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FUSARIUM DIMERUM EFFECT ON CHLOROPHYLL CONTENT AND MORPHOLOGICAL AND ANATOMICAL TRAITS OF DATE PALM

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SUMMARY

This experiment transpired to determine the effect of the fungus *Fusarium dimerum* on the chlorophyll content and some phenotypic and anatomical morphological traits of the leaves of date palms (*Phoenix dactylifera* L.). The study results showed the ability of the fungus *F. dimerum* to cause infection on date palm leaves of five cultivars. The findings revealed that *F. dimerum* can secrete the enzymes cellulase, phenol oxidase, lipase, and amylases, as the area of their enzyme activity reached 6.1, 5.8, 6.2, and 12.4 mm, respectively. The results of histological analysis of the infected leaves also indicated the presence of *F. dimerum* spores in tissues of the infected leaves and the decomposition of cell walls and the loss of their integrated shape. The experiment showed significant differences in the chlorophyll content in healthy leaves and those infected with the fungus *F. dimerum*, wherein the percentage decreased in leaves infected with the fungus compared with the control treatment (healthy leaves).

Keywords: Date palm (*P. dactylifera* L.), *Fusarium dimerum*, histological anatomy, chlorophyll content in leaves, enzymes, fungal disease

Key findings: The results of the pathogenicity test for the fungus *F. dimerum* on the leaves of five cultivars of date palms (*P. dactylifera* L.) showed the ability of the fungus *F. dimerum* to cause infection and its ability to secrete the enzymes cellulase, phenol oxidase, lipase, and amylases. The ability to secrete enzymes plays a primary role in causing infection in the date palms**.**

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INTRODUCTION

The cultivation of the date palm (*Phoenix dactylifera* L.) is one of the most vital economic issues in the dry areas of the Middle East and North Africa. The date palm (*Phoenix dactylifera* L.) occupies a distinguished position economically, especially in the agricultural sector. The date palm is a flowering plant belonging to the palm family Arecaceae, grown for its edible fruits (Ibrahim *et al*., 2018). The nutritional value of dates is the highest compared with other fruits for calories, proteins, carbohydrates, fiber, calcium, potassium, phosphorus, and iron, such as apples, oranges, grapes, and bananas (Al-Adeeb, 2021).

The date palm leaves continue to prevent the fungi. The presence of the fungi on newly growing date palm leaves or on palm trees mainly negatively affects the growth rate and the lack of flowering. The result of the effect of these fungi on the green area of leaves is crucial in the photosynthesis process of the date palm (Djerbi, 1983; Al-Akaidy, 1994). In previous years, many diseases existing before high school, such as palm leaf spot disease, have spread (Al-Zubaidi, 2005). Many recorded fungi specific to date palm leaf spot disease included *Alternaria alternata*, *A. tenuissima*, *Mycosphaerella tassiana,* and *Stemphylium* sp. The *Cladosporium cladosporioides, Drechslera* sp., and *Xylohypha nigrescens* comprised the causes of brown leaf spot disease on date palm leaves (Carpenter and Elmer, 1978; Elarios, 1989; Kassim *et al*., 1983; Jabr *et al*., 2003). The fungal species *Alternaria chlamydospora*, *Nigrospora oryzae*, *Epicoccum purpurascens,* and *Pestalotia* sp. also attained documentation as a cause of spot disease on date palm leaves for the first time in Iraq (Abbas *et al*., 2007). Ahmed (2011) stated that the fungus *A. radicina* was evident for the first time in Basra Governorate as a cause of black spot disease on date palm leaves.

The fungus *F. dimerum* has become a plant-pathogenic soil fungus. It has been noteworthy in different types of soil. The growth of the fungus reaches 90 mm in eight days of incubation at a temperature of 25 ± 2 °C. The fungal colony form on PDA (potato dextrose agar) media is white at the beginning of growth and then turns to gray. Greenish-white mycelium spreads aerially. Macroconidia are similar to conidia, small and blunt, with a curved or foot-shaped end. Conidia are solitary or in the form of a multi-faceted chain. The conidia could be divided or undivided and may have up to 2–3 cells. Their dimensions are 2–3 microns in width and 12.0–16.5 microns in length (Booth, 1971; Nelson *et al*., 1983). The fungus prevails in various soils, as the fungus gets isolated from different types of agricultural and desert soils in Europe, Africa, America, Asia, and Australia (Domsch *et al*. 1980).

