendirilmiştir.
Metod: Toprak ve şerbetçiotu örnekleri Eylül 2022 ve Ağustos 2023 dönemlerinde Pazaryeri'ndeki üretim alanlarından toplanmıştır. *Fusarium* izolatları seçici besiyerleri (SNA ve SFA + Rose Bengal) kullanılarak elde edilmiştir. Tür

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teşhisleri morfolojik özellikler ile ITS ve TEF1 gen bölgelerine ait çoklu lokus dizileme analizlerine dayanılarak gerçekleştirilmiştir. Filogenetik analizler MEGA 11.0 programı kullanılarak yapılmış, topluluk yapısı ve tür çeşitliliği Bray-Curtis benzerlik indeksi ile Shannon ve Simpson çeşitlilik indeksleri kullanılarak değerlendirilmiştir.
Bulgular: Çalışmada toplam 98 *Fusarium* izolatı elde edilmiş olup bunların 71'i toprak, 27'si ise bitki örneklerinden izole edilmiştir. Morfolojik ve moleküler analizler sonucunda izolatların sekiz türe ait olduğu belirlenmiştir: *Fusarium oxysporum, F. venenatum, Neocosmospora solani (*Eski adı: *F. solani), F. acuminatum, F. equiseti, F. camptoceras, F. coeruleum* ve *F. redolens*. En yaygın tür *F. oxysporum* (%25,51) olup bunu *F. venenatum* (%17,35) ve *F. solani* (%15,31) izlemiştir. Biyoçeşitlilik analizleri çalışma alanında yüksek tür çeşitliliği bulunduğunu ve mevsimsel değişimlerin tür baskınlığı ile dağılımını etkilediğini göstermiştir. Ayrıca *F. venenatum* ve *F. camptoceras* türleri Türkiye'de ilk kez kaydedilmiştir.
Sonuç: Bulgular, şerbetçiotu yetiştirilen toprakların çeşitli *Fusarium* türleri için önemli bir rezervuar olduğunu ve bu türlerin bitki gelişimi sırasında bitki dokularına geçebildiğini göstermektedir. Mevsimsel koşullar ve rüzgâr gibi çevresel faktörler türlerin yayılımında etkili olmuştur. Türkiye için ilk kez bildirilen türlerin tespiti, bölgenin mikrobiyal çeşitliliğinin önemini ortaya koymakta ve gelecekteki hastalık yönetimi ile izleme çalışmalarına temel oluşturmaktadır.

Anahtar kelimeler: *Humulus lupulus, Fusarium sp.,* Pazaryeri, biyoçeşitlilik

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1. Introduction

Humulus lupulus L. (hop) is a perennial climbing plant of medicinal and aromatic importance from the *Cannabinaceae* family, widely cultivated for brewing and pharmaceutical uses. Although hop production worldwide dates to ancient times, it began in Turkey in 1930 during the Second World War. Following various research and production efforts, cultivation was restricted after 1965 to a single area of 3.26 km² in Pazaryeri/Bilecik, which remains the most productive hop-growing region in the country. Today, hop are cultivated only in this region. With increasing annual beer consumption, domestic production has become insufficient, and approximately 60% of the hop required are imported [1]. Previous studies have shown that hop diseases are predominantly of fungal origin, supporting the selection of this region as the study area.

The genus *Fusarium*, derived from the Latin “fusus” (referring to conidia with transverse compartments) [2], is widely distributed in organic matter, air, water, and soil. Species of this genus produce toxins of major concern due to their broad host range and ecological adaptability [3]. In 1993, the International Agency for Research on Cancer (IARC, WHO) classified mycotoxins according to their carcinogenic potential. Among those produced by Fusarium spp., fumonisins were categorized as “probable carcinogenic mycotoxins” (Group 2B) [4].

Crop losses attributed to Fusarium are well documented, particularly in cereals, where abiotic and biotic factors, as well as agronomic practices, contribute to disease incidence. *Fusarium oxysporum*, a soil-borne pathogen, is the most common species worldwide and causes diseases in many economically important crops. Legumes such as chickpea (Cicer arietinum), broad bean (Phaseolus vulgaris), pea (Pisum sativum), lentil (Lens culinaris), and alfalfa (Medicago sativa) are among the most affected [5].

Biotic factors, especially fungal pathogens, are major contributors to yield losses in agriculture. Members of the genus *Fusarium* are considered important plant pathogens due to their ability to survive saprophytically on diverse host plants and crop residues under varying environmental conditions. Their capacity to infect root, collar, and spike tissues under favorable conditions makes them globally significant, with Fusarium wilt recognized as one of the most destructive diseases.

The aim of this study was to determine the presence, distribution, and frequency of *Fusarium* spp. in hop plants and cultivated soils of Pazaryeri/Bilecik, Turkey. This represents the first investigation of the genus in hop-growing areas of the country.

