Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 30 (2), 547-559
Mustafa Kemal University Journal of Agricultural Sciences, 30 (2), 547-559
https://dergipark.org.tr/tr/pub/mkutbd
e-ISSN: 2667-7733

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Molecular identification and disease management of false smut disease (*Graphiola phoenicis*) on date palms grown in Türkiye

Türkiye'de yetişen hurma ağaçlarında yalancı sürme hastalığının (*Graphiola phoenicis*) moleküler tanımlanması ve hastalık yönetimi

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ARTICLE INFO

Article history:
Recieved / Geliş: 06.03.2025
Accepted / Kabul: 21.05.2025

Keywords:
Graphiola phoenicis
Date palm
False smut
Fungicides
Türkiye

Anahtar Kelimeler:
Graphiola phoenicis
Hurma ağaçları
Yalancı sürme
Fungusit
Türkiye

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ABSTRACT

The date palm (*Phoenix dactylifera* L.) is a common ornamental tree in Turkey, frequently found in homes, hotels, and public gardens. This study aimed to molecularly identify *Graphiola phoenicis*, the causal agent of false smut in date palms grown in Turkey, and to determine the efficacy of fungicides in controlling the disease. Characteristic false smut symptoms were observed on date palms during 2020–2021 surveys in Adana. These included numerous cup-shaped black fruiting bodies (sori) that appeared on theauricles and the rachis of the fronds, releasing whitish filaments with powdery yellow spores. Molecular identification of the pathogen was carried out based on the internal transcribed spacer (ITS) region and the large subunit of ribosomal DNA (LSU rDNA). NCBI accession numbers assigned to the ITS sequences are PQ060144 and PQ060145, as well as PQ060146 for the LSU rDNA. Under field conditions, four fungicidal treatments—Boscalid (26.7%) + Pyraclostrobin (6.7%), Prothioconazole (175 g/L) + Trifloxystrobin (88 g/L), Copper oxychloride (700 g/L), and Tea tree oil (222.5 g/L)—were evaluated for disease control. In a two-year trial, a combination of Boscalid + Pyraclostrobin was the most effective, reducing disease incidence by 90.40% and 88.48% in consecutive years. This study not only provides the first molecular characterisation and confirms the presence of *G. phoenicis* in Türkiye but also proposes an effective fungicide strategy for its control in ornamental date palms.

ÖZET

Türkiye'de yaygın bir süs ağacı olan hurma ağacı (*Phoenix dactylifera* L.) sıklıkla evlerde, otellerde ve halka açık bahçelerde bulunur. Bu çalışmanın amacı, Türkiye'de yetiştirilen hurma ağaçlarında yalancı sürme hastalığına neden olan *Graphiola phoenicis*'i moleküler olarak tanımlamak ve fungisitlerin hastalığı kontrol etmedeki etkinliğini belirlemektir. Bu kapsamda Adana ilinde 2020-2021 yılları arasında yapılan surveyler sırasında, hurma ağaçlarında yalancı sürme hastalığının karakteristik belirtileri gözlenmiştir. Hastalık belirtileri gösteren ağaçların yaprak ve orta damarında çok sayıda fincan şeklinde siyah meyve gövdeler (sori) ve bu yapılardan beyazımsı ipliksi, toz halinde sarı renkli sporlar gözlenmiştir. Patojenin moleküler tanımlaması, internal transcribed spacer (ITS) ve large subunit ribosomal DNA (LSU rDNA) gen bölgeleri temel alınarak gerçekleştirilmiştir. Fngal etmene ait ITS (PQ060144 ve PQ060145) ve LSU rDNA (PQ060146) gen bölgelerinin dizilerinin Gen Bankası (NCBI) erişim numaraları alınmıştır. Tarla koşulları altında, dört fungisit uygulaması - Boscalid (%26.7) + Pyraclostrobin (%6.7), Prothioconazole (175 g/L) + Trifloxystrobin (88 g/L), Bakır oksiklorür (700 g/L) ve Çay ağacı yağı (222.5 g/L)- hastalık kontrolü için değerlendirilmiştir. İki yıllık bir denemede, Boscalid + Pyraclostrobin kombinasyonu, hastalık insidansını ardışık yıllarda %90.40 ve %88.48 oranında azaltarak en etkili yöntem olmuştur. Bu çalışma, *G. phoenicis*'in Türkiye'de yetişen hurma ağaçlarında hastalık etmeni olduğuna yönelik ilk moleküler çalışma olup, aynı zamanda süs hurma ağaçlarında hastalığın kontrolünde etkili bir fungisit uygulama stratejisi de önermektedir.

Cite/Atıf Lavkor, I. (2025). Molecular identification and disease management of false smut disease (*Graphiola phoenicis*) on date palms grown in Türkiye. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 30* (2), 547-559. https://doi.org/10.37908/mkutbd.1652986

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is widely grown as an ornamental, shade plant in parks, gardens and rural areas throughout Turkey. In addition, *Phoenix dactylifera* has become a major economic crop in the world, providing significant income and nutritional benefits. However, various biotic and abiotic stresses often hampered its cultivation, including fungal diseases. Among these diseases, *Graphiola phoenicis* (Mougeot) Poiteau, commonly known as Graphiola leaf spot or false smut, poses a significant threat to date palm cultivation (Sinha et al., 1970; Chohan, 1972). *G. phoenicis* has been the subject of studies and reports from various locations worldwide, including the United States (Simone, 2004), Qatar (Abbas & Abdulla, 2004), Libya (Edongali, 1996), the Canary Islands (Cabrera et al., 1990), Yemen (Sattar et al., 2013) and India (Guar, 2000). It is the most typical disease worldwide and is found wherever date palms are grown in wet environments - mainly in developing at the edge of the regions (Mediterranean coasts) and also within the southern wet areas of Mali, Niger, Senegal, andmose Mauritania. The disease is most common in Florida, although as hosts for the fungus, some species of palm have been identified, including *Phoenix canariensis* (Canary Island date palm), *Phoenix roebelenii* (pygmy date palm), and *Phoenix dactylifera* (date palm). It is rarely found on *Phoenix sylvestris* (wild date palm) (Signh et al., 2023).