The fungus' recording in Turkey appeared on the leaves of potato (*Solanum tuberosum*), causing yellow to brown spots (Eken *et al.*, 2007). The fungus *F. dimerum* gained isolation from the leaves of the potato plant *S. tuberosum* in America and Australia. The fungus caused spots on the leaves of the potato plant of the same cultivar in the greenhouse after treating the potato plant leaves with the fungal filtrate, and symptoms appeared nine days after the treatment (Farr *et al*., 2007). The fungus *F. dimerum* also entailed isolation from the leaves of wild plants in Upper Egypt (Sabry *et al*., 2014). The fungus' first-time recording in Iraq was a cause of the fall of Al-Sayer date palm fruits (Ahmed *et al*., 2013). This study aimed to test the ability of the fungus *F. dimerum* to cause infection on the leaves of date palms. Likewise, it sought to determine the effect of fungal infection on the chlorophyll content and some phenotypic and anatomical morphological traits of the date palm leaves.

MATERIALS AND METHODS

Isolation and identification of the pathogen

Al-Sayer date palm leaves, showing signs of the spots, came from the Shatt al-Arab Region, Basrah Governorate, Iraq. The removal of leaves from the plant underwent washing well for sterilizing reasons. Then, pieces of affected

areas have a length of 0.5–1 cm. They received sterilization with 10% sodium hypochlorite for 3 min. Their further washing with sterile distilled water continued for 5 min before drying the pieces with sterile filter paper. The transfer of four pieces to a sterilized petri dish containing the potato dextrose agar (PDA) received sterilization and chloramphenicol (250 mg/L) before incubation in an incubator at a temperature of 25 ± 2 °C. The fungi's classification depended on Booth's classification keys (1971, 1977) and Nelson (1983).

F. dimerum infection test on leaves

After identifying the fungus *F. dimerum* successfully, its pathogenic ability to cause artificial infection on date palm leaves in the laboratory began its testing on five cultivars (Al-Sayer, Al-Halawi, Al-Barim, Al-Barhi, and Al-Khadrawi). Several healthy leaves obtained from date palm shoots of previously mentioned cultivars comprised five of the date palm leaves of each cultivar, uniform in size. Upon removing the leaflets from the date palm leaves, the leaves underwent washing with tap water to remove dirt. Then, the dust acquired superficial sterilization with 70% ethyl alcohol before rinsing with sterile distilled water various times to remove traces of the alcohol sterilization. Using a sterile blade, the surface layer of the leaves bore incisions at a distance of 1 cm on each side of the leaves, at a height of 11 cm.

From the bottom of the leaflet connection to the leaf, inoculating both sides of the leaflet with a sterile ear-cleaning tool (ear-cleaning cotton) progressed. This consisted of swabbing from the surface of a dish of a newly growing colony of the fungus *F. dimerum*. Then, passing it over the area designated for inoculation with the fungus and on both sides, the leaflet's transfer continued in appropriately sized test tubes containing 20 ml of sterile distilled water. Afterward, covering the mouth of the tubes with sterile cotton and aluminum foil ensued. The tubes gained incubation in the incubator at a temperature of 25 ± 2 °C for a month. The growth of the fungus and the development of the area inoculated with the fungus entailed monitoring and regularly recording the symptoms. As the expansion of the area inoculated with the fungus by more than 1 cm served as evidence of the development of fungal growth and the occurrence of infection, the experiment continued by taking three replicates of each treatment. Meanwhile, the comparison treatment consisted of applying a swab of the PDA culture media only to both sides of the leaves in three replicates (Ahmed, 2011).

