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Morphological and Molecular Identification of *Fusarium* Wilt Fungi in Tomato Plants in the Desert Farms of Karbala

Layth Shakir Abdullah[1], Jamal Hussein Kadhim[2], and Ameer Kh Yeaser[3]

[1,2] Department of Plant Protection - College of Agriculture - University of Kufa

[٣]Department of Soil and Water Sciences - College of Agriculture - University of Kufa

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*Corresponding Author E-Mail:
layths.alsaadi@student.uokufa.edu.iq

ABSTRACT

Tomato (Solanum lycopersicum L.) production is globally threatened by Fusarium wilt, a disease primarily attributed to Fusarium oxysporum f. sp. lycopersici. However, the diversity of Fusarium species associated with this syndrome in distinct agro-ecosystems remains underexplored. This study aimed to characterize Fusarium species isolated from wilted tomato plants in the desert farming region of Karbala, Iraq, using morphological and molecular approaches. Symptomatic tomato plants were sampled from several fields, and fungal isolates were obtained. Initial identification was performed based on cultural and morphological traits. Molecular identification was conducted through phylogenetic analysis of translation elongation factor-1α (*TEF-1α*) and internal transcribed spacer (ITS) rDNA sequences. The pathogenicity of representative isolates was evaluated on susceptible tomato seedlings. The results revealed the presence of Fusarium oxysporum and Fusarium solani as the primary species associated with wilt symptoms. Molecular analysis confirmed the morphological identification and further delineated the isolates into distinct clades. Pathogenicity assays confirmed that both species were capable of inducing typical wilt symptoms, with F. oxysporum isolates exhibiting higher virulence. Notably, F. solani isolates showed variable pathogenicity, with a germination rate of 10% in the in-vitro dish assay. This study provides the first consolidated report of Fusarium species diversity in tomato in Iraq's desert agriculture, highlighting the importance of precise identification for effective disease management.

1-Introduction

Tomato (*Solanum lycopersicum* L.) is a globally significant vegetable crop, prized for its nutritional value and economic importance [1]. However, its cultivation is persistently threatened by a spectrum of phytopathogens, among which soil-borne diseases pose particularly severe challenges. Fusarium wilt, caused by fungal pathogens of the genus *Fusarium*, stands as one of the most devastating and economically crippling diseases affecting tomato production worldwide [2]. The disease is characterized by symptoms including vascular discoloration, wilting, stunting, chlorosis, and ultimately plant death, leading to substantial yield losses and diminished crop quality [3].

The primary causal agent of Fusarium wilt in tomatoes has been historically attributed to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) [4]. This pathogen exhibits a high degree of host specificity and exists in distinct physiological races (Race 1, 2, and 3), complicating breeding for durable resistance. However, the *Fusarium* species complex associated with wilt and root rot in solanaceous crops is not monolithic [5]. Recent taxonomic revisions and molecular studies have revealed that other species, such as *Fusarium solani* and members of the *F. oxysporum* species complex (FOSC) with potentially different host ranges or virulence profiles, may also be implicated in similar disease syndromes, sometimes in complex interactions. Accurate identification of the etiological agents is therefore not merely academic but is a fundamental prerequisite for developing effective, targeted management strategies, including the deployment of resistant cultivars, biological control, and crop rotation [6].

The need for precise identification is greater in agriculturally demanding environments, such as desert farming systems. The desert farms of Karbala, Iraq, represent a critical region for intensive vegetable production, relying heavily on irrigation and protected cultivation to overcome arid conditions [7]. These unique agro-ecosystems—characterized by specific soil properties, microclimates, and often continuous cropping—may exert distinct selective pressures on pathogen populations. Consequently, the *Fusarium* communities prevalent in these settings may possess unique compositional or pathogenic traits compared to those in temperate or humid regions. Understanding the local pathogen landscape is essential for safeguarding local and national food security [8].

Traditionally, the identification of *Fusarium* species has relied on morphological and cultural characteristics, such as macroconidial and microconidial morphology, colony pigmentation, and growth rates. While these methods provide a foundational basis, they are often time-consuming, require high expertise due to phenotypic plasticity, and can be insufficient for distinguishing among closely related species or formae speciales within the FOSC [9]. The advent of molecular techniques has revolutionized fungal diagnostics. Tools such as sequencing of conserved genomic regions (e.g., translation elongation factor-1α (*TEF-1α*), the internal transcribed spacer (ITS) rDNA, and the RNA polymerase II second largest subunit (*RPB2*)) allow for unambiguous, rapid, and accurate species-level identification, enabling researchers to delineate phylogenetic relationships and assess genetic diversity within pathogen populations.

Despite the global significance of Fusarium wilt, comprehensive studies integrating both morphological and molecular approaches to delineate the specific *Fusarium* species responsible for tomato wilt in the unique context of Iraq's desert agriculture remain scarce. A detailed epidemiological profile of the pathogen(s) in the Karbala region is lacking. Therefore, this study was undertaken with the

following objectives: (1) to isolate and characterize *Fusarium* species associated with wilted tomato plants from desert farms in Karbala using morphological criteria, and (2) to confirm the species identity and explore the genetic diversity of these isolates through molecular phylogenetic analysis based on *TEF-1α* and ITS gene sequences. The findings from this research will provide the first consolidated insights into the etiology of Fusarium wilt in this key agricultural region, forming a vital scientific foundation for future disease management and resistance breeding programs tailored to local conditions.

2- Materials and Methods

2.1. Culture Media Preparation and Fungal Isolation

Potato dextrose agar (PDA) was prepared by dissolving 39 g of commercial powder in 1 L of distilled water according to the manufacturer's instructions, with chloramphenicol (250 mg/L) added to suppress bacterial growth. The medium was autoclaved, dispensed aseptically into Petri dishes, and stored at 4°C. Water agar (WA) was prepared by dissolving 17 g of agar in 1 L of distilled water, autoclaved at 121°C and 15 psi for 20 min, then cooled and held at 4°C prior to use.

