First Record of the Fungus Rhizoctonia Solani that Causes Root Rot and Damping off on Catharanthus Roseas L. In Provinis 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 Babylon and Karbala and to test its pathogenicity in the laboratory and under greenhouse conditions. The results showed that 30 isolates of the fungus *Rhizoctonia solani* were phenotypically diagnosed. Isolate RK22 significantly outperformed the other isolates in reducing the percentage of germination of red radish seeds on Water agar media, as the germination percentage in it was 0.00% and the percentage of inhibition was 100% 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 RK22 was significantly superior in reducing the germination rate, which amounted to 3.33% and an inhibition rate of 96.67% when treating the seeds of *Catharanthus roseas* 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 RK22 belongs to the fungus *Rhizoctonia solani*, and it was deposited in the American Gene Bank underthe entry number.

Keywords: Catharanthus roseas, Rhizoctonia solani, root rot.

1. Introduction

The plant, Cartharanthus roseas, belongs to the oleander family Apocynaceae, its original home is the island of Madagascar located in the African coast [1]. It has different colors, including white, violet and pink with five-petaled flowers, and then its cultivation spread in tropical, semi-tropical and warm temperate regions. It is also widely cultivated in India, Australia, Africa and China [2]. This plant has received increasing attention because it contains some important chemicals, as the alkaloids are the main components of the plant, including Vincristine, Vinblastine, Flavonoids and Phenolic, which are of medical importance as they are antibacterial, anti-cancer, antioxidant, high blood pressure and wound healing [3]. This plant is exposed to many pathogens like other plants, and thus it needs to diagnose these causes using modern methods, the most important of which is the polymerase chain reaction (PCR) Because of the importance of the Catharanthus roseas plant in ornamental nurseries and what the pathogens that afflict it cause great losses, and because there is no study on the root rot disease and the death of seedlings of the Catharanthus roseas 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 in the laboratory and in the conditions of the plastic house.)2021 ,2018)

2. Material and Methods

The fungi were isolated from the roots of the local plant (Cartharanthus roseas), which showed symptoms of the disease represented by yellowing of leaves, root rot and weak plant growth.) and Babil (Al-Musayyib District, Abu Gharq, 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 Nutrient media (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 cork piercing to another dish containing the nutrient medium (PDA). Taxonomic [4].

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

In the pathological ability test, 30 isolates of the fungus Rhizoctonia solani were isolated from the roots of Catharanthus roseas plant by taking Petri dishes containing Water Agar (W.A) culture medium, which was prepared from 20 gm of Agar and 1000 ml of distilled water and placed in glass flasks whose nozzles were blocked With cotton tampons and sterilized in the autoclave for 20 minutes at a pressure of 15 pounds / Eng ² and a temperature of 121 ° C, after the end of sterilization, Tetracycline was added to the antibiotic 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 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=*100

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Total number of seeds
(Jaber, 2020).
As well as calculating the percentage of inhibition according to the Abbott [5] equation.
The number of germinated seeds in comparison – the number of seeds germinated in the treatment
Inhibition percentage=* 100
Number of germinated seeds in comparison
A test of the pathogenicity of fungi isolated from Catharanthus roseas 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 glass beakers with a capacity of 250 ml. Pressure of 15 pounds / inch ² and a temperature of 121 °C. After that, the millet was inoculated with the isolates under study by 5 tablets per beaker from 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 all seeds) (Dewan, 1989) At a rate of 10% for each pot, then covered with polyethylene bags for 48 hours, then the pots were planted with superficially sterilized seeds Catharanthus roseas plant with 1% sodium hypochlorite solution, with 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 the seeds of Catharanthus roseas plant only and with the same number of replicates.
The results were recorded after two months of conducting the experiment by calculating the percentage of seed germination and the percentage of inhibition according to the equations mentioned previously.

3. Results and Discussion

The results of testing the pathogenicity of fungi isolated from the roots Catharanthus roseas plant from some nurseries in the governorates of Karbala and Babil in the laboratory on the WA medium (Table 1 and Figure 1) showed that isolates RK2, RK4, RK22, RB2 significantly outperformed the other isolates in decreasing the germination rate of red radish seeds It reached 0.00% and the percentage of inhibition of 100% compared to the comparison treatment, which amounted to 100% and 0.00%, respectively. The least isolates in their effect on the percentage of seed germination and the percentage of inhibition was RB1, which amounted to 33.43% and 56.66%, respectively.

Table (1) Detection of pathogenic isolates of *Rhizoctonia solani* using red radish seeds on WA culture medium

inhibition ratio	germination percentage	types of fungi	
96.66	3.33	RK 1	1
100.00	0.00	RK 2	2
90.00	10.00	RK 3	3
100.00	0.00	RK 4	4
93.33	6.66	RK 5	5
96.66	3.33	RK 6	6
70.00	30.00	RK 7	7
70.00	30.00	RK 8	8
93.33	6.66	RK 9	9
76.66	23.33	RK 10	10
93.33	6.66	RK 11	11
96.66	3.33	RK 12	12
86.66	13.33	RK 13	13
90.00	10.00	RK 14	14
93.33	6.66	RK 15	15
83.33	16.66	RK 16	16
86.66	13.33	RK 17	17
96.66	3.33	RK 18	18
70.00	30.00	RK 19	19
70.00	30.00	RK 20	20
86.66	13.33	RK 21	21
100	0.00	RK 22	22
73.33	26.66	RK 23	23
90.00	10.00	RK 24	24
73.33	26.66	RK 25	25
80.00	20.00	RK 26	26
93.33	6.66	RK 27	27
56.66	43.33	RB 1	28
100.00	0.00	RB 2	29
80.00	20.00	RB 3	30
0.00	100.00	control	31
1.437	1.437	Lsd	
0.05	0.05	morale level	