Detection of the cellulase activity

The use of media ensured the sustainable development of the *F. dimerum* (Mandels *et al*., 1975). The media consisted of the following materials: KH_2PO_4 (2 g); $(NH_4)_2SO_4$ (1.4 g); urea (0.3 g); $MgSO_4.7H_2O$ (0.3 g); $CaCl_2$ (0.3 g); $FeSO_4.7H_2O$ (0.04 g); $MnSO_4.H_2O$ (0.16 g); $ZnSO_4.7H_2O$ (0.14 g); $COCl_2$ (0.02 g); peptone (0.8 g); carboxymethyl cellulose (CMC) (10 g); agar (20 g); and one liter of distilled water. As for the reagent used to detect the cellulase enzyme, it is HCl-iodine solution, prepared with a mixture of 100 ml of HCl (0.1 M) and 500 ml of I_2 (1%) + KI (2%) in terms of weight/volume. Sterilizing the media with a steam sterilizer took place, except for urea, whose preparation was in the form of a solution in sterile distilled water. Its sterilization ensued by passing the solution through a fine membrane filter with a diameter of 0.45 microns, produced by Millipore, using a vacuum device. After the media's cooling, adding the urea filtrate to it followed before being distributed on petri dishes with a diameter of 9 cm.

After the media had hardened, it received inoculation with a 0.5 cm disc, which was taken using a sterile cork piercer from a newly growing colony of the fungus *F. dimerum.* Then, its placement upside down in the center of each petri dish occurred after three days of incubation at a temperature of 25 °C. The reagent dye solution's adding to the surface of the media took three minutes, after which pouring the dye from the dish succeeded. The ability of the fungus to secrete the cellulase enzyme succeeds by the formation of a yellow halo around the colony.

Table 1. Scale of the efficiency of the *F. dimerum* in secreting the cellulase enzyme.

Degree of activity	Area of activity (area diameter)/mm	Its details
–	Negative	not secreted
±	from 1-3	Weak
+	more than 3-5	Medium
+ +	more than 5-8	Good
+ + +	more than 8-11	Active
+ + + +	more than 11	Very active

The diameter measurement of the halo proceeded, with the rate of enzyme activity calculated by computing the difference between the diameter of the colony growth and the diameter of the halo (mm). Using the scale by Al-Saadoun (1989) helped determine the efficiency of the *F. dimerum* in secreting the cellulase enzyme. The experiment proceeded with three replicates (Table 1).

Detection of the phenol oxidase activity

Gessner (1980) media, as used, consisted of malt extract (15 g), tannic acid (0.8 g), agar (20 g), and one liter of distilled water. Dissolving the tannic acid in 100 ml of sterile distilled water, continue to mix it with the other sterile media components dissolved in 900 ml of sterilized distilled water separately. The same previous method to detect the secretion of the cellulase enzyme in inoculated dishes transpired. The secretion of the phenol oxidase enzyme has an indication of the appearance of a dark brown color in the back and around the colony, indicating the enzyme activity. Its calculation comprised measuring the difference between the diameter of the colony growth and the diameter of the areola in millimeters. The experiment also had three replicates.

Detection of the lipolytic activity

The effectiveness of the fungus in producing the lipase enzyme reached detection according to the method of Sierra (1957). The medium consisted of peptone (8 g/L), calcium chloride (CaCl, 0.1 g/L), Tween 80 (10 ml/L), agar (20 g/L), and distilled water (1 L)**.**

Tween 80 (oleic-mono polyxythylene sorbitan) entailed separate sterilization before adding to the cooled basal media after sterilization. The secretion of the lipase enzyme into the solid media has signs of either the formation of a visible white precipitate under the growth or white crystals immersed in the media around the colony.