G. phoenicis is a fungus that mainly attacks the leaves of date palms, forming characteristic dark brown to black spots on leaf surfaces. Severe infections can result in defoliation, reduced photosynthetic efficiency, and ultimately decreased fruit yield and quality. Subepidermal patches on both sides of the auricle and the rachis are characteristic of the disease. On old fronds, there is an abundance of small black sori, and fructiflora. Sori are more common in the apical regions of the pinnae and are 1-3 mm in diameter. Sori are similar to those of scale insects, but under the microscope, they are whitish filaments with powdery yellow spores. The spores occur spherical to ellipsoidal and are characterised by a smooth hyaline wall and the diameter of the spores ranges from 3 to 6 µm. The disease is endemic to regions characterizsed by high humidity levels where the cultivation of date palms is prevalent, manifesting in instances where date palms are cultivated under such conditions. Conversely, the absence of the disease has been observed in regions exhibiting lower humidity levels, suggesting potential factors contributing to its epidemiology (Djerbe, 1983; Guar, 2000; Zaid et al., 2002; CAB International, 2003; Abbas & Abdulla, 2004; El Deeb et al., 2007; El Gariani et al., 2007; Haldhar et al., 2017).

The species of Graphiolaceae are classified within the Ustilaginomycota, a taxonomic category of fungi. These organisms are distinguished by their capacity to parasitically thrive on palms, the configuration of their basidia (which assemble into a chain of cells), and the manner in which the spores of *Graphiola* spp. undergo division into myxomycete. This organism was subsequently identified by Mougeot and other workers as belonging to a number of different fungal classification systems. It was first categorised as a Discomycete, a rust, a Pyrenomycete and a smut-like fungus. Following a further refinement of these classifications, it was placed within the Hypomycetes and ultimately the Exobasidiales under the Basidiomycota. This fungus belongs to the Heterobasidiomycetes and is classified within the distinct order, Graphiolales. Based on ontogenetic and karyological similarities to the Ustilaginales, *G. phoenicis* is phylogenetically related to the smuts, but through steps of evolution, changes occur in distinct morphogenetic characters which separate this species from other smuts. However, it is important that despite this relationship, changes in distinct morphogenetic characteristics have occurred at various steps of evolution, thus distinguishing this species from other smuts (Pal, 2019).

Severe infection has been demonstrated to have a detrimental effect on tree growth and yield, with the result that leaves may die prematurely. To eliminate the risk of the pathogen, it is recommended to prune the infected leaves and burn them to prevent new infections (Zaid et al., 2002). The practice of pruning leaves in conjunction with the administration of a Bordeaux mixture or a broad-spectrum fungicide (mancozeb, copper(II) hydroxide, copper(II) hydroxide in conjunction with maneb, or copper(II) oxychloride and copper(II) chloride, maneb plus zineb) has been reported as a control measure for field-grown palms (*Sabal* spp.). Such a measure involves the application of three to four treatments on a 15-day schedule following the onset of sporulation (Lodha, 2003). Management of *G. phoenicis* mainly relies on cultural practices and chemical control measures, with fungicide applications being a primary strategy for disease management. However, the efficacy of the fungicides used may depend on several factors such as determination of fungal and bacterial microbiotas and their densities, formulation, application timing, dosage, and the development of fungicide resistance in the pathogen population (Uysal et al., 2022). Therefore, it is essential to evaluate and assess the efficacy of various fungicides to develop efficient disease management strategies. The dearth of available literature on the chemical control of *G. phoenicis* is evidenced by a paucity of research and limited available information. For this reason, this study was conducted to evaluate the effectiveness of various fungicides against leaf spots in date palms. Fungal disease management is a safe alternative to minimize the incorrect use of pesticides and reduce production costs.

The study aimed to manage the prevalence of false smut through the application of fungicides while also undertaking a comprehensive evaluation of their susceptibility to this particular disease within the geographical boundaries of Adana.

MATERIALS and METHODS

Collection and morphological detection of fungal structures

Samples were taken from diseased symptomatic leaves of *P. dactylifera* palms in Adana (36.996265, 35.336898), and randomly selected to ensure representativeness in 2020-2021. The lesions of occurrence have been documented as either isolated instances or in clusters, manifesting on both the adaxial and abaxial sides of the leaves. Morphometric investigations and micrometric leaf sections for light microscopic observations were carried out on samples of diseased leaves under a scanning electron microscope (Pal, 2019).

Molecular identification

Dark lesions on foliar pinnae were collected to isolate Fungal DNA for molecular analysis. The lesions were then cut into 5 mm long pieces for DNA isolation. Afterwards, they were surface sterilized with a 2% NaOCl solution for approximately 3 minutes, followed by thorough washing with sterilized water. The sterilized pieces were transferred to a tube containing 20 ml of pure water. The mixture underwent rigorous agitation for one hour and was centrifuged for 5 minutes. The mixture was vigorously shaken for 1 hour and vortexed for 5 minutes. The residual liquid was disposed of, and the resultant pellet was subjected to a process of centrifugation at a speed of 1,000 × g for a period corresponding to one minute, using an amount of 70% ethanol in volume for 10 minutes at 10000 rpm. The resulting pellet was then subjected to a process of air-drying, spanning a duration of one to two hours (Er & Akgül, 2021).

