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Identification and Control of the Causal Agent of Root and Stem Rot in Cucumber Plants Grown in Open Sandy Soil

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ABSTRACT

This study was conducted to identify the causal agent of root and stem rot in cucumber plants grown in open sandy soil and to control it both in the laboratory and in the field using silicon and urea. The isolation and diagnosis results revealed 39 isolates associated with the disease, including the fungus *Fusarium redolens*. Molecular diagnosis of the most Aggressive Isolate , FR342a and FR342b, confirmed their identification as *F. redolens* with 100% match to global isolates. Laboratory results showed that silicon-amended culture media at concentrations of 2%, 4%, and 6% inhibited the growth of the pathogenic fungal *F.redolens* colony by 66-100%. where the concentration of 4%, 6% superiority with inhibition rate of 100%. In contrast, urea -amended culture media at concentrations of 0.4%, 0.6% and 0.8% achieved 100% inhibition of fungal *F.redolens* growth. In the field, the 4% silicon treatment was most effective, reducing disease severity to 42.65% followed by the 0.6% urea treatment, which reduced disease severity to 45.82%. All treatments provided effective disease control and positively impacted growth parameters, including branch length, number of branches, fruit count, fruit weight, and the wet and dry weights of the root system.

Key words: *fusarium redolens*, PCR, Rotting fungal disease, Silicon, urea.

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INTRODUCTION

Cucumber (*Cucumis sativus*) is one of the most economically and nutritionally important cucurbit crops, cultivated in both spring and fall seasons in open fields and protected agriculture across Iraq (Omar and Jasim,2021). In 2022, global cucumber production reached 94,718,396 tons, while in Iraq, 203,810 dunams were cultivated, yielding 195,924 tons (Faostat,2022). Cucumber crops are susceptible to various diseases at all growth stages, with root and stem rot being one of the most significant, caused primarily by soil-borne pathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, and *Monosporascus cannonballus* (Almaghasla et al., 2023). As a result, various control methods were used, the most commonly used of which is chemical control to control root and stem rot disease, which led to the emergence of resistance in pathogens and their development against many pesticides, and thus the application of pesticides is ineffective in combating pathogens In addition to the emergence of negative effects on the environment and human health. And its entry into the food chain, which greatly affects environmental sustainability (Rampersad, 2020) so researchers have turned to developing new methods to combat the disease that are effective and low in toxicity to solve the problem of resistance in pathogens. By using environmentally friendly materials, silicon is a useful element in protecting plants from stress and plays a role in physiological processes, the most important of which is improving the efficiency of photosynthesis. Adding silicon to the soil can significantly enhance plant resistance to diseases and increase the plant's immune response (Sun et al., 2022) Soil fumigation using nitrogen fertilizers effectively affects soil-borne pathogens and increases beneficial organisms in the soil, to

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develop sustainable disease suppression after fumigation. Nitrogen encourages cell division, cell elongation, and increased biomass in the plant (Sun et al 2015) Recently, severe symptoms of root and stem rot, including wilting, yellowing, stunted vegetative growth, basal stem rot, and complete root decay with browning, were observed in cucumber plants grown in open sandy soils in the Najaf province Due to the severity of the disease and the substantial economic losses it causes to cucumber crops, this study aimed to identify the causal agent of root and stem rot in cucumber plants grown in open sandy soils in Najaf and to control it both in the laboratory and field using silicon and urea.

MATERIALS AND METHODS

Soil Sample Collection: Sandy soil samples were collected from six agricultural fields in Najaf province (Al-Diouk Street, Sector 270) on July 1, 2023. The fields were labeled with the letters A, B, C, D, E, and F. These fields had been planted with cucumbers in the previous season and showed symptoms of root and stem rot disease. The 6samples were collected using a hand trowel and placed in polyethylene bags, each labeled with the date of collection and the field identification code. After collecting the soil samples, each field's soil was mixed separately. A total of 400 grams of soil from each field was evenly placed in clean, sterilized pots with a 7 cm diameter, with three replicates for each field. Five seeds of the *YASEER F1* cucumber variety (Dutch origin) were sown in each pot after surface sterilization of the seeds for two minutes in a sodium hypochlorite solution (1% free chlorine). After two weeks of planting, infected seedlings showing symptoms of wilting, yellowing, and root rot were uprooted from the soil for the purpose of isolating and identifying the pathogen responsible for root and stem rot disease.

Isolation and Identification: The roots and stems of infected seedlings were cut into 0.5–1 cm pieces and surface sterilized in a 1% sodium hypochlorite solution for two minutes. After drying, the pieces were placed in 9 cm Petri dishes containing water agar (WA), with four pieces per plate and three replicates per field. Plates were incubated at 25 ± 2°C for 5–7 days. Fungal isolates were purified and identified based on colony appearance and microscopic examination using a Light Compound Microscope (LCM) at the Plant Disease Laboratory, University of Baghdad. Fungi were identified to the genus and species level using Leslie and Summerell's (2006) taxonomic keys.

