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Orijinal araştırma (Original article)

Detection and prevalence of fungal diseases in kiwifruit cultivation areas in the Western Black Sea Region of Türkiye

Batı Karadeniz Bölgesi kivi ekiliş alanlarında görülen fungal hastalıkların tespiti ve yaygınlıklarının belirlenmesi

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A B S T R A C T

Surveys were conducted in kiwifruit orchards in Bartın, Düzce and Zonguldak provinces in the Western Black Sea Region between 2014 and 2016 to determine the prevalence. To detect fungal diseases, leaf, stem and root samples were collected and isolations were made using PDA and Water Agar media. Pathogenicity tests were performed using different techniques. For leaf pathogens, the "detached leaf inoculation method" was employed; for fruit pathogens, the "fruit inoculation method" was used; and for stem pathogens, the "stem inoculation method" was used. Isolates were re-isolated from infected tissues obtained in pathogenicity tests. Isolates found to be pathogenic were used in molecular studies to identify them at the species level. For molecular studies, DNA extraction, PCR, and gel electrophoresis were performed to sequence the ITS gene region. Nucleotide sequences were analyzed using the BIOEDIT software program on the NCBI website. Pathogenic fungi diagnosed in this study: *Alternaria alternata* (Fr.) Keissl, *A. tenuissima* (Kunze) Wiltshire, *Colletotrichum gloeosporioides* Penzig, *Neopestalotiopsis* spp., *N. vitis* Jayawardena, Maharachch., Yan, Li & Hyde, *Diaporthe actinidiae* N.F. Sommer & Beraha, *D. nobilis* Sacc. & Speg., *Valsa leucostoma* (Persoon) Saccardo, *Botryosphaeria dothidea* (Mougeot) Cesati & de Notaris, *Phaeoacremonium aleophilum* (Tulasne & C. Tulasne) D. Gramaje, L. Mostert & Crous, *Cylindrocarpon macrodidymum* (Halleen, Schroers & Crous) L. Lombard & Crous, *Botrytis cinerea* Persoon *Cladosporium* spp. and *Armillaria mellea* (Vahl) Kummer were found. In prevalence studies, the most common leaf pathogen agents were identified as *Alternaria* spp., *Colletotrichum gloeosporioides*, *Diaporthe* spp., and *Pestalotiopsis* spp.

INTRODUCTION

Kiwifruit production is rapidly increasing both in Türkiye and globally. World kiwi production reached approximately 4,539,471.22 tons. According to FAO 2024 Crops and Livestock Products statistics, approximately 85% of the world's kiwi fruit production was produced in China, New Zealand, Italy, and Greece in 2022. China is the largest producer, with an annual production of approximately 2.4 million tons, followed by New Zealand, Italy, and Greece, in that order. In 2022, Türkiye produced 100,722 tonnes of kiwifruit, making it the seventh-largest producer globally (Anonymous 2024). Kiwi was first tested in Türkiye at the Yalova Atatürk Horticultural Central Research Institute and the Giresun Hazelnut Research Institute in 1988. It was determined that the climate and soil structure of the Marmara and Black Sea Regions were suitable for kiwi cultivation. Kiwi production started in the 1980s in the country (Atak 2021). Türkiye is among the countries that initiated kiwi cultivation relatively late. However, with the increase in the number of newly established and productive gardens, it is now among the most important countries in the world for kiwi production. Yalova is the leading city in terms of kiwi production in Türkiye. The production of this spice in the provinces of Yalova, Bursa, Mersin and Rize is estimated to be approximately 19,000 tons. This statistic accounts for approximately 57.1% of Türkiye's total kiwi production area. A substantial 32% increase in kiwi production areas in Türkiye was observed between 2020 and 2016 (Anonymous 2025).

Surveys have been conducted in kiwi production areas in countries such as New Zealand, California, China, Greece, Iran, and Türkiye. The identified fungal pathogens have been identified as factors limiting kiwi production. These agents include: *Diaporthe nobilis* Saccardo & Spegazzini., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Phytophthora* spp., *Armillaria mellea*, *Cylindrocarpon radicicola* Wollenw., *Fusarium roseum* (Sacc.) Snyd. et Hans., *Pythium splendens* Braun (Waterhouse, 1967)., *Phomopsis* sp., *Colletotrichum gloeosporioides*, *Alternaria dumosa* Simmons, *Epicoccum purpurascens* Ehrenb. ex Schltdl., *Botrytis cinerea*, *Botryosphaeria dothidea*, *Alternaria alternata*, *Pestalotiopsis microspora* Speg., *Nigrospora oryzae* (Berk. & Broome) Petch 1924 and *Macrophomina phaseolina* (Aghajani 2008, Elena 2009, L. Li et al. 2017, Y. Li et al. 2017, Michailides and Elmer 2000, Mohamadalian et al. 2011, Pei et al. 2019, Pennycook and Samuel 1985, Warrington and Weston1990)

There are limited studies on fungal diseases of kiwifruit in Türkiye. Some studies conducted in kiwifruit production areas include: *B. cinerea* and *A. alternata*, *Fusarium oxysporum, F. solani, Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp., *Macrophomina phaseolina, Cylindrocarpon* spp., *Verticillium* spp., *Acremonium* spp., *Clonostachys* spp., *Rhizopus* spp., and *Trichoderm*a spp., *Phytopythium vexans, P. litorale* in Rize province (Karakaya and Bayraktar 2009, Karakaya and Çelik 2012, Polat et al. 2017, Polat et al. 2023, Şahin and Türkkan 2020).