Tomato tissues exhibiting wilting symptoms were sampled, and 0.5 cm segments (3–5 per sample) were excised aseptically. Surface sterilization comprised sequential treatments: 70% ethanol (30 s), sterile distilled water (1 min), 2% sodium hypochlorite (3 min), and a final rinse in distilled water (5 min). Four segments per 9 cm Petri dish were plated on PDA and incubated at 25 ± 2°C for 3–4 days. Pure cultures were obtained by subculturing hyphal tips from emerging colonies onto fresh PDA plates, followed by incubation for 5–7 days at 25 ± 2°C.

2.2. Phenotypic Identification of Fungal Isolates

For phenotypic characterization, a 0.5 cm disc was aseptically excised from the edge of actively growing pure fungal colonies and inoculated at the center of PDA plates. Plates were incubated at 25 ± 2°C for two to three days. Morphological identification was performed at the species level based on colony morphology and pigmentation using a taxonomic key [10] and was carried out by Dr. Jamal Hussein Kadim of the University of Kufa's College of Agriculture.

2.3. Molecular Diagnosis of Pathogenic Fungal Isolates Using Polymerase Chain Reaction (PCR)

Genomic DNA was isolated from 100 mg of 4–6-day-old fungal mycelium grown on PDA using the Geneaid fungal DNA extraction kit following the manufacturer's protocol. Fungal tissue was homogenized in 200 µL of FATG buffer, incubated at room temperature for 5 minutes, and then mixed with 200 µL of FABG buffer and centrifuged for 5 minutes. Samples were incubated at 70°C for 10 minutes with intermittent mixing. After adding 200 µL of 95% ethanol, the mixture was transferred to a FAPG spin column and centrifuged at 14,000 rpm for 5 minutes. The column was washed sequentially with 400 µL and 600 µL of wash buffers, centrifuging between washes to remove contaminants. DNA was eluted in 100 µL of Elution Buffer after a 3-minute room temperature incubation and centrifuged at 13,000 rpm for 1 minute. DNA concentration and purity were assessed spectrophotometrically, and samples were stored at −20°C until use.

2.4. DNA Concentration and Purity Assessment

The concentration and purity of extracted DNA were evaluated using a Nanodrop spectrophotometer by measuring absorbance ratios at 260 nm and 280 nm.

2.5. Polymerase Chain Reaction (PCR) Amplification

PCR amplification was performed using the ITS1 and ITS4 primers. The thermal cycling conditions are detailed in Table 1. PCR products from fungal isolates were bidirectionally sequenced using the same primers at Macrogen Korea. The resulting sequences were analyzed via BLAST

against the NCBI database for identification by comparison with reference sequences.

Table 1. Conditions and steps of the polymerase chain reaction (PCR) used to amplify the DNA of isolated fungi.

Description	Cycles	Temperature	Time	Step
Initial Denaturation	1	95°C	5 min	1
Denaturation	35	95°C	30 sec	2
Primer Annealing	35	60°C	30 sec	3
Primer Extension	35	72°C	40 sec	4
Final Extension	1	72°C	5 min	5

2.6. Pathogenicity Assessment of Fungal Isolates on Tomato Seeds Using Petri Dish Assays

Water agar medium was poured into sterile Petri dishes and inoculated with 0.5 cm plugs from 7-day-old fungal colonies, with triplicate plates per isolate. After incubation at 25 ± 2°C for 3 days, surface-sterilized tomato seeds (treated with 2% sodium hypochlorite, rinsed, and air-dried) were placed near the fungal margins, with 10 seeds per plate. Controls consisted of sterilized seeds placed on water agar without fungi. Plates were incubated for 7 days at 25 ± 2°C to assess the seed germination percentage.

For the seedling mortality assay, sterilized seeds were first germinated on moist filter paper at 25 ± 2°C for 7 days. The resulting seedlings were then transferred to Petri dishes adjacent to 3-day-old fungal colonies (10 seedlings per plate, three replicates). Controls were not exposed to fungi. All plates were incubated for 7 days at 25 ± 2°C. The germination percentage was calculated using the following formula:

Germination Percentage (%) = (Number of Germinated Seeds / Total Number of Seeds) × 100

2.7. Pathogenicity Assessment of Fungal Isolates on Tomato Seeds in Plastic Pots

Sterilized soil (sand:peat moss, 1:1) was autoclaved twice at 121°C and 15 psi for 1 hour. One-kilogram portions were inoculated aseptically with 5 g of millet seeds colonized by the fungal isolates and incubated for 7 days under controlled moisture in a shade net house. Three non-inoculated pots served as controls. The inoculated soils were placed in 5 × 7 cm plastic pots (3 replicates per isolate), and each pot was sown with four surface-sterilized hybrid tomato seeds. Pots were lightly irrigated and covered with perforated polyethylene bags to maintain humidity for 14 days under nursery conditions. Seed germination and rot incidence were recorded.

Simultaneously, surface-sterilized seeds (2 per well) were sown in Styrofoam trays, incubated for 7 days, and then transplanted (4 seedlings per pot, 3 replicates per isolate) for another 14 days. Seedling mortality was

calculated. All data from the experiments were statistically analyzed using Genstat with a completely randomized design (CRD). The least significant difference (LSD) test at a 5% significance level ($p \leq 0.05$) was used to assess treatment differences [11]. The seedling mortality percentage was determined using the following formula:

Seedling Mortality Percentage (%) = (Number of Dead Seedlings/Total Number of Seedlings) ×100

3. Results and Discussion

3.1. Morphological and Molecular Identification

3.1. Morphological Identification of Fungal Isolates

The isolated fungi were primarily identified as *Fusarium* spp., comprising one *Fusarium solani* and four distinct *Fusarium oxysporum* strains. Morphological assessment on culture media revealed variations in colony pigmentation on both fronts and reverses. Microscopic examination identified key diagnostic structures, including septate hyphae, falcate macroconidia with 3–5 septa, microconidia with 0–2 septa, and terminal or intercalary chlamydospores, which contribute to survival under stress conditions [11] (Figure 1). Further microscopic observations highlighted diversity in conidial morphology among isolates, including variations in size, shape, and septation. These phenotypic traits remain valuable for preliminary classification [13] (Figure 2).

