

ARTICLE

Diagnosis and pathogenicity of *Fusarium oxysporum* and *Rhizoctonia solani* associated with root rot and damping-off of eggplant seedlings in Najaf Governorate

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ABSTRACT

Root rot and damping-off caused by soil-borne fungi are among the most destructive diseases of eggplant, causing poor seedling establishment and heavy economic losses. This study was conducted to isolate and identify the causal fungus of root rot and damping-off of eggplant (*Solanum melongena* L.) seedlings in the growing areas of Najaf Governorate, recovering and molecularly identifying *Fusarium oxysporum*, and to compare its pathogenicity with that of a previously registered reference isolate of *Rhizoctonia solani* in the laboratory and in potting soil. A field survey covering the areas of Al-Hira, Al-Haidariyah and Bahr Al-Najaf showed the spread of the disease at all sites, with infection rates ranging from 29% to 62% (Al-Haidariyah recording the highest). The pathogenic fungi were isolated and purified from the surveyed fields. Isolation and morphological identification yielded three isolates belonging to *Fusarium* spp. (F.1, F.2 and F.3), all isolated from eggplant plants infected with root rot and damping-off, while two isolates belonging to *Rhizoctonia solani* (R.1 and R.2) were obtained from the culture collection of the Plant Protection Department. The isolated fungi differed in some morphological characters such as colour, mycelial density and growth rate, as well as in their pathogenicity. Pathogenicity testing on water-agar (WA) medium and in sterilized potting soil showed that isolates R.2 and F.2 were the most pathogenic; *R. solani* R.2 was the most virulent, recording 100% seed rot in Petri dishes and 88.50% disease severity in pots, while *F. oxysporum* F.2 recorded 72.50% disease severity in pots. Molecular diagnosis using PCR and sequencing of the ITS region showed that isolate F.2 belonged to *F. oxysporum* with 100% identity to global isolates, and it was deposited in GenBank under accession number PZ531662.1, whereas isolate R.2 matched reference isolates of *R. solani* registered in GenBank (e.g., PP767138.1).

Keywords: Soil-Borne Fungi; Molecular Diagnosis; damping-off; ITS-rDNA

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INTRODUCTION

The Eggplant (*Solanum melongena* L.), like many solanaceous vegetable crops, is susceptible to a wide range of diseases and pests that cause considerable economic losses, including fungal, bacterial and viral agents that impair plant health and reduce yield (Blancard, 2013). Crop losses due to plant diseases are estimated at about 10–40% (Ghorbanpour et al., 2018), especially those caused by soil-borne pathogenic fungi. Soil-borne fungi such as *Fusarium*, *Rhizoctonia*, *Pythium* and *Verticillium* are among the most common groups of plant pathogens and are responsible for some of the most important and damaging eggplant diseases, such as seed rot, damping-off, root rot and stem-base rot (Yan et al., 2024; Soliman et al., 2026).

Root rot and damping-off caused by *R. solani* and *F. oxysporum* are among the most common fungal diseases worldwide, including in Iraq, and may cause heavy losses in the total seedling stand when conditions favour the pathogen. The two fungi persist in the soil for long periods as dormant resistant structures and infect a wide range of hosts, which complicates their control and encourages the development of resistance to many currently used chemical fungicides. In Iraq, both pathogens have been repeatedly reported on vegetable crops; [Matloob and Al-Baldawy \(2020\)](#) identified *R. solani* as a major cause of eggplant root rot (see also [Soliman et al., 2026](#)), and [Al-Rammahi and Alshimaysawe \(2025\)](#) recently demonstrated in Najaf Governorate that *F. oxysporum* and *R. solani* are the causal agents of tomato seed rot and seedling death, with *F. oxysporum* showing higher pathogenicity. Despite these reports, molecularly-supported information on the fungi causing eggplant root rot and damping-off in Najaf Governorate remains limited, as most local documentation has focused on other hosts such as tomato ([Mwaniki et al., 2016](#)).