Pathogenicity Test of Isolated Fungi on Cucumber Seeds in Laboratory Using WA Medium: The pathogenicity of 39 fungal isolates was tested in vitro on water agar (WA). Each plate was inoculated with a 0.5 cm disc from a 5-day-old fungal colony and incubated at 25°C for 3 days. Six surface-sterilized *YASEER F1* cucumber seeds were sown in each plate, sterilized in 1% sodium hypochlorite for two minutes, then dried on sterile filter paper. Seeds were placed 1 cm from the fungal growth. Three control plates were prepared with seeds only. The plates were incubated at 25°C for 7 days, after which seed germination was recorded, and the most pathogenic isolate was determined using the disease index by Abdullah and Kareem (2021). DSI = 1: 1 mm decay length, 2: >1 to 3 mm, 3: >3 to 5 mm, 4: >5 to 7 mm, 5:≤7 mm. The most pathogenic was determined by converting the decay scale to the Diseases Severity Index (DSI) average, categorizing isolates as follows:

Avirulent isolate = 0 - 0.3, Low Virulent isolate = 0.4 - 1.9, Moderately Virulent isolate = 2 - 2.9, Virulent isolate = 3 - 3.9, Strongly Virulent isolate = 4≤5.

Molecular Identification of the Most Aggressive Virulent Fungal Isolates Using Polymerase Chain Reaction (PCR): Two fungal isolates, which exhibited high pathogenicity on *YASEER F1* cucumber seeds and were identified morphologically, were selected for molecular identification. The targeted fungal gene region was amplified using a pair of universal primers, ITS1/ITS4, through the Polymerase Chain Reaction (PCR) technique. The PCR process was conducted using a thermal cycler.

1. DNA was extracted from the pure mycelium of the pathogenic fungal isolates, which were grown using the single spore isolation method. The extraction was performed using a standard

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kit manufactured by Geneaid, following the manufacturer's protocol. The extraction process took place in the molecular diagnostics laboratory of Jisr Al-Musayyib Company, located in Baghdad.

2. DNA amplification for each sample was carried out using the PCR technique, following the method described by Homa et al., (2018). A total of 2 µL of the ITS1 primer (5'-CCGTAGGTGAACCTGCGG-3') and 2 µL of the ITS4 primer (5'-TCCTCCGCTTATTGATATGC-3') were used. The final volume was brought up to 20 µL by adding 11 µL of sterilized ionic water to small tubes 1.5ml size containing 5 µL of Master Mix. The components were mixed using a Vortex device, and the tubes were then placed in a Thermal cycler to perform the PCR reaction. The DNA amplification was carried out under the following thermal cycling conditions: initial denaturation at 94°C for 1 minute (30 cycles), denaturation at 94°C for 30 seconds (30 cycles), annealing at 55°C for 30 seconds (30 cycles), extension at 72°C for 30 seconds (30 cycles), and a final extension at 72°C for 5 minutes (one cycle).

3.The amplified DNA samples were sent to *Macrogene Corporation* in South Korea for sequencing of the pathogenic fungal isolate's nucleotide sequences using the Sanger Sequencing method. The sequencing results were analyzed using the MEGA11 software and compared with global isolates. The obtained sequences were then deposited in the GenBank database.

Evaluation of the Efficiency of Silicon and Urea in Inhibiting *Fusarium redolens* on PDA Medium: The effectiveness of silicon (Potassium silicate 35%). (2%, 4%, 6%) and urea (0.4%, 0.6%, 0.8%) was tested using the poisoned food technique against the pathogen responsible for root and stem rot in cucumber. Silicon and urea were dissolved in of Potato Dextrose Agar (PDA) at the specified concentrations, sterilized at 121°C and 1.5 kg/cm² for 15 minutes. After cooling, tetracycline (50 mg/L) was added to prevent bacterial growth. The media were poured into Petri dishes, with three replicates per concentration. Control plates contained only PDA without additives. Each plate was inoculated with a 0.5 cm disc from a 5-day-old fungal colony, then incubated at 25 ± 2°C. Once fungal growth was complete in the control plates, the percentage inhibition of fungal growth was calculated using the following formula:

$$\textbf{Percentage inhibition} = \frac{\text{Colony diameter in control} - \text{Colony diameter in treatment}}{\text{Colony diameter in control}} \text{X}100$$

Field Evaluation of the Efficiency of Silicon and Urea in Protecting Cucumber Plants from Root and Stem Rot Disease: The experiment took place in the summer of 2023 in an open field at the Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad. The field was prepared by plowing twice, leveling, and dividing it into three sectors (10 m long, 1 m wide). A 25 cm deep trench was dug along each sector, filled with 15 cm of sandy soil, followed by 5 cm of fermented organic manure, and topped with 5 cm of naturally contaminated field soil from Najaf. A drip irrigation system was installed, and the experiment was designed using a Randomized Completely Block Design (RCBD). Each sector received eight treatments, including three concentrations of silicon (2%, 4%, 6%) applied at 50 mL per plant hole, and three concentrations of urea (0.4%, 0.6%, 0.8%) applied per 10 cm row length. Black plastic mulch covered the soil for 14 days before planting. Silicon was applied in three rounds, 7 days apart, beginning 7 days before planting. The fungicide AOLVER5SC was applied at 20 mL per plant hole with a solution of 0.5 mL/L, 3 days after planting. Regular irrigation and foliar fertilizers were applied for all treatments. Fourteen days post-planting, seed germination percentage was recorded, and at 90 days, plant survival percentage was calculated. Growth parameters measured included wet and dry root weight, branch length and count, fruit weight, and fruit count. Disease severity and index were assessed based on root wet weight, following Zang et al.'s (2010) scale: 0 = <22 g (healthy), 1 = 18–

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21.9 g, 2 = 14–17.9 g, 3 = 10–13.9 g, 4 = <9.9 g or complete plant death. Control efficiency was calculated using the following formula:

$$\text{Control Efficiency} = \frac{(\text{Disease severity in control} - \text{Disease severity in treatment})}{\text{Disease severity in control}} \text{X}100$$

