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Identification of Fusarium oxysporum f.sp. niveum Races Causing Vascular Wilt Disease in Watermelon Plants in Western Iraq

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ABSTRACT

This study aimed to identify and classify races of *Fusarium oxysporum* f.sp. *niveum*, the causal agent of *Fusarium* wilt in watermelon, using precise molecular methods. Nine fungal isolates were collected from different agricultural regions in Iraq and identified using Polymerase Chain Reaction (PCR) techniques with specialized primers targeting specific regions of the fungal DNA. After molecular confirmation, the pathogenicity of these isolates was tested on five hybrids of the *Charleston Gray* watermelon cultivar in a laboratory experiment, helping to assess the pathogenic effects of the isolates. The results showed significant variation in the pathogenic abilities of the isolates, with *Race 3* being the most aggressive, displaying infection rates ranging from 80-100% and infection severity between 76.6-96.5%. This study highlights the importance of using molecular techniques for accurately and rapidly identifying pathogenic races, which can aid in developing effective disease management strategies and reducing agricultural crop losses.

Key words: *Citrullus lanatus* (*Charleston Gray*), *Fusarium oxysporum*, Molecular, Pathogenicit.

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INTRODUCTION

The watermelon crop (*Citrullus lanatus*) is considered one of the most important economic crops in the Cucurbitaceae family (*Cucurbitaceae*) and is widely traded. It is successfully cultivated in tropical and subtropical regions in many countries around the world (Naz et al.,2014; Kyriacou et al.,2018; Manivannan et al.,2020). Watermelon plants are affected by *Fusarium* wilt, caused by the fungus *Fusarium oxysporum* f. sp. *niveum*, which limits the commercial production of watermelon (Fernández-Herrera et al., 2021; Vargas-Arispuro et al., 2023) *Fusarium* wilt causes significant losses in watermelon crops, with loss estimates ranging between 30-80%, and in some cases reaching up to 100%. This disease is a major obstacle to watermelon cultivation in various agricultural regions worldwide (Lal et al., 2024). The fungus *Fusarium oxysporum* was first described by the scientist Smith, who also conducted histological studies to clarify the infection process and the formation of structures within plant tissues known as tyloses in vascular tissues (Martyn, 2014). Many races of *Fusarium oxysporum* f. sp. *niveum* have been discovered. This fungus is specialized in attacking watermelon plants and is considered significant from a physiological perspective, as it has the ability to develop different types of resistant spores that enhance its survival and reproduction. Four different races of this fungus have been identified: Race 0, Race 1, Race 2, and Race 3. These races exhibit varying levels of aggressiveness towards all commercially cultivated watermelon cultivars. Some races show high levels of aggressiveness, meaning they demonstrate a greater ability to overcome the resistance found in watermelon cultivars (Martyn & Bruton, 1989; Martyn &Netzer, 1991; Hudson et al., 2021). The *FON* race 0 was first recorded in Florida, USA, in 1963. This race has since become of little economic importance, as the majority of commercially

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grown watermelon cultivars today are resistant to it. This race causes wilting only in watermelon cultivars that lack resistance genes, such as the cultivar Sugar Baby, which is susceptible to infection by this race and all other *FON* races. In contrast, the cultivar *Charleston Gray* is resistant to *FON 0* but not to the other races of the fungus *FON* (Martyn. 2014). It has been found that *Race 1* is the most widespread race in watermelon-growing regions around the world. Seedless cultivars (triploid) are usually susceptible to *Race 1*, while the cultivar *Calhoun Gray* is resistant to both *FON* races (Race 0 and Race 1) but is susceptible to *Race 2* and *Race 3* of the *FON* fungus (Elmstrom& Hopkins. 1981; Kleczewski & Egel. 2011; Martyn & Bruton. 1989; Zhou, &Everts, 2003). *Race 2* was first reported in Palestine in 1976 and later recorded in 1981 in the United States. This race is considered the most harmful and aggressive towards both seeded (diploid) and seedless (triploid) watermelon plants. The absence of a high-level resistance gene against *Race 2* in commercial watermelon cultivars is a significant concern for farmers in all watermelon-growing regions, including the United States, due to the losses and damage caused by this race. The cultivar *PI-296341-FR* is resistant to the other races but is susceptible only to *Race 3* (Martyn & Bruton. 1989; Petkar et al., 2019). Race 3 of FON was first recorded in 2009 in Maryland, USA, and later in Florida. It is considered more aggressive and damaging than the other three races (0, 1, 2) identified so far. This race causes symptoms of Fusarium wilt in all watermelon cultivars. Initially, Race 1 was believed to cause the highest infection rate and was This study aimed to identify the races of the watermelon wilt pathogen caused by *Fusarium oxysporum* f. sp. *niveum* using molecular methods with both general and specialized primers. Additionally, the study tested the susceptibility of several watermelon hybrids (*Anguria F1*, *Gurranty F1*, *King Charles F1*, *Astera F1*, *Elegant F1*) available in the Iraqi markets.

