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Research Article

Identification and characterizations of a few species of *Fusarium* infecting cucumber in greenhouse conditions

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Abstract
One of the most problematic and devastating diseases affecting cucumber production is *Fusarium* wilt (*Cucumis sativus* L.). The present study aimed to characterize *Fusarium* species isolated from three different regions of cucumber fields in Babylon province, Iraq and identify them based on morphological features and phylogenetic analyses. This study showed the presence of *Fusarium incarnatum, F. solani and F. oxysporum* in cucumber roots. *Fusarium* species were phylogenetically analyzed based on internal transcribed spacer (ITS) regions. All *Fusarium* isolates were pathogenic to cucumber cultivars but varied significantly in their growth and pathogenicity toward cucumber seeds during the experiment. *F. solani*-2 was greater in growth and pathogenicity than all other *Fusarium* species. In contrast, *F. solani*-1 was the lowest. The data obtained from morphological and molecular studies sufficiently supported each other, and the phylogenetic trees based on ITS were distinguished. Closely related species and distinctly separated all morphological taxa. These findings are reported for the first time for the cucumber plant in Babylon province. All these ITS sequences showed homologous to those of *Fusarium* species isolates in the GenBank database with a similarity percentage of 99%. To the best of present knowledge, this is the first molecular record of *F. incarnatum* on the cucumber plants in Iraq. The study concluded that *F. incarnatum* was reported for the first time in Iraq and worldwide as a causal agent of wilt disease in cucumber plants. The three species of *Fusarium* have different pathogenic abilities, highlighting their disease incidence, growth, and pathogenicity.

Keywords: Cucumber, *Fusarium* species, Morphological, Molecular identification, Pathogenicity

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a vegetable crop with an annual production value of approximately $ 9.76 billion worldwide. Cucumbers are cultivated and produced primarily in Asia (87.2%), with China dominating the world's cucumber production. It is a popular fruit crop in Iraq, and it is grown in both greenhouses and open fields, with Babylon Province being one of the most valuable producing areas. Due to favourable environmental conditions, particularly the presence of fertile soils and high demand for cucumbers, most farmers have applied continuous cucumber cultivation. Cucumber yield and quality are affected by continuous cropping. Disease pathogens are common in constantly cultivated soil (Meng *et al*., 2018).

Fusarium is considered to be one of the most important genera of plant pathogens, as it is known to cause serious diseases in several economical plants, including cucumbers, watermelons, tomatoes, etc. (Alnuaimy *et al*., 2017; Oyedeji *et al*., 2022; Ajmal *et al*., 2023). Cucumber *Fusarium* wilt disease caused a significant problem in cucumber production. This disease can devastate cucumber plants and cause considerable economic losses for farmers. The disease is prevalent in most countries, with yield losses ranging from 40–70%

(Din *et al*., 2020; Sharma and Shukla, 2021). According to Fareed *et al*. (2016), the cucumber wilt is common in most of the world's cucumber-growing regions.

Fusarium solani and *F. oxysporum*, cause root rot and damping off in cucumber plants reducing the production of these plants (Din *et al*., 2020). *F. incarnatum* causes serious disease in the young cucumber fruit that has withered and is coated in white mycelium (Mao *et al*. 2020). The wilting symptoms are also accompanied by chlorosis, necrosis of the inter-veinal parts of the leaves, and a failure to produce fruit. Later infection causes abnormal fruits to be produced (Al-Tuwaijri, 2015). The pathogenicity test revealed that yellowing old leaves was the primary symptom of *Fusarium* wilt disease. Over time, the diseased plant will wilt and eventually die (Din *et al*., 2020).

The conventional approaches for describing and distinguishing between *Fusarium* species are mostly focused on their appearance (Rahjoo *et al*., 2008). Identifying the closely related *Fusarium* species simply on morphological distinctions is impossible because there are so many different morphological variations (Leslie and Summerell, 2006). Molecular techniques are therefore required to identify *Fusarium*s specifically at the species level. However, according to multiple earlier research, employing just the ribosomal DNA gene is insufficient to identify *Fusarium* species (O'Donnell *et al*., 2015). As a result, recent techniques for accurately identifying *Fusarium* species combine morphological traits with multi-gene molecular phylogenetic analyses (Jedidi *et al*., 2021). *Fusarium* species generate a variety of secondary metabolites, including mycotoxins and extracellular cell wall-degrading enzymes (CWDEs), which aid in weakening and successfully invading the host plant (Perincherry *et al*., 2021).

The present study aimed to investigate the disease prevalence of *Fusarium* wilt pathogens that attach cucumber grown in greenhouses in different regions of Babylon Province, and to identify the causal agents using morphological characteristics and sequencing of Internal transcribed spacer (ITS) region and pathogenicity of the causative agents of *Fusarium* wilt disease.

MATERIALS AND METHODS

Preparation of fungal growth medium

Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) were prepared according to the manufacturer's instructions and fixed on their containers supplemented with chloramphenicol (25 µg/mL) to both media. They were sterilized by autoclave at 15 psi/inch^2 in 121ºC for 15 min., 15 ml of sterilized medium were poured in disposable Petri dishes (9 cm diameter) and they were incubated at 25±2^{o}C overnight to ensure sterility and stored at 4^{o}C until used. For all the experiments described below, a plug of culture (5 mm in diameter), was cut from the actively growing margin of stock culture using a cork borer and incubated at 25 ^{o}C ± 2 for 7 days.

Field disease assessment

A field investigation was carried out to study the occurrence of *Fusarium* wilt disease in cucumbers within the primary cultivation regions of Babylon Province, Iraq. The extent of the disease was determined by calculating the disease incidence (%) using a random sampling technique. A minimum of 100 plants were examined per farm, and the recorded data reflected the percentage of infected plants relative to the total number of plants surveyed.

Sample collection

A field survey was conducted for this research project from December 2022 to April 2023. The cucumber *Fusarium* wilt disease was studied in the main growth locations in Al-Azizia, Al-Badah, and Al-Tahiria in the Al-Musayyib District in Babylon Province, Iraq.