RESULTS AND DISCUSSION

Isolation and Identification: The results from collecting samples of roots and stems of cucumber seedlings grown in sandy soil from Najaf province revealed the widespread occurrence of root and stem rot pathogen in across all six sites where sandy soil was collected. The isolation and identification process, conducted through microscopic examination of infected plant tissue grown on PDA medium, identified 39 fungal isolates belonging to various genera isolated from cucumber roots and stems. The most frequently isolated fungus was *Fusarium* sp., with a frequency of 61.53%, followed by *Didymella bryoniae* with a frequency of 10.25%. Other fungi isolated included *Chaetomium* sp. and *Rhizoctonia solani* (5.12%), *Macrophomina phaseolina*, *Alternaria* sp., *Mortierella* sp., *Bipolaris* sp., *Curvularia* sp., *Pithomyces* sp., and *Phoma* sp. (2.56%), (Table 1). The isolation of these fungal species from infected plant tissues may be attributed to their high parasitic ability, enabling them to grow and penetrate the cells of infected tissues. This internal growth may have provided them with protection from the surface sterilized process (Roy et al., 1997; Kareem et al., 2015).

Table 1. Fungi isolated from the roots and stems of infected cucumber plants and their frequency percentages

ID	Isolated Fungi	Number of isolates	Frequency %
1	*Fusarium* sp.	24	61.53
2	*Didymella bryoniae*	4	10.25
3	*Chaetomium* sp.	2	5.12
4	*Rhizoctonia solani*	2	5.12
5	*Macrophomina phaseolina*	1	2.56
6	*Alternaria* sp.	1	2.56
7	*Mortierella* sp.	1	2.56
8	*Bipolaris* sp.	1	2.56
9	*Curvularia* sp.	1	2.56
10	*Pithomysis* sp.	1	2.56
11	*Phoma* sp.	1	2.56
$LSD_{0.05}$	--		2.93

The results of the morphological identification of fungal isolates obtained from the roots and stems of cucumber plants revealed several species belonging to the genus *Fusarium* sp. The color of the fungal colonies ranged from cottony white, transparent white, pale, pink, red, purple, and violet to brown. Among these was *Fusarium redolens*, which was isolated from cucumber roots and identified based on its morphological and microscopic characteristics. *Fusarium redolens* colonies typically range in color from white to pink, with some brown pigmentation on PDA medium. The mycelium is relatively flat with a fibrous, cotton-like appearance. The fungus forms small sporodochia structures that range in color from creamy to pale brown, and it produces abundant, robust macroconidia connected to the sporodochia. These macroconidia are thick-walled, segmented by three to five septa, with the upper third expanding into a curved apical cell and a foot-shaped basal cell. The fungus also produces smaller microconidia, which are either single-septate or non-septate, oval to cylindrical in shape, and connected to single conidiophores. These are borne singly or in small false heads. Additionally, *Fusarium redolens* produces abundant, rough-walled, and spherical to oval chlamydospores that form quickly with age, either in terminal chains or between the hyphal cells (Figure 1). These characteristics align with the description of *F. redolens* provided by Leslie and Summerell (2006).

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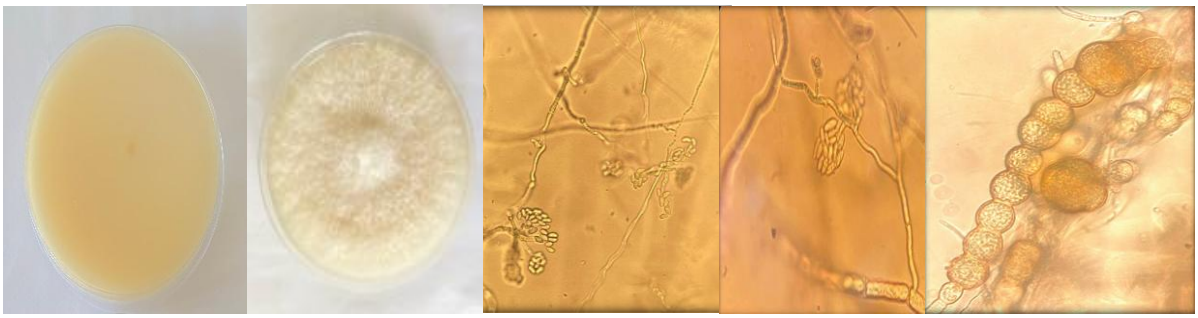

A B E C F

Figure 1. A - Bottom side of the plate at 7 days of age, B - Top side of the plate, C and E - Displaying the fungal mycelium and microconidia, F - Displaying the chlamydospores