MATERIALS AND METHODS

1. Sample Collection: Samples were collected from watermelon plants showing symptoms of Fusarium wilt from several Governorates in Iraq during the 2021-2022 month (5-6) growing season. These Governorates included Salahuddin and Anbar. The samples consisted of infected watermelon roots and stems, which were placed in polyethylene bags. All necessary information was recorded for each sample The samples were then transferred to the Plant Pathology Research Laboratory at the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, and stored in a refrigerator at 4°C until isolation and diagnosis were performed.

2. Isolation and Identification of the Fungus Causing Fusarium Wilt in Watermelon: The plant samples were thoroughly washed with running water to remove any attached soil. Roots and sections from the crown area of the watermelon plants showing symptoms of Fusarium wilt were cut into 0.5 cm segments. The cut plant parts were surface sterilized using a 6% sodium hypochlorite solution (1% free chlorine) for 2 minutes. The plant parts were then rinsed three times with sterile water to remove any residual sterilizing solution and dried on sterile blotting paper to remove excess moisture. Four pieces of plant tissue were transferred to each 9 cm diameter Petri dish containing 15-20 cm^3 of Potato Dextrose Agar (PDA) medium. The PDA medium was prepared by adding 41 grams of PDA powder to 1 liter of water and sterilizing it in an autoclave at 121°C and a pressure of 1.5 kg/cm^2 for 20 minutes. An antibiotic, Tetracycline, was added at a concentration of 50 mg/L to prevent bacterial growth. The plates were then incubated at a temperature of 25 ± 2°C. After three days, fungal purification began by taking a portion from the edge of the fungal growth and transferring it to other sterile Petri dishes containing PDA medium. The plates were incubated for five days, and *Fusarium* isolates were selected based on the taxonomic characteristics described by Booth, including the shape and color of the fungal colony, the structures of the conidial carriers, and their morphology. Observations were made using a light microscope at magnifications of X10 and X40. The fungal isolates were stored in plastic tubes (slants)

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containing sterile PDA medium with a slanted surface for use in subsequent experiments.

3. Pathogenicity test of *Fusarium* spp. Isolates on Watermelon Seedlings in the Laboratory: Watermelon seeds of the *Sugar Baby* cultivar were sown in a nursery. After the emergence of the third true leaf on the seedlings, the laboratory experiment was conducted by transferring the seedlings to the Plant Pathology Research Laboratory at the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad. The seedlings were removed from the foam trays in which they were planted, and the roots were thoroughly washed under running water to remove any adhering planting medium (peat moss). The seedlings were then divided into two groups: the first group had their roots cut at the crown area using a sterile scalpel, while the second group's roots were left intact (uncut). Plastic containers with a volume of 20 mL were prepared, each containing 18 mL of a spore suspension of the fungal isolates to be tested for their pathogenicity against *Sugar Baby* watermelon seedlings, at a concentration of 10^6 spores mL^{-1}. Both the root-cut and intact seedlings were transferred into the plastic containers containing the fungal spore suspension. Five root-cut seedlings and five intact seedlings were used for each fungal isolate, with three replicates for each fungal isolate. The experiment was left for 12 days at room temperature, and the fungal suspension was supplemented with 0.05% aqueous agar solution as needed (Freeman and Rodriguez, 1993; Fulton et al., 2021). The percentage of infection was calculated using the following formula:

$$\text{Infection Percentage} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{x} 100$$

The severity of the infection was calculated using a five-point disease scale as follows:
0 = Healthy plant, 1 = 25% yellowing or wilting of the seedling, 2 = 2650% yellowing or wilting of the seedling, 3 = 5175% yellowing or wilting of the seedling, 4 = 76% or more yellowing, wilting, or complete death of the seedling. The disease severity was calculated using McKinney's (1923) formula:

$$\text{Disease Severity } \% = \frac{(0 \times \text{No. of plants with rating } 0) + \cdots + (4\text{x No. of plants with rating } 4)}{\text{Total number of plants x Highest rating}} \text{x} 100$$

4. Molecular Diagnosis of the Pathogenic Fungus: To confirm the morphological diagnosis of the *Fusarium* spp. pathogenic isolate, molecular identification was conducted using Polymerase Chain Reaction (PCR) technology. The DNA extraction was carried out by Al-Musayyib Bridge Company in Baghdad. The DNA was extracted from the pure fungal growth of the isolate using a standard kit provided by Geneaid, following the manufacturer's instructions. A pair of primers was used: *ITS1* as the forward primer (F: 5′- TCCGTAGGTGAACCTGCGG- 3′) and *ITS4* as the reverse primer (R: 5′- TCCTCCGCTTATTGATATGC- 3′), which amplified the target region of *Fusarium oxysporum* using a PCR machine (Najem et al., 2018).