As production areas have expanded, complaints related to diseases have increased, and it is necessary to identify these problems, find solutions, and develop control programs. This study, conducted in kiwi orchards in Bartın, Düzce and Zonguldak provinces between 2014 and 2017, identified fungal diseases as a problem and therefore aimed to determine the prevalence of fungal disease in kiwi production areas in the region (Western Black Sea).

MATERIALS AND METHODS

The material of the study consisted of diseased kiwi plant samples (fruit, leaf, branch, root and stem) collected from kiwi vineyards in Düzce, Bartın, and Zonguldak provinces; isolation media; Hayward variety kiwi seedlings used in pathogenicity tests; leaves; moist chamber materials; fungal isolates used in molecular studies; DNA isolation kit; primers; devices; and other chemical materials.

Field studies

In surveys conducted in May-June and September-October 2014-2015, samples were taken from leaves, fruits, shoots, branches and roots of diseased plants in Bartın, Düzce and Zonguldak provinces (Table 1). In the surveys, all of the total kiwi areas were examined. Survey areas and coordinate information are given in Table 1.

Calculation of the prevalence ratio

The percentages of leaf diseases, averaged over the 2014 and 2015 seasons, were determined by the ratio of the number of plants to the total number of diseased plants. Next, using the formula below, the prevalence rate for each surveyed region was calculated (Bora and Karaca 1970).

$$\text{Prevalence rate} = \frac{\Sigma(\text{Percentage of Orchard Disease X Area of Orchard (da)X100}}{\text{Maximum Disease Probability (total area examined area (da) X 100)}}$$

Isolation of fungal agents from plant tissue

Potato Dextrose Agar (PDA) and Water Agar (WA) were used as fungal isolation and growth media. 3-4 cm pieces were cut from diseased plant samples with a sterile scalpel to include diseased and healthy tissue. The pieces were kept in 1% NaOCl for 2-3 min. and subjected to superficial disinfection, and then excess water was removed between sterile blotting papers. Then, small pieces were cut from the edges of the plant pieces with a sterile scalpel and discarded. The remaining diseased plant pieces were cut into 3-5 mm pieces and planted in the medium. Approximately 7 days after incubation at 24±1 °C, mycelial tips of the developing colonies were transferred to new medium and subcultures were obtained. Agar discs obtained from pure cultures of the isolates were stored in slant agar at +4 °C.

Table 1. Surveyed province, district, village, area, total number of trees, number of examined trees and coordinate information

Province	County	Village	Total Number of Trees	Number of Trees Examined	Garden Area (da)	GPS Coordinates of Gardens
Bartın	Merkez	Akçalı	11000	1650	140	41.60290 North/32.39172 East
		Terkehaliller	4000	600	48	41.56661 North/32.39613 East
		Yanaz	220	150	3	41.58243 North/32.41686 East
Düzce	Yığılca	Gürcü Çiftlik	150	150	2	40.91106 North/31.24006 East
	Akçakoca	Arabacı	80	80	1	-
			35	35	0.7	-
		Kabalar	80	80	1	41.09610 North/31.40761 East
Zonguldak	Alaplı	Yeşilyurt	60	60	1	41.12646 North/31.41373 East
	Ereğli	Elmacı	300	200	4	-
			150	150	2	41.34992 North/31.63831 East
			50	50	1	-

The identification of fungal isolates

The isolates were purified on PDA media conducive to the growth of each fungus, after which they were grouped based on colour, growth characteristics, and spore structure observed under a microscope. Subsequently, they underwent morphological and molecular identification.

Morphological diagnostics

The fungi were identified both macroscopically and microscopically characteristics such as colony colour, growth rate, hyphae colour and presence of septa, phialides shape, presence and size of macro-micro conidia and chlamydospores, and formation of pycnidium, perithecium, and macro-micro sclerotia (Ellis 1976, Gerlach and Nirenberg 1982, Samson et al. 1996, Tousson 1995).

Molecular diagnostics

In addition to morphological identification, molecular analyses were performed to evaluate the outcomes. For this purpose, fungal DNA isolation and PCR tests were performed. The methods followed for molecular studies were as follows.

DNA extraction from samples

DNA extraction was performed according to the Qiagen Blood and Tissue Kit protocol (Madison, WI, USA).

250 mg (0.25 g) of mycelia developed in the medium were taken, crushed in a mortar with liquid nitrogen and transferred to an Eppendorf tube. It was vortexed with 180 µl buffer ATL (Tissue Lysis Buffer), and 20 µl Proteinase K was added and mixed once more using a vortex. The resulting suspension was incubated at 65 °C for 90 minutes, and then 200 µl of buffer AL (Lysis Buffer) was added. Then 200 µl of 100% ethanol was added and mixed once more, and transferred to specially produced filtered tubes for extraction (DNeasy Mini Spin Columns) and centrifuged at 8,000 rpm for 1 minute. The supernatant in the collection tube was discarded. 500 µl of buffer AW1 (Wash Buffer 1) was added to the remaining part of the filtered section containing DNA and centrifuged at 8 000 rpm for 1 minute, and the portable tube remaining at the bottom was discarded, and a new sterile clean tube was placed. After this process, 500 µl of buffer AW2 (Wash Buffer 2) was added to the pellet and centrifuged at 20 000g (14 000 rpm) for

3 minutes, and the resulting pellet was placed in a new Eppendorf tube and centrifuged with 100 µl of buffer AE at 8 000 rpm for 1 minute to obtain DNA. The resulting DNA was stored in a deep freezer at -20 °C.