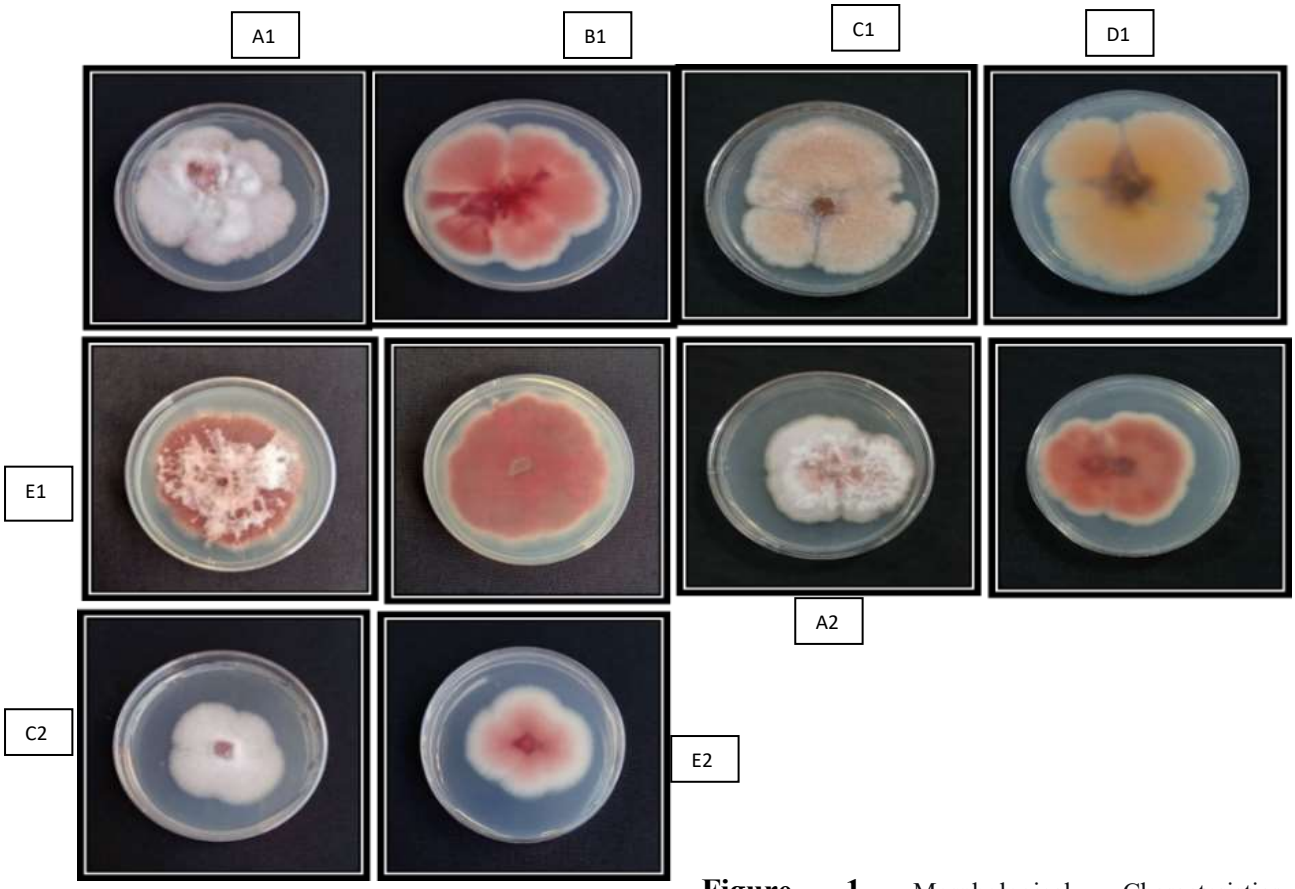

Figure 1 Morphological Characteristics of *Fusarium* spp

A1: Front side of *F. solani* Colony **A2**: Back side of *F. solani* Colony

B1: Front side of *F. oxysporum*1 Colony **B2**: Back side of *F. oxysporum*1 Colony
C1: Front side of *F. oxysporum*2 Colony **C2** :Back side of *F. oxysporum*2 Colony
D1: Front side of *F. oxysporum*3 Colony **D2**: Back side of *F. oxysporum*3 Colony
E1: Front side of *F. oxysporum*4 Colony **E2**: Back side of *F. oxysporum*4 Colony

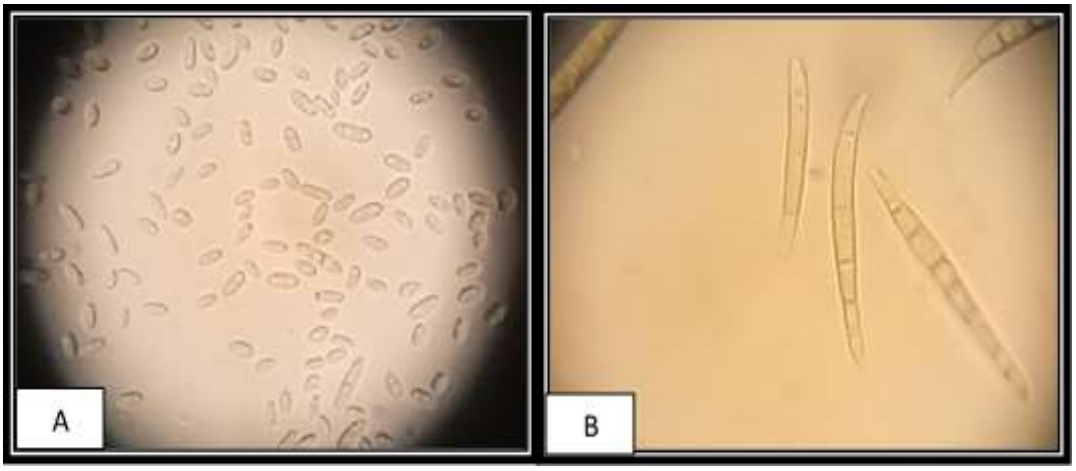

Figure 2 Microscopic visualization of *Fusarium* spp. spores under 40X magnification **A=** Microconidia ,**B**= Macroconidia