2. Material and Method

2.1. Sampling Area

The sampling area is the Pazaryeri district of Bilecik Province, Türkiye, encompassing the agricultural fields where *Humulus lupulus* (hop) is cultivated. Pazaryeri, located in the eastern part of the Marmara Region (Figure 1), covers a surface area of 396 km². Approximately 55% of this area is forested, while the remainder consists of productive agricultural lands [5]. The most striking feature of the study area is that hops are grown exclusively in this region in Türkiye [1, 5]. A map and location details of the sampling sites are provided in Figure 1.

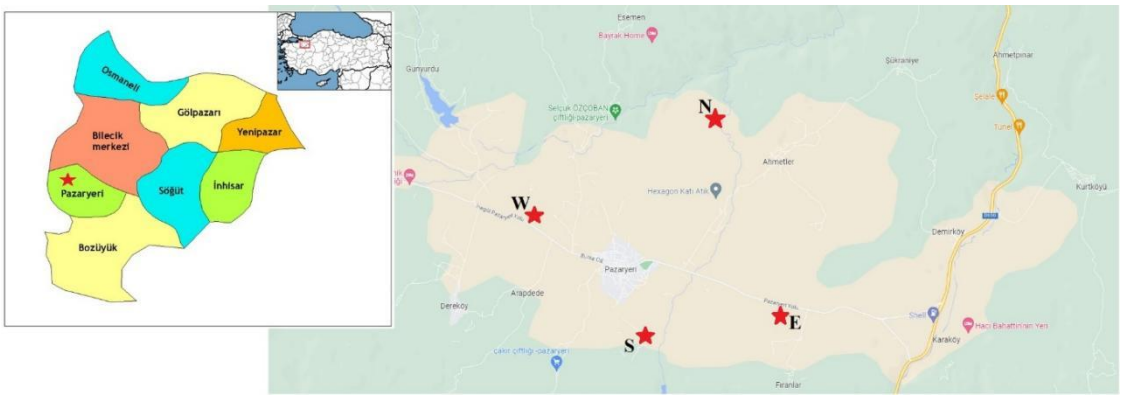

Figure 1. Map of Pazaryeri province, sampling area and locations; N: North DD: 40.018792 29.912277, S: South DD: 39.982318 29.912812, E: East DD: 39.985724 29.933285, W: West DD: 40.007351 29.873321

2.2. Sampling

In our study, hop plants grown in the Pazaryeri and the agricultural lands where they were grown were examined. Soil samples were collected according to Brown's (1958) technique in in September 2022, when the plant was not yet active, and in August 2023, when the plant started to bear fruit. Sampling was conducted from four locations, with one sample taken from each location in two different seasons, resulting in a total of eight soil samples analyzed in this study. Importantly, sampling was carried out from the same designated locations during both periods to ensure consistency and comparability of results. Soil samples (total of 8) were collected with a sterilized trowel from 5 profiles in 0–10 cm depth within 50 m away from each other. Samples were transported in sterile polyethylene bags and stored at +4 °C until analysed. Plant samples were collected at the same time and from the same fields, using random selection among both visibly infected and apparently healthy plants. This approach ensured representation of different plant conditions, although the emphasis on symptomatic material is acknowledged as a limitation of the sampling strategy. These samples were commonly obtained from the leaf and stem parts of the plant. In addition, healthy-looking plant leaves and stems were sampled in relevant locations and time periods and used for fungal biodiversity control in the study. The samples taken were placed in sterile polyethylene bags and brought to the laboratory environment. The samples were preserved at +4 °C.

2.3. Isolation of Microfungi

For the isolation of microfungi from soil samples, the soil dilution plate method was used [2]. Briefly, the percentage moisture content of the soil was first determined using the formula: Moisture % = (c – g) / g, where "c" is the weight of wet soil and "g" is the weight of soil dried at 105 °C for 24 h. Based on the moisture content calculation, the wet weight corresponding to 10 g dry weight of each soil sample was diluted (1:10) in sterile physiological saline solution (0.85%) and homogenized by shaking at room temperature for approximately 30 minutes.

Serial dilutions were then prepared up to 1/10,000,000. For each dilution, Spezieller Nährstoffarmer Agar (SNA) and Selective Fusarium Agar (SFA) supplemented with Rose Bengal [3] were used for selective isolation and determination of colony-forming units (CFU) [6]. Plates were incubated at 25 °C for 14 days. After incubation, colonies with characteristic features of the genus Fusarium were counted and calculated as CFU/g dry soil. These colonies were subsequently subcultured on Potato Dextrose Agar (PDA) medium.

Plant samples were cut into small pieces, washed three times in sterile distilled water for 15 minutes, and then placed on the same media used for isolation (SNA and SFA + Rose Bengal). After incubation at 25 °C for 14 days, colonies exhibiting the characteristic features of the genus Fusarium were subcultured on PDA medium [3].

2.4. Morphological and Multi-locus Gene Identification of Microfungi

Taxonomic identification of fungal isolates was based on their cultural characteristics and morphological structures. Isolates were inoculated at three points on Potato Dextrose Agar (PDA), Oatmeal Agar (OA), Carnation Leaf Agar (CLA), and Malt Extract Agar (MEA), and incubated at 25 °C for 7 days.