Isolation of *Fusarium spp.*

This work was started by collecting naturally infected cucumber plants showing wilt symptoms from root-rotted plants. Collected samples were sent to the Advanced Mycology Lab./ Department of Biology, College of Sciences, University of Babylon. Diseased tissues were cut with a disinfected blade. Every five pieces of diseased tissues were sterilized by dipping in 1% Sodium hypochlorite solution (NaOCl) for 3–5 min and washed thoroughly with distilled water (5 mm), dried with sterilized filler papers (No.1) to remove surplus moisture, All five segments were inserted on 9 cm diameter of sterilized plastic Petri dishes with disinfected PDA culture media, which were then incubated at 25±2 ^{o}C while being periodically checked (Hussein *et al*., 2022).

Purification

Fusarium isolates were purified by cutting off a piece of the fungal growth edge, replanting it in different Petri dishes, and incubating it for five days. The fungus was then purified using the single spore method, and it was identified at the species level based on its cultural and morphological traits and the spores it produced, as described by (Leslie and Summerell, 2006).

Morphological characterization of the isolated fungi

For morphological identification, culture features were determined at seven day old PDA cultures at 25º 2 ± C. Cultural features of *Fusarium* species were determined at seven-day-old PDA cultures at a temperature of 25± 2 ^{0}C. Fungus tissues were taken out and placed on disinfected PDA plates using a sterilized toothpick. Af-

ter growing on PDA and being incubated for 9 days at 25± 2 ^{0}C, the characteristics of the fungus colony and its microscopic characteristics were examined.

Under a light microscope, a mature culture was observed. The presence or absence of chlamydospores, macro and microconidia, and sporodochia were recorded (Leslie and Summerell, 2006; Mohsen *et al*., 2017). The whole process took place in a laminar hood under sterile circumstances. Each sample was examined at a 400X magnification after five days of incubation.

Maintenance of fungal isolates

Sterilized PDA medium was prepared, poured into Petri dishes, 15 ml each, and left until solidification. The medium in Petri dishes was inoculated with a fungal isolate taken from the edge of the recently produced colony and incubated at 25±2^{o}C for 7 days and then kept in the refrigerator at 5^{0}C. All isolates were maintained on PDA slants at 5 ^{o}C and subculture monthly throughout this study. The total isolates were cultured on pieces of sterile filter paper on PDA for long-term storage and incubated for 7 days. The cultures were then stored at -20ºC. Also, fungal isolates were grown and kept in sterile mallet seeds in small bottles. Furthermore, fungal isolates were grown and stored in small bottles of sterilized mallet seeds.

Measurement of mycelial diameter

Mycelium disks of 0.5 cm diameter were taken and placed in the center of five Petri dishes containing PDA media to measure the mycelial diameter for each *Fusarium* species, then incubated at 25±2°C for 9 days. The growth was measured at intervals of days, starting after 72 h. Colon diameter was divided into two orthogonal directions, and the average was determined for each replication. Once one of the isolates had completely covered the plate, the measurements were stopped after nine days.

Molecular identification of *Fusarium spp.*

To validate the identity of a specific representative sample, this was further confirmed through ITS amplification using universal primers, namely ITS1 and ITS4, which target the ITS regions of the *Fusarium sp.* The extraction of the Internal Transcribed Spacer (ITS) gene from *Fusarium sp.* was conducted following the DNeasy Plant Mini kits protocol. The PCR amplification and gene sequencing steps employed the universal primer pairs ITS1 and ITS4, with their corresponding nucleotide sequences being ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as defined by White *et al*. (1990).

For the PCR amplifications, a 30 µL reaction mixture was prepared, comprising 2 µL of DNA template, 1.5 µL of each forward and reverse primer, 15 µL of PCR master mix (utilizing Taq DNA polymerase from BIO-MAX Company), and 10 µL of nuclease-free water. The reaction mixture was gently vortexed for 30 seconds after combining the components. The PCR amplification was carried out using a VITAR SEGATEC-Bio Rad Company Thermocycler, involving an initial denaturation at 95°C for 4 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 58.8°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. Subsequently, the reaction mixture was maintained at 4°C.

A 1% agarose gel was prepared by mixing 1 g of agarose with 100 mL of 1x TBE buffer (pH 8.0), and 4 µL of red-safe stain was added to enhance visualization. The gel tray was placed within a tank filled with 1x TBE buffer. A molecular marker ladder (100bp DNA Ladder H3 RTU, 1st base company, Malaysia) was loaded initially, followed by loading 7 µL of the PCR product samples. Electrophoresis was conducted at a rate of 80 V cm−1 for 60 minutes. The gel was then observed under UV light using a gel documentation system (version 6.03, Syngene Laboratories) to visualize the amplified DNA bands.

Gene sequencing analysis

PCR amplicons were forwarded to MyTACG Bioscience Enterprise for the process of purification and DNA sequencing. The cleaned PCR products were subjected to sequencing using an automated DNA sequencer, ABI PRISM®. The gene sequences of ITS were matched and organized utilizing Bioedit software version 7.27, accessible at http://www.mbio.ncsu.edu/bioEdit/bioEdit. A similarity search was conducted for comparison against *Fusarium* species sequences present in the GenBank database. This comparison was performed through the BLAST network services available in the National Center for Biotechnology Information (NCBI) database at http://www.ncbi.nlm.nih.gov.

Genomic DNA extraction and PCR amplification

Genomic DNA extraction

The Favor PrepTM Genomic DNA Mini Kit was employed to extract genomic DNA from fungal samples by the provided instructions. Initially, 1 to 5 x 10 fungal cell cultures were transferred into 1.5 ml microcentrifuge tubes. Subsequently, FA Buffer (1 ml) was added to the cells and re-suspended through pipetting. The cells were then centrifuged at 7,500 rpm (5,000 x g) for 2 minutes, and the resulting supernatant was discarded. The cell pellet was re-suspended in 550 µl of FB Buffer, followed by the addition of 50 µl of lyticase solution, and the mixture was adequately vortexed. The sample was incubated at 37°C for 30 minutes and then centrifuged at 7,500 rpm (5,000 x g) for 10 minutes. After discarding the supernatant, pipetting re-suspended the cell pellet in 350 µl of TG1 Buffer.