3.2. Molecular Identification and Phylogeny of Fusarium spp. via ITS-PCR

For precise identification, PCR amplification of the internal transcribed spacer (ITS) region using primers ITS1 and ITS4 yielded fragments ranging from 538 to 567 bp (Figure 3). Sequencing and subsequent submission to GenBank (accession numbers PV355877–PV355881) confirmed these as novel Iraqi strains. The ITS sequences were trimmed, assembled, and aligned using BioEdit and MEGA6. BLAST analysis confirmed genetic distinctness from previously reported Iraqi strains. A neighbor-joining phylogenetic tree constructed in MEGA7 illustrated that the Iraqi isolates clustered closely with known pathogenic reference strains, exhibiting 99.63–100% homology, which underscores their close genetic relatedness within pathogenic lineages (Figure 4) [14]. Sequence alignment revealed polymorphic sites within *F. solani* and nucleotide divergences in *F. oxysporum* isolate 2 compared to its closest NCBI match.

3.3. Broader Implications for Pathogen Study and Control

Pathogenicity tests on tomato seeds and seedlings were conducted under both controlled Petri dish and pot-based conditions to simulate laboratory and near-natural environments.

3.3.1. Petri Dish Assays

In Petri dish assays, all *Fusarium* isolates proved pathogenic, but their aggressiveness varied significantly. *F. solani* was the most virulent, reducing seed germination to 10% compared to 93.33% in the control. It also caused the highest seed rot (90%) and seedling mortality (63.33%). *F. oxysporum* isolates exhibited moderate to mild effects, with germination rates ranging from 26.66% to 40.00% (Table 2). Statistical analysis revealed significant differences among isolates ($p < 0.05$) (Table 2).

3.3.2. Pot Assays

In pot assays, which better simulate natural soil conditions, the overall trends remained consistent. *F. solani* continued to cause the greatest reduction in germination (25.00% vs. 91.66% in the control) and the highest seed rot (75.00%) and seedling mortality (66.66%). Among *F. oxysporum* isolates, isolate 4 was the least aggressive, with

germination reaching 75.00% (Table 3). Statistical analysis confirmed significant differences among treatments ($p < 0.05$) (Tables 2 and 3).

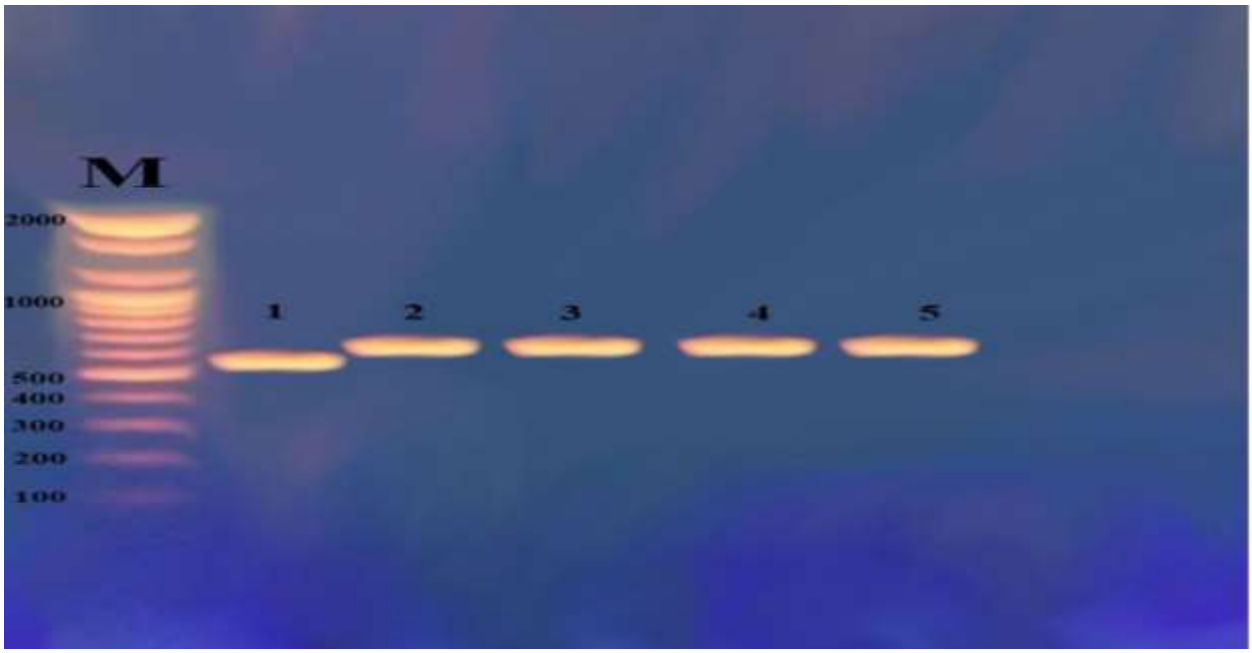

Figure 3 PCR-amplified DNA: 1. *F. solani*;۲ . *F. oxysporum* 1;۳ . *F.oxysporum* 2; 4**.** *F.oxysporum* 3 5. *F.oxysporum* 4