5. Identification of *Fusarium spp.* Races Using Specialized Primers: The fungal isolates obtained in this study, totaling 9, were identified using specialized primers: *FNR3-F/FNR3-R*, *FONSIX6F/FONSIX6R*, and *Fon-1/Fon-2* (Hudson et al., 2021). The *Fon-1/Fon-2* primer was first used to identify the fungal isolate. If a DNA band appeared on the agarose gel, it indicated a positive reaction, meaning that the isolate belonged to *Fusarium oxysporum* f. sp. *niveum* (*Fon*). If no DNA band appeared, the reaction was considered negative, indicating that the isolate was not *Fusarium oxysporum* f. sp. *niveum*. In the second phase, the *FONSIX6F/FONSIX6R* primer was used to differentiate *Race 2* of *Fon*. If no DNA band appeared after the PCR reaction, the reaction was negative, indicating that the isolate belonged to *Race 2*. However, if a DNA band appeared, the reaction was positive, indicating that the isolate was either *Race 1* or *Race 3*. To distinguish between *Race 1* and *Race 3*, the third differential primer, *FNR3-F/FNR3-R*, was used. If a DNA band appeared on the agarose gel after the PCR reaction, the reaction was positive, meaning the isolate belonged to *Race 1*. If no DNA band appeared, the reaction was

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negative, indicating that the isolate belonged to *Race 3* (Hudson et al., 2021).

6. Testing the Susceptibility of Some Genetic Lines of the Watermelon Cultivar *Charleston Gray* to the Pathogenic Fungus in a Pot Experiment: *A* pot experiment was conducted to test the susceptibility of watermelon cultivars using five hybrids of the *Charleston Gray* variety (*Anguria*, *Gurranty F1*, *King Charles F1*, *Astera F1*, *and Elegant F1*). The inoculum of the pathogenic fungal isolate (A4) was prepared at a concentration of 1×10^6 spores/mL for use in the experiment. The seeds of the five hybrids of *Charleston Gray* watermelon were sown in the nursery. After the emergence of the third true leaf, the laboratory experiment was conducted by transferring the seedlings to the Plant Pathology Research Laboratory at the Department of Plant Protection, College of Agricultural Engineering Sciences, and University of Baghdad. The seedlings were removed from the foam trays in which they were planted, and the roots were thoroughly washed under running water to remove any adhering planting medium (peat moss). The seedlings were then divided into two groups. The first group of seedlings had their roots cut at the crown area using a sterile scalpel, while the second group remained intact (roots uncut). Plastic containers of 20 mL were prepared, each containing 18 mL of a spore suspension of the selected fungal isolates at a concentration of 10^6 spores/mL to test their pathogenicity against the seedlings of the five hybrids of the *Charleston Gray* watermelon variety. Both the root-cut and intact seedlings were transferred into the plastic containers with the fungal suspension. Five root-cut seedlings and five intact seedlings were used for each hybrid, with three replicates per hybrid, comparing each hybrid with both root-cut and intact seedlings in containers containing only 0.05% aqueous agar solution. The experiment was left for 12 days, and the fungal suspension was supplemented with 0.05% aqueous agar solution as needed (Freeman & Rodriguez, 1993; Fulton et al., 2021).