Pathogenicity Test: The results of the pathogenicity test for the 39 fungal isolates show in (Table 2) ,whin indicate variability in their ability to affect the germination of *YASEER F1* cucumber seeds (Dutch origin) in laboratory conditions. The percentage of seed germination ranged from 0% to 100%. As evident from the pathogenicity test results and the measurement of the disease index for the tested fungal isolates, there were significant differences in the ability of the isolates to infect cucumber seeds and seedlings on PDA medium in vitro. The fungal isolates were categorized into low virulent 0.4–1.9 and strong virulent 4-5 groups based on their pathogenicity. The results showed that one fungal isolate was classified as low virulent, with a disease index of 1.05. Four fungal isolates were found to be moderately virulent, with a disease index ranging between 2.5 and 2.8. Four other isolates were classified as virulent, with a disease index ranging between 3 and 3.8. Meanwhile, 30 fungal isolates were categorized as strong virulent, with a disease index between 4 and 5. The *Fusarium redolens* isolates, labeled FR342a, FR342b, FR21, and FR24, achieved a 0.0% seed germination rate and a disease index of 5 (Figure 2), showing significant differences compared to the other tested fungal isolates. This high pathogenicity can be attributed to their ability to produce secondary toxic metabolites that kill the embryos, as well as enzymes that degrade plant tissues, leading to seed rot and hindering seedling growth (Ekwomadu and Mwanza ,2023). Studies by Morphy et al., (1984) demonstrated that most *Fusarium* species and other fungi responsible for seed and seedling rot produce enzymes such as cellulase and pectinase, in addition to several mycotoxins, including deoxynivalenol, zearalenone, and fusaric acid. These compounds have the capability to degrade plant cell walls, facilitating fungal entry into plant tissues and exploiting the metabolic activities within plant cells. These effects are likely to be exacerbated in the case of highly virulent fungal isolates due to increased production of these degrading enzymes and toxin (Ekwomadu and Mwanza ,2023).

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Table 2. Pathogenicity test of fungal isolates on cucumber seeds in vitro**.**

ID	Isolate	Code	Average germinated seeds	Seed germination %	Disease index	Pathogenicity index
1	*Fusarium* sp.	AR24	5.33	88.83	4.05	Strong virulent
2	*Fusarium* sp.	AR31	6.00	100.00	4.55	Strong virulent
3	*Fusarium* sp.	AR21	6.00	100.00	5.00	Strong virulent
4	*Fusarium* sp.	BR34	6.00	100.00	1.05	Low virulent
5	*Fusarium* sp.	BR11	5.00	83.33	4.61	Strong virulent
6	*Fusarium* sp.	BR31	6.00	100.00	5.00	Strong virulent
7	*Fusarium* sp.	BR22	5.33	88.83	4.44	Strong virulent
8	*Fusarium* sp.	CR31	6.00	100.00	5.00	Strong virulent
9	*Fusarium* sp.	CR33	5.66	94.33	5.00	Strong virulent
10	*Fusarium* sp.	CR12	5.33	88.83	5.00	Strong virulent
11	*Fusarium* sp.	CR22	5.00	83.33	3.38	Virulent
12	*Fusarium* sp.	CR24	6.00	100.00	3.83	Virulent
13	*Fusarium* sp.	CR13	4.00	66.66	5.00	Strong virulent
14	*Fusarium* sp.	CR21	5.00	83.33	3.72	Virulent
15	*Fusarium* sp.	CR34	5.33	88.83	3.05	Virulent
16	*Fusarium* sp.	DR13	4.66	77.66	2.55	Moderately virulent
17	*Fusarium* sp.	DR14	5.66	94.33	2.61	Moderately virulent
18	*Fusarium* sp.	ER34	6.00	100.00	4.11	Strong virulent
19	*Fusarium* sp.	ER13	6.00	100.00	2.88	Moderately virulent
20	*Fusarium* sp.	ER22	5.33	88.83	4.44	Strong virulent
21	*Fusarium* sp.	FR342a	0.00	0.00	5.00	Strong virulent
22	*Fusarium* sp.	FR342b	0.00	0.00	5.00	Strong virulent
23	*Fusarium* sp.	FR21	0.00	0.00	5.00	Strong virulent
24	*Fusarium* sp.	FR24	0.00	0.00	5.00	Strong virulent
25	*Didymella bryniae*	DR32	5.66	94.33	5.00	Strong virulent
26	*Didymella bryniae*	DR33	5.00	83.33	5.00	Strong virulent
27	*Didymella bryniae*	DR34	6.00	100.00	5.00	Strong virulent
28	*Didymella bryniae*	DR31	6.00	100.00	5.00	Strong virulent
29	*Chaetomium* sp.	ER212	6.00	100.00	5.00	Strong virulent
30	*Chaetomium* sp.	ER231	5.66	94.33	5.00	Strong virulent
31	*Rhizoctonia solani*	DR24	6.00	100.00	5.00	Strong virulent
32	*Rhizoctonia solani*	ER14	6.00	100.00	5.00	Strong virulent
33	*M.Phaseolina*	DR23	6.00	100.00	4.77	Strong virulent
34	*Alternaria* sp.	FR12	5.66	94.33	5.00	Strong virulent
35	*Mortierella* sp.	AR34	5.00	83.33	4.55	Strong virulent
36	*Biborals* sp.	AR23	5.66	94.33	4.77	Strong virulent
37	*Curvularia* sp.	ER12	5.66	94.33	5.00	Strong virulent
38	*Pithomysis* sp.	BR24	5.33	88.83	2.55	Moderately virulent
39	*Phoma* sp.	ER24	6.00	100.00	5.00	Strong virulent
LSD 0.05	--	--	0.45	4.72	0.39	--