The DNA extraction was performed successfully using the Fungi/Yeast Genomic DNA Isolation Kits (Norgen Biotek Corp.) following the stipulated directives provided by the manufacturing authority. The ITS region, alongside the D1/D2 domain of the large subunit ribosomal DNA 28S (LSU rDNA), was subjected to amplification through the utilization of specific primers, designated as ITS1-4 (5'-TGAACCTGCAGAAGGATCATTA-3' and 5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990; Barnes & Szabo, 2007) in addition to NL1m and NL-4m primers (5'-GCATATCAATAAGCGGAGGAAAAG-3' and 5'-GGTCCGTGTTTCAAGACG-3') (O'Donnell, 1993). The protocol of amplification was comprised of an initial denaturation stage at 94°C for five minutes, followed by 35 cycles of denaturation at 94°C for one minute, annealing for one minute at 55°C and extension for one minute at 72°C, and a final extension at 72°C for two minutes. A quantity of 2 µL of the PCR product was loaded onto a 1% agarose gel (Oğuz et al., 2024). Subsequently, the amplification products were dispatched to Medsantek for purification and DNA sequencing purposes.

A comparison was made between the aligned sequences and the database at GenBank, a division of the National Center for Biotechnology Information (NCBI), employing the basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/). The present study employed MEGA version 6.0 to conduct both the necessary sequence alignment and subsequent analysis (Tamura et al., 2013). The maximum likelihood algorithm was utilized in the undertaking of the phylogenetic reconstruction. The robustness of the branches was determined using 1,000 replicates from a bootstrap analysis (Felsenstein, 1985).

Pathogenicity test

In this study, procedures for the inoculation with Graphiola spore culture and incubation requirements were modified as described by Parveen et al., (2015) and Manu et al., (2017).

Sterilization with 70% ethanol followed by washing with sterile distilled water was performed on plant material collected from the diseased surface of the date palm. Black fruiting bodies were harvested from the diseased date palm leaf area, suspended in sterile distilled water, and kept in a shaker (125 rpm) for 2 days. The sori produced mature yellow spores. The black bodies produced rise to short, pale, filamentous structures. Small strips of 1-2 cm^2 were cut from healthy leaves of 10-year-old date palms. The leaves were washed in sterile distilled water for 10 minutes and then surface sterilized by soaking in 1% NaOCl for 5 minutes a triple rinse is necessary, with the water used for this purpose being sterile and distilled. Subsequently, the leaves were placed on %5 water agar in petri plates. Leaves were injected with 1 ml suspension containing 10^4 spores ml^{-1}. Control samples were inoculated with pure distilled water. The petri dish was hermetically sealed with parafilm before being subjected to a temperature of 37 ± 1 °C and an ambient humidity level of 85% for 60 days then assessed for infection. The frequency and size of necrotic lesions on the infected leaves were recorded after 15 of infection. Koch's postulates were evaluated by recovering sori-inoculated spores from Adana-collected sori.

Determining the efficacy of fungicides on disease occurrence

The study was conducted in 2020/2021 on naturally infected date palm cultivars with *G. phoenicis* in Adana. Ten-year-old trees were selected. The experiment was designed using a randomised block-design approach, which was then replicated three times to ensure robust statistical analysis. Three trees for each block were randomly selected. A total of 15 trees were used in an experiment. The treatments were conducted in natural field conditions, with one tree kept as one replicate. This study was conducted to investigate the efficacy of four fungicides in the management of date palm leaf spot. Table 1 shows the fungicides used to control date palm leaf spot. 26.7 % boscalid + 6.7 % pyraclostrobin, 175 g/l prothioconazole + 88 g/l trifloxystrobin and 222.5 g/l tea tree oil are used for chemical control of smut. Copper oxychloride is also useful in controlling the fungal growth of *Graphiola phoenix*.

Table 1. The fungicides against false smut disease in date palms and their properties

Çizelge 1. Hurma ağaçlarında yalancı sürme hastalığına karşı kullanılan fungisitler ve özellikleri

Fungicides	Company	Active Ingredient	Chemical Group	Application Dose g/ 100 L
Signum WG	BASF	26.7 Boscalid% + 6.7 % Pyraclostrobin	Pridine-carboxamides + Methoxy-carbamates	150
Madison® SC 263	BAYER	175 g/l rothioconazole + 88 g/l Trifloxystrobin	Triazole+ Strobilurin	100
ZZ-Cuprocol SC	Syngenta	700 g/l Copper oxychloride (metallic copper)	Inorganic compound	300
Timorex Gold	Nufarm	222.5 g/l Tea tree oil	Terpene hydrocarbons, terpene alcohols and Terpene phenols	700

To address the issue, the management of the plants in question underwent a process referred to as pruning, which entailed the removal of leaves that had been infected. In addition to this, a process referred to as spraying was initiated every 15 days from July to September with Boscalid + Pyraclostrobin, Copper oxychloride, Prothioconazole + Trifloxystrobin, and Tea tree oil applied. In addition, a control treatment was implemented, which entailed the application of water to the plants at equivalent intervals. All plants in the trial received a total of six fungicide sprays for disease control.

The disease on the leaves was evaluated on four compound leaves on each of the four sides of the tree when approximately 20% of small yellow lesions turned dark brown. Infected leaves were selected 2^{nd} and ^{3rd} year old leaves and marked for disease observation. To calculate the disease severity (DS), a 0-5 scale; zero indicates a state of absence of infection, one indicates a 1-10% percentage, two indicates an 11-25% percentage, three indicates a 26-50% percentage, four indicates a 51-75% percentage, and five indicates a 76-100% percentage. The infection rate for each leaf was recorded (Metha et al., 1989). The DS was calculated using scale scores according to the Townsend-Heuberger formula, an analysis of variance was conducted and the mean values of the treatments were statistically compared, employing the least significant difference (LSD) method to identify any statistically significant variations (LDS).