Colony properties were first examined under a stereomicroscope (Olympus SF20) and then under a high-resolution light microscope (Olympus BX51) to determine the morphological structures of the microfungi. Preparations were stained with Lactophenol Blue [8]. In addition to colonial features such as colony diameter, color, mycelial color and structure, conidial heads, and pigmentation released into the agar, microscopic characteristics including the morphology of macro- and microconidia, phialides, and mycelial structures were examined. For species-level identification, relevant mycological literature was consulted: The Fusarium Laboratory Manual [3], Food and Indoor Fungi [7], and Toprak Mikrofungusları (Volumes I–VII) [8].

For DNA extraction, isolates were grown on MEA for 7 days at 25 °C. DNA was extracted using the UltraClean® Microbial DNA Isolation Kit (Qiagen 12224) following the manufacturer's protocol. Multilocus gene identification was performed by sequencing the internal transcribed spacer (ITS) regions and the translation elongation factor 1 (TEF1) gene. The ITS regions were amplified using previously described methods [9], while TEF1 amplification followed Karlsson et al. (2016). PCR products were separated by agarose gel electrophoresis (1% w/v in 1× TAE) and visualized with GelRed staining. Subsequent steps were carried out by Genoks Company as a service provider. PCR products were purified using EXOSAP-IT (Affymetrix) and used for sequencing. Sequencing reactions were performed with BigDye Terminator v3.1 (Applied Biosystems) and analyzed using the 3130 XL DNA Analyzer.

2.5. Data Analysis

The sequences obtained in this study, together with type culture sequences retrieved from GenBank, were aligned using Muscle in MEGA 11.0 program [10]. Maximum composite likelihood (MCL) analysis as performed by the MEGA 11.0 program was used for investigation of positions on phylogram with 1000 bootstrap replications. The obtained sequence data have been deposited in GenBank with accession numbers (Table 2). Species assignments based on ITS and TEF1 sequences were confirmed using multiple criteria. Sequence similarity thresholds (≥98–99% identity) were applied by comparison with type reference sequences in GenBank. Phylogenetic analyses were conducted using Maximum Likelihood with bootstrap support (≥70%) to validate clustering with reference taxa. Concordant results between ITS and TEF1 loci were required for final species-level identification. In cases of ambiguity, morphological characteristics were also considered to support molecular assignments.
Biodiversity analyses were conducted using multiple complementary indices to capture different aspects of community structure. The Bray–Curtis Similarity Index [11] was applied to compare species composition between sampling periods, as it is widely used for assessing ecological similarity. Simpson's Index [12] and its derivatives were employed to evaluate species dominance and prevalence, providing insight into the extent to which a few species may dominate the community. In addition, the Shannon Diversity Index [13] was used to quantify overall species diversity, while the Shannon Evenness Index [14] assessed the distribution of individuals among species. Together, these indices allowed for a balanced interpretation of diversity, dominance, and similarity, and comparisons were made to highlight temporal changes in fungal community structure.

3. Result

In this study, sampling was carried out in two different seasons, and the percentage moisture content of the soil samples was determined as follows: West 20.02%, East 21.95%, South 19.46%, and North 24.08% in September 2022; and West 14.52%, East 10.35%, South 10.21%, and North 18.56% in August 2023. Colony-forming units (CFU) of Fusarium spp. per gram of soil were calculated (Table 1).

In the selective isolation study using SNA and SFA + Rose Bengal (RB) media, the highest CFU/g value on SFA + RB (5.7×10^4 CFU/g) was recorded from the South and East locations in September 2022. On SNA medium, colony counts ranged between 1.0×10^4 and 1.5×10^4 CFU/g. However, these numbers decreased significantly in August 2023 (SFA + RB: 1.2×10^4 CFU/g; SNA: $1.2–1.1 \times 10^4$ CFU/g) (Table 1).

Table 1. *Fusarium* sp. cfu/g values in soil at September 2022 and August 2023

Location	Sept 2022 – SFA+RB	Sept 2022 – SNA	Aug 2023 – SFA+RB	Aug 2023 – SNA
North	4.9×10^4	1.5×10^4	0	5.5×10^4
South	5.7×10^4	1.0×10^4	0	1.2×10^4
East	5.7×10^4	1.0×10^4	0	1.1×10^5
West	2.9×10^4	1.0×10^4	1.2×10^4	5.5×10^4

- Values expressed as colony-forming units per gram (cfu/g).
- 0 = No fungal growth detected.
- SFA+RB = Selective *Fusarium* Agar + Rose Bengal; SNA = Spezieller Nahrstoffarmer agar

The isolates were analyzed periodically. It was determined that, out of a total of 141 soil isolates collected in September 2022, 41 belonged to the genus *Fusarium*. Similarly, out of 74 soil isolates collected in August 2023, 30 were identified as *Fusarium*. In total, 71 members of the genus *Fusarium* were obtained from soil samples across both sampling periods.