RESULTS AND DISCUSSION

1. Isolation and Morphological Identification of the Fungus Causing Fusarium Wilt in Watermelon: The results of the isolation process revealed the presence of several fungal isolates obtained from infected plant parts, specifically from the stems and crown areas of watermelon plants collected from different provinces of Iraq. A total of 15 fungal isolates were obtained, of which 9 isolates were morphologically identified based on the appearance of the fungal colonies. These isolates exhibited variation in their growth rates and the density of fungal mycelium on the PDA medium, with colors ranging from white and cottony to pink or dark purple. The isolates displayed three main types of asexual reproductive structures. The first type was microconidia, which are typically non-septate spores produced on short, single conidiophores. These microconidia were cylindrical to oval or kidney-shaped and formed false heads on a short, single conidiophore known as a monophialide. The second type of spores is macroconidia, which are divided into 3-6 septa. These spores are spindle-shaped with tapered ends, sometimes hooked, and asymmetrical in their dimensions. Macroconidia are formed on multi-branched conidiophores that cluster together to form structures known as sporodochia. The third type is chlamydospores, which are spores that form either individually, in pairs, on small lateral branches, within the fungal mycelium, or in chains. These spores enable the fungus to survive under unfavorable conditions and are characterized by their thickened walls, which may be rough or smooth. These characteristics match those of *Fusarium oxysporum*, which have been previously described in various studies, as illustrated in Figure (1) (Guadet et al., 1989; Amaradasa et al., 2018; Hirano & Arie 2006; Niu et al., 2016; Kadhim et al., 2019).

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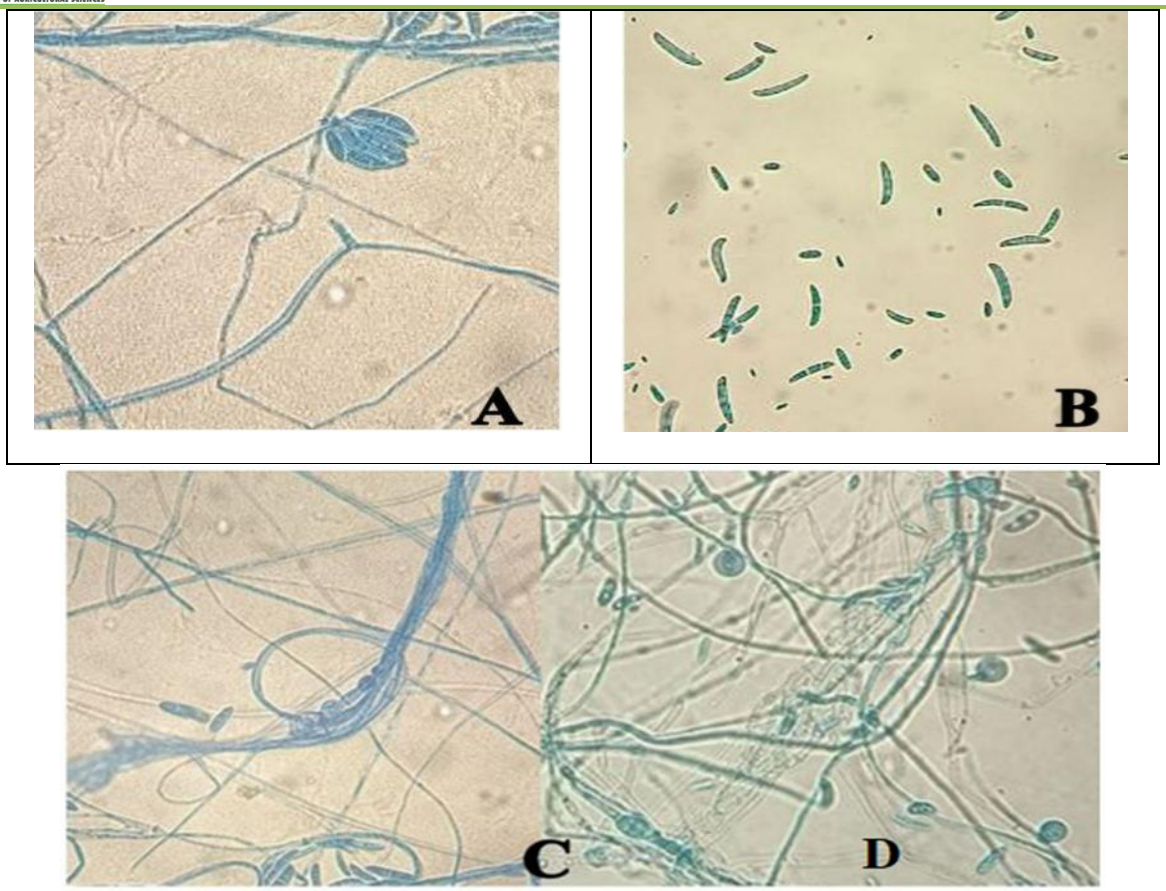

Figure 1. *F. oxysporum* under the microscope, isolated from the roots and stems of watermelon plants (400X). A- Short single conidiophores, B- Microconidia, C, D- Macroconidia, and Chlamydospores