Observations on the development of Graphiola leaf spot in the form of sori and disease severity (%) were recorded before every spray and 15 days after the last spray on 2nd and 3rd-year-old leaves on each side of each tree, which were randomly selected and tagged for disease observation. Disease severity percentage (DS) was determined using this formula:

$$DS\ (\%) = \left(\sum (\text{Disease scala} \times \text{Number of Leaves}) / (\text{Total Number of Leaves} \times \text{Maximum Disease scala})\right) \times 100$$

The percentage of disease control (DC) was determined through the utilisation of the following formula (Lodha, 2003):

$$DC\ (\%) = \frac{\%\text{infection in the final observation} - \%\text{infection at the initial observation}}{\%\text{disease infection at the initial observation}} \times 100$$

Statistical analysis

The analysis of the data was conducted using a two-way analysis of variance (ANOVA) with treatment as the independent variable. The use of the LSD (Least Significant Difference) test was imperative in achieving the separation of the means at the 5% level of significance. The collected data were statistically analysed using the JMP program.

RESULTS AND DISCUSSIONS

Morphological identification

The initial symptoms are characterised by the presence of diminutive yellow lesions that subsequently become discoloured, with a dark brown hue manifesting at the centre and blurred edges. This condition primarily affects the leaves that are the most mature. The presence of lesions manifests either in isolated instances or in the form of clusters, with such lesions being observed on both sides of the leaves. The foliar pinnae exhibit minor yellow to dark lesions on both sides of the leaf blade, accompanied by brown to black sori that are globular, cylindrical or irregular in shape (Figure 1A). Sori are defined as fruiting bodies with an average diameter of between 0.5 and 1.2 millimetres. They originate from beneath the epidermis and are characterised by a dark, hard exterior shell (Figure

1A). As the sori reach maturity, filaments emerge through the ostiole of each sorus. These filaments characteristically display a white to creamy hue and a thread-like appearance (Figure 1B-C).

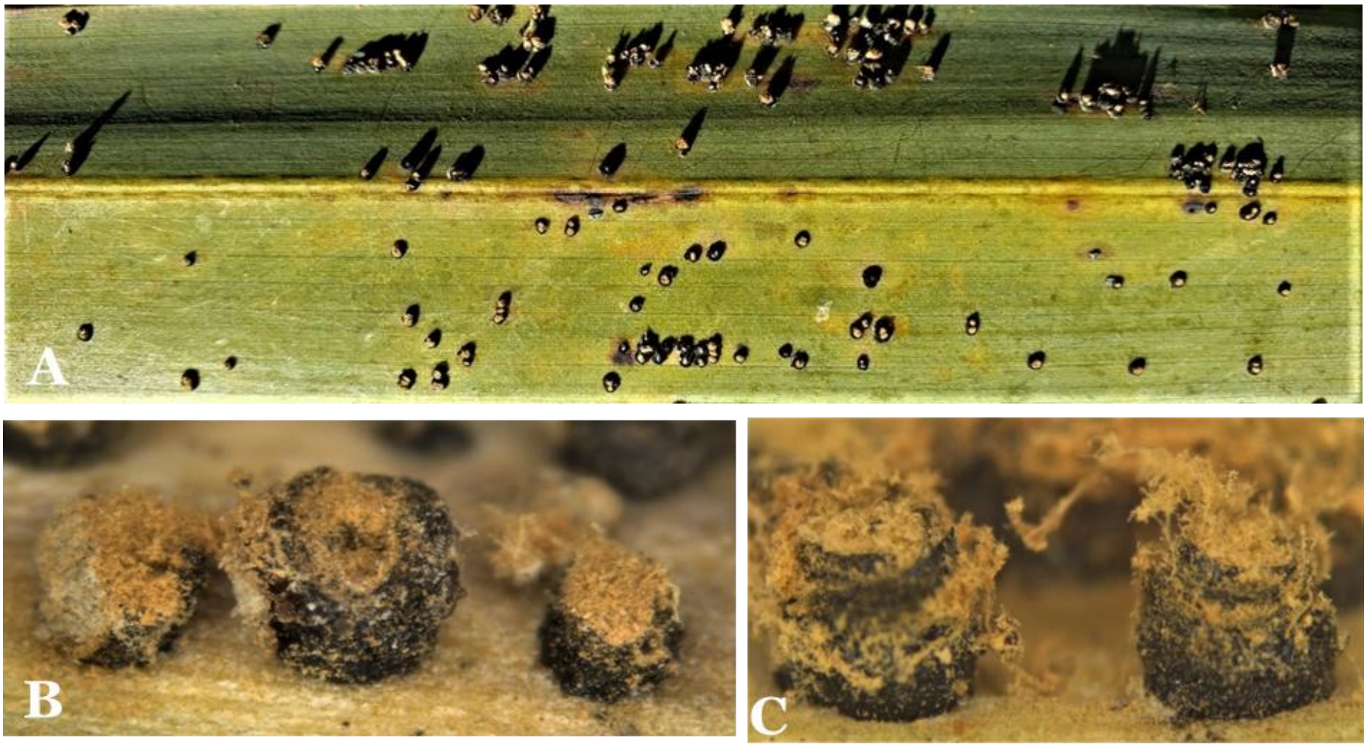

Figure 1. Reproductive structures of the false smut fungus (*Graphiola phoenicis*) on Date palms (*Phoenix dactylifera*) in Adana, Türkiye. (A: Heavy infestation of the false smut fungus on the surface of *P. dactylifera* leaves. B and C: Profile view of sorus, with abundant thread filaments protruding. Bars=1-1.5 mm.)
Şekil 1. Türkiye, Adana'da, hurma ağaçlarında (Phoenix dactylifera) yalancı sürme hastalığına neden olan fungusun (Graphiola phoenicis) üreme yapıları. (A: P. dactylifera yapraklarının yüzeyinde yalancı sürme hastalığının yoğun enfeksiyonu. B ve C: Sorus'un bol miktarda iplik liflerinin çıkıntı yaptığı profil görünümü. Kalınlık=1-1,5 mm.)

