SHORT ARTICLE / INVESTIGACIÓN

Isolation and diagnosis of some associated fungi with cowpea root rot disease and testing its pathogenicity

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Abstract: Execute search by date 1/4/2021, The results of collecting samples from the regions of Anbar, Baghdad, Salah al-Din and Wasit showed that cowpea root rot disease is widespread in all studied areas, and The results of isolation phenotypic and molecular diagnosis showed the presence of different isolation of fungi that infected cowpea root, such as *Fusarium nygamai* (Fu1), *F. nygamai* (Fu2), *F. solani* (Fu3), *F. solani* (Fu4), *Rhizoctonia solani* (Rh5), and *Fusarium solani* (Fu6), The results of the pathogenicity test on radish seeds showed that tested isolates were significantly decreased germination percentage of radish seeds of water Ager, and the most effective isolation was *F. solani* (Fu4). The infection rate was 90% compared to 0.00% of the control media treatment, which was uncontaminated by the pathogenic fungus. Isolated fungi showed a difference in the percentage and severity of infection on cowpea seedlings and seedlings, as the isolate of *F.nygamai* (Fu1) achieved the highest infection rate of 66.67 % and the severity of disease at 75%. All fungal isolates significantly increased the rate and severity of infection on seed radish compared with the control treatment not contaminated with pathogenic fungi by 0.0%.

Key words: *Vegan unguiculata*, *Rhizoctonia solani* and *Fusarium solani*, PCR.

Introduction

Cowpea (Vegan unguiculata) is a multi-purpose crop belonging to the legume family, and it is grown in dry and semi-dry tropical regions[1]. The global cowpea production is estimated at 4.5-6.5 million tons, and 80% of production is from Africa[2]. In Iraq, the cultivated area of cowpeas reached 72,507 m^2, and the output of one dunam ranged between 2600-2768 kg^3. Copwea is affected by many diseases, such as root rot and damping off, as these diseases cause losses that may reach 55% of the cowpea production[4,5]. Moreover, the losses can get 100% yield in the appropriate environmental conditions[6,7]. Root and stem rot disease is caused by fungi such as *Fusarium solani* and *Rhizoctonia solani*, the most dangerous fungi in cowpea[8,9], because they contain toxins and enzymes (fludarabine, fusaric acid, javanicine, pectin methyl esterase and galacturonic enzymes), as well as their ability to tolerate toxins and antibiotics secreted by other organisms[10,11]. *Fusarium solani* and *Rhizoctonia solani fungi* are classified based on their phenotypic characteristics[12-14]. However, several studies have been using Polymerase Chain Reaction technology (PCR) as a modern application in diagnosing fungi based on the nitrogenous bases sequence traits in a single DNA strand[15-17]. Found[18,19] that the root rot disease and damping off of cowpea seedlings, caused by *Fusarium solani* and *Rhizoctonia solani*, have caused significant losses in yield, and they are the most common pathogens that infect cowpea.

Materials and methods

Sample collection

Samples of infected cowpea plants by fungi were collected from different governorates of Iraq, Anbar, Baghdad and Wasit and Salah Al-Din, in June, July, August and September 2021. that showed symptoms of root rot diseases were collected, such as leaves wilting, burning of the leaves edges, discoloration of the roots and plant death[20].

Isolation of associated fungi with cowpea root rot disease

Infected parts of cowpea plants were cut into small pieces 0.5-1 cm in length and superficially sterilized using a solution of 6% sodium hypochlorite (Naocl) free chlorine, then washed with sterilized distilled water to remove the remnants of the sterilizing solution and placed on a filter paper, then transferred into Petri dishes 9 cm containing Potato Dextrose Agar (PDA) and incubated at 25 ± 2 °C for 5-7 days until a formation of the fungal growth, the growing fungi were purified by taking a small part of the edge of The fungal growths, and transferred in the center of another Petri dishes containing (PDA) average of three replicates then incubated at a temperature of 25 ± 2 °C.

Phenotypic diagnosis

Fungi isolates were diagnosed after purification of the growth of fungal colonies was completed on the PDA medium., Microscopic slides were prepared and checked using a light microscope; the fungi were diagnosed to the genus level depending on the phenotypic characteristics by using the approved taxonomic keys[21,22].

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Molecular Diagnosis

DNA of six isolates, *F. nygamai* (Fu1), *F. nygamai* (Fu2), *F. solani* (Fu3), *F. solani* (Fu4), *R. solani* (Rh5) and *F. solani* (Fu6), Scientific Progress Laboratory – Baghdad- Al-Harithiya, was extracted by using standard kit ABI OPure from the American company., The extraction was performed in the Scientific Progress Laboratory, located in Baghdad,/Iraq, and diagnosis steps were described by (23). The polymerase chain reaction (PCR) was prepared according to (24) and the primersITS1.5'-TCCGTAGGTGAACCTGCGG-3. and.ITS4 5'-TCCTCCGCTTATTGATATGC-3(Macrogen Company, Korea) were used. The lyophilized primers were dissolved in nuclease-free water to give a final concentration of 100 µl. After completing the DNA amplification and migration steps on agarose gel, the samples were sent to the Korean Macrogen Company to obtain Sequencing (Nitrogenous bases sequence).