2. Pathogenicity test of *Fusarium* spp. isolates on watermelon seedlings in the laboratory: The results of the patho*genicity test on watermelon seedlings in the laboratory showed that all tested Fusarium* spp. *isolates* had pathogenic abilities, with significant differences on *Sugar Baby* watermelon seedlings with intact roots, as shown in Table (1). The percentage of infection ranged between 80-96.5% compared to the control treatment (without the pathogenic fungus), which had an infection rate of 0.00%. There was significant variation in the pathogenic abilities of the isolates. The highest pathogenic isolate was *A4*, isolated from Fallujah – Anbar, with a disease index of 3.8 based on the scale used in the experiment and an infection rate of 96.5%. The disease severity for this isolate was 95%, with significant differences. This was followed by isolate *S5*, isolated from Dujail – Salahuddin, which had an infection rate of 93.3%, a disease severity of 93.3%, and a disease index of 3.7, with significant differences compared to the control treatment without the fungus. The other tested pathogenic fungal isolates, including *S1*, *S2*, *S3*, *S4*, *A1*, *A2*, and *A3*, showed infection rates between 80-90%, disease severity between 76.6-87.5%, and a disease index between 3-3.5 based on the scale used in this experiment, with significant differences compared to the control treatment without the fungus, as shown in Figure (2).

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Table 1. Pathogenicity test of *Fusarium oxysporum* isolates on *Sugar Baby* watermelon seedlings with intact roots in the laboratory

No.	Isolate Code (FOX)	Isolation Area	Disease Index	Infection Percentage (%)	Infection Severity (%)
1	S1	Salahuddi n/Tikrit	3.4 d	87.6 c	85.0 b
2	S2	Salahuddin / Samarra	3 f	80.0 d	76.6 c
3	S3	Salahuddin / Ishaqi	3 f	80.0 d	76.6 c
4	S4	Salahuddin / Balad	3.4 d	87.7 c	85.0 b
5	S5	Salahuddin / Dujail	3.7 b	93.3 ab	93.3 a
6	A1	Anbar / Khalidiya	3.4 d	87.6 c	85.0 b
7	A2	Anbar / Amiriya	3.5 c	90.0 bc	87.5 b
8	A3	College of Agriculture	3.3 e	86.6 c	82.5 b
9	A4	Anbar / Fallujah	3.8 a	96.5 a	95.0 a
Control	Control		0 g	0.0 e	0.00 d
LSD 0.05	LSD 0.05		0.075	3.92	5.08

***Means followed by different letter are significantly different at P ≤ 0.05**

Figure 2. A - Shows intact watermelon seedlings not treated with the fungus,B - Shows the experiment with intact watermelon seedlings treated with the pathogenic fungus *Fusarium oxysporum*

The results of the pathogenicity test on root-cut watermelon seedlings showed that all tested *Fusarium spp.* isolates exhibited high pathogenicity with significant differences compared to the control treatment without the fungus, as shown in Table (2). The infection percentage for all tested isolates ranged between 80-100%, which was significantly different from the control treatment (without the pathogenic fungus), where the infection percentage was 0.00%. The isolate *A4* (from Fallujah – Anbar) showed the highest pathogenicity, with an infection percentage of 100.00% and an infection severity of 96.5%. Its disease index was 4, based on the scale used in the experiment. This isolate significantly differed from all other fungal isolates. The second highest pathogenic isolate was *S5*, with an infection percentage of 96.6%, an infection severity of 95%, and a disease index of 3.8, which also showed significant differences compared to the control treatment without the fungus. The remaining tested fungal isolates (*S1*, *S2*, *S3*, *S4*, *A1*, *A2*, *A3*) had infection percentages ranging between 80-93.3%, with infection severity between 76.6-93.3%, and a disease index between 3-3.7, based on the scale used in this experiment. These results showed significant differences compared to the control treatment without the fungus, as illustrated in Figure (3). The variation in pathogenicity among the isolates, which affected the infection percentage and severity, may be due to the differences in the ability of the fungal isolates to secrete the enzyme Polygalacturonase. Pathogenic fungal isolates have the ability to secrete this enzyme, whereas non-pathogenic isolates either do not secrete it or secrete it in small amounts. This enzyme helps pathogenic isolates break down

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and penetrate plant cell walls, facilitating infection. It is considered one of the key factors contributing to the variation in the pathogenic ability of fungal isolates and the increase in infection percentage (Hudson et al., 2021).