For plant samples, 16 isolates from September 2022 and 11 isolates from August 2023 were identified as *Fusarium*, yielding a total of 27 plant-derived isolates (Figure 2). Notably, no isolates of the genus *Fusarium* were recovered from the healthy plant samples examined in this study (Figure 3).

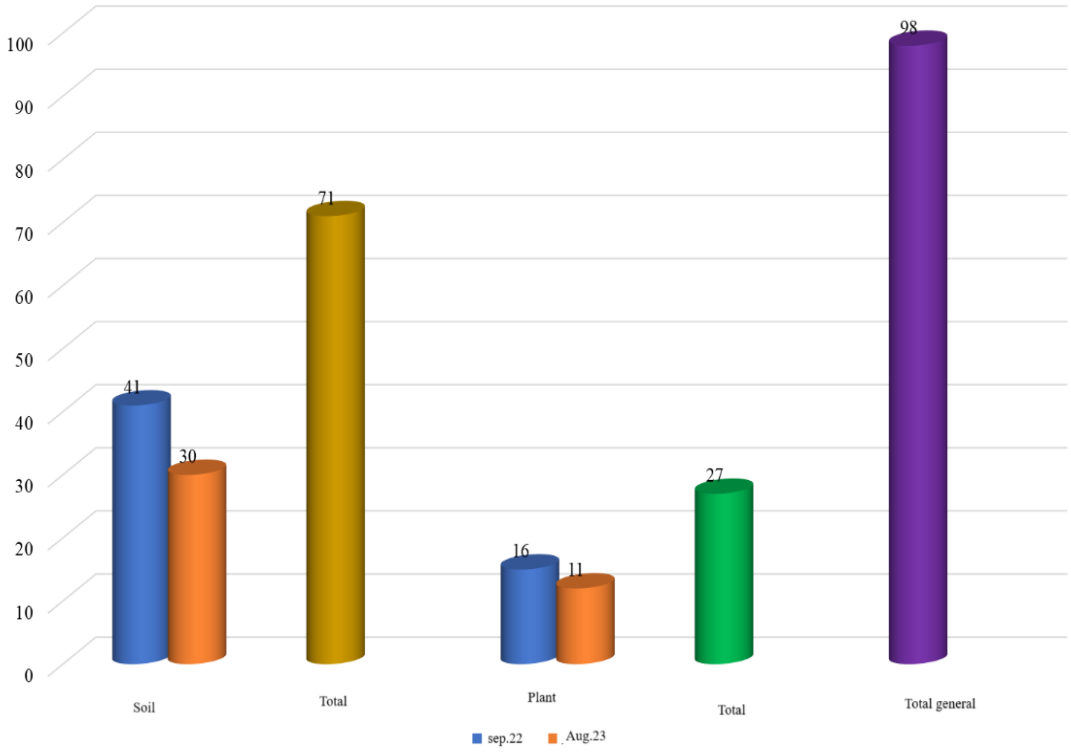

Figure 2. The number of isolates obtained from the genus *Fusarium* and the total number of *Fusarium* isolate

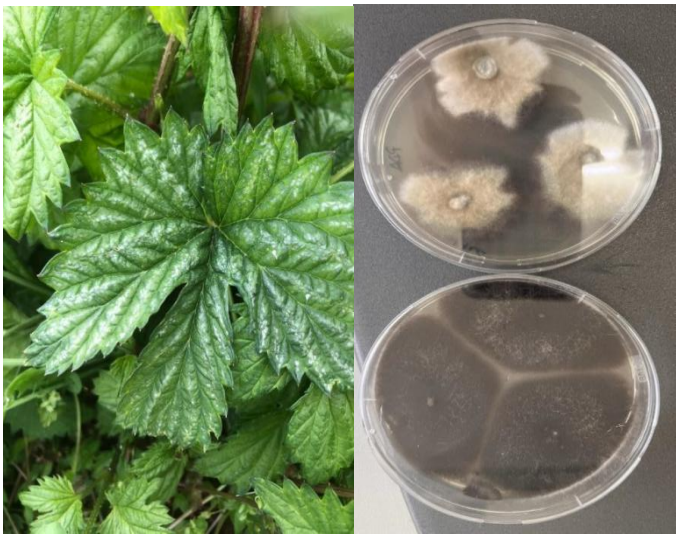

Figure 3. A sample of a non-*Fusarium* isolate (right) that developed after isolation from a healthy hop plant (left) and purification (Petri front and back view on PDA medium)

In addition, when the healthy and severely infected plant samples examined in this study were compared visually, it was concluded that the plant-associated *Fusarium* members detected here could be responsible for significant plant infections (Figure 4).