Pathogenicity test on radish seeds

The fungus Fusarium spp and *Rhizoctonia solani* isolations were tested on radish seed in the Plant Protection Department, /College of Agriculture, /Anbar University, Iraq. The test of pathogenicity was carried out according to the method of (25). Data were statistically analyzed according to the completely randomized design (CRD), and the germination percentage of radish seeds average of three replicates calculated after 14 days of sowing seeds on PDA medium was calculated and compared with the control treatment them by following the equation below: Germination percentage = (number of germinated seeds)/(total seed number) × 100.

Pathogenicity test on cowpea seed and seedling

Inoculum of fungal isolates was prepared according to the (26) by using millet seeds, the soil was sterilized with commercial formalin, then sterilized soil was placed in sterilized plastic pots with a capacity of 2 kg, and pathogenic fungi inoculum was added at a rate of 1% (weight-weight), the process was repeated average of three replicates on all fungal isolates, and then the pot was rinsed with water and covered with polyethylene bags for 3 days after that cowpea seeds were planted at a rate of three seeds per pot, the sterilized cowpea seeds were placed without adding the pathogenic fungus inoculum as the control treatment, three replicates were used for each treatment. The test was carried out by using a CRD design, and the percentage of germination was calculated according to the following equation:

Germination percentage = (number of infected plants)/(total number of plants) x 100

The percentage of infected severity was calculated according to equation[27] as follows:

% Infected severity= ×100

0= no injury (healthy roots)

1 light brown coloration in the secondary roots

2 light brown coloration in the secondary roots, with a small part of the main root

3 dark brown coloration in the main roots, with no rotting of the stem bases

4 dark brown coloration of the roots, with rotting of the stem bases

5 plant death

Results

Sample collection

The results in Table (1) showed that cowpea root rot diseases were widespread in all studied sample collection areas, which is consistent with what was mentioned by (9 and 28); the condition is dangerous and widespread in most countries where the crop is grown.

Phenotypic diagnosis

The results of the phenotypic diagnosis in Table (2) and Figure 1 showed the presence of fungi (*Fusarium spp.*, and *Rhizoctonia solani*), which were associated with cowpea root rot disease, which is the leading cause of the disease[29,30].

Molecular Diagnosis

The results of the molecular diagnosis by using the PCR technique in Table (3) and Figure (2), according to the arrangement of the nitrogen bases in the single DNA strand, showed that most types of fungi that cause cowpea root rot disease belong to *Fusarium* spp and *Rhizoctonia solani*. These results reinforce what was indicated by (34,35), that these fungi are the leading cause of the disease.

Pathogenicity test on radish seeds

The results in Table (4) and Figure (3) showed that the pathogenicity test for some isolates of fungi (*Fusarium nygamai* (Fu1)- Khalidiya, *Fusarium nygamai* (Fu2)- Amriya, *Fusarium solani* (Fu3)- Essaouira, *Fusarium solani* (Fu4) Radwaniya, *Rhizoctonia solani* (Rh5), *Fusarium solani* (Fu6), *Fusarium solani* (Fu7) achieved a significant reduction in the percentage of germination of radish seeds compared to the control treatment (untreated with pathogenic fungi) in which the infection rate was 0.0%[20], (36,37) or. (38,39)

Sample number	Location	Sampling date for 2021
1	Anbar / Khalidiya	13/6
2	Anbar / Amriya	14/6
3	Wasit / Essaouira	21/6
4	Baghdad / Radwaniyah	23/6
5	Al-Anbar / Al-Bodhiab	13/7
6	Salah Al-Din / Sheikh Ahmed	4/9

Table 1. The infected sample of cowpea plants collection areas and the collection date.