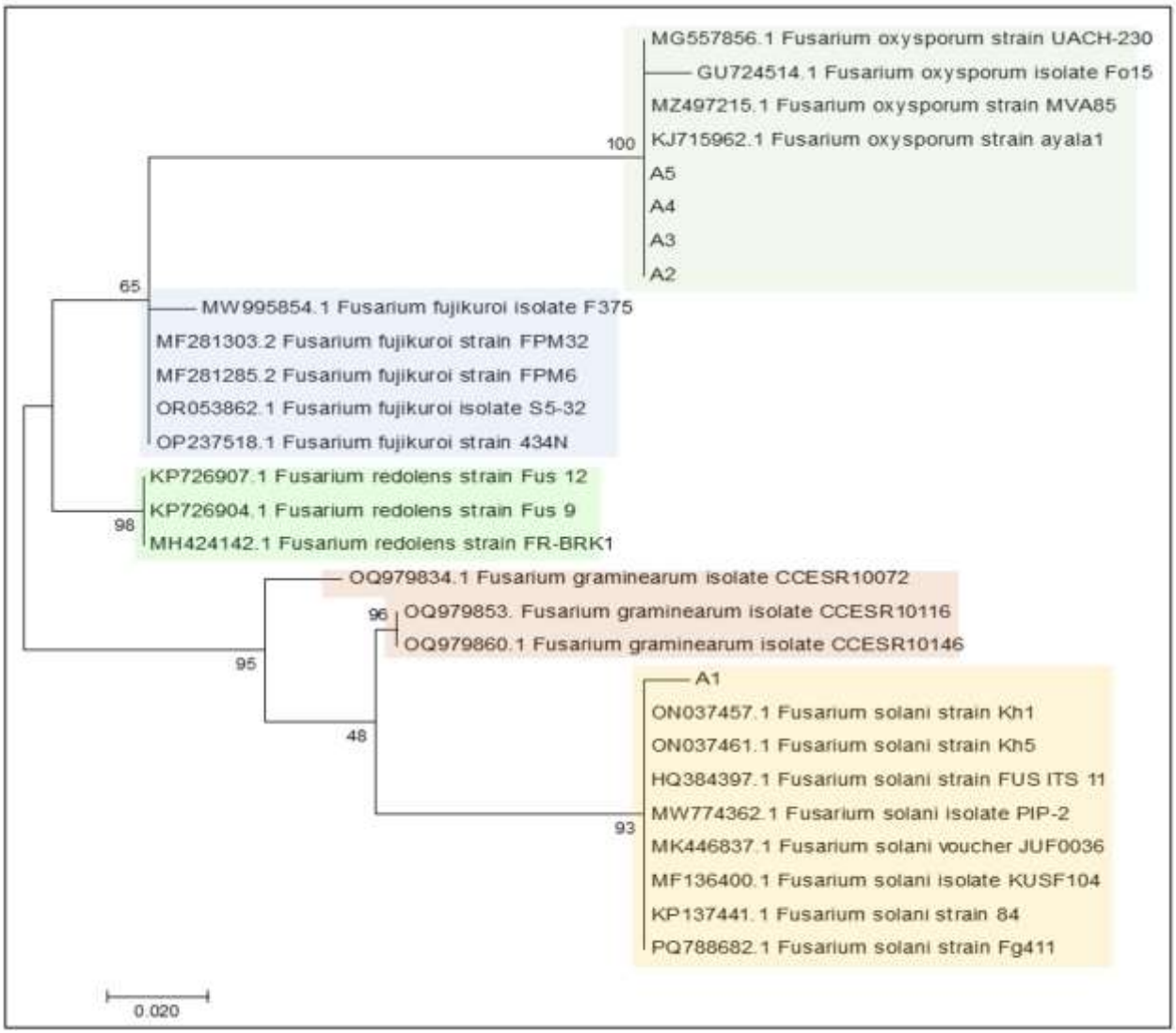

Figure 4 Neighbor-Joining phylogenetic tree illustrating the genetic relationships among *Fusarium* spp. isolates obtained in this study and reference isolates of the same genus previously registered in the National Center for (NCBI).

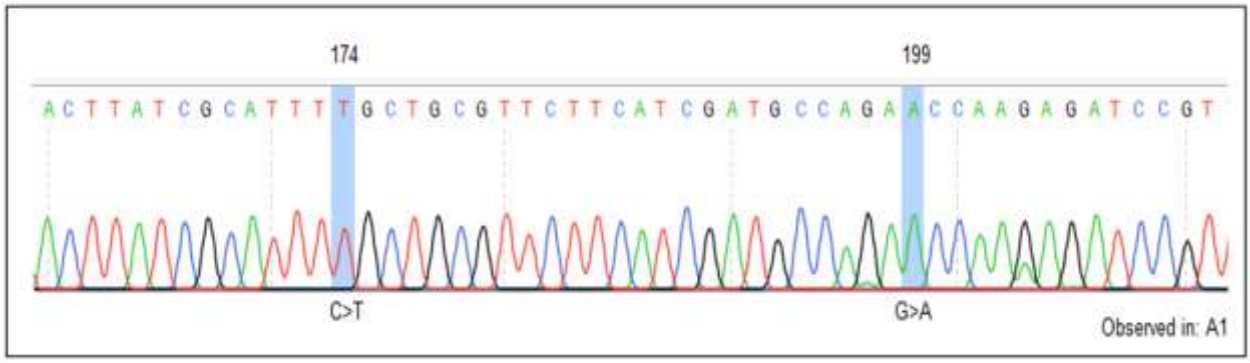

Figure 5 Sequence variation at a specific site in the nucleotide sequences of the PCR-amplified products from the *F. solani* isolate obtained in this study.