The morphological characteristics exhibited a high degree of consistency with the existing literature on *G. phoenicis* (Tubaki & Yokoyama, 1971; Cole, 1983, Selpulveda et al, 2017; Pal, 2019; Singh et al., 2023).

Molecular identification

PCR amplification and sequencing of ITS 1-4 and LSU rDNA from Adana isolates were performed to verify the morphological identity of *G. phoenicis*. Fragments of 517 bp and 560 bp were obtained for ITS and LSU rDNA, respectively. These fragments have been deposited in GenBank (accession numbers PQ060144, PQ060145 and PQ060146).Blast analysis using ITS 1-4 sequences showed 99.80% similarity between the Adana isolate and *G phoenicis* from Chile (KX344499), 99.42% with Germany (KY424483), 98.66% similarity with the Netherlands and Taiwan (MH855106, MK336541), while the LSU rDNA sequences showed a similarity of 99.83% with *G. phoenicis* from the USA (AY818965), 99.64% with *G. phoenicis* from Chile (KX344500) and 99.50% with *G. phoenicis* from Brazil (GU566017). Further analysis based on the principle of phylogenetic analysis confirmed the existence of a distinct Graphiola cluster which received support from ITS 1-4 analysis of 82% of participants. Among the *Graphiola* spp., the SILI variant demonstrated the most significant degree of resemblance to *G. phoenicis*, supporting its classification with this species (Figure 2). Figure 3 indicates a close relationship (99%) between the DNA sequences of the LSU rDNA regions and all isolate sequences. A bootstrap value of 99% provides support for the Graphiolales and the Exobasidiales (Figure 3).

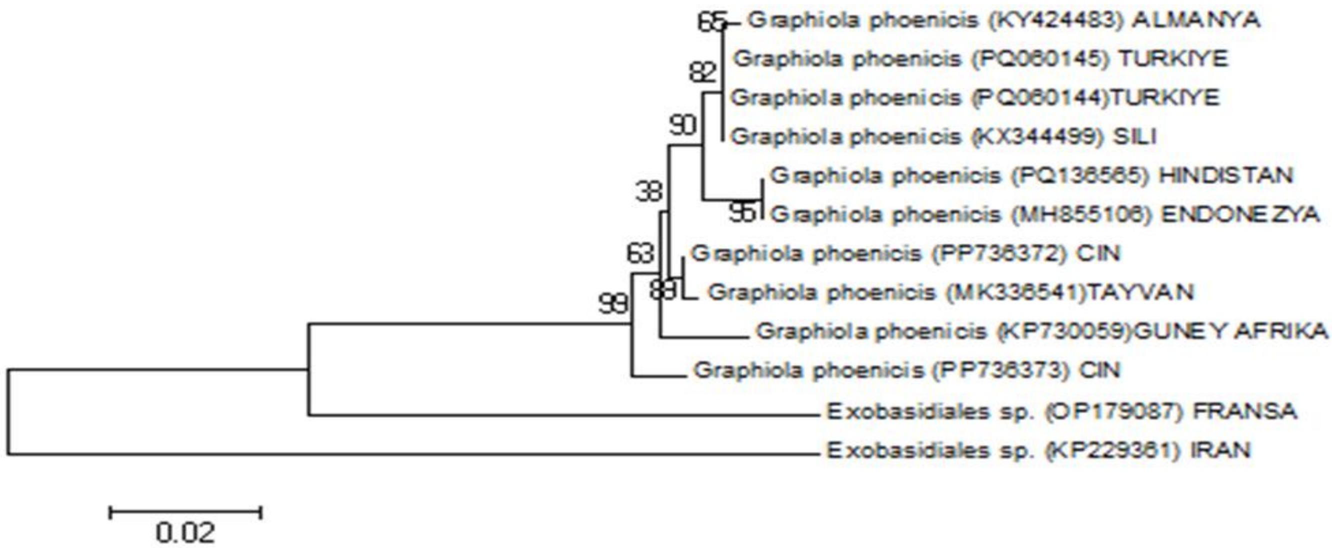

Figure 2. Phylogenetic tree based on ITS region of *Graphiola phoenicis* strain and nearest relatives

