First Recording of the Fungus Fusarium Oxysporum that Causes Root Rot and Seedling Death on Catharanthus Roseas L. in the Governorates of Karbala and Babylon/Iraq

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Abstract

This study was conducted with the aim of isolating and diagnosing the fungus that causes root rot and seedling death of Catharanthus roseas L. in some nurseries in the governorates of Karbala and Babil, and to test its pathogenicity in the laboratory and under greenhouse conditions. The results showed that 11 isolates of Fusarium oxysporum were phenotypically diagnosed and outperformed. Isolate FK8 significantly lowered the percentage of germination of red radish seeds on the water agar medium, as the percentage of germination in it was 3.33% and the inhibition rate was 96.66% compared to the comparison treatment in which the percentage of seed germination was 100%. The results of the plastic pots experiment in the greenhouse also showed that the results matched the laboratory experiment, as isolate FK8 was significantly superior in reducing the germination rate, which amounted to 13.33%, and an inhibition rate of 86.67% when treating the seeds of Catharanthus roseas L. plant with the isolated fungi compared to the comparison treatment, in which the percentage of germination reached 100%. . The results of the molecular diagnosis showed that the most pathogenic isolate FK8 belongs to the fungus Fusarium oxysporum and it was deposited in the American Gene Bank under the entry number on025785.

Keywords: Catharanthus roseas, Fusarium oxysporum, root rot

1. Introduction

Ornamental plants are one of the botanical groups that are distinguished by their beautiful shapes and colors, as they work to beautify places and are used as decorations in homes or public offices because they add joy and pleasure to the soul and provide pleasure and comfort, in addition to their environmental benefits as they contribute to reducing environmental pollution by absorbing toxic gases. Catharanthus roseas L. plant is cultivated in Iraq as an ornamental plant, as its flowers are distinguished by their different colors and is considered one of the medically important plants [1-3]. There are many nurseries in the governorates of Karbala and Babil, which are interested in cultivating the multiplication of ornamental plants, including Catharanthus roseasL. Plant. Nitrogenous base sequences of pathogen-specific genetic markers to facilitate control. In view of the importance of Catharanthus roseasL. plant in ornamental nurseries and the great losses of the pathogens that afflict it, and the lack of a study on root rot disease and the death of seedlings of Catharanthus roseas L. plant in the governorates of Karbala and Babylon and perhaps in Iraq, so this study aimed to isolate and diagnose the cause of this disease and test its pathogenic ability Laboratory and in the conditions of the plastic house.

2. Material and Methods

Samples collection and isolation

The fungi were isolated from the roots of the local plant Catharanthus roseas L., which showed symptoms of disease represented by weak plant growth, yellowing of leaves and root rot. The samples were collected from some nurseries located in Karbala governorate (Al-Hussainiya, Al-Atishi, Al-Hur neighborhood, Al-Abbas neighborhood, Al-Moalemeen neighborhood, Al-Hafiz, Al-Hur district, Al-Mutahideen) and Babel (Al-Musayyib district, Al-Hilla, Al-Mahaweel). The roots were washed well with water to remove dust from them for half an hour, then they were cut into small pieces of size (0.5-1) cm and sterilized with sodium hypochlorite solution at a concentration of 1% for two minutes and then washed with sterile distilled water and were dried by sterile blotting paper and transferred the pieces by forceps to the dishes containing The food medium (PDA) then the dishes were incubated at a temperature of 25 ± 2 °C for three days, after which the fungi were purified by transferring part of the fungal colony by means of a cork piercing to another dish containing the food medium (PDA). Phenotypic traits using taxonomic keys [4, 5].