Therefore, this study was conducted to isolate and molecularly identify the causal fungus (*Fusarium oxysporum*) of root rot and damping-off of eggplant seedlings in the growing areas of Najaf Governorate, and to compare its pathogenicity on eggplant with that of a previously registered reference isolate of *Rhizoctonia solani* in the laboratory and in potting soil. The identity of the pathogens was further supported by molecular tools, including sequencing of the ITS region and deposition of the *F. oxysporum* sequence in the GenBank database.

MATERIALS AND METHODS

The study comprised a field survey to detect the presence of root-rot and damping-off fungi, particularly *R. solani* and *F. oxysporum*. The resulting isolates were purified, identified and confirmed by morphological and molecular diagnosis. The pathogenicity of the two pathogens was also tested on WA medium and in a plastic-pot experiment.

Field survey and sample collection

The field survey was carried out in mid-April 2025 in eggplant-growing fields of Najaf Governorate, covering the areas of Al-Hira, Al-Haidariyah and Bahr Al-Najaf (two fields). Sampling was restricted to the principal eggplant-producing localities in the governorate, which represent the main commercial eggplant production accessible during the growing season. Infected eggplant plants showing weakness, wilting, damping-off and root rot were recorded. From each site, five infected plants were sampled; each sample was placed in a labelled bag (area, sampling date and grower's name), and the grower was asked about the planting date, the pesticides used and the crops grown in the field. Samples were transferred to the laboratory and kept at 4 °C for isolation on the following day. The percentage of infected plants was calculated from the symptoms as: $\text{infection \%} = (\text{number of infected plants} \div \text{total number of plants}) \times 100$.

Isolation, purification and identification of the pathogenic fungi

Infected parts were cut into small pieces (0.5–1 cm), surface-sterilized with 1% sodium hypochlorite (NaOCl) for 3 minutes, rinsed several times with sterile distilled water, dried on sterile filter paper, and four pieces were transferred to sterile Petri dishes (9 cm) containing potato Dextrose Agar (PDA), incubated at 25 ± 2 °C for 2–4 days. The fungi were then purified by transferring a small piece from the colony margin to a fresh plate and incubating for 5–7 days. The pure isolates were identified to the genus level based on colony growth and the type, shape and colour of the spores, according to the diagnostic keys of [Cubeta and Vilgalys \(1997\)](#) for *R. solani* and [Leslie and Summerell \(2006\)](#) for *Fusarium*, with the assistance of specialists at the Department of Plant Protection, Faculty of Agriculture, University of Kufa.

Pathogenicity of the isolates on WA medium and in potting soil

The pathogenicity of the isolates was tested on eggplant-seed germination on water-agar (WA) medium and in 250 ml plastic pots containing 150 g of sterilized soil mixed with 1 g of inoculum of each *F. oxysporum* or *R. solani* isolate produced on a sterilized grain-seed medium (Dewan, 1989), with four replicates per isolate, in addition to a control of sterilized, non-inoculated soil. After three days, each pot was sown with ten surface-sterilized eggplant seeds (cv. Kroy) disinfected in 1% NaOCl for 3 min, and arranged randomly under natural conditions. Germination percentage, healthy-seedling percentage, seedling-death percentage, seed-rot percentage and the growth indicators (plant height and shoot/root fresh and dry weights) were recorded. Disease severity was assessed in the pot experiment using a four-grade index (Grade 0 = healthy plants; 1 = seed rot/failure to germinate; 2 = temporary wilting; 3 = damping-off of seedlings), and the percentage disease severity was calculated using equation according to McKinney (1923).

Molecular diagnosis of the isolates using PCR

Molecular identification was performed on the most virulent, newly field-recovered pathogen (F.2) to confirm its morphological identity; isolate R.2 was a previously identified and GenBank-registered reference isolate (*R. solani* AG-4, accession no. PP767138.1) and therefore did not require re-sequencing. Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research) following the manufacturer's instructions. The ITS region was amplified by PCR using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990) in a 20 µl reaction (1 µl of each primer, 5 µl DNA, 13 µl deionized water and AccuPower PCR PreMix, Bioneer). The thermal programme was 95 °C for 5 min, followed by 35 cycles (95 °C/30 s, 55 °C/30 s, 72 °C/1 min) and a final extension at 72 °C for 5 min. The products were separated by electrophoresis on 2% agarose gel in 1× TBE buffer with 2 µl ethidium bromide at 135 V for 30 min, using a Geneaid DL007 DNA ladder (100–3000 bp, with an enhanced 500 bp band), and visualized with a Gel Documentation system. The amplicons were sent to Macrogen (South Korea) for sequencing, and the sequences were compared against the NCBI database using BLAST; a phylogenetic tree was constructed by the neighbour-joining method.