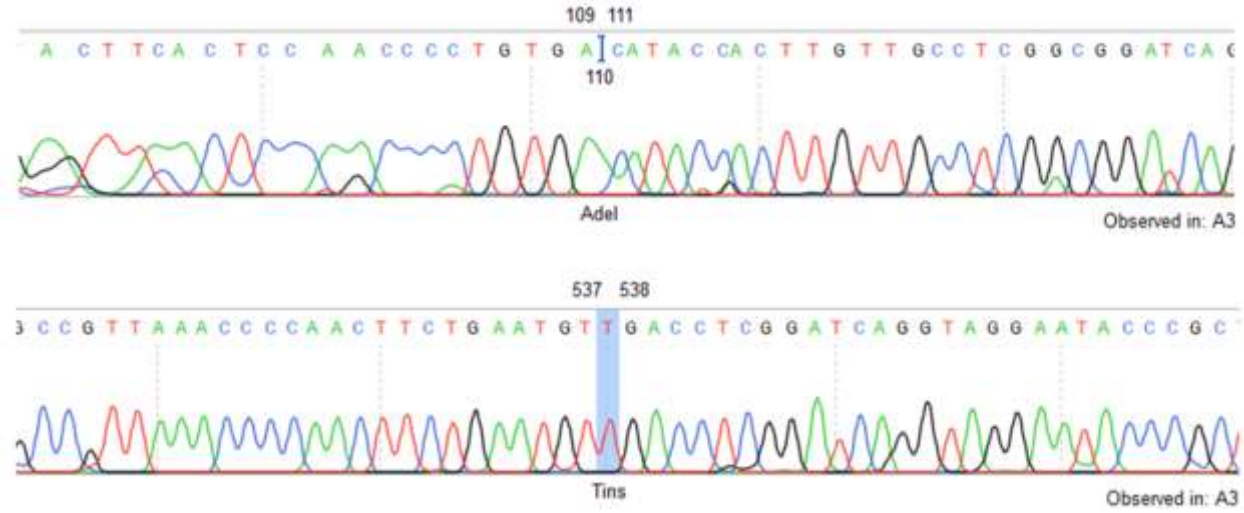

Figure 6 Sequence variation at a specific site in the nucleotide sequences of PCR-amplified products from the *F. oxysporum* 2 isolates obtained in this study and its closest matching isolate registered in the National Center (NCBI)

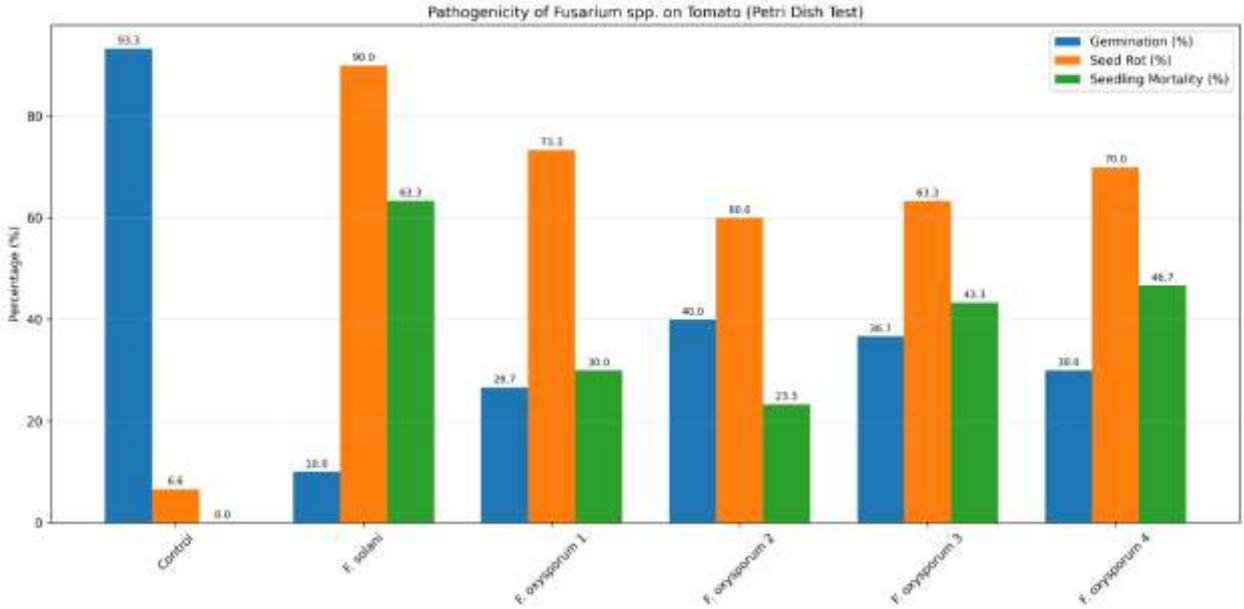

Figure 7: Pathogenicity of *Fusarium* spp. On Tomato (Petri Dish Test)

Table 1: Pathogenicity Test of *Fusarium* spp. Isolates on Tomato Seed Germination Percentage, Seed Rot, and Seedling Mortality in Petri Dishes

Treatments	Seed Germination %	Seed Rot %	Seedling Mortality %
Control	93.33*	6.64	00.00
F. solani	10.00	90.00	63.33
F. oxysporum 1	26.66	73.34	30.00
F. oxysporum 2	40.00	60.00	23.3
F. oxysporum 3	36.66	63.34	43.33
F. oxysporum 4	30.00	70.00	46.66
L.S.D=0.05	11.35	13.56	6.66

Each value represents the mean of three replicates. Different superscript letters within the same column indicate significant differences (p < 0.05).