Each number in the table represents an average of three replicates* RK: 27 isolates of the fungus Rhizoctonia solani from Karbala governorate* RB: 3 isolates of the fungus Rhizoctonia solani from Babylon Governorate*

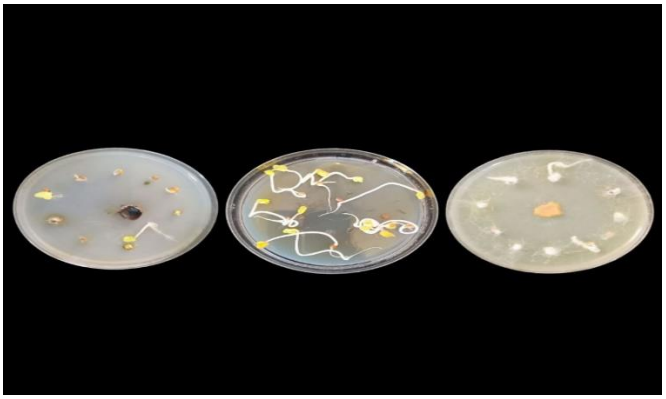

Figure (1) Pathological determination of the fungal isolate RK22 and RB1 of Rhizoctonia solani with comparison with the use of red radish

The results of the plastic pots experiment in the greenhouse matched with the results of the laboratory experiment, as isolate RK22 significantly outperformed the other isolates in reducing the germination rate of Catharanthus roseas seeds (Table 2 and Figure 2), as the germination percentage reached 3.33 and the inhibition rate was 96.67% 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

RB1, which amounted to 86.67%, and the percentage of inhibition reached 13.33%, respectively.

Table (2) Detection of germination and inhibition ratios by using *Catharanthus roseas* plant seeds resulting from the effect of isolates of the fungus *Rhizoctonia solani* on plastic pots in the greenhouse

inhibition ratio	germination percentage	types of fungi	
83.33	16.67	RK 1	1
73.33	26.67	RK 2	2
73.33	26.67	RK 3	3
63.33	36.67	RK 4	4
50.00	50.00	RK 5	5
83.33	16.67	RK 6	6
33.33	66.67	RK 7	7
80.00	20.00	RK 8	8
33.33	66.67	RK 9	9
33.33	66.67	RK 10	10
40.00	60.00	RK 11	11
93.33	6.67	RK 12	12
33.33	66.67	RK 13	13
30.00	70.00	RK 14	14
83.33	16.67	RK 15	15
26.67	73.33	RK 16	16
26.67	73.33	RK 17	17
66.67	33.33	RK 18	18
46.67	53.33	RK 19	19
66.67	33.33	RK 20	20
63.33	36.67	RK 21	21
96.67	3.33	RK 22	22
56.67	43.33	RK 23	23
60.00	40.00	RK 24	24
36.67	63.33	RK 25	25
53.33	46.67	RK 26	26
66.67	33.33	RK 27	27
13.33	86.67	RB 1	28
63.33	36.67	RB 2	29
76.67	23.33	RB 3	30
0.00	100.00	Control	31
2.779	2.443	Lsd	
0.05	0.05	morale level	

Each number in the table represents an average of three replicates* RK: 27 isolates of the fungus *Rhizoctonia solani* from Karbala governorate* RB: 3 isolates of the fungus *Rhizoctonia solani* from Babylon Governorate *

This study agreed with where all isolates showed a high pathogenicity against red radish seeds with an infection rate of 93.3% compared to the control treatment, which amounted to 0%

The fungus Rhizoctonia solani is one of the most important pathogens of plant hosts that causes root rot diseases and seedling death [6]. Also, the fungus Rhizoctonia solani has a Necrotrophic parasitic ability and infects plant tissues by secreting enzymes that break down cell walls and penetrate into plant tissues. Cells (Hamid, 2010; Bradley, and Ajayi-Oyetunde et al. [7].

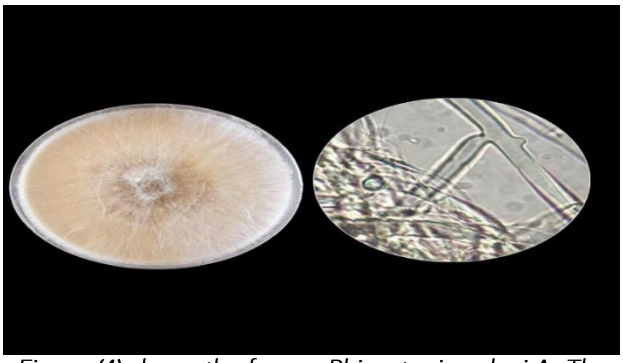

Figure (4) shows the fungus Rhizoctonia solani A- The appearance of the fungus B- The shape of the fungus under a light microscope

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