Figure 4. **A)** Infected hop plant leaf, **B)** Infected hop plant fruits**, C)** Healthy hop plant leaf

The isolates were identified according to their macroscopic and microscopic features as well as ITS and elongation factor α genes sequencing, it was determined that 98 isolates belonged to 8 species (NCBI GenBank accession numbers of ITS sequences; between PP907843-PP907862). Among the isolates examined, the most encountered species was determined as *F. oxysporum* with 25.51% (25 isolates). This was followed by *F. venenatum* (17) with 17.35% and *Neocosmospora solani* (15) with 15.31%. *F. acuminatum* and *F. equiseti* were determined as 13.27% (13) and 10.20% (10), respectively, while the other recorded species, *F. coereleum* and *F. redolens*, were determined as 2.04-3.06% (2-3). In addition to determining the presence of members of the *Fusarium* genus, an important plant pathogen, in hop plants grown in Turkey, according to; *Fusarium camptoceras* (Wollenw. & Reinking 1925) and *Fusarium venenatum* Nirenberg (1995) were new records for Turkey (Table 2).

Comparisons of soil and plant samples collected in September 2022 and August 2023 revealed consistent biodiversity patterns. Soil samples from both periods showed high similarity, with coefficients of 0.86 and 0.80, respectively. Moreover, biodiversity in soil and plant samples was strongly correlated (similarity coefficient = 0.86), supporting the conclusion that Fusarium members detected on perennial hop plants originated from the cultivated soils (Table 3).

When biodiversity indices were compared across sampling periods, the highest diversity was recorded in August 2023, with a Simpson Diversity Index (1–D) of 0.819, compared to 0.676 in September 2022. Simpson's dominance index (1/D) indicated that *F. venenatum* and *N. solani* were dominant in September 2022 (3.09), whereas *F. oxysporum* became dominant in August 2023 (5.00).

Overall biodiversity across the study was high, with a Simpson Diversity Index of 0.818. This was further supported by Shannon Diversity (H = 1.893) and Shannon Evenness (E(H) = 0.911), indicating both richness and balanced distribution of species. Six *Fusarium* species (*F. oxysporum, F. venenatum, N. solani, F. acuminatum, F. equiseti*, and *F. camptoceras*) were consistently recorded as dominant taxa, each with Simpson's prevalence index values above 5.52 (Table 3).

Table 2. Distribution of members of the genus *Fusarium* according to location and sample types

Species (GenBank Accession)	**Isolates (n)**	**Sept 2022 (E/W/N/S)**	**Aug 2023 (E/W/N/S)**	**% of Total**
F. acuminatum (PP907858.1, PP907851.1)	13	– / – / 2(P:1,S:1) / 4(P:1,S:3)	3(P:1,S:2) / 1(S:1) / 1(S:1) / 2(P:2)	13.27
***F. camptoceras* (PP907861.1, PP907857.1, PP907850.1, PP907849.1)**	10	3(S:3) / 1(S:1) / 2(S:2) / 3(S:3)	– / 1(S:1) / – / –	10.20
F. equiseti (PP907852.1)	13	5(P:1,S:4) / – / – / –	3(P:1,S:2) / 1(P:1) / 2(S:2) / 2(P:2)	13.27

Table 2. Continued

***F. oxysporum* (PP907862.1, PP907860.1, PP907847.1, PP907844.1)**	**25**	**4(S:4) / 4(P:4) / 1(P:1) / –**	**1(S:1) / 6(P:2,S:4) / 4(S:4) / 5(P:2,S:3)**	**25.51**
F. caeruleum (PP907845.1)	2	– / – / – / –	1(S:1) / 1(S:1) / – / –	2.04
F. redolens (PP907855.1, PP907848.1)	3	3(P:1,S:2) / – / – / –	– / – / – / –	3.06
***F. venenatum* (PP907859.1, PP907854.1, PP907853.1, PP907846.1, PP907843.1)**	**17**	**3(S:3) / 3(P:1,S:2) / 2(P:1,S:1) / 5(P:1,S:4)**	**1(S:1) / 2(S:2) / – / 1(S:1)**	**17.35**
***Neocosmospora solani (Syn.: F. solani)* (PP907856.1)**	**15**	**3(S:3) / – / 6(P:3,S:3) / 2(S:2)**	**1(S:1) / 1(S:1) / 1(S:1) / 1(P:1)**	**15.31**
Total isolates	**98**	**21 / 8 / 13 / 14**	**10 / 13 / 8 / 11**	**100.00**

• E/W/N/S: East/West/North/South
• P = Plant isolate; S = Soil isolate
• "–" indicates absence of Fusarium species in samples from that location.
• Percentages are calculated based on total isolates (n = 98).

Table 3. Biodiversity assessment index results

	SEPTEMBER/2022	**AUGUST/2023**	**SEPTEMBER/2022 + AUGUST/2023**
TOTAL NUMBER OF ISOLATES	56	42	98
S (NUMBER OF SPECIES)	7	7	8
D	0,324	0,18114875	0,166
(1-D)	0,676	0,800232	0,81885125
(1/D)	3,08641975	5,005806736	5,52032515
H	1,893	1,813	1,893
E (H)	0,93213988	0,918110322	0,911

In addition to the losses these plant pathogenic species cause in crops, it has been reported that they also pose risks to consumers through the mycotoxins they produce. These conditions compel producers to protect themselves against such pathogens. Indeed, some of the packaging waste observed in the fields during our surveys indicated that fungicides were being used by farmers, and that local communities were actively struggling with fungal contamination (Figure 5).