Table 2. Pathogenicity test of *Fusarium oxysporum* isolates on root-cut *Sugar Baby* watermelon seedlings in the laboratory

No.	Isolate Code (FOX)	Isolation Area	Disease Index	Infection Percentage (%)	Infection Severity (%)
1	S1	Salahuddi n/Tikrit	3.6 ab	91.5 b	90.0 ab
2	S2	Salahuddin / Samarra	3.7 ab	93.3 ab	93.3 ab
3	S3	Salahuddin / Ishaqi	3.0 c	80.0 c	76.6 c
4	S4	Salahuddin / Balad	3.7 ab	93.3 ab	93.3 ab
5	S5	Salahuddin / Dujail	3.8 ab	96.6 b	95.0 a
6	A1	Anbar / Khalidiya	3.6 ab	93.3 ab	90.0 ab
7	A2	Anbar / Amiriya	3.7 ab	93.3 b	93.3 ab
8	A3	College of Agriculture	3.5 b	90 a	87.5 b
9	A4	Anbar / Fallujah	4.0 a	100 d	96.5 a
Control	Control		0 d	0 b	0 d
LSD 0.05	LSD 0.05		0.495	7.30	7.20

***Means followed by different letter are significantly different at P ≤ 0.05**

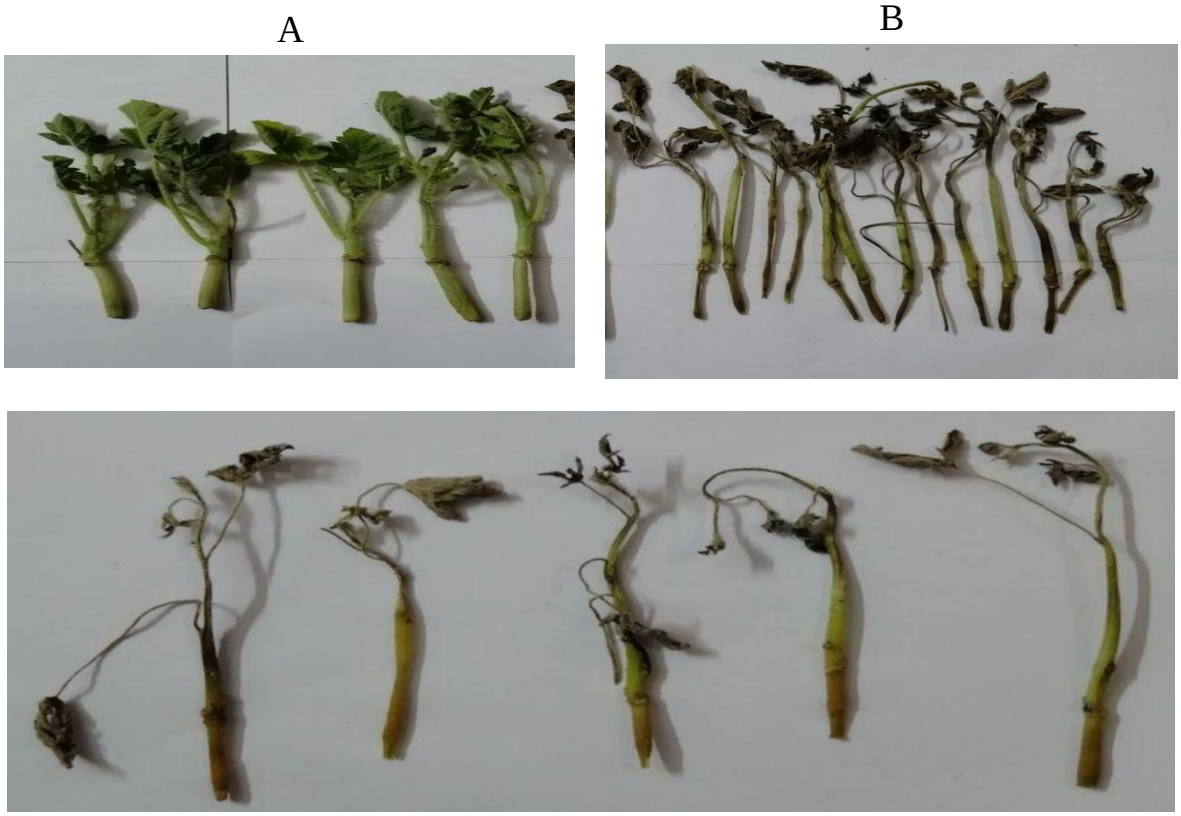

Figure 3. A - Root-cut control seedlings, B, C - Root-cut watermelon seedlings treated with the pathogenic fungus *Fusarium oxysporum*

3. Molecular Diagnosis of the Pathogenic Fungus: The results of the gel electrophoresis of the DNA extracted from the *Fusarium* spp. isolates, after amplification using Polymerase Chain Reaction (PCR) with the general primer *ITS1/ITS4*, showed the formation of bands with a molecular weight of approximately 550 base pairs. Additionally, the nucleotide sequences of the ITS region of the tested isolates were determined and compared with global isolates in the NCBI Gene Bank. It was found that all 9 isolates examined molecularly belonged to *Fusarium oxysporum* f. sp. *niveum*, with a 100% match. These isolates were deposited in the Gene Bank with accession numbers, as shown in Table (3).