The sample mixture was transferred to a bead tube, vigorously vortexed for 5 minutes, and then supplemented with 20 µl of Proteinase K (10mg/ml), followed by further vortexing. Incubation at 60°C for 15 minutes was performed, with vortexing every 5 minutes. Subsequently, cells were centrifuged at 7,500 rpm (5,000 x g) for 1 minute, and 200 µl of the supernatant was carefully moved to a fresh 1.5 ml microcentrifuge tube. 200 µl of TG2 Buffer was added and mixed by pipetting. An addition of 200 µl of ethanol (96-100%) was mixed by pulse-vortexing for 10 seconds. The sample mixture, including any precipitate, was transferred to a TG Mini Column. After centrifuging for 1 minute, the TG Mini Column was transferred to a new Collection Tube. The column was washed with 450 µl of W1 Buffer by centrifugation, and the flow-through was discarded. This step was repeated using 750 µl of Wash Buffer, and the column was centrifuged for 3 minutes to dry. The TG Mini Column was then placed in an Elution Tube, and 50 to 100 µl of Elution Buffer or ddH2O was added to the center of the membrane on the TG Mini Column. The column was left standing for 3 minutes and then centrifuged for 2 minutes to elute the total DNA. The quality and quantity of the DNA were assessed using a NanoDrop 2000 spectrophotometer. The eluted genomic DNA was stored at -20ºC until needed.

Primer pairs

About the primer pairs, the ones used in the study (provided by Macrogen/Korea) were dissolved using sterile ddH2O. A 100 pmol/µl stock solution was prepared by combining ddH_2O with the lyophilized primer vial. A 10 pmol/µl working stock was then produced by mixing 10 µl of the stock primer with 90 µl of sterilized d.H2O.

Pathogenicity of *Fusarium spp*. isolates using cucumber seeds

The pathogenicity of *F. incarantum, F. solani*1*, F. solani* 2*, F. oxysporum* on cucumber seeds in Petri dish was examined. Twenty seeds were treated with 2% sodium hypochlorite for 4 min, rinsed three times with sterile distilled water then dried with filter paper (Whitman 1). Petri dishes of 9 cm in diameter were prepared, containing 15 ml of water agar culture, with the addition of the antibiotic chloramphenicol. The Petri dishes were inoculated with discs 0.5 cm in diameter taken from the edge of the recent pure colonies of *Fusarium* isolates, grown separately on WA, at the age of five days. The Petri-dishes were incubated at 25 ± 2°C for 3 days. Then it was planted with cucumber seeds (surface sterilized with sodium hypochlorate solution) 1% free chlorine) in a circular motion near the edge of the plate at a rate of 20 seeds/plate. Each treatment was repeated in four plates for each isolation with a control treatment (without fungus). The Petri-dishes were incubated at a temperature of 25±2°C. The design of the experiment was CRD and the percentage of germination was calculated seven days after sowing the seeds (Carling *et al*., 1987).

Statistical analyses

The mean and standard error (SE) were used to present statistical data. Data were analyzed using a one-way analysis of variance (ANOVA). A least significant difference range test with the SE was used to compare treatment means when the P-value was less than 0.05.

RESULTS AND DISCUSSION

Field disease prevalence

The infected areas displayed symptoms of the *Fusarium* wilt disease during the relevant period. The survey results show that leaf yellowing and considerable wilting were observed in every district surveyed. These signs and symptoms were typical of *Fusarium* wilts (Fig. 1). Table 1 summarises field data regarding disease incidence and symptoms. Field disease incidence ranged from 22% to 52.3%. The areas with the highest disease incidence were Al-Tahiria (5 (%2.3and Al-Azizia 4 %2.3followed by Al-Badah, which had the lowest disease incidence (22%). This data indicated that *Fusarium* wilt disease varied significantly among the three areas and was more prevalent in mature cucumber plants.

Cultural and morphological features of *Fusarium* species

Causal organisms were isolated by cultivating them on a PDA medium at 25°C ± 2 for 7 days. Through an analysis of their morphological features, a total of 40 isolates were categorized into three distinct *Fusarium* species (Table 3). Among these, 5 isolates were identified as *F. incarnatum,* 22 as *F. solani,* and the remaining 13 isolates were determined to be *F. oxysporum*. The morphological traits of these three species are visually depicted in Fig. 2.

Fusarium incarnatum

Colonies of *F. incarnatum* produce profuse mycelium that was initially white, then the colony appearance (surface) looks light pink in the periphery and brown in the center. Sporodochia were eventually produced. Machroconidia had three to five septa. Microconidia were fusoid in form, mostly with two to four septa {Fig. 2 (A1, A2 and A3)}.

Fusarium solani

Two different isolates from *F. solani* (*F. solani*-1 and *F. solani*-2) were detected, both produced creamy pig-

Table 1. ITS primer pair

Primer name	Sequence 5′-3′	Reference
ITS3 F	GCATCGATGAAGAACGCAGC	Wahyuningsih et al., 2000
ITS4 R	TCCTCCGCTTATTGATATGC	

Table 2. *Fusarium* wilt disease incidence reported in cucumber farms in three geographical regions in Al-Musayab District (Al-Azizia, Al-Badah, and Al-Tahiria)

Regions	Symptoms	Disease incidence %
Al-Azizia	Leaf yellowing, wilt	42.3 ± 1.4 b
Al-Badah	Leaf yellowing, wilt	22 ± 1.4 a
Al-Tahiria	Wilt	52.3 ± 1.4 c

Table 3. Radial growth (cm) of *Fusarium incarnatum*, *F. solani*-1, *F. solani*-2 and *F. oxysporum* grown on PDA at 25 ± 2°C after 3, 5, 7 and 9 days after inoculation.

Isolates	3 days M ± SE	5 days M ± SE	7 days M ± SE	9 days M ± SE
Fusarium incarnatum AJA	4.0 ± 0.1B	5.0 ± 0.1 b	6.7 ± 0.1b	8.1 ± 0.1c
Fusarium solani AJA1	3.1± 0.2A	4.0 ± 0.06a	5.0 ± 0.1a	5.5 ± 0.2a
Fusarium solani AJA2	4.1 ± 0.1B	5.3 ± 0.1b	7.3 ± 0.1c	8.8 ± 0.08d
Fusarium oxysporum AJA	3.6 ± 008.B	3.9 ± 0.08a	6.4 ± 0.1b	7.4 ± 0.08b

Data are expressed as mean ±SE of three replicates for each treatment. Similar letters indicate no differences at P≤0.05. All *Fusarium* species were individually inoculated at the center of the Petri plate.