Detection of *the* amylolytic activity

The method described by Hankin and Anagnostakis (1975) was the technique adopted, and the media consisted of soluble starch (8 g/L), yeast extract (0.1 g/L), agar (18 g/L), and distilled water (1 L). The detector used a solution of potassium iodide, KI (I_2 3 gm/L + KI 5 gm/L), by adding it to the media for the amylase enzyme and leaving it for 10 min, then pouring it out and leaving it for 5 min. A yellow halo formed around the colony, and the rest of the media with blue signifies the production of the enzyme. The diameter of the halo indicates the activity of the fungus.

Histological anatomy of healthy leaves infected with *F. dimerum*

Taking four leaves of the Sayer cultivar (healthy and infected) each separately, cutting them into small parts before performing a fixation process on them in FAA solution for 48 h. Then, passing the parts through increasing concentrations of ethyl alcohol and embedding the samples in paraffin wax followed at 58 °C before cutting them again. The models made used a rotary microtome with a thickness of 10 micrometers. The specimens placed on slides incurred staining with Safranin dye before placing them in Fast Green dye, then loading with drops of DPX before being covered. Afterward, studying the slides progressed, taking micrometer measurements using an

ocular micrometer in an Olympus optical microscope equipped with a camera connected to an electronic calculator (Al-Attar *et al*., 1982).

Total chlorophyll content in healthy leaves infected with *F. dimerum*

As successfully approved in Abbas and Mohsen (1992), taking the total chlorophyll content in the leaves of date palms of the Al-Sayer cultivar (healthy and infected) included samples from leaves nearing growth. A weight of 2 g of the sample (palm leaves) underwent mixing with 100 ml of acetone at a concentration of 80%. Mixing it with a blender for 3 min, then filtering it, the filtrate proceeded to be added into 100 ml of 80% acetone. Performing the central stir on the sample three times, 3 ml of the thin solutions succeeded in transferring into the vital systems of the wave spectrometer programs (spectrophotometer). The display started reading. Optical light at wavelengths of 645 and 665 nm had the total filth of the sample estimated as follows:

Total chlorophyll (mg/L) = 20.2 × the optical hand at wavelength 645 + 8.02 × the optical hand at wavelength 665. The amount of chlorophyll, as converted from mg/L to mg/100 g, employed the method of Harborne (1984).

Statistical analysis

Laboratory experiments proceeded according to a completely randomized design with single-factor experiments. The means' comparison was according to the adjusted least significant difference (R.L.S.D.) method under a significance level of 0.01 (Al-Rawi and Khalaf Allah, 1980)***.***

RESULTS AND DISCUSSION

Pathogenicity of *F. dimerum* on the leaves

Table 2 shows the difference in the rate of artificial infection according to the cultivars, where the Al-Sayer cultivar recorded the highest rate of infection development, reaching 3.4 cm, followed by the Al-Halawi cultivar, with a rate of 3.2 cm. The lowest rate of infection was in the Al-Barim and Al-Barhi cultivars, as the infection rate reached 1.8 and 1.9 cm, respectively.

The results of the pathogenicity for the fungus *F. dimerum* on the leaves of the Al-Sayer date palm indicated the ability to cause infection. Symptoms of infection on the leaves resulted in brown spotting that extended to a greater distance than the area inoculated with the fungus. The edges of the spot area bore yellow stains extending further from the fungal inoculation area to both sides (Figure 1-A).

The difference in the artificial infection rate according to the cultivars is due to the varying components of the date palm leaves of the studied cultivars. Ghali (2001) showed the presence of cellulose and carbohydrates in the Sayer and Al-Halawi cultivars makes them the most responsive to infection with the fungus *Chalaropsis paradoxa* compared with the Barim and Barhi cultivars. They contain a high percentage of protein and calcium in their leaves, which respond less to fungal infection.

Table 2. Effect of the *F. dimerum* on infecting the leaves of different cultivars of date palms.

Cultivars	Rate of development of artificial infection with *F. dimerum* (cm)
Al-Sayer	*3.4
Al-Halawi	3.2
Al-Barim	1.8
Al-Barhi	1.9
Al-Khadrawi	2.4
R.L.S.D 0.01	0.13

*Each number represents an average of three replicates.