Laboratory test of pathogenicity of fungi isolated on red radish seeds on WA culture medium (Water Agar)

In the pathogenicity test, 11 isolates of the fungus Fusarium spp were used. They were isolated from the roots of Catharanthus roseas L. plant by taking Petri dishes in which Water Agar (WA) was prepared, which was prepared from 20 gm of agar and 1000 ml of distilled water and placed in sealed glass flasks. Its nozzles are covered with tampons and sterilized in the autoclave for 20 minutes at a pressure of 15 pounds / ing ² and a temperature of 121 ° C. After the sterilization was completed, the antibiotic Amoxicillin was added to the medium at an amount of 125 mg / liter). Inoculated with a disc with a diameter of 5 mm from a pure fungal colony at the age of 7 days and placed in the middle of the prepared nutrient medium. Seeds were distributed on the outskirts of the fungal colony in a circular manner

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and by three dishes for each fungal isolate as replicates, as well as the comparison treatment, which planted the seeds only without fungal isolate. The number of germinated seeds

Germination percentage=x100 Total number of seeds. As well as calculating the percentage of inhibition according to the equation. The number of germinated seeds in comparison-the number of seeds germinated in the treatment Inhibition percentage=x100 Number of germinated seeds in comparison

A test of the pathogenicity of fungi isolated from the Catharanthus roseas L. plant in plastic pots under the conditions of the greenhouse

The experiment was carried out in one of the greenhouses affiliated to the Plant Protection Department, College of Agriculture / University of Karbala, where a mixture of mixture soil and peat moss was sterilized 1:1 at a temperature of 121 and under pressure of 15 pounds / inch ² for one hour a day and for two consecutive days, after which they were placed in plastic containers with a capacity of 1 The soil was moistened and fungal isolates grown on seeds of local millet (Panicum miliacem) were added(By cleaning and removing the dust and impurities present in the millet seeds, then washed well and soaked for six hours with water, then dried from the excess water and placed in 250ml glass beakers, 50 gm of millet seeds were placed for each beaker and sterilized in the oxidizer for 20 minutes under pressure 15 pounds / inch ² at a temperature of 121 °C, after which the millet was inoculated with the isolates under study by 5 tablets for each beaker of the nutritional medium containing the fungal colonies, and then incubated at a temperature of 25 ± 2 °C for 14 days with moving the beakers every three days in order to distribute the fungal inoculum on the All seeds) [6] at a rate of 10% per pot and then covered with polyethylene bags for 48 hours, then the pots were planted with superficially sterilized Ain Al-Bazoon seeds with 1% sodium hypochlorite solution and 10 seeds per pot .

The experiment was designed according to the complete random design (CRD), with three pots for each treatment as replicates, with a comparison treatment planted with Ain Al-Bazon seeds only and with the same number of replicates.

The results were recorded after 60 days of conducting the experiment by calculating the percentage of seed germination and the percentage of inhibition according to the equations mentioned above.

Molecular diagnosis of pathogenic fungi

The polymerase chain reaction (PCR) test, which is the technique used in the study for the purpose of confirming the phenotypic diagnosis of the pathogenic fungal isolates that cause root rot and seedling death, is Catharanthus roseas L. plant according to the method of [2, 7].

Genomic DNA was extracted and purified from fungal isolates, as the commercial D Neasy Plant Kits were used to extract and purify genomic DNA from the isolates of pathogenic fungi according to the instructions of the supplied company. The polymerase chain reaction (PCR) was tested using 2 microliters of genomic DNA for each pathogenic fungal isolate isolated from the infected Catharanthus roseasL. Plants as a template in the PCR reaction tests. ITS1 and ITS5 5' (TCC GTA GGT GAA CCT GCG G) 3', 5' (TCC TCC GCT TAT TGA TAT GC) 3' respectively Which duplicates the internal transcribed spacer (ITS) regions located within the genes of the small and large unit of ribosomes in the fungal chromosome (White et al., 1990). The same kit was also used in the PCR reaction for the actin gene duplication of the studied fungi, using the primers ACT-512 F and ACT- 783 R (5'-ATG TGC AAG GCC GGT TTC GC-3', 5'-TAC GAG TCC TTC TGG CCC AT-3') respectively [2].