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Table 3. Accession numbers of *Fusarium oxysporum* f. sp. *niveum* isolates deposited in the Gene Bank

No.	FOX	Isolation location	Accession Number in NCBI
1	S2	Salahuddin / Samarra	PP266986
2	S3	Salahuddin / Ishaqi	PP266987
3	S5	Salahuddin / Dujail	PP266988
0	A4	Anbar / Fallujah	PP266993

4. Molecular Identification of *Fusarium oxysporum* f.sp. *niveum* Races Using Specialized Primers: The results of the molecular identification of *Fusarium oxysporum* f.sp. *niveum* races using a specialized set of primers showed that the first primer set, known as the initial set (*Fon-1/Fon-2*), differentiates between isolates that belong to *Fusarium oxysporum* f.sp. *niveum* and those that do not. The results of the PCR reaction using the *Fon-1/Fon-2* primer, after running the PCR products on agarose gel, revealed the appearance of 9 bands with a molecular weight of approximately 174 base pairs. These were considered positive reactions, indicating that the isolates belong to *Fusarium oxysporum* f.sp. *niveum*. However, the PCR results using the second primer set (*Fonsix6F/R*), after gel electrophoresis, showed no band formation for 5 isolates (negative reaction), indicating that these isolates belong to *Race 2* of *Fon*. This is because the *Fonsix6F/R* primer does not amplify any genomic region in the DNA of *Fon* race 2. In contrast, the remaining 4 isolates showed a positive reaction with the second primer (*Fonsix6F/R*), producing bands with a molecular weight of approximately 450 base pairs. This prompted the use of the third specialized differential primer (*FNR3F/R*) in this test, which is known for not amplifying any genomic region in the DNA of *Fon* race 3. The results of the PCR reaction with the third differential primer (*FNR3F/R*), after gel electrophoresis on agarose, showed no DNA bands, confirming that all the tested isolates (4 isolates) belong to *Fon* race 3. Differentiating between races of *Fusarium oxysporum* f.sp. *niveum* and determining specific *forme speciales* typically requires the cultivation of multiple differential plant hosts using traditional methods. However, relying on molecular methods can save time and effort by identifying these races based on their genetic variation. The results of differentiating between *Fusarium oxysporum* f.sp. *niveum* races (Table 4) showed the presence of 5 fungal isolates belonging to *Race 2* of the *Fon* fungus, distributed across different agricultural regions. Isolate *S1* was identified in Salahuddin - Tikrit, isolate *S4* in Salahuddin - Balad, isolate *A1* in Anbar - Khalidiya, isolate *A2* in Anbar - Amiriya, and isolate *A3* in Anbar - College of Agriculture. As for the fungal isolates belonging to *Race 3* of the *Fon* fungus, 4 fungal isolates were found in the following ***agricultural regions: isolate S2 in Salahuddin - Samarra, isolate S3 in Salahuddin - Ishaqi, isolate S5*** in Salahuddin - Dujail, and isolate *A4* in Anbar - Fallujah (Martyn & Bruton. 1989; Hirano & Arie, 2006; Martyn, 2014; Tan et al., 2016).

Table 4. Type of Race Belonging to *Fusarium oxysporum* f.sp. *niveum*

No.	Isolate Code	Location sample	Race
1	S1	Salahuddi n/Tikrit	2 Race
2	S2	Salahuddin / Samarra	3 Race
3	S3	Salahuddin / Ishaqi	3 Race
4	S4	Salahuddin / Balad	2 Race
5	S5	Salahuddin / Dujail	3 Race
6	A1	Anbar / Khalidiya	2 Race
7	A2	Anbar / Amiriya	2 Race
8	A3	College of Agriculture	2 Race
9	A4	Anbar / Fallujah	3 Race

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5. Testing the susceptibility of five hybrids of the watermelon cultivar *charleston gray* to infection by *Fusarium oxysporum* f.sp. *niveum* (Race 3) in a pot experiment: The laboratory results of testing the susceptibility of five genetic lines (*Anguria F1*, *Gurranty F1*, *King Charles F1*, *Astera F1*, *Elegant F1*) of the watermelon cultivar *Charleston Gray* against the most pathogenic fungal isolate of *Fusarium oxysporum* f.sp. *niveum* (*Race 3*), as shown in Table (5), using intact seedlings, revealed that the infection percentage ranged between 80-96.5% for all tested watermelon hybrids, compared to the control treatment without the pathogenic fungus, which showed an infection percentage of 0.00%. The infection severity in all five tested hybrids of the *Charleston Gray* watermelon cultivar ranged between 76.6-95%, with a disease index ranging between 3-3.8.