PCR study

PCR reactions were performed with a total of 50 µl; 5 µl DNA (approximately 10 ng), 26 µl Ampliqon (Denmark) RED Master mix, 15 µl Nuclease Free Water (Merck), 2 µl primer (ITS1:5'TCCGTAGGTGAACCTGCGG3', ITS4:5'TCCTCCGCTTATTGATATGC3') (White et al.1990). PCR was performed at 94 °C 4 min for 1 cycle, 94 ᵒC for 1 min, 58 ᵒC for 1 min, 72 ᵒC for 1.5 min for 35 cycles final 72 ᵒC 5 min.

Pathogenicity tests of Alternaria spp., *Botrytis cinerea, Diaporthe* spp., *Neopestalotiopsis* spp., and *Colletotrichum gloeosporioides* on leaves

In pathogenicity, "detached leaf inoculation method" was implied to use a total number of six isolates of *Alternaria* spp., *Botrytis cinerea*, *Diaporthe* spp., *Neopestalotiopsis* spp., *Colletotrichum gloeosporioides* (Dolar et al. 1994, Ertek et al. 2018, Ertek et al. 2022). Fungi stored in slant agar medium were transferred to PDA and incubated at 24±1 °C for 10-12 days. Leaf samples taken from kiwi trees were disinfected in 1% NaOH for 3 min. Then, they were placed on blotting paper and excess water was removed. Moist chambers were prepared by wetting two layers of blotting paper in plastic Petri dishes. 3-4 wounds were made with a scalpel on both sides of the leaf blade of each leaf sample, and 10-12-day-old fungi grown in PDA were removed in the form of a disk with a cork and placed on the wounds opened with a needle. The stems of kiwi leaves were wrapped with cotton. Sterile distilled water was used to moisten the cotton and the wounds with agar. The Petri dishes were placed in a lighted compartment, and sterile distilled water was dropped onto the agar discs on the leaves once every 2 days. Disease development was observed on the leaves, and the results were evaluated 14 days after inoculation.

Pathogenicity tests of Botrytis cinerea on fruits

"Fruit inoculation method" was used for *Botrytis cinerea*. Healthy kiwi fruits were immersed in 5% NaOCl for 1 min. for surface disinfection, then transferred to blotting paper and stored in a sterile cabinet, and inoculated with mycelial agar discs of *B. cinerea*. Control fruits were inoculated with mycelium-free agar discs. Fruits placed in sterile plastic containers were incubated at 22±1 ᵒC. Results were evaluated 1-2 weeks after inoculation.

Pathogenicity tests of Phaeoacremonium aleophilum, Botryosphaeria dothidea, and Cylindrocarpon macrodiymum on stems

"Stem inoculation method" was used for *Phaeoacremonium aleophilum*, *Botryosphaeria dothidea, Cylindrocarpon macrodiymum* 5 mm diameter mycelial agar discs taken from the edges of one-week cultures grown on agar media were placed on the wounds opened on the stems of the plants. The wounds were wrapped with moist cotton wool and covered with stretch film to prevent moisture loss. Mycelia-free agar discs were used in the control plants. At least five plants were used for each isolate and control in the study. Plants were kept in a controlled greenhouse at 25 °C and irrigated once a week. Four weeks after inoculation, the bark around the wounds was peeled off with a sterile scalpel and necrotic areas were examined, and lesion length was measured.

RESULTS

Isolation

The pathogens were identified as *Neopestalotiopsis vitis, Neopestalotiopsis* spp., *Neopestalotiopsis protearum, Botryosphaeria dothidea, Alternaria alternata, Alternaria tenuissima, Armillaria mellea, Colletotrichum gloeosporioides, Botrytis cinerea, Diaporthe actinidae, Valsa leucostoma, Diaporthe nobilis, Phaeoacremonium* spp., *Cylindrocarpon* spp., and *Cladosporium* spp.

Obtained 157 isolates from 83 plant samples were placed on slant agar medium and stored in the fridge at +4 °C (Table 2).

Table 2. Number of isolates obtained in 2014 and 2015

Isolates	Number of Isolates in 2014	Number of Isolates in 2015	Total
Colletotrichum gloeosporioides	8	5	13
Neopestalotiopsis vitis	18	20	40
***Neopestalotiopsis* spp.**	4	4	8
Neopestalotiopsis protearum	7	2	9
Botryosphaeria dothidea	8	6	14
Alternaria alternata	9	5	14
Alternaria tenuissima	6	5	11
Botrytis cinerea	8	9	17
Diaporthe actinidiae	5	6	11
Diaporthe nobilis	4	3	7
Valsa leucostoma	2	3	5
Phaeoacremonium aleophilum	2	1	3
Cylindrocarpon macrodiymum	3	1	4
***Cladosporium* spp.**	2	1	3
			157

Morphological features of the pathogens obtained in this work

As shown in Figure 1, the causal agent isolated from each leaf is indicated.