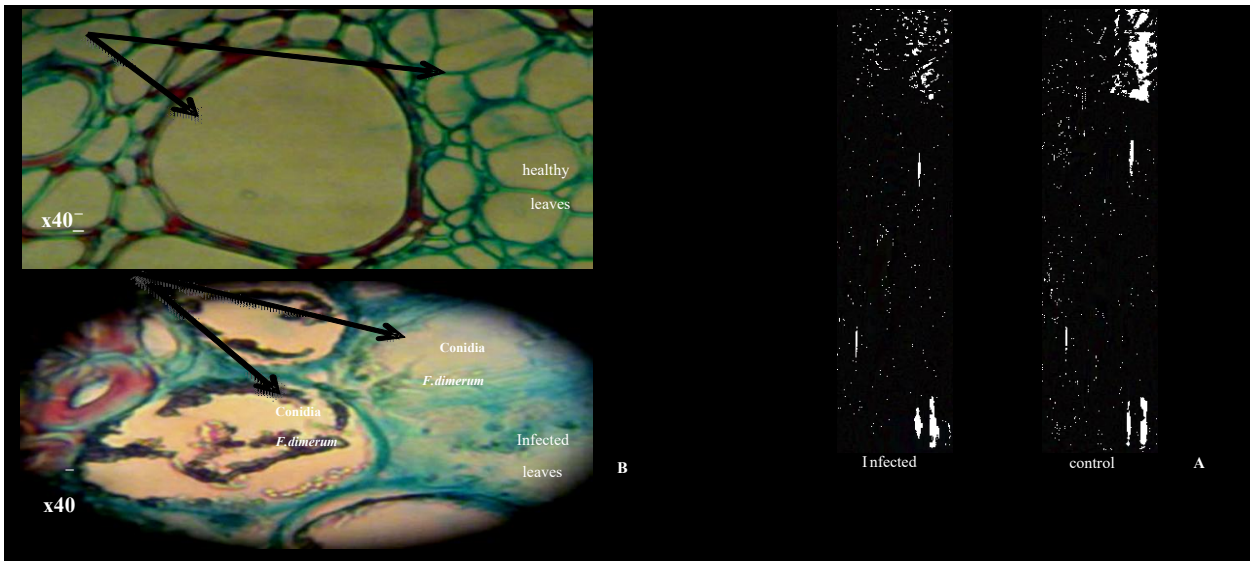

Figure 1 A. Pathogenicity of the *F. dimerum* leaves (control) and leaves (Infected).
B. Anatomically, leaves (healthy and infected).

The ability of *F. dimerum* to secrete enzymes

The results in Table 3 showed the ability of the *F. dimerum* to secrete the enzymes cellulase, phenol oxidase, lipase, and amylases, as the area of their enzyme activity reached 6.1, 5.8, 6.2, and 12.4 mm, respectively**.**

Detection of *F. dimerum* in leaf tissues

Outcomes of the histological analysis of the leaves (healthy and infected) showed the presence of *F. dimerum* spores in the tissues of infected leaves, the presence of decomposition of cell walls, and the loss of their integrated shape (Figure 1-B). The decomposition of the cell walls could be because the fungus can secrete cell wall-degrading enzymes, such as the cellulase enzyme (Bhat and Bhat, 1997). Their test of the effectiveness of the fungus in secreting cellulase and phenol oxidase proved that the fungus was good at secreting the two enzymes. This explains the decomposition of tissues in the results of histological anatomy versus healthy leaves, revealing the leaf tissue is free of fungal spores and has an integrated appearance for cell walls.

Domsch *et al*. (1980) also reported some species of fungi, *Fusarium* sp., have the ability to decompose cellulose in host plant cells due to their high capacity to secrete the cellulase enzyme. The power to secrete the enzymes cellulase and phenol oxidase plays a key role in causing the infection. The enzymes have a fundamental role in causing the disease on the infected plant as they destroy the structural content of the plant cells and work to decompose the non-living materials in the cell (Agrios, 1997). Cellulose is the main component of plant cell walls, composed of several glucose molecules as a structural material in the form of fine fibers. As for lignin, which is the second compound of plant cells after cellulose, it is a highly organic complex that is resistant to attack by most microorganisms and the ability of fungi to decompose it (Agrios, 1997; Saparat *et al*., 2000).