Şekil 2. Graphiola phoenicis izolatının ITS bölgesine dayalı filogenetik ağacı

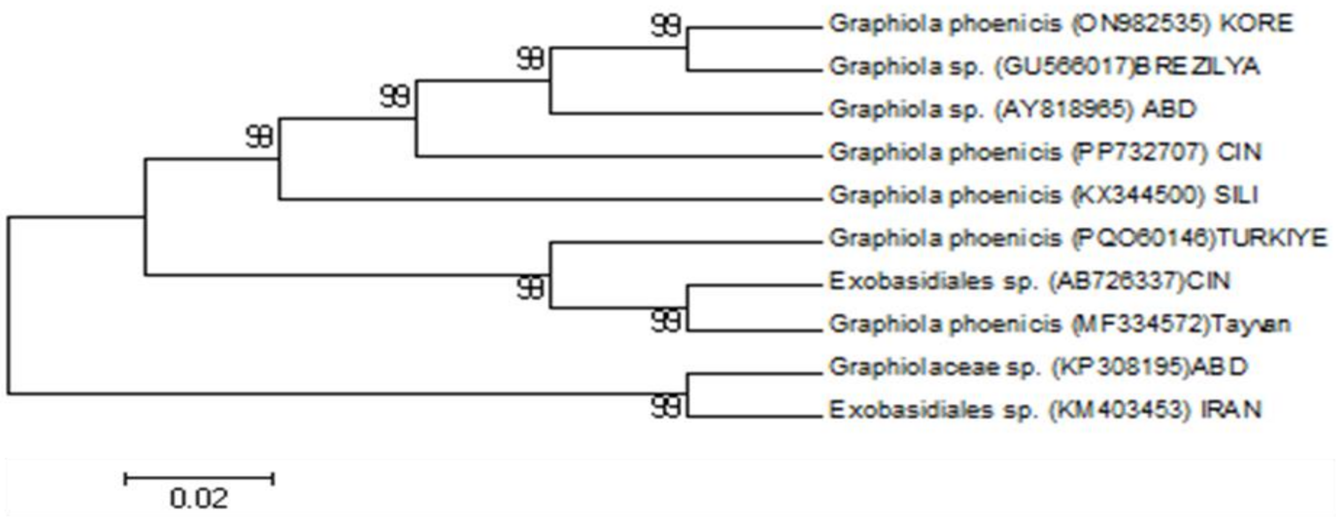

Figure 3. Phylogenetic tree indicating the relatives of *Graphiola phoneix* isolates to NCBI

Şekil 3. NCBI'a göre Graphiola phoneix izolatlarının akrabalarını gösteren filogenetik ağaç

There are complex similarities between the Exobasidiales and the Graphiolales. *Graphiola phoenix* represents the Graphiolales and Exobasidiales (Begerow et al., 1997). Blast analysis shows that the ancestor of Graphiolaceae evolved from palms, the first extant monocotyledonous family, and may represent the Exobasidiomycetidae within the Ustilaginomycotina. Some taxonomists have included the palm parasitic family Graphiolacaceae in Ustilaginales, for example, Oberwinkler et al. (1982). In the new order Graphiolales Donk ex. Oberwinkler and Bandoni classified the Graphiolaceae as basidiomycetes (Vanky, 1987). Recent molecular data suggest that although the species lack the thick-walled ustilospores common to the Ustilaginales, the Graphiolaceae are closely related to the smuts (Hawksworth et al., 1995).

The findings conclusively identified *G. phoenicis* also commonly known as a member of the basidiomycete fungus family, as being responsible for causing false smut on Canary date palm trees in Adana. According to Piepenbring (2012), *G. phoenicis* is known to be a plant pathogen affecting various palm species globally. For instance, there is evidence that *P. roebelenii* has been found in Argentina (Cúndom, 2009) and Florida (Martinez, 1966). Similarly, *P. dactylifera* has been identified in Brazil, Egypt, India, Kenya, Libya (Edongali, 1996) and Qatar (Abbas & Abdulla, 2004). There is evidence to suggest that this species was first introduced into glasshouses in the cooler climates of countries such as Belgium, Denmark, England, France, Germany, Hungary and the Netherlands as an ornamental plant, following the introduction of the *P. canariensis* (Pal, 2019).

The fungus under consideration is known to thrive in conditions characterised by levels of moisture in the atmosphere and an abundance of dense foliage. This constitutes the first recorded instance of *G. phoenicis* on Phoenix canariensis in the Mediterranean region of Turkey. For molecular analysis of this basidiomycete, we utilized ITS and LSU rDNA sequences, ultimately focusing on LSU rDNA due to its widespread recommendation for identifying rust fungi at both genus and species levels (Hyde et al., 2014). Blast analysis of ITS and LSU rDNA sequencing positioned the strain isolated from Adana, within a similarity of *G. phoenicis*. As previously noted by Piepenbring et al. (2012), a comparable cluster was previously identified.

Based on these findings, we can confidently conclude that the strain that has been isolated from the eastern Mediterranean and is affecting the date palms of the Canary Islands in Adana belongs to *G. phoenicis*. It is anticipated that further findings and new records of Graphiola will be detected with more detailed studies.

Pathogenicity test

Results from 60 days after inoculations with spores from sori were characterised by round or oval spots on leaves, varying in colour from yellowish brown to black and measuring 0.5-1 mm. These spots were surrounded by dark brown rings on the leaf from which the fungus emerged and broke down the leaf surface (Figure 4). This method was repeated to induce spore germination and inoculate *P. dactylifera* leaves, confirming *G. phoenicis* as the causal agent of the symptoms.