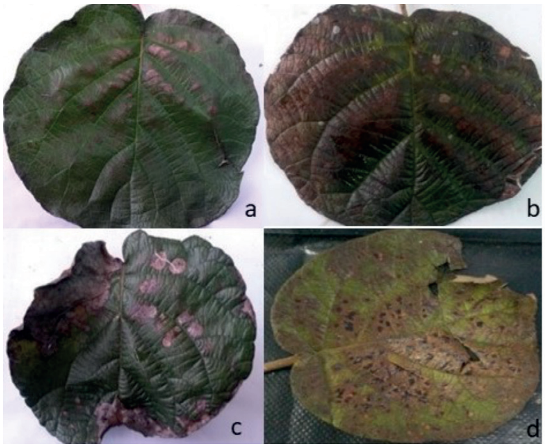

Figure 1. Symptoms of different leaf pathogens of kiwifruit a) *Neopestalotiopsis* spp., b) *Colletotrichum gloeosporioides*, c) *Alternaria* spp., d) *Phomopsis* spp.

Alternaria alternata, Alternaria tenuissima: Alternaria spp. caused leaf spots on kiwi leaves (Figure 1c). Alternarias grow dark and concentric on PDA (Figure 2a). Conidia are obclavate to obpyriform or ellipsoid, with a short conical beak at the tip, or beakless, with a smooth to verruculose surface and a size of 20–63 x 9–18 µm (Figure 2b). Despite causing the same pathogenic necrosis on the leaves, they are only distinguishable by microscopic identification. While *A. alternata* isolates produced branched conidia chains with 8 to 12 spores and numerous secondary or occasionally tertiary branching chains, *A. tenuissima* had dark, septate, and sparsely branched conidiophores as well as dark brown conidia with short conical beaks at their tips, 7 to 17 spores in chains.

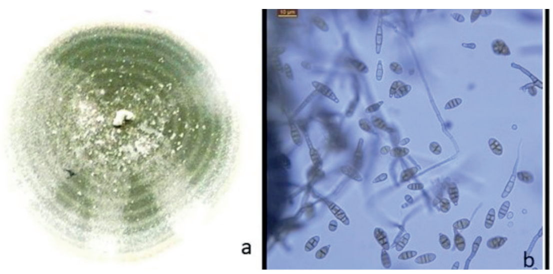

Figure 2. Some appearance of *Alternaria alternata*, a) Consantric and dark growth of Alternaria in PDA, b) Typical muriform conidia of *Alternaria alternata*

Neopestalotiopsis spp.: *Neopestalotiopsis* spp. caused leaf blight disease in leaves (Figure 1a). White mycelia produced black globular acervuli with conidia on PDA after 7 days of incubation at 28 ± 2 °C with a 12-h light and 12-h dark period (Figure 3a). Under a microscope, conidia appear as five-celled with hyaline end cells and darker median cells. Acervular conidiomata are abundant in PDA culture (Figure 3b).

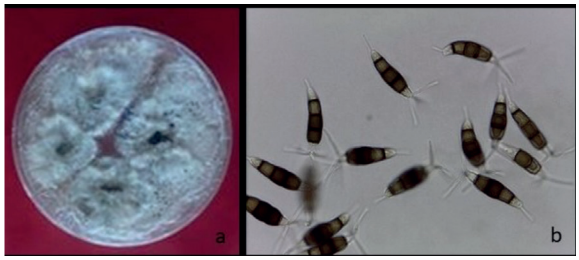

Figure 3. Various aspects of *Neopestalotiopsis vitis*, a) PDA growth characteristics, b) Up to five-celled conidia

Colletotrichum gloeosporioides: This fungus caused anthracnose disease in the kiwifruit leaves. *C. gloeosporioides* caused irregularly shaped lesions, which then turned into brown or black spots on infected leaves. At the last stage of the disease, the centers of the lesions had turned gray, and the edges were dark brown (Figure 1b). The fungus produced white mycelia, which turned gray after 5 days. The colonies produced abundant conidia that were hyaline, one-celled, straight, and cylindrical (Figure 4).

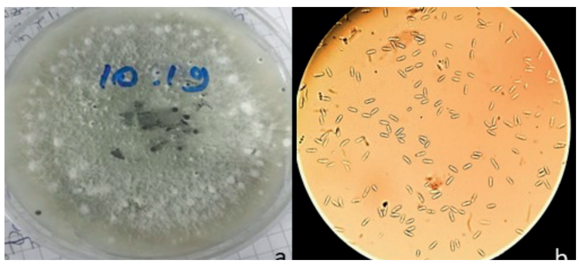

Figure 4. Colony morphology, conidial characteristics, and pathogenicity of *Colletotrichum gloeosporioides*, a) Colony growth on potato dextrose agar (PDA), b) Conidia observed under light microscopy

Valsa leucostoma: Leucostoma canker was isolated from infections of small twigs that appear as sunken, discoloured areas with concentric circles of dead tissue. Pycnidia are black on the outside.

Phaeoacremonium aleophilum: The symptoms exhibited discolouration, necrotic wood, and death of shoots. Phaeoacremonium species were identified based on colony coloration, conidiophore and phialid structures. The fungus PDA culture medium showed pale brown, beige, or brownish-gray growth (Figure 5a). The mycelium consisted of septate, pale brown hyphae, found singly or in groups of up to 7 hyphae. The phialides were transparent and cylindrical in shape. All three types of phialides were observed. Conidia form at the tips of the phialids. They are transparent, unseptate, elongated ellipsoidal, cylindrical, or reniform in shape (Figure 5b).