Experimental design and statistical analysis

The experiments were carried out according to a Completely Randomized Design (C.R.D.) with four replicates. Data were analysed using the GenStat program, and means were compared using the least significant difference (L.S.D.) at 5% probability (Al-Rawi and Khalaf Allah, 2000); Excel was used for drawing the figures. Means followed by the same letter within a column do not differ significantly at $P \leq 0.05$.

RESULTS AND DISCUSSION

Field survey and isolation of the causal fungi

The field survey (Figure 1) showed the spread of eggplant root rot and damping-off at all surveyed sites in Najaf Governorate in 2025, with infection rates ranging from 29% to 62%. Al-Haidariyah recorded the highest infection (62%), followed by Al-Hira (38%), Bahr Al-Najaf field 2 (34%) and Bahr Al-Najaf field 1 (29%). Infected plants showed weakness, wilting, damping-off and root rot, in addition to the brown discoloration observed in a longitudinal section of the infected stems. The spread of the disease may generally be attributed to the use of contaminated irrigation water and the repeated cultivation of the same solanaceous hosts in the same fields during the season, which allows the pathogens to persist and their inoculum to accumulate in the soil until weather conditions and the presence of the host

become suitable. Isolation, morphological identification and microscopic examination of the pure colonies on P.D.A. medium yielded three isolates belonging to *F. oxysporum* F.1 (Bahr Al-Najaf), F.2 (Al-Hira) and F.3 (Al-Haidariyah), recovered from infected eggplants. In addition, two previously identified and registered *R. solani* isolates (R.1 and R.2) were obtained from the culture collection of the Plant Protection Department and included for comparative pathogenicity testing on eggplant; the most virulent of these, R.2, corresponds to *R. solani* AG-4 registered in GenBank under accession number PP767138.1.

The two *R. solani* isolates showed colonies ranging in colour from light to dark brown with dense mycelium; the vegetative hyphae branched at right angles to the main hypha with a slight constriction at the points of attachment characteristic features of the fungus with rapid growth on the medium (Cubeta and Vilgalys, 1997). The three *F. oxysporum* isolates showed colours ranging from light to dark pink with dense cottony mycelium, septate hyphae, curved sickle-shaped macroconidia tapering at both ends, oval microconidia, and large, thick-walled, dark, spherical chlamydospores (Leslie and Summerell, 2006). The viability test of the eggplant seeds used in the study (cv. Kroy) showed a germination percentage of 98.33%, indicating the high viability of the seeds.

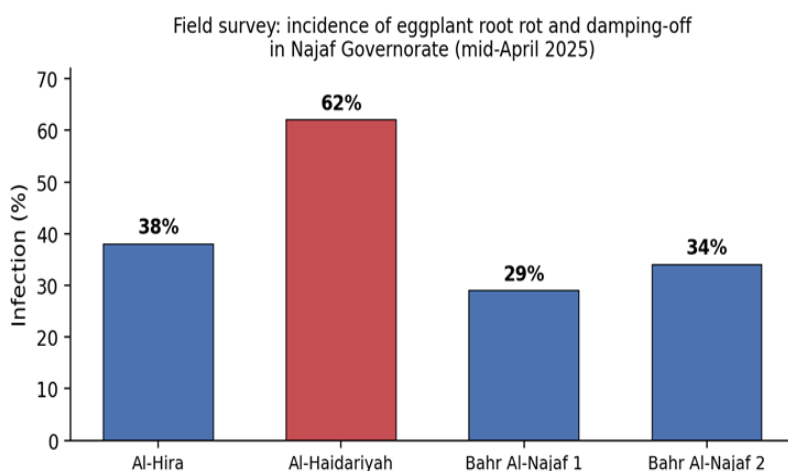


Figure (1) Incidence (%) of eggplant root rot and damping-off at the survey sites in Najaf Governorate (mid-April 2025).