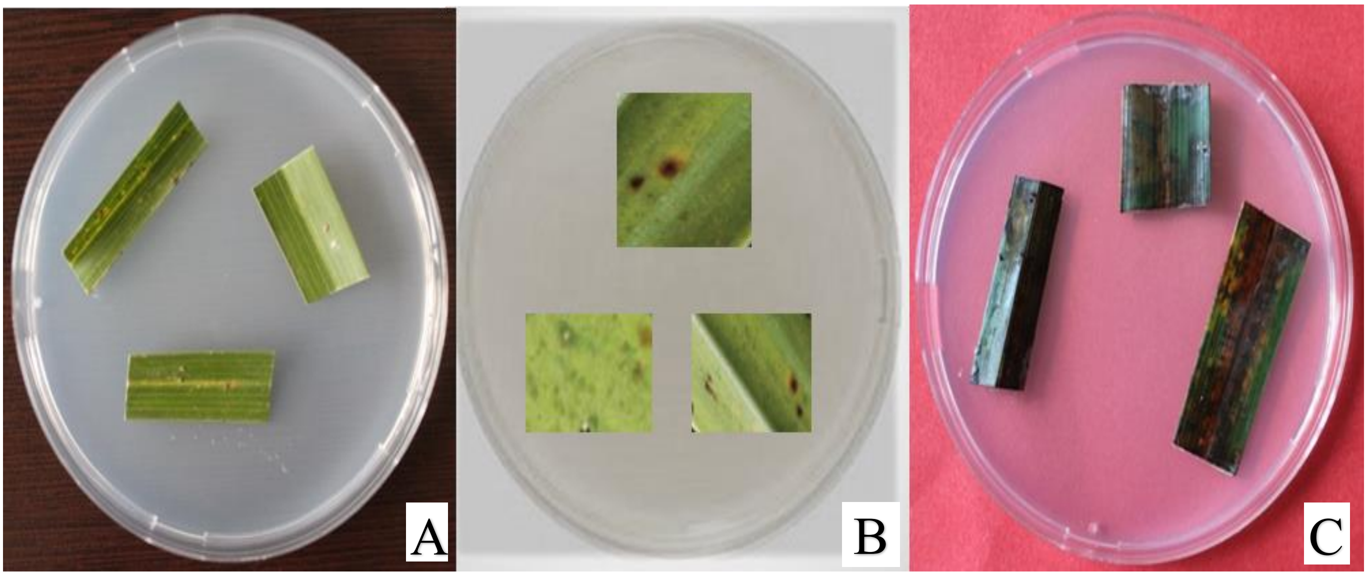

Figure 4. Pathogenicity of *P. dactylifera* leaf with the spore of *G. phoenicis* evolution. A: 30 days after, B: 45 days after, C: 60 days after inoculations with spores.(Credits: I. Lavkor)

Şekil 4. P. dactylifera yaprağının G. phoenicis sporları ile patojenitesi. A: inokülasyondan 30 gün sonra, B: 45 gün sonra, C: 60 gün sonra. (Referans: I. Lavkor)

The spores from sori recovered from inoculations produced infectious, thus verifying Koch's postulates. There were no apparent differences in the percentage of infected leaves, statically.

Fungicide assessments

Four fungicides were used over two years. Observations (Figure 5) showed that 26.7% boscalid + 6.7% pyraclostrobin gave maximum disease control (90.40-88.48%), which was closely followed by 175 g/l prothioconazole + 88 g/l trifloxystrobin (81.36-77.46%). In general, statistical analysis of the data showed that 70.64% and 58.94 % of patients treated with pyraclostrobin and 175 g/L prothioconazole + 88 g/L trifloxystrobin outperformed the remaining chemistries in terms of disease control in both 2020 and 2021. 700 g/l Copper oxychloride and 222,5 g/l Tea tree oil behaved equally during both years.

Double-active fungicide (26.7% boscalid + 6.7% pyraclostrobin, 175 g/l prothioconazole + 88 g/l trifloxystrobin) was more effective than a single-active fungicide (copper oxychloride, tea tree oil) in controlling Graphiola leaf spot in the field and reducing disease severity (Figure 6). The fact that 700 g/l copper oxychloride and 222.5 g/l tea tree oil are in contact fungicides, whereas 175 g/l prothioconazole + 88 g/l trifloxystrobin are systemic fungicides, may explain the observed differences in the behaviour of these fungicides.

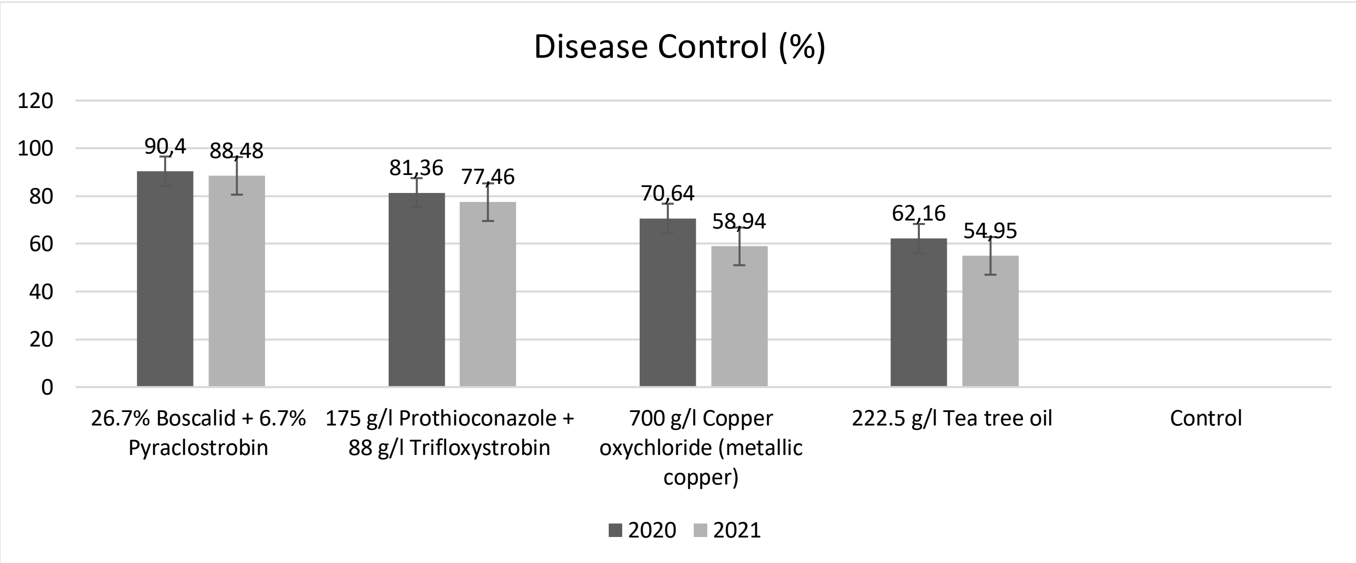

Figure 5. *Disease control of different fungicides against false smut disease on date palms in the field conditions*
Şekil 5. Farklı fungisitlerin tarla koşullarında hurma ağaçlarında yalancı sürme hastalığına karşı hastalık kontrolü