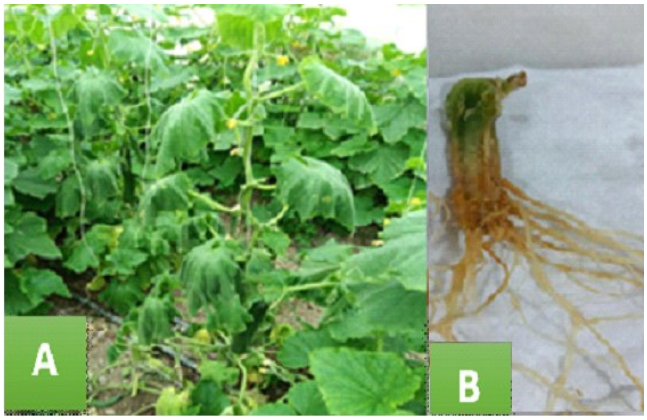

Fig. 1. *Symptoms of Fusarium wilt disease in sampling areas of cucumber field in the Al-Azizia, Al-Badah, and Al-Tahiria regions of Al-Musayab District, Babylon province, have been found to include yellowing leaves and wilting symptoms. A: Arial part, B: Root system*

mentation (Fig. 2), and they differed in shape, reproductive structures, growth behaviour and growth rate. The colony of the *F. solani*-1 {Fig. 2(B1, B2 and B3)}, produced soft woolly hairs. The shapes of macroconidia of this isolate were fairly limited. Microconidia shapes were normally straight to cylindrical, and the chlamydospores were formed in chains. The fungal strain *F. solani*-2 exhibits a profuse growth of white creamy mycelium in its colony. The larger spores, known as macroconidia, typically possessed an average of three to four partitions and displayed a slight curvature, with the end cell being rounded and the basal cell having a mycelium foot-like shape that was usually straight to cylindrical. Abundant microconidia were present, having an oval, ellipsoidal, or kidney-like appearance. Chlamydospores were produced either individually or in chains. *F. solani*-2 had a rapid growth rate, nearly twice that of the initial isolate, and could cover Petri dishes completely within a week of being introduced (Figure 2, panels C1, C2, and C3).

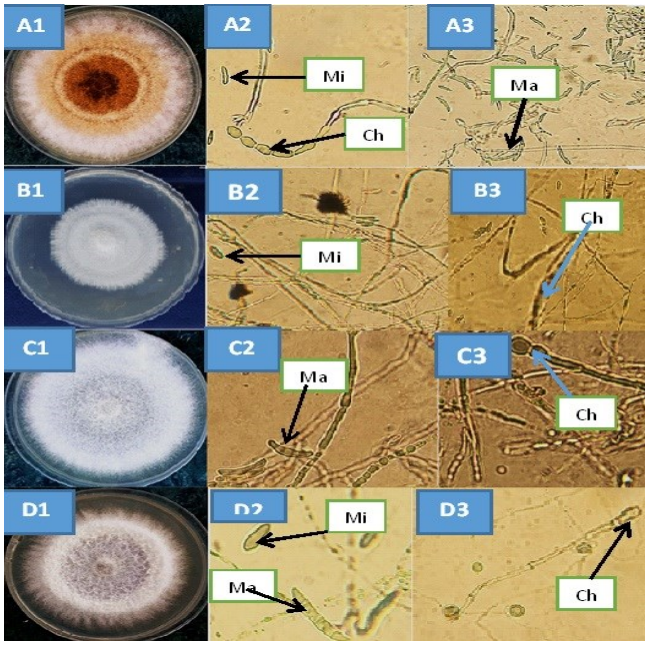

Fig. 2. *Cultural and morphological characteristics of Fusarium isolates: Colonies (A1 to D1) and morphological characters (A2 to D3) of F. incarnatum (A1), F. solani-1 (B1), F. solani-2 (C1) and F. oxysporum (D1), grown on PDA for 9 days at 25±2°C . Morphological characteristics of F. incarnatum (A2: Microconidia and Chlamodospores, A3: chlamidospores), F. solani 1(B2: Microconidia, B3: Chlamodospres), F. solani 2 (C2: Macroconidia and C3: chlamidospres), F. oxysporum (D2: Macroconidia and microconidia, D3: Chlamidospores). Under 40 X.*

Fusarium oxysporum

Morphological analysis of *F. oxysporum* colonies {Fig.2 (D1, D2 and D3)}, were characterized by an abundant white cottony mycelium and a dark-purple undersurface on PDA. Macroconidia were oval tapering and separated into 3-5 cells. The isolates displayed varying shapes of conidia, ranging from elongated and straight to curved. Microconidia exhibited forms that varied between oval and kidney-shaped. The number of septa present ranged from one to five. Chlamydospores were generated both individually and in chains. Complete plate coverage by mycelia occurred within 5 to 7 days.

Radial growth of *Fusarium* species

The radial growth of *Fusarium* species (*F. incarnatum, F. solani-1, F. solani-2,* and *F. oxysporum*) significantly increased (0.05) during the experiment. *F. solani*-2 revealed the highest growth (4.1, 5.3, 7.3 and 8.84 cm) after 3, 5, 7 and 9 days of the experiment, respectively. Followed by *F. incarnatum* as its radial growth reached 4, 5, 6.7 and 8.1 cm after the same period, respectively. The radial growth of *F. oxysporum* was: 3.67, 3.94, 6.4 and 7.47 cm after the same periods, respectively. The lowest growth was recorded in *F. solani*-1 (3.17, 4, 5 and 5.67 cm after a similar period of inoculation, respectively. This experiment revealed no significant differences between *F. incarnatum, F. solani-2* after 3 and 5 days, as both fungi were significantly higher than *F. solani*-1 at the time of the experiment.

Molecular identification

All 4 isolates of *Fusarium* species produced intense bands at 300 bp–400 bp (Fig. 3). The ITS gene sequence of the four isolates was successfully obtained. These sequences were deposited into the Genbank database for validation. Based on BLAST analysis, the similarity index ranged from 99% to 100%. The details of the similarity index data are summarized in Fig 5. PCR products amplified with ITS1/ITS4 primers. Lane 1: *Fusarium incarnatum* strain AJA; Lane 2: *Fusarium solani strain* AJA1 *;* Lane 3: *Fusarium solani* strain-AJA2; Lane 4: *F. oxysporum strain AJA*; Lane M: The ladder at 300bp.