Figure 5. Fungicide boxes found in the fields during field work in September 2022 and August 2023

We examined the meteorological maps for September 2022, when the fieldwork was conducted. The average temperature in the region was recorded as 30 °C, with humidity levels between 65–70%. Precipitation data indicated cloudy weather, with rainfall ranging from 20–30 mm during this period. Considering wind speed and direction, the average wind speed was approximately 30 km/h, predominantly from the northwest (Figure 6).

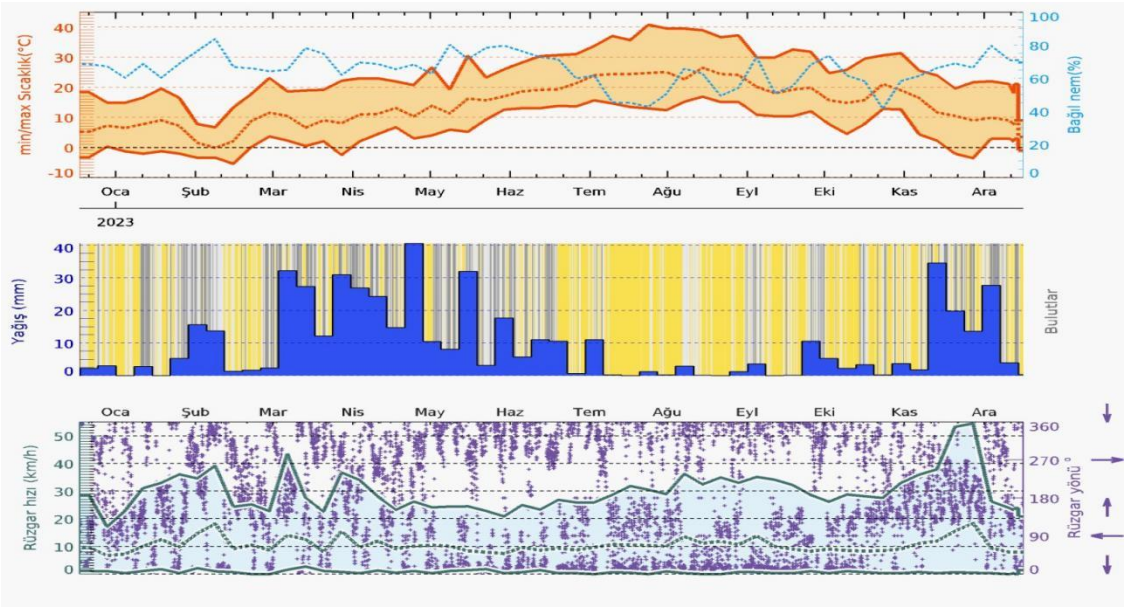

Figure 6. Meteorological records for the year 2022 covering the period September 2022 in Bilecik [23]

We examined the meteorological maps for August 2023, when the fieldwork was conducted. The average temperature in the region was recorded as 40 °C, with humidity levels ranging between 50–60%. Precipitation data indicated clear weather, with rainfall amounts of approximately 5–10 mm during this period. Considering wind speed and direction, the average wind speed was about 30 km/h, predominantly from the north to the west (Figure 7).

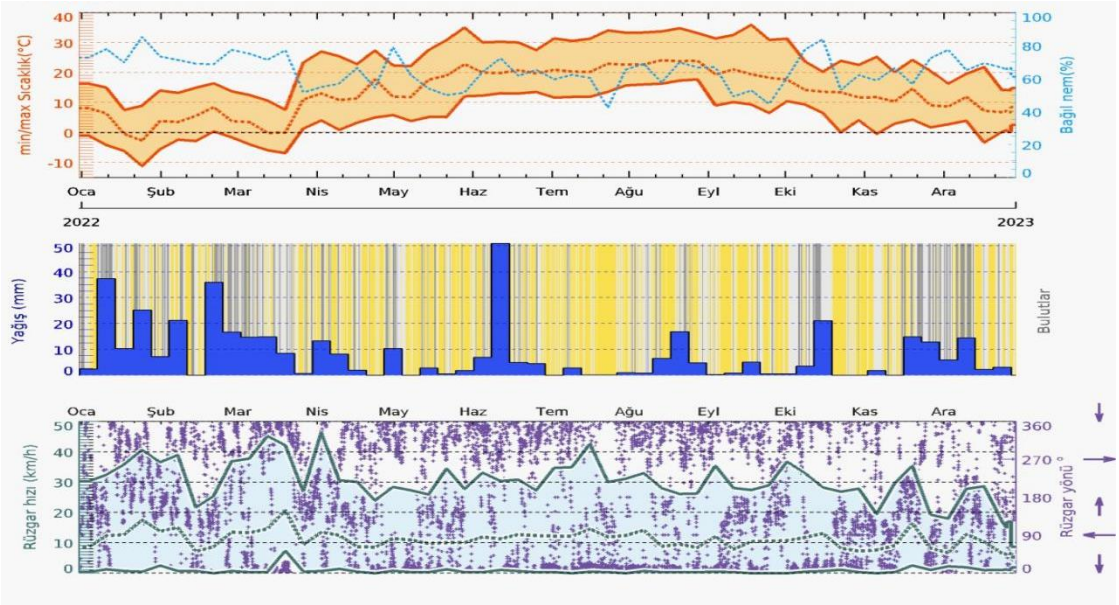

Figure 7. Meteorological records for the year 2023 covering the period August 2023 in Bilecik [23]