The results of this study are corroborated by some of the findings of the researchers' management. There is little information in the literature on controlling leaf spots on date palms. However, it has been demonstrated that the application of a Bordeaux mixture following the pruning of infected pinnae is an effective method of treatment (Nixon, 1957; Chohan, 1972). Nonetheless, there is some evidence that for various leaf spot diseases, such as *Graphiola phoenix*, the combination of copper oxychloride, carbendazim and mancozeb is more effective (Lodha, 2003). Carbendazim applied as a foliar spray (Bavistin) and benomyl (Benlate, 0.2%) was suggested by Gupta and Mehta (1985) as a control measure. But, as posited by Raj Bhansali (1987), Mancozeb (Dithane M-45, 0.2 %) and copper oxychloride (Fytolan, 0.2 %) constitute the most prevalent chemicals employed for the management of the disease, exhibiting efficacies of 71.20% and 65.20%, respectively, when administered through four applications at 15-day intervals under Jodhpur conditions. Furthermore, Mehta et al. (1989) found that the effectiveness of carbendazim was effectively controlled, whereas the results with mancozeb were not consistent. Moreover, in the EU and our country, carbendazim and maneb are banned fungicides.

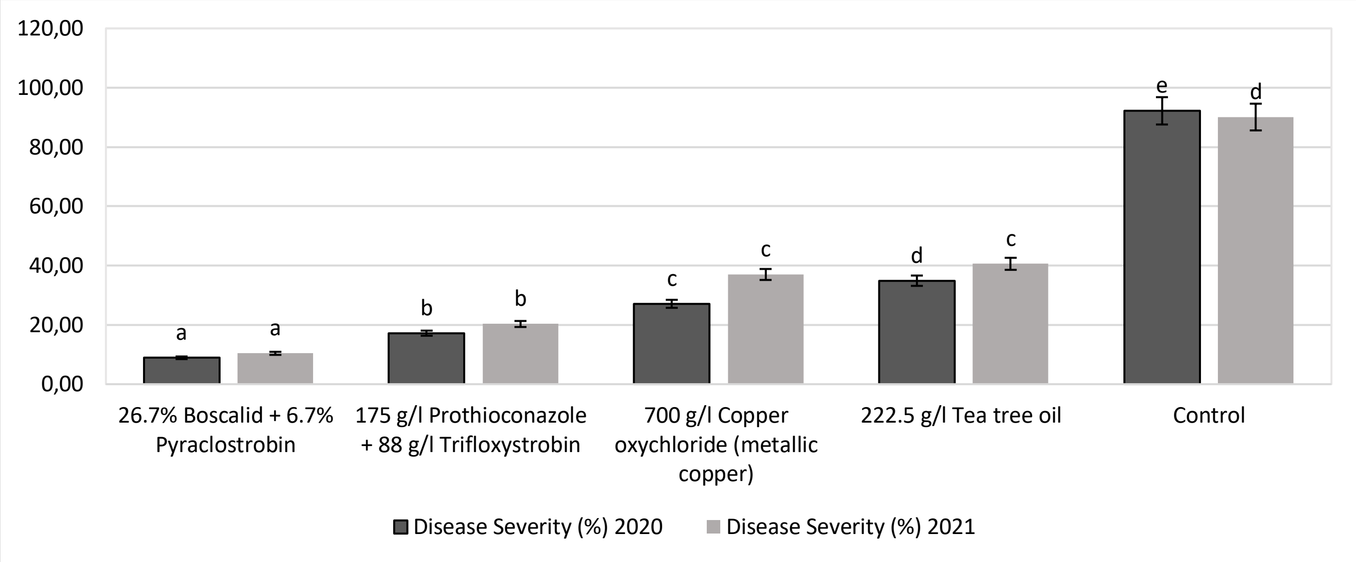

Figure 6. Effect of different fungicides on disease severity against false smut disease in date palm trees under field conditions. The same letters are similar in terms of statistical significance level by Tukey's multiple range test at $p< 0.05$

Şekil 6. Tarla koşullarında hurma ağaçlarındaki yalancı sürme hastalığına karşı farklı fungisitlerin hastalık şiddetine etkisi. Aynı harfler, Tukey'in çoklu aralık testi ile p< 0,05'te istatistiksel önem düzeyi açısından benzerdir

In vitro antifungal effect of different concentrations of six fungicides, fluazinam (0.001-0.015 µg/ml), thiophanate-methyl (0.1-10.0), boscalid + pyraclostrobin (1.0-50.0 µg/ml), trifloxystrobin (0.5-50.0 µg/ml), kresoxim-methyl (0.1-50.0), and tebuconazole (0.5-50.0 µg/ml) were also evaluated against walnut stem canker disease agent *Botryosphaeria dothidea* (Kurt et al., 2020). According to EC_{50} values, fluazinam (0.002 µg/ml) was recorded as the most effective fungicide and it was followed by thiophanate-methyl (0.275 µg/ml), tebuconazole (0.994 µg/ml), boscalid + pyraclostrobin (3.993 µg/ml) and trifloxystrobin (4.262 µg/ml).

Graphiola infection occurs year-round on date leaves, and the disease's spread varies with the leaf's age and position In regions where Graphiola leaf spot is a serious disease of date palms, these findings may be of practical use. There is a need to develop new strategies to create reliable, environmentally sustainable and effective approaches to managing this disease.

ACKNOWLEDGEMENTS

This study was financially supported by the Ministry of Agriculture and Forestry, General Directorate of Food and Control of the Republic of Türkiye. I would like to thank Deda BÜYÜKÖZTÜRK, Okan ÖZGÜR, Tahsin AY and Şakir ŞAHİN for their support of this study.

STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest with anybody.

STATEMENT OF ETHICS CONSENT

This article does not require ethical approval as there are no experiments with human or animal subjects.

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