The sequence's results were studied and the obtained sequence was submitted to GenBank. The results were released in the NC-hosted International Nucleotide Sequence Database Collaboration. Significant amplification of anticipated band size has been seen in all isolates (Table 4), supporting the identification of the following *Fusarium species*: *Fusarium incarnatum* strain AJA (MN460366), *Fusarium solani* strain AJA1 (MN460657), *Fusarium solani* strain AJA2 (MN460664) and *Fusarium oxysporum* AJA (MN460665).

The phylogenetic analysis showed the close genetic relationship among *Fusarium incarnatum* AJA strain MN460366 in this study with those worldwide deposited in Genbank database table 5 and Fig. 4 strains accession Nos . GQ505705.1, MG857469.1, MN233577.1, MT431637.1, MN170477.1, MT410507.1, LC510361.1, LC505036.1, KY120358.1 and EU111657.1 from USA (100%), New Zealand (98%), China (98%), India (100%), Iran (100%), Mexico (100%), Bangladesh (100%), Thailand (98%), Brazil (99%)and Germany (100) respectively. The Babylon district isolates' ITS region nucleotide sequence has been assigned to GenBank with Accession No. MN460366.1. Thus, *F. incarnatum* AJA was identified as the causative agent of *Fusarium* wilt of cucumbers in Iraq (Fig. 4). This study

Table 4. Molecular identification of *F. incarnatum*, *F. solani*-1, *F. solani*-2 and *F. oxysporum* in Al-Musayyib District, Iraq.

No.	Fungal scientific name	Accession No.	Geographic region
1	*Fusarium incarnatum* AJA	MN460366	Al-Azizia
2	*Fusarium solani* AJA1	MN460657	Al-Badah and Al-Tahiria
3	*Fusarium solani* AJA2	MN460664	Al-Azizia, Al-Badah and Al-Tahiria
4	*Fusarium oxysporum* AJA	MN460665	Al-Azizia, Al-Badah and Al-Tahiria

Table 5. Homology sequence identity for local isolates of *Fusarium* incarnatum AJA strain MN460366 using the NCBI-Blast GenBank database

	ACCESSION	Country	Source	Compatibility
1	ID: MN460366.1	IRAQ	*Fusarium incarnatum*	100%
2	ID: GQ505705.1	USA	*Fusarium incarnatum*	100%
3	ID: MG857469.1	New Zealand	*Fusarium incarnatum*	98%
4	ID: MN233577.1	China	*Fusarium incarnatum*	98%
5	ID: MT431637.1	India	*Fusarium incarnatum*	100%
6	ID: MN170477.1	Iran	*Fusarium incarnatum*	100%
7	ID: MT410507.1	Mexico	*Fusarium incarnatum*	100%
8	ID: LC510361.1	Bangladesh	*Fusarium incarnatum*	100%
9	ID: LC505036.1	Thailand	*Fusarium incarnatum*	98%
10	ID: KY120358.1	Brazil	*Fusarium incarnatum*	99%
11	ID: EU111657.1	Germany	*Fusarium incarnatum*	100%

Table 6. Homology sequence identity for local isolates of *Fusarium solani* strain AJA1 (MN460664) and strain AJA2 (MN460657) using the NCBI-Blast GenBank database

	ACCESSION	Country	Source	Compatibility
1	ID: MN460664.1	IRAQ	*Fusarium solani*	100%
2	ID: MN460657.1	IRAQ	*Fusarium solani*	99%
3	ID: MT641200.1	India	*Fusarium solani*	100%
4	ID: MN646254.1	China	*Fusarium solani*	99%
5	ID: MT453275.1	Germany	*Fusarium solani*	100%
6	ID: MT396412.1	Turkey	*Fusarium solani*	100%
7	ID: MT261780.1	Korea	*Fusarium solani*	100%
8	ID: MW076176.1	Iraq	*Fusarium solani*	100%
9	ID: MW063483.1	Pakistan	*Fusarium solani*	100%
10	ID: MT139626.1	Brazil	*Fusarium solani*	100%
11	ID: MT032385.1	Egypt	*Fusarium solani*	99%

Table 7. Homology sequence identity for local isolates of *Fusarium* oxysporum strain AJA (MN460665) using the NCBI-Blast GenBank database.

	ACCESSION	Country	Source	Compatibility
1	ID: MN460665.1	IRAQ	*Fusarium oxysporum*	100%
2	ID: MW260118.1	Nigeria	*Fusarium oxysporum*	100%
3	ID: MW250868.1	India	*Fusarium oxysporum*	99%
4	ID: MT819945.1	Portugal	*Fusarium oxysporum*	100%
5	ID: LC592361.1	Korea	*Fusarium oxysporum*	98%
6	ID: MW227311.1	China	*Fusarium oxysporum*	100%
7	ID: MW207951.1	Belgium	*Fusarium oxysporum*	100%
8	ID: MW165780.1	Egypt	*Fusarium oxysporum*	98%
9	ID: MH866024.1	South Africa	*Fusarium oxysporum*	100%
10	ID: MH857852.1	Germany	*Fusarium oxysporum*	100%
11	ID: MH856610.1	Netherlands	*Fusarium oxysporum*	99%

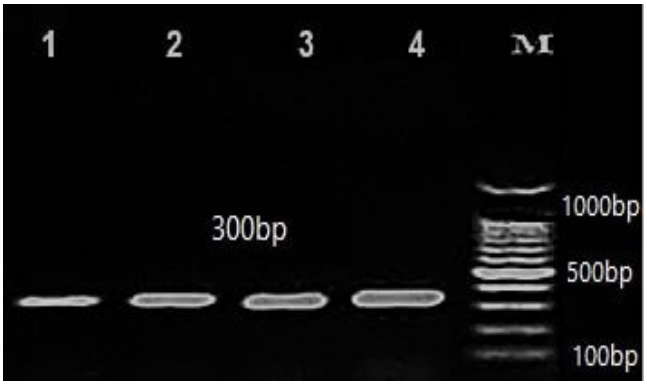

Fig. 3. *Agarose gel electrophoresis amplified from ITS Region in Fusarium sp. L-300 bp ladder, all samples have shown significant amplification of expected band size: 1-Fusarium incarnatum strain AJA, 2- Fusarium solani strainAJA1, 3-Fusarium solani strainAJA2 and 4- Fusarium oxysporum strain AJA), 5- the ladder.*