4. Discussion

The hop plant has considerable economic and medicinal importance, and Pazaryeri/Bilecik represents the only cultivation area in Türkiye. This study therefore provides the first record of *Fusarium* species associated with HOP plants and soils in this region, highlighting their role as microfungus-borne plant pathogens and potential contamination factors for end products [15]. Previous research on hop has focused mainly on antimicrobial, antifungal, cytotoxic, and phytochemical properties, as well as reviews of its role among medicinal and aromatic plants in Türkiye and worldwide [16]. However, systematic investigations of fungal pathogens, particularly *Fusarium*, have been lacking.

The genus *Fusarium* is recognized as a broad host phytopathogen causing significant losses in many crops, including hop. Species such as *F. avenaceum, F. culmorum, F. equiseti, F. oxysporum, F. poae, F. sambucinum, N. solani, F. sporotrichioides*, and *F. tricinctum* are commonly encountered in hop plants and have been the focus of biological control strategies [17]. Our findings address this gap by documenting six species frequently reported as plant pathogens (*F. oxysporum, F. venenatum, N. solani, F. acuminatum, F. equiseti*, and *F. camptoceras*). Notably, *F. camptoceras* and *F. venenatum* represent new records for Türkiye, consistent with Sesli et al. (2020) [18], Asan et al. (2022) [19], and Asan et al. (2024) [20].

Among these, *F. oxysporum* was the most prevalent species, in line with its well-documented role as a major causal agent of wilt disease, responsible for significant yield losses in agriculture. Although *F. oxysporum* can occasionally act as an endophyte, its pathogenic impact dominates agricultural contexts [17]. The co-occurrence of *F. equiseti, N. solani,* and *F. oxysporum* suggests overlapping infection mechanisms and ecological niches, as reported previously [21]. Beyond plant damage, the production of mycotoxins by these species poses risks to human health [22], underscoring the broader significance of monitoring their presence. Evidence of fungicide use observed in the fields further highlights the practical challenges faced by local farmers in managing fungal contamination (Figure 5).

Spatial and temporal analyses revealed shifts in isolate abundance between sampling periods and locations. In September 2022, the East location yielded the highest total number of isolates, while the West had the lowest. Soil isolates followed the same pattern, whereas plant isolates were most abundant in the North and least in the East. In August 2023, the distribution shifted: the West location exhibited the highest total number of isolates, while the North had the lowest. Soil isolates were again most abundant in the West but least in the South, whereas plant isolates were most frequent in the South and least in the East.

Meteorological and isolation data suggest that wind direction may have influenced the dispersal of Fusarium spp., with higher isolate counts recorded along prevailing wind paths. Specifically, spores appeared to be transported from West to East in September 2022 and from North to West in August 2023 (Figures 6 and 7). While this observation is intriguing, it remains speculative without experimental confirmation. Accordingly, the claim has been presented more cautiously, emphasizing the need for further studies to rigorously test the role of wind in fungal spread.

5. Conclusion

In summary, this study establishes baseline knowledge of *Fusarium* diversity in Turkish hop cultivation systems, highlights species of economic and biological importance, and acknowledges limitations regarding causal

inference. Future work should integrate molecular tracking, controlled inoculation experiments, and environmental monitoring to strengthen understanding of pathogen dispersal and plant–soil interactions.

Artificial Intelligence Statement
(X) This article did not use artificial intelligence.
( ) This article does use artificial intelligence.

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Data Availability Statement
( ) Option A (Public Repository): "The research data supporting this study are publicly available in [Name of Repository: e.g., Zenodo, Figshare, Dryad] with the permanent DOI link [URL/DOI]."
(**X**) Option B (Upon Request): "The raw data supporting the findings of this study are available from the corresponding author [Rasime Demirel: Eskişehir Technical University, Faculty of Science, Department of Biology, Eskişehir, Türkiye] upon reasonable request and within ethical boundaries."
( ) Option C (Third-Party Data): "The data used in this study were obtained from [Name of Institution/Source] and are subject to third-party restrictions regarding redistribution."
( ) Option D (No Data): "No new data were created or analyzed in this study. Data sharing is not applicable to this article."