Table 5. Testing the susceptibility of five watermelon hybrids to *Fusarium oxysporum* f.sp. *niveum* (Race 3) using intact watermelon seedlings in the laboratory

No.	Watermelon hybrids	Location	Disease Index		Infection Percentage (%)		Infection Severity (%)	
1	Anguria f1	Salahuddi n/Tikrit	3.0	e	80.0	c	76.6	D
2	Gurranty f1	Salahuddin / Samarra	3.2	d	86.0	bc	80.0	Cd
3	King Charles f1	Salahuddin / Ishaqi	3.4	c	87.6	b	85.0	Bc
4	Astera f1	Salahuddin / Balad	3.6	b	91.5	ab	90.0	Ab
5	Elegant f1	Salahuddin / Dujail	3.8	a	96.5	a	95.0	A
	Control	0	0	f	0	d	0	E
LSD 0.05			0.141		6.98		6.20	

The results of testing the susceptibility of five watermelon hybrids (genetic lines) of the Charleston Gray cultivar using root-cut seedlings against the fungal isolate Fusarium oxysporum f.sp. niveum (Race 3), as shown in Table (6), revealed that the infection percentage for all tested watermelon hybrids ranged between 86.3% and 100%, compared to the control treatment without the pathogenic fungus, which showed an infection percentage of 0.00%. The infection severity in all five tested *Charleston Gray* watermelon hybrids ranged between 82.5% and 96.5%, with a disease index ranging between 3.3 and 4

Table 6. Testing the susceptibility of five watermelon hybrids to *Fusarium oxysporum* f.sp. *niveum* (Race 3) using root-cut watermelon seedlings in the laboratory

No.	Watermelon hybrids	Location	Disease Index		Infection Percentage (%)		Infection Severity (%)	
1	Anguria	Salahuddi n/Tikrit	3.3	d	86.3	d	82.5	C
2	Gurranty f1	Salahuddin / Samarra	3.5	c	90.0	c	87.5	Bc
3	King Charles f1	Salahuddin / Ishaqi	3.7	b	93.3	bc	93.3	Ab
4	Astera f1	Salahuddin / Balad	3.8	b	96.6	b	95.0	A
5	Elegant f1	Salahuddin / Dujail	4.0	a	100	a	96.5	A
	Control	0	0	e	0	e	0	D
LSD 0.05			0.13		3.35		6.74	

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CONCLUSION

Conclusions should answer the research objectives of the research and should be clearly explained.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

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تحديد سلالات الفطر *Fusarium oxysporum* f.sp. *niveum* المسبب لمرض الذبول الوعائي في نبات الرقي في مناطق غرب العراق

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المستخلص

هدفت هذه الدراسة إلى تشخيص وتصنيف سلالات *Fusarium oxysporum* f.sp. *niveum*، المسبب لمرض الذبول الفيوزاريومي في نبات الرقي، باستخدام الطرق الجزيئية الدقيقة. تم جمع 9 عزلات فطرية من مناطق زراعية مختلفة في العراق وشُخِصَت باستخدام تقنيات تفاعل البلمرة المتسلسل (PCR) باستخدام بوادئ متخصصة تستهدف مناطق معينة في الحمض النووي للفطر. بعد التأكيد الجزيئي، تم اختبار المقدرة الإمراضية لهذه العزلات على خمسة هجن من صنف الرقي *Charleston Gray* في تجربة مختبرية، مما ساعد على تقييم التأثيرات الممرضة للعزلات. أظهرت النتائج تفاوتاً كبيراً في المقدرة الإمراضية للعزلات، حيث كانت السلالة *Race 3* الأكثر شراسة، إذ تراوحت نسب الإصابة بين 80-100%، بينما تراوحت شدة الإصابة بين 76.6-96.5%. تسلط هذه الدراسة الضوء على أهمية استخدام الطرق الجزيئية في تحديد السلالات الممرضة بدقة وسرعة، مما يمكن من تطوير استراتيجيات فعالة لإدارة الأمراض وتقليل الخسائر في المحاصيل الزراعية.

الكلمات المفتاحية: الرقي، *Fusarium oxysporum*، التشخيص الجزيئي ،الامراضية.