***Each number in the table represents three replicates, and each replicate contains six cucumber seeds..**

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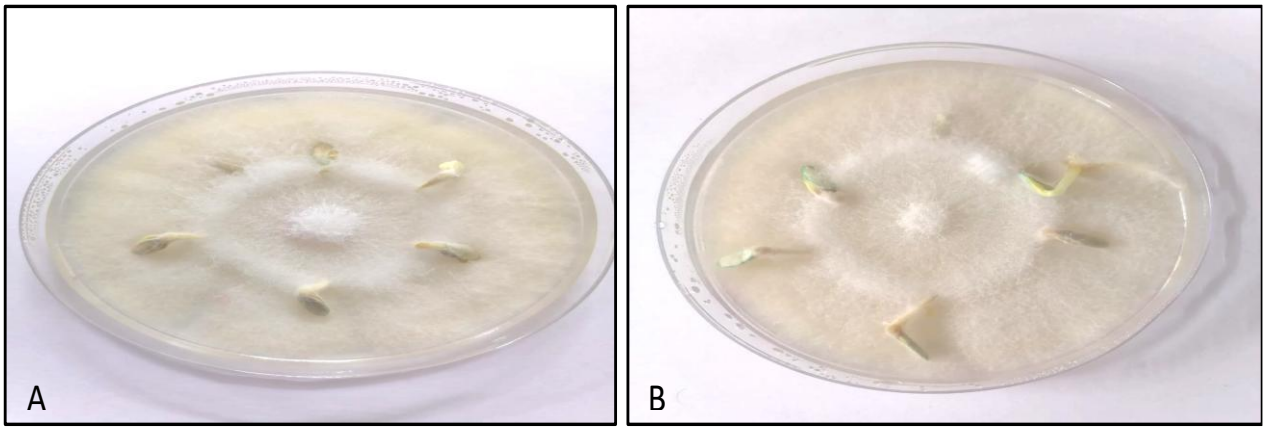

Figure 2. Pathogenicity test of *Fusarium redolens* A - Isolate FR342a, B - Isolate FR342b

Molecular Identification of the Most Aggressive Virulent Fungal Isolates: The molecular identification results, based on PCR and the ITS rDNA region of the DNA from the highly pathogenic isolates FR342a and FR342b, confirmed that they belong to *Fusarium* sp. The purity of the DNA was estimated at 2 based on its absorption ratio at 260/280 nm, and gel electrophoresis revealed a single band for the amplified gene, indicating that the primers successfully targeted the specific gene without amplifying other regions of the extracted DNA, as shown in (Figure 3). The fungal isolates were sequenced, and their nucleotide sequences were submitted to GenBank, receiving accession numbers.

Figure 3. Gel electrophoresis results of DNA fragments from *Fusarium redolens* on agarose gel

Isolate FR342a, with a molecular weight of 545 bp, was assigned the accession number PP461490 , and isolate FR342b, with a molecular weight of 543 bp, received the accession number PP461491 . The nucleotide sequences of the amplified isolates matched 100% with the ITS gene region sequences of global isolates of *Fusarium redolens* deposited in the National Center for Biotechnology Information (NCBI) database, as confirmed by BLAST (Basic Local Alignment Search Tool) analysis. These results are consistent with the morphological identification of the pathogenic isolates *Fusarium redolens*, the causal agent of root and stem rot in cucumber plants grown in open sandy soil in Najaf province.The phylogenetic tree (Figure 4), constructed using the Maximum Likelihood method, includes the Iraqi isolates PP461490 and PP461491 of *Fusarium redolens*, along with 11 global isolates retrieved from GenBank. The maximum composite likelihood (MCL) genetic distances were calculated using the neighbor- joining and BioNJ methods within the Molecular Evolutionary Genetics Analysis7 (MEGA7) software. Isolate FR342a (PP461490) aligned with the Canadian isolate GU934525, retrieved from GenBank and belonging to *F. redolens*, within the same branch. Meanwhile, isolate FR342b

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(PP461491) aligned with the Indian isolate KC924920, also retrieved from GenBank and belonging to *F. redolens*, within the same branch. The two Iraqi isolates, FR342a and FR342b, clustered together with other global isolates of *Fusarium redolens* retrieved from GenBank, forming a single cluster separate from the outgroup branch represented by *Rhizoctonia solani* isolate KF372657 (Kumar & Tamura, 2015). The Iraqi isolates also exhibited genetic differences between them, possibly due to geographic variations related to the isolation location of the fungal isolates. This is the first recorded instance of *Fusarium redolens* on cucumber plants in Najaf province.