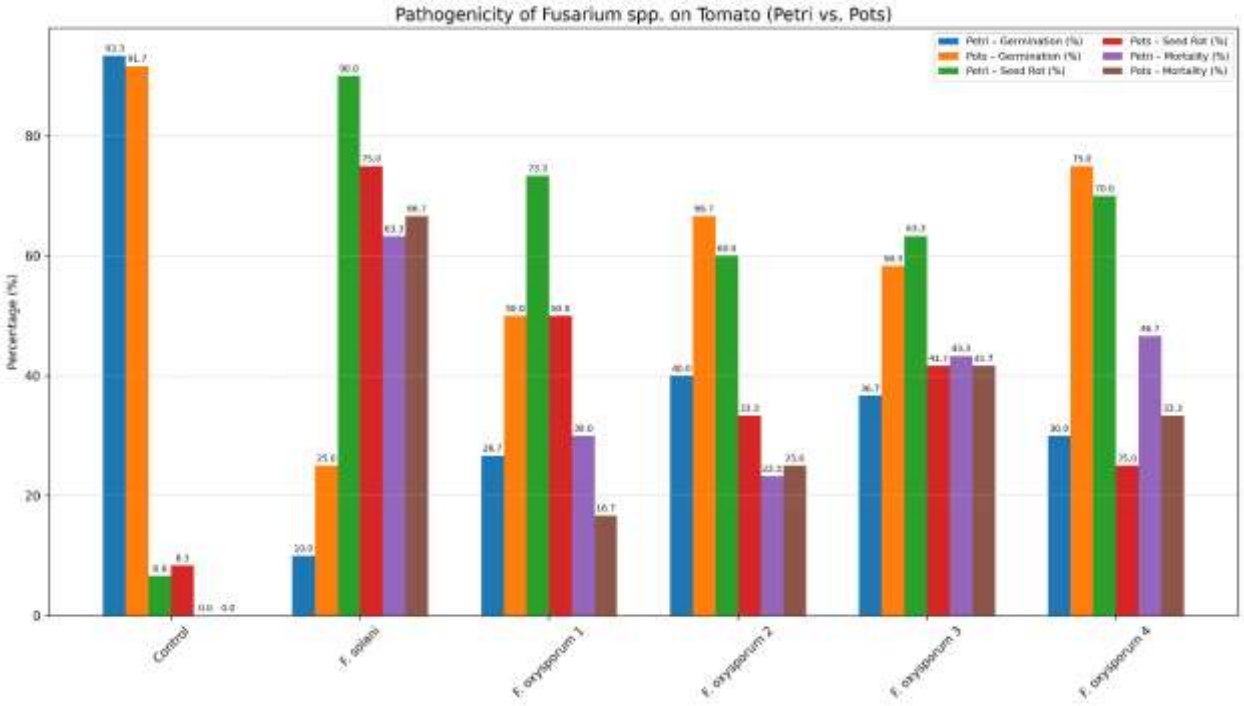

Figure 8: % Pathogenicity of *Fusarium* spp. On Tomato (Petri vs. Pots)

Table 2 Pathogenicity Test of *Fusarium* spp. Isolates on Tomato Seed Germination Percentage, Seed Rot, and Seedling Mortality in Pots

Treatments	Seed Germination %	Seed Rot %	Seedling Mortality %
Control	91.66*	8.34	00.00
F. solani	25.00	75.00	66.66
F. oxysporum 1	50.00	50.00	16.66
F. oxysporum 2	66.66	33.34	25.00
F. oxysporum 3	58.33	41.67	41.66
F. oxysporum 4	75.00	25.00	33.33
L.S.D=0.05	19.77	19.77	22.81

Each value represents the mean of three replicates. **Different superscript letters within the same column indicate significant differences (p < 0.05).**

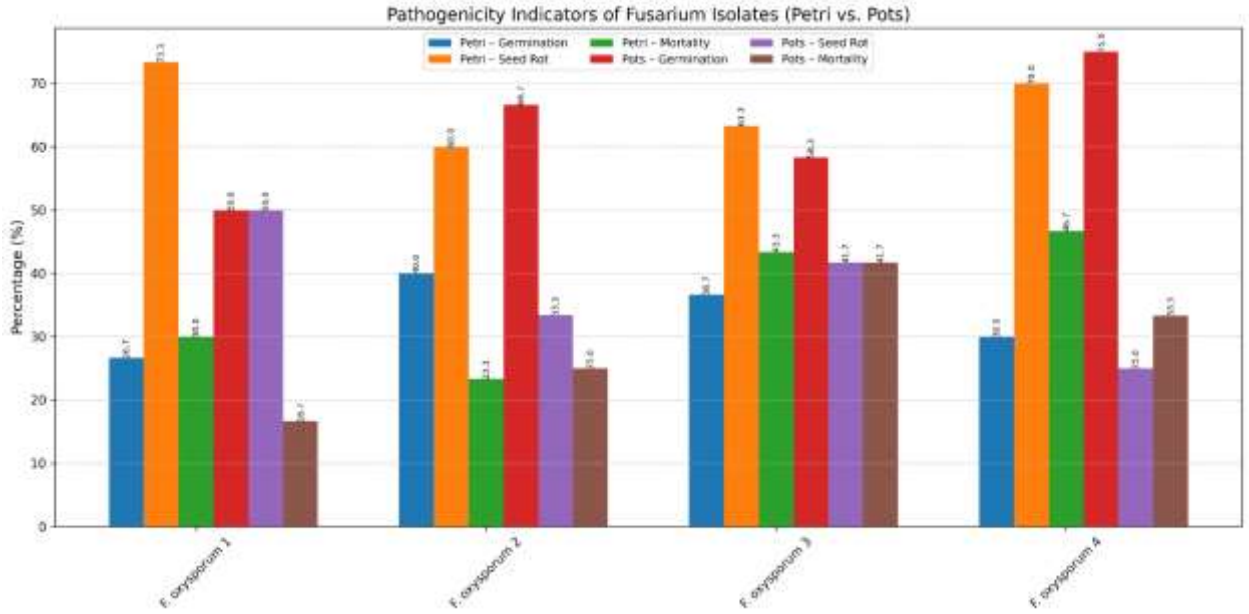

Figure 9: Pathogenicity indicators of *Fusarium* isolates (Petri vs. Pots)

Table 3: Averages from three replicates; *F. solani* consistently most virulent, *F. oxysporum* 4 least in pots.

Isolate	Petri: Germination (%)	Petri: Seed Rot (%)	Petri: Mortality (%)	Pots: Germination (%)	Pots: Seed Rot (%)	Pots: Mortality (%)
F. oxysporum 1	26.66	73.34	30.00	50.00	50.00	16.66
F. oxysporum 2	40.00	60.00	23.33	66.66	33.34	25.00
F. oxysporum 3	36.66	63.34	43.33	58.33	41.67	41.66
F. oxysporum 4	30.00	70.00	46.66	75.00	25.00	33.33

***Each value represents the mean of three replicates. *F. solani* was consistently the most virulent isolate, while *F. oxysporum* 4 was the least aggressive in pot assays.**

4- Discussion

4.1. Morphological and Molecular Characterization

The morphological characteristics observed in this study—including septate hyphae, falcate macroconidia with 3–5 septa, and the presence of chlamydospores—are consistent with established taxonomic descriptions of *Fusarium* species [11]. These features facilitated preliminary identification but required molecular confirmation due to phenotypic plasticity within the genus.