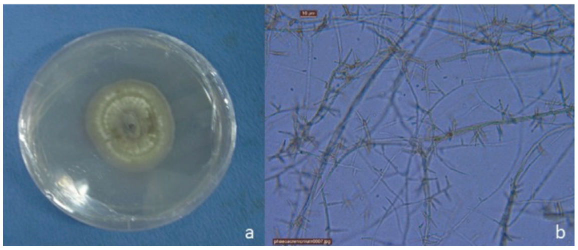

Figure 5. Colony morphology, conidiophore and phialid of *Phaeoacremonium minimum*, a) Colony growth on potato dextrose agar (PDA), b) Conidia observed under light microscopy

Cylindrocarpon species were identified based on colony color, macroconidia, microconidia, conidiophores. The fungus PDA showed orange-dark brown colony growth on medium (Figure 6a). Two types of conidia were observed: macroconidia and microconidia. The conidia did not form chains. Macroconidia were colorless, 1–3 septate, straight or slightly curved, cylindrical or rounded at the ends (Figure 6b). Microconidia were colorless, oval or ellipsoidal, unsegmented or single-segmented. Conidia are formed as heads within conidiophores.

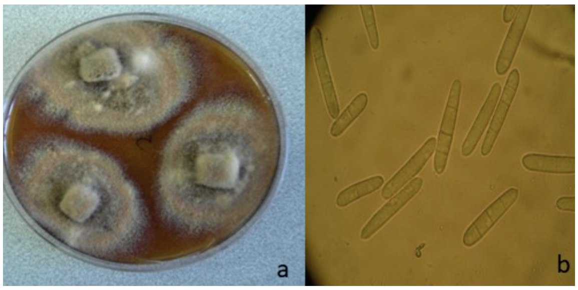

Figure 6. Colony morphology, macroconidia and microconidia of *Cylindrocarpon macrodidymum*, a) Colony growth on potato dextrose agar (PDA), b) Conidia observed under light microscopy

Armillaria mellea: The symptoms exhibited by the plant were consistent with root rot caused by Armillaria, which had resulted in complete defoliation. The presence of white mycelial fans, extensive wood rot and rhizomorphs was observed in the root collars and woody roots of the affected plants, thus serving as diagnostic signs of the disease.

Botryosphaeria dothidea: This pathogen is related to stem canker, dieback, and fruit rot in woody plants (Marsberg et. al. 2017, Vučković et. al. 2024). Colonies on PDA were cushiony, surface light to dark gray-brown, and reverse black in the center and gray-brown towards the irregular margin after 7 days at 25 °C in the dark (Figure 7a). Young conidia were hyaline, aseptate, fusiform, subtruncate at the base, subobtuse at the apex, and measured (19.56–) 24.12 to 26.31 (–28.99) × (5.13–) 5.94 to 6.54 (–7.44) µm, old cells were dark and consistent with the description of *B. dothidea* (Phillips et al. 2013) (Figure 7b).

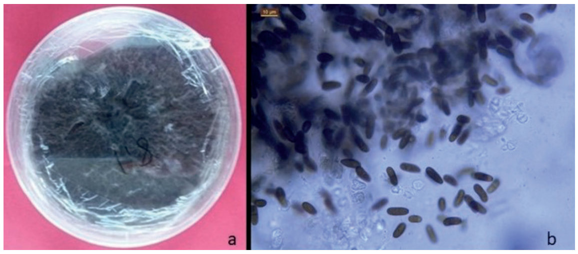

Figure 7. Various aspects of *Botryosphaeria dothidea*, a) PDA growth characteristics, b) conidia

Diaporthe actinidiae, Diaporthe nobilis: *D. actinidiae* caused cankers, red-brown colour and shoots showing blight, and some trees eventually died (Bai et al. 2017). The colonies were grayish. Isolates produced α-conidia, measuring 3.9 to 6.6 × 1.0 to 2.7 µm and 5 to 8.1 × 1.7 to 3.2 µm, respectively, and β-conidia measuring 11 to 20 × 0.5 to 1.5 µm. All α-conidia were one-celled, hyaline, fusiform, and β-conidia were one-celled, hyaline, and filiform with straight or curved ends. *D. nobilis* caused cankers like *D. actinidiae*. Conidiomata are pycnidial, subcuticular, scattered to confluent, uniloculate, dark brown to black, uniloculate, broadly spherical to flattened. Alpha conidia were 8 × 3.8 µm, non-septate, straight or curved, cylindrical or ellipsoidal, obtuse at both ends, hyaline, generally biguttulate. Beta conidia were 24 × 0.5 µm, filiform; blunt at one end, pointed and usually curved at the other, hyaline, one-celled (L. Li et al. 2017) (Figure 8 a, b, c).

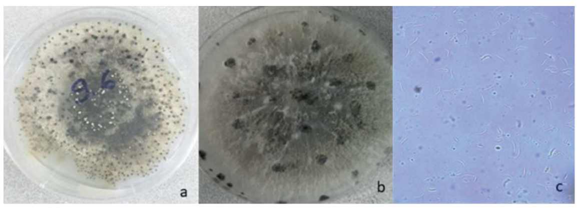

Figure 8. Various aspects of *Diaporthe* spp., a) PDA growth characteristics of *Diaporthe actinidiae*, b) PDA growth characteristics of *Diaporthe nobilis*, c) α-conidia and β-conidia of *Diaporthe actinidae*

Botrytis cinerea: *B. cinerea* grew grayish in PDA media by producing abundant conidia (Figure 9). The conidiophores are characterised by their branched nature and the presence of rounded apical cells, which are arranged in clusters.