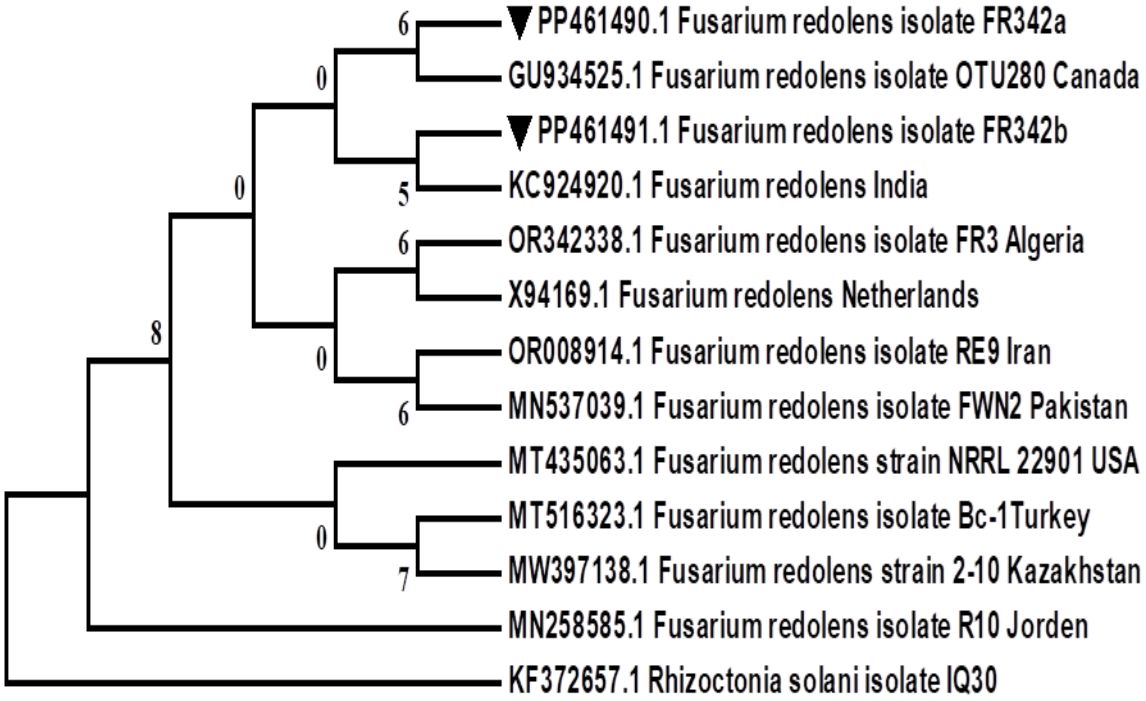

Figure 4. Molecular Phylogenetic analysis by Maximum Likelihood method constructed using ITS1/ITS4. Initial tree(s) for the heuristic search were obtained automatically BY Neighbour-joining phylogeny test and maximum composite likelihood model by using MEGA 7 among fungal species with the use of Rhizoctonia solani sequence as the outgroup comparison

Evaluation of the Efficiency of Silicon and Urea in Inhibiting the Pathogenic Fungus *Fusarium redolens* on PDA Medium: The results of evaluating the efficiency of silicon and urea using the poisoned food technique against the pathogenic fungus *Fusarium redolens* showed that silicon-amended media at all concentrations (2%, 4%, and 6%) had varying effects on the colony growth rate of *F. redolens*, with inhibition percentages ranging from 66% to 100%. The 4% and 6% concentrations were the most effective, achieving a 100% inhibition rate, while the 2% concentration resulted in a 66% inhibition rate, showing significant differences compared to the control treatment, where the inhibition rate was 0.00% (Table 3, Figure 4). The effectiveness of silicon in controlling the pathogenic fungus is likely due to its ability to inhibit several enzymes responsible for essential cellular reactions and its capacity to alter the structure and composition of the fungal cell walls, which impedes the growth of the pathogen and eventually leads to its death.These findings are supported by the study of Similarly, Al-Ani (2023) which reported that the addition of calcium silicate ($CaSiO_3$) at concentrations of 250, 500, 750, and 1000 mg/L to the growth medium significantly inhibited the mycelial growth of *Fusarium solani* found that The 500 mg/L concentration resulted in complete inhibition of the fungal mycelium, outperforming the other concentrations. As for urea, it exhibited exceptional efficiency and a significant inhibitory effect on the colony growth rate of

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Fusarium redolens. The inhibition percentages for the 0.4%,0.6%, and 0.8% concentrations all reached 100%, with no significant differences among the tested concentrations compared to the untreated control, which had an inhibition rate of 0.00% (Table 3, Figure 5). The efficacy of urea in inhibiting the pathogenic fungus may be attributed to its breakdown products, primarily ammonia, which creates an unfavorable environment for fungal growth. The release of ammonia alters the pH of the medium, making it more alkaline, which hinders the growth and spread of the pathogenic fungus, as *Fusarium* species generally prefer acidic to slightly alkaline conditions (Castellano-Hinojosa et al., 2022) Moreover, ammonia may disrupt cell membranes by affecting their permeability and inhibit essential biological processes required for the pathogen's growth, ultimately leading to its death. (Sun et al., 2015) This result further demonstrates the high effectiveness of urea in controlling pathogenic agents, aligning with the findings of Hussein and Hussain (Hussein and Hussein, 2024), who observed 100% inhibition of *Aspergillus flavus* when 3% regular urea was added to the medium.

Table 3. Evaluation of the efficiency of silicon (Si) and urea in inhibiting the growth of the pathogenic fungus *Fusarium redolens* on PDA medium**.**

Materials	Concentration (%)	Average colony diameter (cm)	Inhibition percentage
Si	2	3.00	66.6
	4	0.00	100.0
	6	0.00	100.0
Urea	0.4	0.00	100.0
	0.6	0.00	100.0
	0.8	0.00	100.0
Control	0	9.00	0
$LSD_{0.05}$	--	--	3.10

***Each number in the table represents the average of three replicates**

Figure 5. The effect of silicon-amended medium on inhibiting *Fusarium redolens*

A - Effect of silicon at 2% concentration,
B - Effect of silicon at 4% concentration,
C - Effect of silicon at 6% concentration, CON - Control plates for *Fusarium redolens*

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Figure 6. The effect of urea-amended medium on inhibiting *Fusarium redolens*
A - Effect of urea at 0.4% concentration, B - Effect of urea at 0.6% concentration, C - Effect of urea at 0.8% concentration, CON - Control plates for *Fusarium redolens*