Pathogenicity of the isolates on eggplant seeds and seedlings

The pathogenicity tests showed a clear and significant variation among the isolates in their effect on seed germination and on the growth indicators of eggplant seedlings (Tables 1–3). Isolate R.2 was generally the most virulent, recording no germination (0.00%) and 100% seed rot on WA medium (Table 1), and 100% seedling death, 79.80% seed rot and 88.50% disease severity in the pot experiment (Table 2). Among the *F. oxysporum* isolates, F.2 was the most pathogenic, with 60.00% seedling death, 37.50% seed rot and 72.50% disease severity in pots, compared with 37.69% and 6.25% disease severity for isolates F.1 and F.3, respectively, while no infection appeared in the control. The two most virulent isolates (R.2 and F.2) also caused the greatest reduction in plant height and in the fresh and dry weights of the shoot and root systems (Table 3). These results are consistent with Al-Rammahi and Alshimaysawe (2025), who reported *R. solani* as the more virulent pathogen and *F. oxysporum* as highly pathogenic on tomato in the same governorate, and with Matloob and Al-Baldawy (2020) for eggplant root rot in Iraq; comparable eggplant studies have reported both pathogens causing severe seedling wilt and root rot (Soliman *et al.*, 2026; Komeil *et al.*, 2025).

A notable difference was observed in the behaviour of isolate R.2 between the two pathogenicity assays: on water-agar medium no seed germinated (0.00% germination and 100% seed rot), whereas in the pot experiment 20.20% of the

seeds germinated but the resulting seedlings died completely (100% seedling death and 88.50% disease severity). This difference can be attributed to the nutrient-free water agar in a closed dish placing the seeds in direct and continuous contact with the highly virulent AG-4 inoculum, causing their rot before germination, whereas in the soil the inoculum was distributed heterogeneously, allowing a proportion of the seeds to germinate before the seedlings were affected by post-emergence damping-off (Abdelghany *et al.*, 2022).

Seed rot and damping-off generally result from the penetration of the pathogen's hyphae into the plant tissue and its browning, which is often attributed to the ability of the fungi to secrete lytic enzymes and toxins that decompose the roots of young seedlings, killing the seed embryos and preventing their germination or killing the seedlings. The variation in virulence among isolates is generally due to genetic and environmental differences and to differences in the production of degradative enzymes and toxins that weaken the plant's defence mechanisms (Perincherry *et al.*, 2019), which is consistent with previous reports indicating that *Fusarium* species are among the most important fungi causing damping-off in eggplant (Mwaniki *et al.*, 2016). The variation in virulence among isolates is further associated with differences in the production of cell-wall-degrading enzymes and secondary metabolites (Zuriegat *et al.*, 2021; Cai *et al.*, 2022; Akber *et al.*, 2023).

Table (1) Pathogenicity of the isolated fungi on eggplant seeds on water-agar (WA) medium.

Treatment	Germination (%)	Healthy seedlings (%)	Seedling death (%)	Seed rot (%)
Control	100.00 a	100.00 a	0.00 e	0.00 e
F.1	70.00 cd	71.43 bc	28.57 b	30.00 bc
F.2	62.50 d	64.00 c	36.00 a	37.50 b
F.3	87.50 b	91.43 a	8.57 d	12.50 d
R.1	77.50 bc	77.42 b	22.58 c	22.50 cd
R.2	0.00 e	0.00 d	0.00 e	100.00 a
L.S.D. (0.05)	12.00	12.00	4.32	12.00

Means of four replications; df error = 18. Same letter within a column = no significant difference ($P \leq 0.05$).

Table (2) Pathogenicity of the isolated fungi on eggplant seeds and seedlings in sterilized potting soil.

Treatment	Germination (%)	Healthy seedlings (%)	Seedling death (%)	Seed rot (%)	Disease severity (%)
Control	100.00 a	100.00 a	0.00 d	0.00 e	0.00 e
F.1	68.75 cd	72.73 b	27.27 c	31.25 bc	37.69 c
F.2	62.50 d	40.00 c	60.00 b	37.50 b	72.50 b
F.3	81.25 b	100.00 a	0.00 d	18.75 d	6.25 e
R.1	75.00 bc	83.33 b	16.67 c	25.00 cd	25.10 d
R.2	20.20 e	0.00 d	100.00 a	79.80 a	88.50 a
L.S.D. (0.05)	9.576	12.00	12.00	9.576	10.62

Means of four replications; df error = 18. Same letter within a column = no significant difference ($P \leq 0.05$).