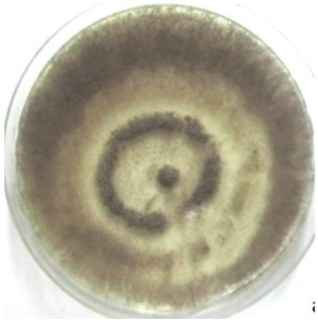

Figure 9. PDA growth characteristics of *Botrytis cinerea*

Molecular tests

A total of 15 representative isolates obtained from pure cultures were selected for molecular analyses. The internal transcribed spacer (ITS) region was amplified from all pathogenic isolates using the universal primers ITS1 and ITS4. The amplification products were visualized by agarose gel electrophoresis, and clear, single bands of the expected size confirmed successful amplification. Representative gel electrophoresis images showing the ITS amplicons are presented in Figure 10. The PCR products were subsequently sequenced, and the obtained sequences were compared with those deposited in GenBank (NCBI). Molecular identification of fungal pathogens isolated from kiwi plants was performed based on ITS sequence similarity, enabling species-level identification for most isolates. The nucleotide sequences generated in this study were deposited in the NCBI GenBank database, and the corresponding accession numbers are provided in Table 3.

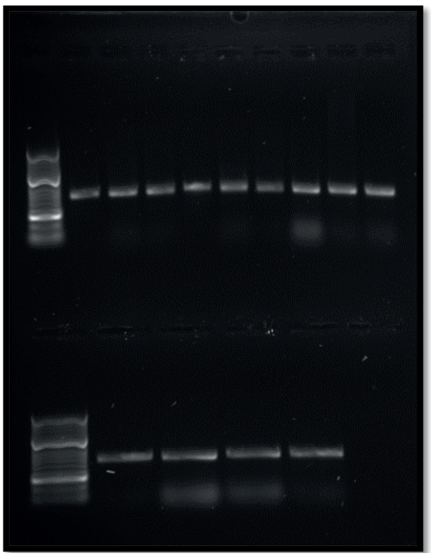

Figure 10. Agarose gel electrophoresis showing PCR amplification of the ITS region (~590 bp) using ITS1 and ITS4 primers from representative fungal isolates. M: 100 bp DNA ladder; lanes 1–15: fungal isolates from kiwi plants

Table 3. Some of the pathogens in kiwifruit and accession numbers in NCBI database

Isolate number	Accession number	Pathogen
ek_1	MK571250	*Neopestalotiopsis vitis*
ek_2	MK571251	*Neopestalotiopsis vitis*
ek_3	MK571252	*Neopestalotiopsis vitis*
ek_4	MK571253	*Diaporthe actinidiae*
ek_5	MK571254	*Botryosphaeria dothidea*
ek_6	MK571255	*Neopestalotiopsis vitis*
ek_7	MK571256	*Neopestalotiopsis vitis*
ek_8	MK571257	*Neopestalotiopsis vitis*
ek_9	MK571258	*Botrytis cinerea*
ek_10	MK571259	*Botrytis cinerea*
ek_11	MK571260	*Neopestalotiopsis* spp.
ek_12	MK571261	*Colletotrichum gloeosporioides*
ek_13	MK571262	*Diaporthe actinidiae*
ek_14	MK571263	*Alternaria alternata*
ek_15	PV874247	*Neopestalotiopsis protearum*

Pathogenicity tests

Pathogenicity tests were conducted on leaves, fruits, and shoots depending on the plant tissue from which each pathogen was originally isolated, as described in the Materials and Methods section.

Leaf pathogenicity assays were performed with *Alternaria* spp., *Colletotrichum gloeosporioides, Botrytis cinerea, Diaporthe* spp., *Botryosphaeria* spp. and *Neopestalotiopsis* spp. Initial lesion development, representing the first disease symptoms, was observed approximately 4 days after inoculation, confirming the pathogenicity of the

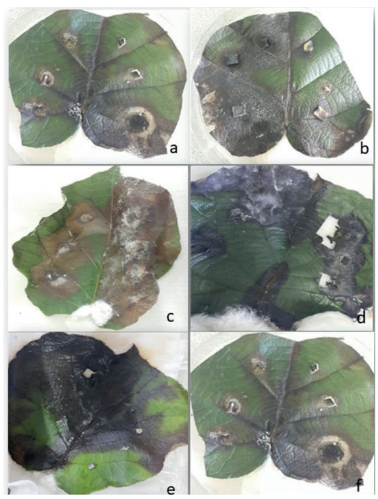

Figure 11. Leaf pathogenicity test results in 7th days a) *Alternaria alternata*, b) *Colletotrichum gloeosporioides*, c) *Botrytis cinerea*, d) *Diaporthe* spp., e) *Botryosphaeria* spp. and f) *Neopestalotiopsis* spp.

tested isolates. Disease symptoms progressed during the incubation period, and leaves were maintained for up to 14 days to complete the pathogenicity assessments (Figures 11a, b, c, d, e, f). No wounds was observed in the control (Figure 12).