Field Test of the Efficiency of Silicon and Urea in Protecting Cucumber Plants from Root and Stem Rot Disease: The results of the field test on the efficiency of silicon and urea in protecting cucumber plants from root and stem rot disease (Table 4) showed that the treatments using silicon and urea, applied to cucumber plants grown in artificially contaminated field soil with *Fusarium redolens* (collected from naturally contaminated fields in Najaf province), were effective in controlling root and stem rot. Significant reductions in disease severity were observed. The 4% silicon treatment showed the best results, with a disease severity of 42.65%, a disease index of 1.6, and a disease control efficiency of 53.01%. This was followed by the 0.6% urea treatment, along with the 6% and 2% silicon treatments, and the 0.8% and 0.4% urea treatments, with disease severities of 45.24%, 45.82%, 51.76%, 52.50%, and 54.12%, respectively. The corresponding disease indices were 1.80, 1.83, 2.07, 2.14, and 2.16, and the disease control efficiencies were 49.10%, 48.32%, 41.61%, 39.58%, and 38.94%, respectively. All these treatments showed significant differences when compared to the control (naturally contaminated field soil) and the fungicide treatment. In the naturally contaminated soil control and the fungicide treatment, disease severities were 88.68% and 83.93%, with disease indices of 3.55 and 3.35, respectively, based on the scale used in this experiment. The treatments with silicon and urea significantly increased the seed germination percentage after 14 days. The silicon treatments at 4% and 6% concentrations, along with urea treatments at 0.6% and 0.8% concentrations, achieved a 100% seed germination rate. This was followed by the 0.4% urea treatment, which had a germination rate of 92.85%, and the 2% silicon treatment with a germination rate of 85.71%. These results showed significant differences compared to the control (naturally contaminated soil) and the fungicide treatment, where the seed germination rates after 14 days were 64.28% and 71.41%, respectively. After 90 days of planting, the 6% silicon treatment recorded the highest percentage of surviving plants at 92.85%, showing significant differences compared to all other treatments. This was followed by the 2% silicon and 0.8% urea treatments, with a survival rate of 85.71% after 90 days. The 4% silicon and 0.4% and 0.6% urea treatments maintained a survival rate of 71.43%, 71.42%, and 71.41%, respectively, after 75 days. These treatments provided significant protection to the plants against the root and stem rot disease in cucumber, with notable differences compared to the control and the fungicide treatment, where the survival rate after 90 days was 50.00%. The results also demonstrated the positive impact of silicon and urea treatments on several studied growth parameters, including branch length and number, fruit count and weight, as well as the wet and dry weight of the root system. The average branch length in the silicon treatments ranged between 248.6 cm and 273.7 cm, while in the urea treatments, it ranged between 240.1 cm and 258.6 cm, showing significant differences

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compared to the control and the fungicide treatment, where the average branch length was 160.0 cm and 174.0 cm, respectively. Urea treatments achieved an average branch number between 3.00 and 5.00 branches per plant, while silicon treatments had an average of 3.07 to 4.66 branches per plant, both significantly higher than the control and fungicide treatment, which had an average of 1.60 branches per plant. Silicon and urea treatments also significantly increased fruit count and weight. Silicon treatments recorded the highest fruit count, ranging between 8.18 and 18.66 fruits per plant, with an average fruit weight between 1109g and 2243g per plant. Urea treatments followed, with a fruit count ranging between 10.28 and 15.42 fruits per plant, and an average weight between 1443g and 1786g per plant. These results showed significant differences compared to the control and fungicide treatments, where the average fruit count was 5.81 and 3.26 fruits per plant, and the average fruit weight was 582g and 383 g per plant, respectively. For the root system, silicon treatments outperformed the others with a wet weight between 17.20 g and 23.94 g, and a dry weight between 1.29 g and 2.33 g. Urea treatments followed, with a wet weight between 16.44 g and 21.19 g, and a dry weight between 1.34 g and 1.97 g. These values were significantly higher than the control and fungicide treatments, where the wet weight of the root system averaged 10.35 g and 12.67 g, and the dry weight was 0.62 g and 0.91 g, respectively (Table 4). These results confirmed the high effectiveness of the nutrients (silicon and urea) in reducing disease severity and improving growth indicators, which positively impacted the productivity of the treated plants compared to the control and fungicide treatments. This can be attributed to the role of silicon in participating in essential metabolic processes within the plant by increasing chlorophyll content, enhancing nutrient availability, and improving plant tolerance to stress conditions such as drought, salinity, and cold, while reducing the toxicity of heavy metals. The addition of silicon to soil containing various root and stem rot pathogens strengthens the plant's cell walls, providing protection against attacks by soil-borne pathogens and reducing infection rates. A lack of silicon in plants can decrease water and nutrient absorption by the roots and increase the susceptibility of fungi to invade xylem tissue by affecting cell membranes, leading to an increased leakage of sugars and amino acids from the cytoplasm into xylem vessels, making the plants more susceptible to infection by pathogens. These pathogens degrade the cell walls of the conductive tissues, resulting in diseases such as rot and wilting, ultimately causing plant death (Xue et al., 2021; Dahal *et al.*, 2023). Moreover, soil fumigation with urea, combined with the volatilization of gases under plastic cover and drip irrigation, can effectively impact soil-borne pathogens (Dahal et al., 2023). Urea also enhances soil quality by reducing salinity, increasing the availability of nutrients, and promoting beneficial soil microorganisms, which collectively improve cucumber plant productivity and the quality of the resulting fruit. Sun et al. (2015) reported that the addition of ammonia to soil infested with *Fusarium oxysporum* f. sp. *niveum* (FON) and planted with watermelon seedlings improved soil conditions and suppressed the growth of FON, which positively impacted crop yield and watermelon fruit quality. Nitrogen is considered a fundamental element for the growth, development, productivity, and quality of fruit trees. It significantly influences plant growth, disease resistance, and yield improvement. Nitrogen is one of the most important nutrients, playing a vital role in plant growth and productivity, as well as enhancing enzymes and proteins related to plant defenses. It also has a direct effect on the pathogens, improves the physical, chemical, and biological properties of the soil, and is an important method for controlling both pathogen growth and wilting. Nitrogen is a crucial nutrient for the growth and development of both plants and beneficial microorganisms in the soil, which are key factors in plant disease interactions (Sharma, 2020; Liu et al., 2022).