Table (3) Effect of the isolated fungi on the growth indicators of eggplant seedlings (pot experiment)

Treatment	Plant height (cm)	Shoot fresh wt (mg)	Shoot dry wt (mg)	Root fresh wt (mg)	Root dry wt (mg)
Control	2.45 a	12.30 a	1.50 a	7.40 a	1.50 a
F.1	1.65 c	8.30 c	0.90 c	3.70 c	0.90 c
F.2	1.34 d	6.70 d	0.70 d	3.10 c	0.70 d
F.3	2.02 b	10.10 b	1.10 b	5.10 b	1.10 b
R.1	1.68 c	8.40 c	0.90 c	3.40 c	0.90 c
R.2	0.25 e	1.90 e	0.20 e	0.40 d	0.20 e
L.S.D. (0.05)	0.264	1.248	0.156	0.84	0.156

Means of four replications; df error = 18. Same letter within a column = no significant difference ($P \leq 0.05$).

Molecular Diagnosis

Electrophoresis of the PCR products on agarose gel showed a single clear band of the ITS region of about 500–600 bp for isolate F.2 relative to the DNA ladder (Figure 2), confirming successful amplification of the target region. Nucleotide-sequence analysis of this region, using BLAST against the NCBI database, showed that isolate F.2 belonged to *Fusarium oxysporum*, with 100% identity in the ITS sequence to global isolates previously registered in the database (Yan *et al.*, 2024), and it was deposited in GenBank under accession number PZ531662.1 (strain F.S. and O.A.Iraq1). The neighbour-joining phylogenetic tree (Figure 3) placed isolate F.2 within the *F. oxysporum* clade, clustering with global reference sequences of the genus *Fusarium*, including *Fusarium oxysporum* f. sp. *capsici* (OP881817.1), *Fusarium proliferatum* and *Fusarium* sp. T19 (KT351618.1). Isolate R.2 matched reference isolates of *R. solani* (anastomosis group AG-4) registered in GenBank (e.g., PP767138.1). The neighbour-joining tree of the registered isolate R.2 (Figure 4) placed it firmly within the *R. solani* AG-4 group, clustering with global AG-4 reference sequences from GenBank and clearly separated from the outgroup (*Rhizopus arrhizus*), which confirms its identity at the anastomosis-group level. The ITS region is of great importance in identifying fungi to the species or strain level and in understanding their phylogenetic relationships, and differences within this region on the ribosomal DNA (rDNA) have contributed accurately to the diagnosis of fungi such as *Fusarium* spp. and *R. solani* (Schoch *et al.*, 2012; Lücking *et al.*, 2020; Yang *et al.*, 2024).

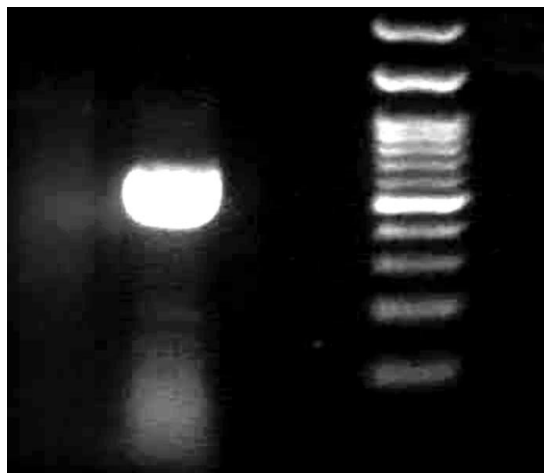


Figure (2) Agarose-gel (2%) electrophoresis of the ITS amplicon of isolate F.2 (~500–600 bp) relative to the Geneaid DL007 DNA ladder (100–3000 bp).