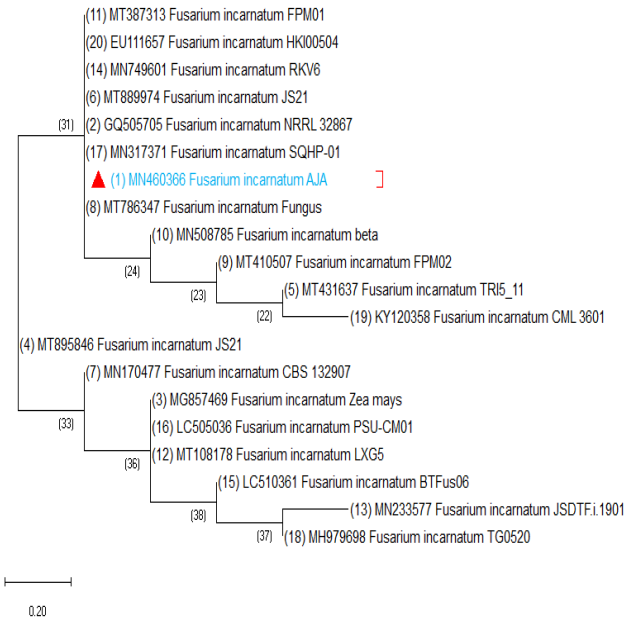

Fig. 4. *Phylogenetic tree of internal transcribed spacer rRNA sequences demonstrating that the isolation of Fusarium. incarnatum AJA was closely related to several geographical isolates of the same fungus*

confirms that no prior reports of *F. incarnatum* on cucumber have been made in Iraq. To the best of present knowledge, this pathogen has been identified in cucumber by molecular analysis.

The homology sequence identity for local isolates of *Fusarium solani* strain AJA1 (MN460664) and strain AJA2 (MN460657) using the NCBI-Blast GenBank database revealed a close genetic relationship between both strains in this study with those worldwide deposited in Genbank database Table 6 and Fig. 5. The strains accession Nos. MT641200, MN646254, MT453275, MT396412, MT261780, MW076176, MW063483, MT139626, MT032385, from India (100%), China (99%), Germany (100%), Turkey (100%), Korea (100%), Iraq (100%), Pakistan (100%),

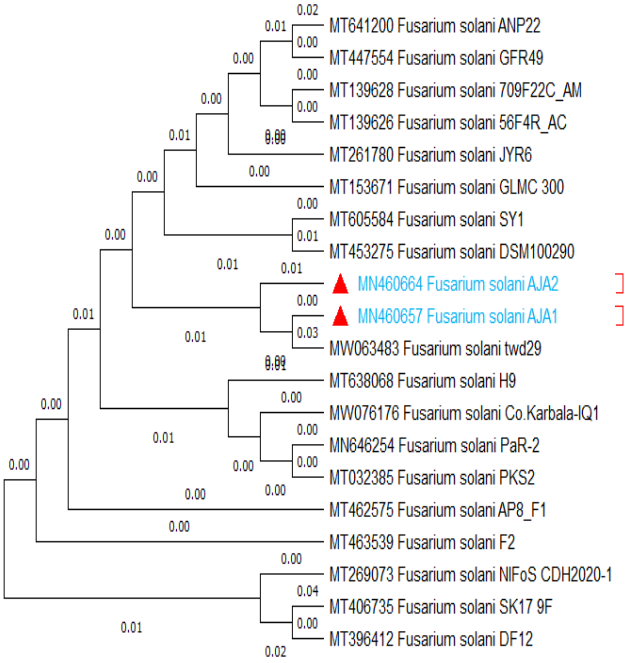

Fig. 5. *Phylogenetic tree of internal transcribed spacer rRNA sequences demonstrating that the isolates of Fusarium solani strain AJA1 (MN460664) and strain AJA2 (MN460657) were closely related to several geographical isolates of the same fungus.*

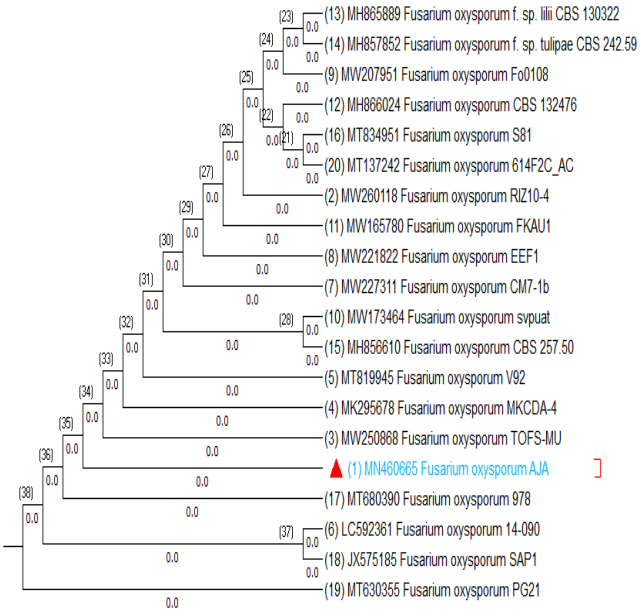

Fig. 6. *Phylogenetic tree of internal transcribed spacer rRNA sequences demonstrating that the isolate of Fusarium oxysporum strain AJA (MN460665) using the NCBI-Blast GenBank database was closely related to several geographical isolates of the same fungus.*

Brazil (100%) and Egypt (99%) respectively. The Babylon district isolates' ITS region nucleotide sequence has been assigned to GenBank with Accession No. MN460366.1. Thus, both isolates of *Fusarium solani* strain AJA1 and strain AJA2 were identified as the causative agent of *Fusarium* wilt of cucumber in Iraq by molecular analysis (Fig. 5). This study confirms that no prior reports of both strains on cucumber are made in Babylon province. To the best of present knowledge, this pathogen has been identified in Iraq's cucumber (Beit Alpha).