References

[1] Şahin, G., & Erbilen, S. Ü. (2012). Türkiye'de yetiştirilen keyf bitkiler içerisinde özel bir tür: Şerbetçiotu (*Humulus lupulus L.*). *Zeitschrift für die Welt der Türken*, *4*(3).
[2] Waksman, S. A. (1922). A method for counting the number of fungi in the soil. *Journal of Bacteriology*, 7(3), 339–341. https://doi.org/0.1128/jb.7.3.339-341.1922
[3] Leslie, J. F., & Summerell, B. A. (2006). Fusarium laboratory workshop—A recent history. *Mycotoxin Research*, 22(2), 73–74. https://doi.org/10.1007/BF02956766
[4] Hussein, H. S., & Brasel, J. M. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, 167(2), 101–134. https://doi.org/10.1016/s0300-483x(01)00471-1
[5] Başkaya, Z. (2011). Bilecik ilinde şerbetçiotu üretiminin coğrafi esasları. *Doğu Coğrafya Dergisi*, 16, 209–230.
[6] Lee, R. J., & Cole, S. R. (1994). Internal quality control samples for water bacteriology. *Journal of Applied Microbiology*, 76(3), 270–274. https://doi.org/10.1111/j.1365-2672.1994.tb01627.x
[7] Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2019). *Food and indoor fungi* (2nd ed.). Westerdijk Fungal Biodiversity Institute.
[8] Hasanekoğlu, İ. (1991). *Toprak mikrofungusları* (Cilt I–VII). Atatürk Üniversitesi Yayınları.
[9] Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., ... & Samson, R. A. (2014). Identification and nomenclature of the genus *Penicillium. Studies in Mycology*, 78(1), 343–371. https://doi.org/10.1016/j.simyco.2014.09.001
[10] Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

[11] Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs,* 27(4), 326–349. http://dx.doi.org/10.2307/1942268
[12] Simpson, E. H. (1949). Measurement of diversity. *Nature,* 163, 688. http://dx.doi.org/10.1038/163688a0
[13] Shannon, C. E., & Weaver, W. (1949). *The mathematical theory of communication.* Urbana, IL: University of Illinois Press.
[14] Margalef, R. (1972). Homage to Evelyn Hutchinson or why is there an upper limit to diversity. *Transactions of the Illinois State Academy of Science*, 44, 221–235. https://doi.org/10.1111/j.1523-1739.2011.01736.x
[15] Rossini, F., Virga, G., Loreti, P., Iacuzzi, N., Ruggeri, R., & Provenzano, M. E. (2021). Hop (Humulus lupulus L.) as a novel multipurpose crop for the Mediterranean region of Europe: Challenges and opportunities of their cultivation. *Agriculture*, 11(6), 484. https://doi.org/10.3390/agriculture11060484
[16] Avan, M. (2021). Türkiye'de ve Dünya'da görülen önemli tıbbi ve aromatik bitkiler, özellikleri ve hastalıkları üzerine araştırmalar. *Uluslararası Doğu Anadolu Fen Mühendislik ve Tasarım Dergisi*, 3(1), 129–156. https://doi.org/10.47898/ijeased.864877
[17] Porteous-Álvarez, A. J., Fernández-Marcos, A., Ramírez-Lozano, D., Mayo-Prieto, S., Cardoza, R. E., Gutiérrez, S., & Casquero, P. A. (2023). Native Trichoderma isolates from soil and rootstock to Fusarium spp. control and growth promotion of *Humulus lupulus L.* plantlets. *Agriculture*, 13(3), 720. https://doi.org/10.3390/agriculture13030720
[18] Sesli, E., Asan, A., & Selçuk, F. (Eds.). (2020). *Türkiye mantarları listesi* (The checklist of fungi of Turkey). İstanbul: Ali Nihat Gökyiğit Vakfı Yayını.
[19] Asan, A., Karabıyık, H., & Giray, G. (2022). Türkiye Mantarları Listesi'ne ilaveler-1. *Bahçe Bitkileri Dergisi,* 9, 65–89. https://doi.org/10.35163/bagbahce.1130135
[20] Asan, A., Karabıyık, H., & Giray, G. (2024). Türkiye mantarları listesi'ne eklentiler-2. *Bağbahçe Bilim Dergisi,* 11(1), 25–43. https://doi.org/10.35163/bagbahce.1375946
[21] Hami, A., Rasool, R. S., Khan, N. A., Mansoor, S., Mir, M. A., Ahmed, N., & Masoodi, K. Z. (2021). Morpho-molecular identification and first report of *Fusarium equiseti* in causing chilli wilt from Kashmir (Northern Himalayas). *Scientific Reports*, 11(1), 3610. https://doi.org/10.1038/s41598-021-82854-5
[22] Sárkány, D., Bata-Vidács, I., Dobolyi, C., Dlauchy, D., Szabó, G., & Kukolya, J. (2024). Effect of lactic acid bacteria inoculation on the aflatoxin B1 contamination and the diversity of yeast communities in Aspergillus flavus-contaminated experimental corn silage. *Acta Alimentaria*, 53(3), 487–505. https://doi.org/10.1556/066.2024.00157
[23] URL1-https://www.meteoblue.com/tr/hava/hafta/bilecik_t%C3%BCrkiye_750598 Access date: 18.06.2026