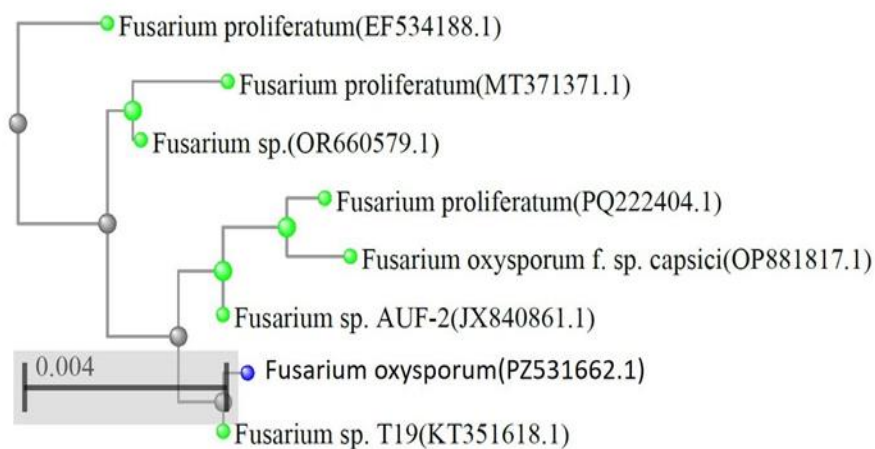


Figure (3) Neighbour-joining phylogenetic tree of *Fusarium oxysporum* isolate F.2 (PZ531662.1), constructed from the ITS-rDNA sequence together with global reference sequences obtained from GenBank.

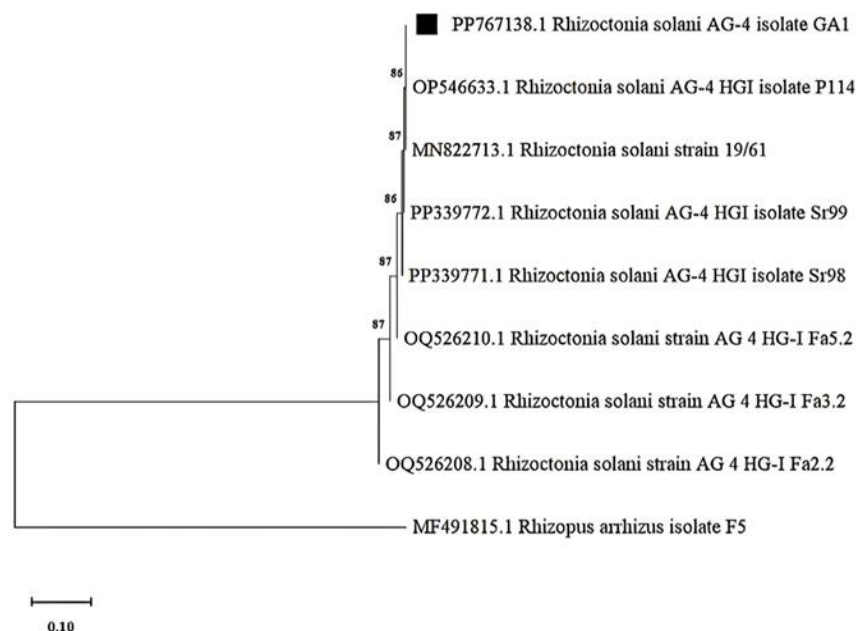


Figure (4) Neighbour-joining phylogenetic tree of *Rhizoctonia solani* AG-4 isolate GA1 (marked with a black square; accession no. PP767138.1), constructed from ITS-rDNA sequences together with global reference sequences of the same pathogen obtained from GenBank; genetic distances were computed by the neighbour-joining method.

CONCLUSIONS

The study showed that root rot and damping-off of eggplant seedlings in Najaf Governorate are caused by *Fusarium oxysporum* and *Rhizoctonia solani*, and that isolates F.2 and R.2 were the most virulent, with *R. solani* R.2 being the most damaging (88.50% disease severity in pots). Molecular diagnosis of the ITS region confirmed the identity of the two isolates, and the *F. oxysporum* sequence was deposited in GenBank under accession number PZ531662.1. Further studies on the integrated management of this disease in Najaf Governorate are recommended. The evaluation of biological and chemical control agents against these pathogens is addressed in a companion study.

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