Abbas (2005) noted that the fungus *F. solani*, which causes the Sykes palm deterioration disease, has a high ability to secrete the cellulase enzyme, as its enzymatic activity space reached 11.12 mm. However, it has a moderate ability to secrete the phenol oxidase enzyme, as its enzymatic activity

Table 3. Ability of the fungus *F. dimerum* to secrete the enzymes.

Enzyme	Fungus enzymatic activity rate (mM)	Degree of activity	Its details
Cellulase	6.1*	++	Good
Phenol oxidase	5.8	++	Good
Lipase	6.2	++	Good
Amylases	12.4	++++	Very active
R.L.S.D 0.01	0.15		

*Each number represents an average of three replicates.

Table 4. Effect of infection with the *F. dimerum* on the chlorophyll content of date palm leaves.

Treatments	Chlorophyll content of leaves: mg/100 g
Fusarium dimerum	1.67*
Control	2.88
R.L.S. D 0.01	1.19

*Each number represents an average of three replicates.

space reached 4.1 mm. This difference refers to the type of isolation and its source. Al-Ameri (2009) confirmed the ability of *F. solani* to secrete cellulase and phenol oxidase enzymes at the rates of 4.08- and 6.05 mm, respectively. Ahmed (2015) mentioned the capability of *F. equiseti* to secrete the enzymes cellulase and phenol oxidase, as the enzymatic activity space for them reached 5.6 and 6.3 mm, respectively. Ahmed *et al*. (2020) indicated the power of *F. equiseti* to secrete the enzymes cellulase and phenol oxidase, with an enzymatic activity range of 5.8 and 6.5 mm, respectively.

As for the lipase enzyme, it has become one of the important enzymes in causing the disease because it attacks the second component of the host's cell surfaces, which is the plasma membrane (Griffin, 1981). Table 3 shows the good activity of the fungus in its production of the hydrolytic enzyme, and the activity area reached 6.2 mm. Abbas (2005) indicated the ability of the fungus *F. solani* to produce the enzyme, and the activity area of the fungus reached 5 mm.

Most plant pathogens can metabolize starch as a source of carbon in their metabolic activities, breaking down starch by the enzymes called amylases (Agrios, 1997). The results showed the highest effectiveness in producing the amylase enzyme, as the activity area reached 12.4 mm (Table 3)**.** Hankin and Anagnostakis (1975) reported the amylase activity of several pathogenic fungi, including *F. oxysporum*, indicating its high capacity to secrete the amylase enzyme. Abbas (2005) confirmed the high activity of the fungus *F. solani*, and the activity area of the fungus reached 12.2 mm.

Total chlorophyll in healthy and infected leaves

Table 4 presents a significant difference that existed in the chlorophyll content in healthy leaves and those infected with *F. dimerum*, wherein the chlorophyll content decreased compared with the control treatment. The decrease in chlorophyll content in leaves infected with the fungus may be due to the effect of the fungal infection on the decomposition of chlorophyll in the leaves. Consequently, it caused an imbalance in the photosynthesis process of leaves versus the healthy leaves (Al-Ameri, 2009).

CONCLUSIONS

The beneficial activity of the fungus *F. dimerum* in secreting the enzymes cellulase, phenol oxidase, lipase, and amylases explains the decomposition of tissues in the results of histological analysis compared with healthy leaves. It showed the leaf tissue is free of fungal spores and the integrated shape of the

cell walls. These enzymes enable the fungus to invade the plant tissue and thus develop the infection, with the fungal infection leading to decomposition. Chlorophyll declines in the infected leaves, which in turn decreases the chlorophyll content in leaves infected with the fungus. Eventually, it leads to a disruption in the process of photosynthesis in the leaves compared with the healthy leaves.

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