3. Results and Discussion

results of the pathological ability test of fungi isolated from the roots of Catharanthus roseas L. plant from some nurseries in the governorates of Karbala and Babylon laboratory on the WA medium (Table 1 and Figure 1) showed that isolate FK8 was significantly superior to the other isolates in reducing the germination rate of red radish seeds, which amounted to 3.33% and an inhibition rate of 96.66% Compared to the comparison treatment, which amounted to 100% and 0.00%, respectively. As for the least isolates in their effect on the percentage of seed germination and the percentage of inhibition, it was FB2, which amounted to 33.33% and 66.66%, respectively.

Table (1) Detection of pathogenic isolates of Fusarium oxysporum using red radish seeds on WA culture medium

inhibition ratio	germination percentage	types of fungi	
73.33	26.66	FK 1	1
80.00	20.00	FK 2	2
93.33	6.66	FK 3	3
73.33	26.66	FK 4	4
83.33	16.66	FK 5	5
86.66	13.33	FK 6	6
86.66	13.33	FK 7	7
96.66	3.33	FK 8	8
73.33	26.66	FB 1	9
66.66	33.33	FB 2	10
86.66	13.33	FB 3	11
0.00	100.00	control	12
1.242	1.242	Lsd	
0.05	0.05	morale level	

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

*FK: isolate of the fungus Fusarium oxysporum from Karbala governorate, which numbered 8 isolates.

*FB: isolate of the fungus Fusarium oxysporum from Babylon Governorate, and there are 3 isolates.

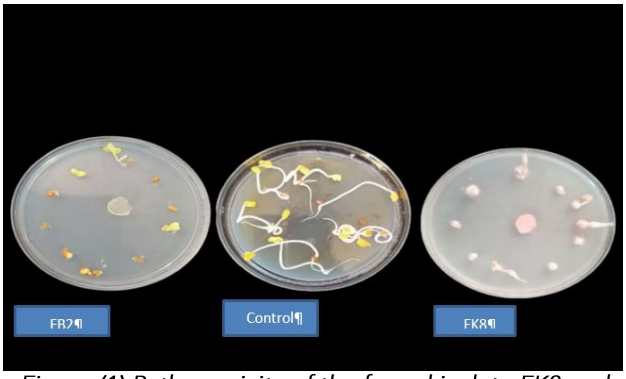

Figure (1) Pathogenicity of the fungal isolate FK8 and

FB2 Fusarium oxysporum with comparison with the use of red radish

The results of the plastic pots experiment in the greenhouse matched with the results of the laboratory experiment, as isolate FK8 significantly outperformed the other isolates in reducing the germination rate of Catharanthus roseas seeds (Table 2 and Figure 2), as the germination percentage reached 13.33% and the inhibition rate was 86.67% compared to the comparison treatment which amounted to 100 % and 0.00%, respectively. As for the weakest isolates in their effect on the percentage of seed germination and the percentage of inhibition, it was FB2, which amounted to 90.00% and the percentage of inhibition reached 10.00%, respectively.

Table (2) Detection of germination and inhibition rates using Catharanthus roseas plant seeds resulting from the effect of isolates of the fungus Fusarium oxysporum on plastic pots in the greenhouse

inhibition ratio	germination percentage	types of fungi	
63.33	36.67	FK 1	1
73.33	26.67	FK 2	2
50.00	50.00	FK 3	3
43.33	56.67	FK 4	4
50.00	50.00	FK 5	5
30.00	70.00	FK 6	6
30.00	70.00	FK 7	7
86.67	13.33	FK 8	8
70.00	30.00	FB 1	9
10.00	90.00	FB 2	10
73.33	26.67	FB 3	11
0.00	100.00	control	12
2.046	2.046	Lsd	
0.05	0.05	morale level	

Each number in the table represents an average of three replicates*
*FK: isolate of the fungus Fusarium oxysporum from Karbala governorate, which numbered 8 isolates.
*FB: isolate of the fungus Fusarium oxysporum from Babylon Governorate, and there are 3 isolates.