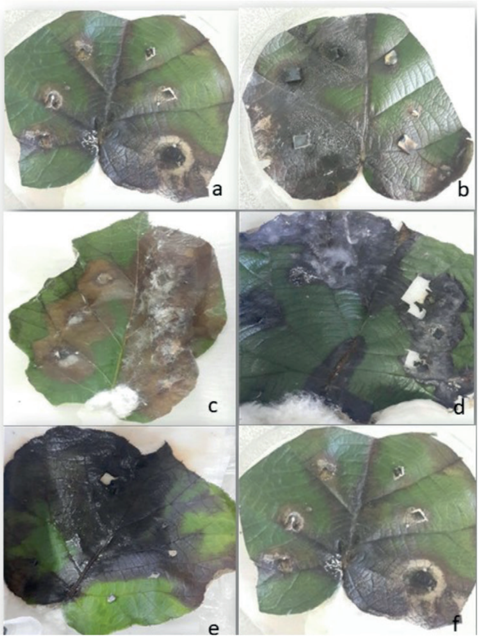

Figure 11. Leaf pathogenicity test results in 7th days a) *Alternaria alternata*, b) *Colletotrichum gloeosporioides*, c) *Botrytis cinerea*, d) *Diaporthe* spp., e) *Botryosphaeria* spp. and f) *Neopestalotiopsis* spp.

Figure 12. Control of leaf pathogenicity test

Fruit pathogenicity tests were conducted using *Botrytis cinerea* on kiwifruit. Brownish lesions and soft rot developed within two days after inoculation, followed by characteristic gray sporulation on in parallel assays the fruit surface. The pathogen was successfully re-isolated from symptomatic fruit tissues using the agar disk method, thereby fulfilling Koch's postulates. In addition to fruit decay, *B. cinerea* also caused notable damage to leaf tissues (Figures 13).

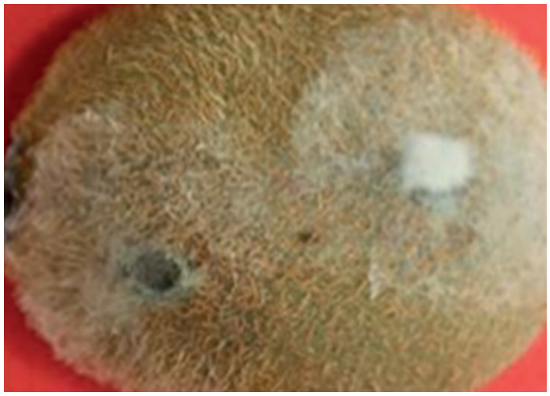

Figure 13. Fruit pathogenicity test result of *Botrytis cinerea* (7th day)

Shoot pathogenicity assays revealed that *Phaeoacremonium* spp. and *Cylindrocarpon* spp. induced light brown to brown longitudinal lesions on inoculated stems, whereas *Botryosphaeria* spp. caused distinct black necrosis. Re-isolation of all pathogens was successfully achieved from discolored stem tissues following pathogenicity tests using the agar disk method.

In all assays, the successful reproduction of disease symptoms and subsequent re-isolation of the causal agents confirmed that Koch's postulates were fulfilled.

Prevalences

In the survey carried out in 2014-2015, the most common agents were *Diaporthe* spp. (anamorph *Phomopsis*), *Pestalotiopsis* spp., *Alternaria* spp., *Colletotrichum* spp. (Table 4). According to this study, the prevalence rates of disease agents were *Diaporthe* spp. (*D. actinidiae, D. nobilis*) 63.63%, *Pestalotiopsis* spp. 18.18%, *Alternaria* spp. (*A. alternata, A. tenuissima*) 13.63% and *C. gloeosporioides* 9.09% in Düzce province. The prevalence rates of the pathogens were *Diaporthe* (*Phomopsis*) spp. 40.68%, *Pestalotiopsis* spp. 28.81%, *Alternaria* spp. 33.89% and *Colletotrichum* spp. 23.74% in Zonguldak province. The prevalence rates of the pathogens were *Diaporthe* (*Phomopsis*) spp. 51.22%, *Pestalotiopsis* spp. 24.39% and *Alternaria* spp. 58.54% in Bartın province.

DISCUSSION

Fungal agents identified in surveys conducted throughout the project included: *Alternaria alternata, A. tenuissima, Botryosphaeria dothidea, Botrytis cinerea, Colletotrichum gloeosporioides, Diaporthe actinidiae, D. nobilis, Neopestalotiopsis* spp., and *N. protearum* which were both morphologically and molecularly identified. *P. aleophilum, Cladosporium* spp., *Valsa leucostoma* and *Armillaria mellea* were morphologically identified (Donati et al. 2020, Haleem et al. 2011, Lv et al. 2023, Zhang et al. 2014). Parallel to the

Table 4. The incidence and prevalence rates of disease agents detected in Düzce, Bartın, and Zonguldak provinces average value of 2014-2015 (%)

Surveyed area			***Phomopsis* spp. (*Diaporthe actinidae, D. nobilis*)**		***Pestalotiopsis* spp. (*Neopestalotiopsis vitis, N. egyptiaca*)**		***Alternaria* spp. (*Alternaria alternata, A. tenuissima*)**		***Colletotrichum gloeosporioides***	
District	**County**	**Village**	**IDG**	**IDC**	**IDG**	**IDC**	**IDG**	**IDC**	**IDG**	**IDC**
Düzce	Yığılca	Gürcü Çiftlik	100	100	-	-	-	-	-	-
	Akçakoca	Arabacı	42.86	41.18	28.57	41.18	21.43	17.65	14.28	11.76
			-		100		-		-	
		Kabalar	32	37.5	36	32.5	28	35	32	30
Zonguldak	Alaplı	Yeşilyurt	46.67		26.7		46.67		26.67	
	Ereğli	Elmacı	42.86	44.9	19.05	12.25	33.33	22.45	14.28	18.37
			46.67		6.67		20		13.33	
			46.15		7.69		7.69		30.77	
Bartın	Merkez	Terkehaliller	50	51.22	25	24.39	55	58.54	-	-
		Yanaz	50		20		60		-	
		Akçalı	54.54		27.27		63.63		-	

IDG: The incidence (=disease) rates of the agents in the examined gardens (%)
IDC: Prevalence rate of disease agents in counties (%)

data collected in the study, research carried out in various years in New Zealand identified *Sclerotinia sclerotiorum*, *B. dothidea* and *B. cinerea* (Pennycook and Samuels 1985), *Phomopsis* spp. and *B. cinerea* (Manning and Lallu 1997) as the agents responsible for fruit rot in kiwifruits.