***Fusarium oxysporum* strain AJA**

The homology sequence identity for local isolates of *Fusarium oxysporum* strain AJA (MN460665) using the NCBI-Blast GenBank database revealed a close genetic relationship between the strain in this study with those worldwide deposited in Genbank database Table 7 and Fig. 6. The strains accession Nos. MW260118, MW250868, MT819945, LC592361, MW227311, MW207951, MW165780, MH866024, MH857852 and MH856610 from Nigeria (100%), India (99%), Portugal (100%), Korea (98%), China (100%), Belgium (100%), Egypt (98%), South Africa (100%), Germany (100%) and Netherlands (99%) respectively. The Babylon district isolates' ITS region nucleotide sequence has been assigned to GenBank with Accession No. MN460366.1. Thus, this *isolate of Fusarium oxysporum* strain AJA as the causative agent of *Fusarium* wilt of cucumber in Iraq by molecular analysis (Fig. 6). This study confirms that there is no prior report of this strain on cucumber in Babylon province. To the best of our knowledge, this pathogen has been identified in Iraq's cucumber (Beit Alpha).

Pathogenicity

A pathogenicity study showed that all *Fusarium* species had a significant reduction in the percentage of germination of cucumber leaves compared with that in control seeds, which showed no symptoms and remained healthy after seven days of incubation. On the other hand, the inoculated cucumber seeds significantly varied in their percentage germination among *Fusarium* species (Fig.7 G1 and G2). The results showed that *F. solani* AJA2 was the most aggressive isolate among all examined species with 5.27% followed by F. oxysporum AJA (8.67%) then *F. incarnatum* AJA (11.4 %), But, the isolate *F. solani* AJA1 recorded the least degree of aggressiveness (20.67%). In contrast, control treatment showed no disease symptoms throughout the assessment periods.

The vegetable crops like cucumber can be produced year-round in open fields or greenhouses. *Fusarium* wilt disease is indeed a significant problem in cucumber production and can have devastating effects on cucumber plants and cause considerable economic losses for farmers, which impairs both yields and fruit quality (Al-Tuwaijri, 2015 ; Din *et al*., 2020).

Cucumber *Fusarium* wilt was highly prevalence in Babylon Province, with field disease incidence ranging from 22% to 55.3%. Based on field survey data, the current study's findings show that this disease was considera-

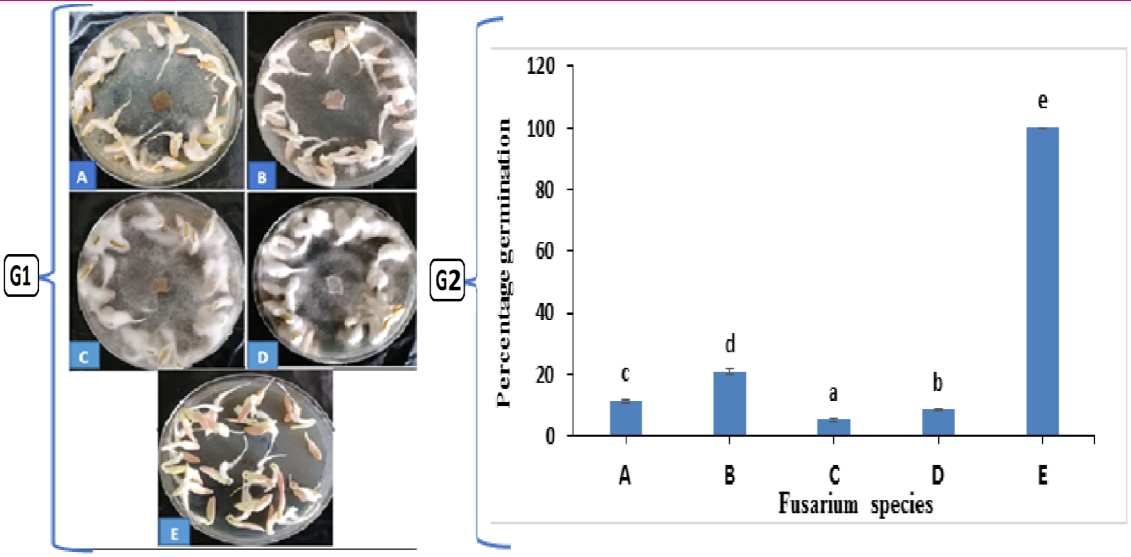

Fig. 7. *Pathogenicity of A: Fusarium incarnatum AJA, B: F. solani AJA1, C: F. solani AJA2, D: F. oxysporum AJA and E: Control (cucumber seeds sterilized with distilled water only) on cucumber seeds after seven days of growth on Petri plates containing 15 ml of WA agar at 25 ± 2 °C.* ***G1****- Colonization of cucumber seeds by Fusarium species during germination and* ***G2****: Percentage germination of cucumber seeds, data are expressed as mean ±SE of three replicates for each treatment. Similar letters indicate no differences at P≤0.05. All Fusarium species were individually inoculated at the center of the Petri plate.*

bly prevalent (up to 52.3%) in the investigated cucumber field of Al-Tahiria. However, Al-Badah (22%) had the lowest disease prevalence.

According to the present study, the symptoms of *Fusarium* wilt on infected cucumbers started with leaf yellowing, mainly on the old leaves, then spread to the top of plants, followed by the entire plant beginning to wilt and proceeding to severe wilting. These results follow previous studies (Al-Tuwaijri, 2015; Singh *et al*., 2021).

The morphological attributes of *Fusarium* species play vital roles during the early stages of distinguishing between different *Fusarium* species (Din *et al*., 2020). Conventional methods for characterizing and identifying *Fusarium* species primarily rely on their morphological features (Rahjoo *et al*., 2008). These distinctive traits corresponding to various *Fusarium* species were in concurrence with findings by Leslie and Summerell (2006).

The causative agents of this ailment were identified through morphological characteristics using various techniques to differentiate among *Fusarium* species (Rahjoo *et al*. 2008. Suresh *et al*. 2019). In terms of macroscopic traits such as colony appearance, pigmentation, and microscopic attributes, including the shapes and sizes of macroconidia, microconidia, and the presence of chlamydospores (Leslie and Summerell, 2006).