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Table 4. Efficiency of silicon and urea in controlling root and stem rot disease in the field using naturally contaminated soil with *Fusarium redolens* and its impact on cucumber plant growth parameters

Treatments	Disease Severity (%)	Disease Index	Control Efficiency (%)	Seed Germination (%) after 14 days	Surviving Plants (%) after 90 days	Branch Length (cm)	Number of Branch	Fruit Count	Fruit Weight (g)	Fresh Root Weight (g)	Dry Root Weight (g)
Silicon 2% + Naturally Contaminated Field Soil	51.76	2.07	41.61	85.71	85.71	253.8	4	13.50	1705	17.20	1.55
Silicon 4% + Naturally Contaminated Field Soil	42.65	1.60	53.01	100.00	71.43	273.7	4.66	18.66	2243	23.94	2.33
Silicon 6% + Naturally Contaminated Field Soil	45.82	1.83	48.32	100.00	92.85	248.6	3.07	8.18	1109	18.09	1.29
Urea 0.4% + Naturally Contaminated Field Soil	54.12	2.16	38.94	92.85	71.42	240.1	3.00	15.42	1786	18.46	1.54
Urea 0.6% + Naturally Contaminated Field Soil	45.24	1.80	49.10	100.00	71.41	240.7	5.00	10.28	1443	21.19	1.97
Urea 0.8% + Naturally Contaminated Field Soil	52.50	2.14	39.58	100.00	85.71	258.6	4.71	12.00	1600	16.44	1.34
Fungicide + Naturally Contaminated Field Soil	83.93	3.35	5.37	71.41	50.00	174.0	1.60	3.26	383	12.67	0.91
Naturally Contaminated Field Soil (only)	88.68	3.55	--	64.28	50.00	160.0	1.60	5.81	582	10.35	0.62
$LSD_{0.05}$	4.11	0.34	4.88	4.31	5.99	9.03	0.44	2.35	14.05	4.80	0.059

CONCLUSION

The fungus *Fusarium redolens*, the causal agent of root and stem rot, was isolated for the first time from cucumber plants grown in sandy soil in Najaf province. The silicon and urea treatments demonstrated high efficiency in inhibiting *F. redolens* both in laboratory and field conditions. All treatments significantly reduced the disease severity index, which positively impacted growth parameters such as branch length, number of branches, fruit count, fruit weight, and the wet and dry weight of the root system, showing significant differences compared to the control treatment.

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CONFLICT OF INTEREST

No disclosed conflicts in this manuscript

AUTHOR/S DECLARATION

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

Author/s signature on Ethical Approval Statement.
Ethical Clearance and Animal welfare
Funds:

AUTHOR'S CONTRIBUTION STATEMENT

Both authors (Z.A. Hashim and T.A. Kareem) contributed equally to the isolation and molecular identification of *F. redolens*, implementation the treatments, data collection, statistical evaluation of growth parameters, and wrote the manuscript.

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تحديد و مكافحة مسبب مرض تعفن جذور و سيقان نباتات الخيار المزروع مكشوف في التربة الرملية

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المستخلص

اجريت هذه الدراسة بهدف الكشف عن مسبب مرض تعفن جذور و سيقان نباتات الخيار المزروع مكشوف في التربة الرملية و مكافحته مختبريا و حقليا باستعمال السليكون و اليوريا اظهرت نتائج العزل و التشخيص وجود 39 عزله مرافقة للمرض بضمنها الفطر *Fusarium redolens* و اكدت نتائج التشخيص الجزيئي للعزلات الأكثر ضراوة FR342a و FR342b بانها تعود للفطر *F. redolens* بنسبة مطابقة بلغت 100% مع العزلات العالمية وبينت النتائج مختبريا بان الأوساط الغذائية المعاملة بمادة السليكون بالتركيز 2 ، 4 ، 6% قد حققت نسبة تثبيط تراوحت بين 66 – 100% لنمو مستعمرة الفطر الممرض *F.redolens* حيث تفوق التركيز 4 ، 6% بنسبه تثبيط بلغت 100%، بينما تميزت الأوساط الغذائية المعاملة بمادة اليوريا بالتراكيز 0.4 ، 0.6 ، 0.8% بنسبه تثبيط بلغت 100% لنمو مستعمرة الفطر الممرض *F. redolens*، وبينت النتائج حقليا تفوق معاملة السليكون بالتركيز 4% التي أدت لخفض شدة الإصابة الى 42.65% جاءت بعدها معاملة اليوريا بتركيز 0.6% والتي أدت لخفض شدة الإصابة الى 45.82. وأعطت جميع المعاملات كفاءة مكافحة جيدة للمرض وانعكس تأثيرها على معايير النمو حيث زادت من طول الفرع وعدد الافرع وعدد الثمار ووزنها والوزن الرطب والجاف للمجموع الجذري.

الكلمات المفتاحية: *fusarium redolens*،PCR ، مرض العفن الفطري، سليكون، يوريا.