The ITS region proved to be a reliable molecular marker, resolving ambiguities associated with morphological identification alone and allowing clear differentiation at the species and strain level within

the *Fusarium* genus. **The high-resolution capability of the ITS region supports its utility in fungal diagnostics**, not only for *Fusarium* but also for other genera such as *Trichoderma*, *Rhizoctonia*, and *Pythium* [17–20]. **The variations present in the ITS region, specifically within the ribosomal DNA (rDNA) of eukaryotic organisms, have contributed to precise and important diagnostic applications for various organisms** [17–20]. Previous studies have demonstrated that PCR-based identification can correct errors associated with morphological identification alone [21], **confirming the efficacy of the diagnostic pipeline used** in this study.

4.2. Genetic Diversity and Phylogenetic Relationships

The neighbor-joining phylogenetic tree, constructed using MEGA7 from ITS rDNA sequences, positioned the novel Iraqi *F. solani* (PV355877) and *F. oxysporum* strains (PV355878–PV355881) in tight clusters with NCBI reference strains [14]. **The tree was constructed utilizing the MEGA7 tool** [15,16]. This clustering reflects shared evolutionary histories, likely driven by common ecological pressures in tomato pathosystems, where sequence conservation in ITS regions facilitates precise taxonomic delineation beyond morphological variability.

Sequence analysis revealed that the *Fusarium* isolates in this study are not genetically identical. The variations in the DNA sequence of the ITS region have proven highly effective for diagnosing numerous fungi belonging to genera such as *Fusarium* and *Cladosporium* [22]. **Specifically, nucleotide polymorphisms were observed in *F. solani* and in *F. oxysporum* 2 when compared to their closest database matches. Such polymorphisms can influence how strains interact with the host—for example, by modifying enzymes or toxins—and may therefore explain why some isolates are more aggressive or better adapted to local conditions in Iraqi tomato fields.**

The sequence variation analysis for the *F. solani* isolate indicates polymorphic sites within the PCR-amplified regions, which reflects genetic diversity at specific loci. These variations **may** impact gene function, altering traits such as virulence, adaptation to host defenses, or resistance to chemical treatments. Such polymorphisms provide insight into microevolution within populations, revealing how environmental pressures or host interactions drive genetic diversification. **These variations can influence key traits, such as the production of enzymes involved in host tissue degradation or toxins that compromise plant defenses. Identifying and mapping these variations is critical for understanding pathogenicity mechanisms and for developing targeted management strategies** such as fungicides or resistant crop varieties (Figures 5 and 6). **Mapping these polymorphisms with tools such as Clustal Omega enables the identification of strain-specific markers for rapid detection of virulent variants.**

In *F. oxysporum*, small but detectable variations in DNA sequence between isolates that are otherwise categorized as belonging to the same species are referred to as sequence divergence. When the ITS sequence of *F. oxysporum* 2 in this study was aligned with the closest reference strain from sequence databases, nucleotide changes were observed, suggesting that the isolate represents a genetically distinct variant rather than an identical copy. Although these base substitutions are **typically** dispersed at particular locations and do not interfere with species identity, they **nevertheless** indicate intraspecific diversity that may result in biological variations.

Such divergence **may** have an impact on the pathogenic behavior of *F. oxysporum* from a functional standpoint. The ability to overcome plant defenses, tissue colonization efficiency, and host recognition can all be affected by even small changes in genes related to the synthesis of toxins, enzymes, or surface proteins. This clarifies why, despite their close kinship, isolates categorized as *F. oxysporum* may differ in virulence, host range, or fungicide sensitivity. These sequence variations can accumulate over time due to local selection pressures in tomato-growing environments, such as host genotype, climate, and management techniques, **potentially**

resulting in locally adapted lineages that are more problematic in particular areas.

4.3. Pathogenicity and Virulence Variation

The pathogenicity tests revealed that all *Fusarium* isolates could infect tomato seeds and seedlings, but their aggressiveness varied notably between species and experimental conditions. **This variation is consistent with previous reports indicating that rapid fungal growth and high spore production are often associated with increased aggressiveness, allowing better tissue invasion at different host stages** [23–25].

F. solani demonstrated the highest virulence across both experimental systems. **This finding is significant because** *F. solani* **has historically been considered a less common causal agent of tomato wilt compared to *F. oxysporum*. The** confirmed its strong virulence under controlled lab conditions **suggests that its role in disease etiology in desert farming systems may be more substantial than previously recognized.**

The observation that disease severity was generally lower in pot assays compared to Petri dish assays suggests that soil microbiological and physical factors may partially suppress disease progression. However, the consistent high virulence of *F. solani* across both systems **indicates that** soil factors do not fully eliminate the threat posed by the most virulent strains. **This differential response highlights the importance of considering experimental conditions when interpreting pathogenicity data and extrapolating to field conditions.**

The variability observed among *F. oxysporum* isolates—particularly the relatively low virulence of isolate 4 in pot assays—**underscores the** genetic and physiological factors, such as each isolate's ability to produce enzymes and toxins that break down seed tissues and hinder early growth [26]. **These differences have practical implications for disease management, as** the variability among *F. oxysporum* strains **must be considered** when developing control measures and selecting resistant tomato varieties.

4.4. Implications for Disease Management and Future Research

The findings of this study establish a foundation for linking specific genetic differences to phenotypic traits such as aggressiveness, host adaptation, and fungicide sensitivity. Given the observed variation in pathogenicity among isolates under controlled conditions, **future research should focus on** disease progression in different tomato varieties and environmental settings. **Priority areas include** identifying tomato genotypes with resistance to these isolates and exploring biological control strategies using beneficial fungi, rhizobacteria, or plant-based treatments.

Integrating molecular diagnostics with field surveys in commercial farms will enable longitudinal monitoring of strain distribution and evolution. This approach will be crucial for designing targeted, region-specific management strategies tailored to the unique conditions of desert agriculture in Iraq.

4- Conclusion

To mitigate yield losses in tomato production, a dual approach integrating molecular monitoring with integrated pest management is recommended. Future research should prioritize three key areas: (1) linking specific genetic markers to virulence phenotypes to enable rapid diagnostic tools; (2) screening tomato germplasm for resistance against locally prevalent *Fusarium* strains; and (3) evaluating biological control agents and cultural practices under field conditions to develop sustainable, region-specific management strategies.

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