The results of this study are in agreement with many previous studies, as James [8], Berg et al. [9] mentioned that many types of Fusarium spp cause seed and root rot disease and seedling death on many plant families due to their parasitic nature and the abundance of mycelium growth. Inside the vascular tissue, which causes obstruction of water and salts from reaching the leaves. In addition to the effect of the enzymes and toxins formed by this fungus that work on decomposing plant cell walls [2].

The results of the genetic analysis tree (Figure 3) showed that the study isolate registered in the Gen Bank as Fusarium oxysporum, using the ITS1 and ITS4 primers [10]. The similarity between it and other isolates recorded in the gene bank was 99%.

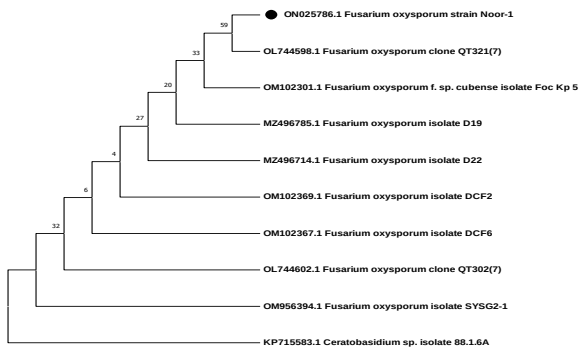

Figure (3) The genetic tree of the pathogenic fungus Fusarium oxysporum strain Noor-1 (marked with a black dot) which was established based on the nitrogenous base sequences of the ITS-rDNA region as well as the sequences of global known strains of the same pathogen obtained from the GenBank data repository. The genetic distances were calculated using the neighbor-joining method.

Figure (4) shows the fungus Fusarium oxysporum A- The appearance of the fungus B- The shape of the fungus under a light microscope

References

1. Nejat N, Valdiani A, Cahill D, Tan Y-H, Maziah M, Abiri R. Ornamental exterior versus therapeutic interior of Madagascar periwinkle (Catharanthus roseus): the two faces of a versatile herb. The Scientific World Journal. 2015;2015.

2. Lahuf AA, Abdullah KM, Mohammadali MT. Assessment of the nanosized particles of ZnO and MgO and some cultivars in control of Alternaria solani causing tomato early blight. Ecology, Environment and Conservation. 2020;26:89-95.

3. Pham HNT, Vuong QV, Bowyer MC, Scarlett CJ. Phytochemicals derived from Catharanthus roseus and their health benefits. Technologies. 2020;8(4):80. https://doi.org/10.3390/technologies8040080

4. Leslie J, Summerell B. Fusarium laboratory workshops--A recent history. Mycotoxin Research. 2006;22(2):73-4.

5. Watanabe T. Pictorial atlas of soilborne fungal plant pathogens and diseases. Can J Res. 2018;11:18-31.

6. Dewan M, Sivasithamparam K. Effect of colonization by a sterile red fungus on viability of seed and growth and anatomy of wheat roots. Mycological Research. 1990;94(4):553-7.

https://doi.org/10.1016/S0953-7562(10)80022-X

7. Narayanasamy P. Microbial Plant Pathogens-Detection and Disease Diagnosis:: Viral and Viroid Pathogens, Vol. 3. Springer Science & Business Media, 2010.

8. James RL. 34. Fusarium Root and Stem Diseases. Forest Nursery Pests. 2012:117.

9. Berg LE, Miller SS, Dornbusch MR, Samac DA. Seed rot and damping-off of alfalfa in Minnesota caused by Pythium and Fusarium species. Plant disease. 2017;101(11):1860-7. https://doi.org/10.1094/PDIS-02-17-0185-RE

10. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 1990;18(1):315-22.