Karakaya and Bayraktar (2009) reported that *B. cinerea* caused leaf blight in kiwifruit cultivation areas in the Western Black Sea Region. Mari et al. (2015) determined that the most common postharvest diseases in Italy were *B. cinerea, B. dothidea* and *D. actinidiae*.

The most common leaf fungal agents identified were *Alternaria alternata*, *Colletotrichum* spp., *Diaporthe (Phomopsis)* spp., and *Pestalotiopsis* spp. (Table 4). Hawthorne et al. (1982) identified *Alternaria, Colletotrichum, Diaporthe (Phomopsis)* and *Phoma* spp. as the most common agents in their survey study in New Zealand. L. Li et al. (2017) identified the most prevalent pathogens found in kiwifruit production areas in China during the 2014 and 2015 seasons as *Phomopsis* sp. 52.6%, *B. dothidea* 23.7%, *A. alternata* 13.2% and *Pestalotiopsis microspora* 10.5%. Mohamadalian et al. (2011) isolated *A. alternata, C. gloeosporioides, Alternaria dumosa, Epicoccum purpurascens, B. cinerea* and *Nigrospora oryzae* from the samples collected during the surveys, in order of prevalence.

Survey studies revealed that root and wood tissue pathogens were found at a negligible prevalence level (Table 4). The most common agents were *Diaporthe* spp. (anamorph *Phomopsis*), *Pestalotiopsis* spp., *Alternaria* spp., *Colletotrichum* spp. However, although the agents isolated from leaves and whose pathogenic activities were detected with a certain prevalence rate, it was determined that leaf symptoms in kiwi orchards appeared after August-September. However, since the fruits reached a certain maturity in this period close to harvest, it was observed that the leaf agents in question did not affect the yield and did not cause any yield loss. Therefore, it is thought that there is no need to control these pathogens in kiwi production areas.

This study reports, for the first time, the detection of *Alternaria tenuissima, Neopestalotiopsis spp., Neopestalotiopsis protearum, Neopetalotiopsis vitis, Colletotrichum gleosporoides, Phaeoacremonium aleophilum, Valsa leucostoma, Diaporthe actinidae, Diaporthe nobilis, Botryospheria dothidea* and *Armillaria mellea* in kiwi-growing areas of Türkiye.

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ÖZET

Batı Karadeniz Bölgesinde, Bartın, Düzce ve Zonguldak illerindeki kivi bahçelerinde, 2014-2016 yılları arasında, hastalıkların ve yaygınlıklarının belirlenmesi amacıyla sürveyler yürütülmüştür. Fungal hastalıkları tespit etmek amacıyla yaprak, gövde ve kök örnekleri toplanmış ve izolasyonlar PDA ve Su Agar besiyerleri kullanılarak yapılmıştır. Patojenisite testleri farklı teknikler kullanılarak yapılmıştır. Yaprak patojenleri için "koparılmış yaprağa inokulasyon yöntemi", meyve patojenleri için "meyve aşılama yöntemi", gövde patojenleri için "gövde aşılama yöntemi" kullanılmıştır. İzolatlar, patojenisite testlerinde elde edilen enfekte dokulardan tekrar izole edilmiştir. Patojenik bulunan izolatlar tür düzeyinde tanımlanmak için moleküler çalışmalarda kullanılmıştır. Moleküler çalışmalarda ITS gen bölgesinin dizi analizi için; DNA ekstraksiyonu, PCR ve jel elektroforezi yapılmıştır. BIOEDIT yazılım programı kullanılarak nükleotid dizileri NCBI web sitesinde analiz edilmiştir. Bu çalışmada teşhis edilen patojen funguslar; *Alternaria alternata* (Fr.) Keissl, *A. tenuissima* (Kunze) Wiltshire, *Colletotrichum gloeosporioides* Penzig, *Neopestalotiopsis* spp., *N. vitis* Jayawardena, Maharachch., Yan, Li & Hyde, *Diaporthe actinidiae* N.F. Sommer & Beraha, *D. nobilis* Sacc. & Speg., *Valsa leucostoma* (Persoon) Saccardo, *Botryosphaeria dothidea* (Mougeot) Cesati & de Notaris, *Phaeoacremonium aleophilum* (Tulasne & C. Tulasne) D. Gramaje, L. Mostert & Crous, *Cylindrocarpon macrodidymum* (Halleen, Schroers & Crous) L. Lombard & Crous, *Botrytis cinerea* Persoon *Cladosporium* spp. ve *Armillaria mellea* (Vahl) Kummer olarak bulunmuştur. Yaygınlık çalışmalarında ise en yaygın yaprak patojeni etmenleri *Alternaria* spp., *Colletotrichum gloeosporioides*, *Diaporthe* spp. ve *Pestalotiopsis* spp. olarak tespit edilmiştir.

Anahtar kelimeler: kivi, *Actinidia deliciosa*, toprak patojenleri, hastalık oranı

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