This study isolated and recognised four *Fusarium* strains from cucumber cultivation areas—namely *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA. Despite being collected from the same geographical region, these isolates exhibited considerable morphological variations. Variations were observed in cultural traits among *Fusarium* species, such as colony color (ranging from white to pink, purple, and violet), appearance of colonies' center and periphery, pigmentation, sporodochia production, macroconidia, microconidia, and their shapes. Microscopic analysis of the isolates indicated variations among *Fusarium* species in terms of macroconidia and microconidia sizes and shapes, as well as growth rates. These findings align with the research conducted by (Sonkar *et al*. 2014) and are consistent with (Leslie and Summerell's ,2006; Ha *et al*., 2023). To the best of current knowledge, the documentation of *F. incarnatum* species and the other three *Fusarium* strains in Alpha-Beta cucumber marks the initial records in Iraq and other *Fusarium species* (Al-Taae and AL-Taae 2019) investigated *Fusarium* wilt in Armenian cucumber in Iraq attributed to *Fusarium oxysporum*. This study is the first to report *F. incarnatum* as a novel pathogen causing wilt disease in cucumbers in Iraq and globally. This discovery has aided in identifying the underlying cause of wilting symptoms.

Based on the outcomes of this study, both *F. solani* isolates exhibited aerial mycelium that ranged from sparse to abundant, with colony colour ranging from white to light purple. These characteristics aligned with the findings (Sonkar *et al*., 2014). Additionally, the morphology and microscopy features of *F. oxysporum* were consistent with those reported by (Ibrahim *et al*., 2015; Abu Bakar *et al*.,2013) noted that *F. oxysporum* pigmentation on PDA ranged from white to purple, with microconidia having a tapered apical cell and a foot-shaped basal cell. Microconidia were observed to be

oval and kidney-shaped with 0–1 septa.

Fusarium oxysporum has been recognized as a severe pathogen affecting numerous plants worldwide, including, (Din *et al*. 2020) reported the prevalence of *Fusarium* Wilt Disease of Cucumber in Peninsular Malaysia caused by *Fusarium oxysporum* and *F. solani*. Based on field survey data, the disease exhibited high prevalence rates of up to 60% in the fields.

Pathogenicity

Following a seven-day incubation period, a pathogenicity investigation revealed that all *Fusarium* species significantly reduced the proportion of cucumber seeds that germinated compared to the control group, which revealed no symptoms and remained healthy. In contrast, the inoculated cucumber seeds significantly varied in their percentage germination among *Fusarium species*. Among all the species that were studied, *F. solani* AJA2 was the most aggressive isolate, showing a percentage decline to 5.27%, followed by *F. oxysporum AJA* (8.67%) and *F. incarnatum* AJA (11.4%). However, isolate *F. solani* AJA1 showed the least germination (20.67%). The connection between the origin of isolates and their level of harmfulness was absent. This marks the initial documentation of *F. incarnatum,* a previously unknown type responsible for causing *Fusarium* wilt in cucumber plants in Iraq. The defense system of the cucumber plants was weak, rendering them ineffective at warding off *Fusarium* from infiltrating their vascular system. Additionally, the root cells couldn't adequately restrict the movement of the pathogen, consequently heightening the plant's vulnerability to pathogenic invasion (Al-Tuwaijri, 2015) .

The present result does not agree with the results of Asma *et al*. (2018), which showed that the four isolates of *F. incarnatum* reported were non-virulence with no visible wilt symptoms in the cucurbit plant. Also, disagreed with Gao *et al*. (2020), who revealed that this fungus causes spots on cucumber-produced lesions that are densely distributed on the leaves but do not cause wilt disease.

Molecular characteristics

Leslie and Summerell's (2006) conclusions are that morphological features can not be enough to differentiate between the closely related species of the *Fusarium* genus. The morphological characteristics of *Fusarium* species were used as an additional tool for precise identification of the *Fusarium* species, which ensured the association between morphological and molecular characterization. But the correlation between morphological and molecular characterization ensures the accuracy and consistency of the study's findings (Khuna *et al*., 2022 ; Rolim *et al*., 2023)**.** Therefore, it is necessary to identify *Fusarium species* by applying a molecular approach (Khuna *et al*., 2022) .

Internal transcribed spacer (ITS) analysis is a reliable method for determining the species of *Fusarium* (Geiser *et al*., 2004). But as of right now, morphological and molecular ancestry traits are combined to correctly identify the *Fusarium species* (Geiser *et al*., 2004). In this study, two novel strains of *F. solani* and *F. incarnatum* emerged from cucumber wilt lesions that were previously collected. Based on their morphological features, all isolates were first classified as belonging to the genus *Fusarium* (Wang *et al*., 2019) . Multi-gene phylogenetic studies were used to confirm the fungi's identity.

All of these ITS sequences were identical to isolates with 99% to 100% identity of *F. incarnatum* AJA, *F. solani* AJA1, *F. solani* AJA2, and *F. oxysporum* AJA in the GenBank database. For *F. incarnatum* AJA, *F. solani* AJA1, and *F. solani* AJA2 on cucumber in Iraq, this finding represents the first molecular record to the best of our knowledge. The PCR approach has been preferred for the identification of fungal pathogens. This was due to its quickness, specificity, effectiveness, and cost-effectiveness (Zhang *et al*. 2012). According to a phylogenetic study based on ITS alignment, the isolate belonged to the same clade as *F. incarnatum*. A phylogenetic study using ITS alignment revealed that the isolate belonged to the same group as *F. incarnatum* (Tamura *et al*., 1993). According to Kumar *et al*.(2018), branch lengths are given in terms of the number of substitutions per site, and the tree is displayed to scale.

Conclusion

According to a survey done on cucumbers in various fields, *F. incactarum* strains of *F. solani* and *F. oxysporum* were associated with wilt disease of cucumbers. All isolates were recognized as the causal agents of the wilting disease of cucumber using a combination of morphological and molecular methods based on morphological observation and ITS sequence data. The most important finding is identifying *F. incarnatum* (MN460366.1) (as a new record for cucumber wilt disease worldwide as far as we know and according to the literature). It is suggested that more studies be conducted using more gene regions to study the identification of *Fusarium* isolates at the species complex. The present study presents a new opportunity for the improved control of cucumber wilt disease. The results will be crucial in establishing a disease management program that effectively manages outbreaks and decreases crop production loss.

ACKNOWLEDGEMENTS

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Conflict of interest

The author declare that they have no conflict of interest.

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