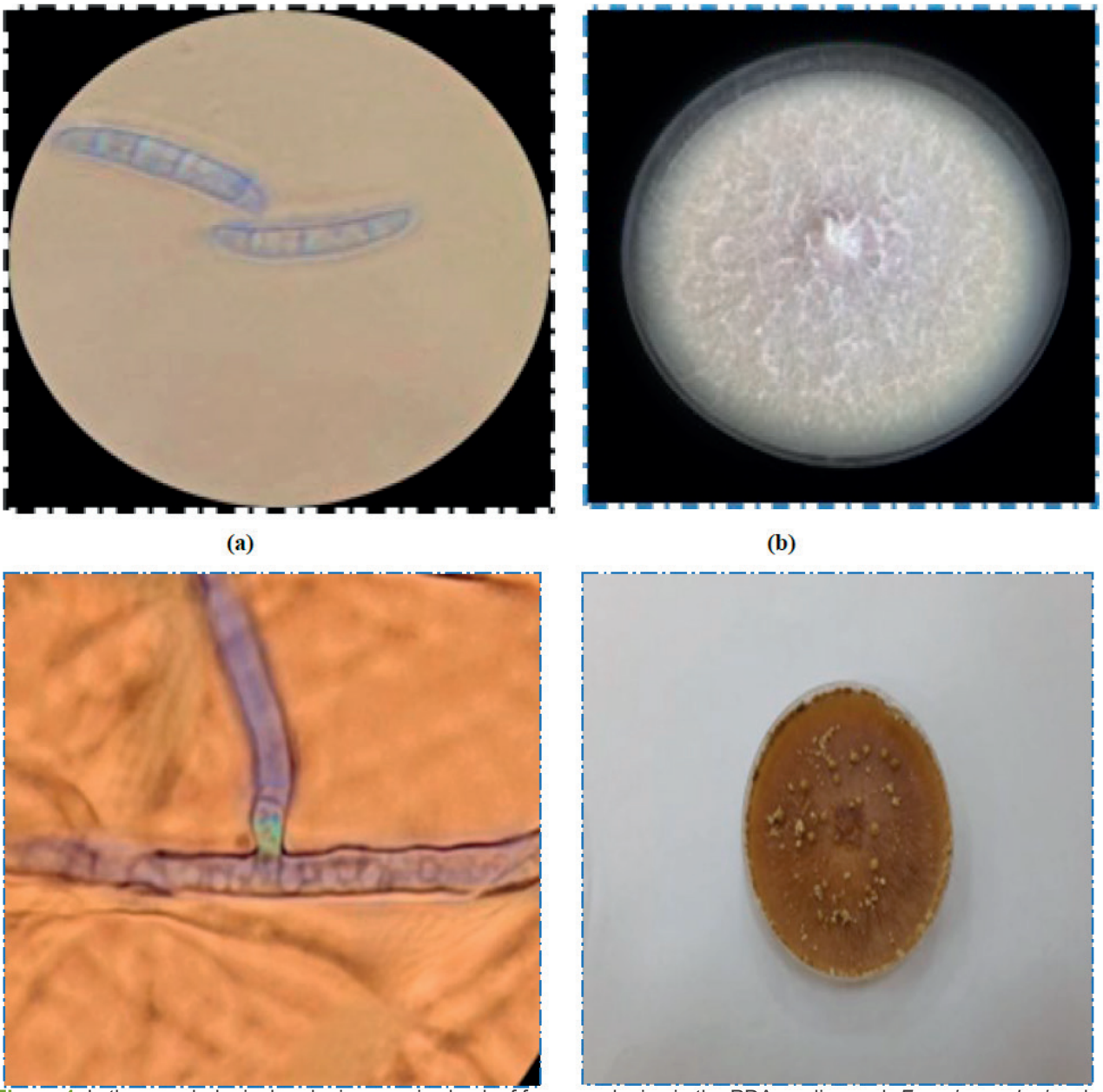

Figure 1. Is the morphological and microscopic check of fungus colonies in the PDA medium. a.b *Fusarium solani* and c. d *Rhizoctonia solani*.

Pathogenicity test on cowpea seed and seedling

The results of pathogenicity examination testing of cowpea seeds in Table (5) and Figure (4) showed that all the tested isolates (*Fusarium solani, Fusarium nygamai, Fusarium nygamai, Fusarium solani, Fusarium solani, Rhizoctonia solani*) varied in their impact on the ratio and severity of cowpea root rot disease, where, the isolate of Al-Anbar - Khalidiya Island (*Fusarium nygamai*) (Fu1) achieved the highest percentage and severity of infection, which amounted to 66.67% and 75%, respectively, and all tested isolates achieved a significant increase in the ratio and severity of disease compared to the control treatment (without adding pathogenic fungi), which reached 0.0% for both.

Discussion

The phenotypic diagnosis of *F. solani* was made based on the shape of the large conidia (Macrioconidia), the presence or absence of small conidia, and the presence of Chlamydiospores whose location is terminal or intertwined, (31,32)—*F.solani* based on the shape of the crescent-shaped Macroconida[33]. As for the fungus *Rhizoctonia solani*, the thread is branched at right angles in the area of genesis with the presence of a constriction, and it is considered one of the diagnostic features of the fungus over other fungi[29]. The difference in the effect of these isolates is due to their difference in the speed of penetration of plant cells and their ability to produce toxins and enzymes that break down plant cell walls; the results of this study agree[11].

Conclusions

The cowpea root rot disease spread in all the governorates from which the plant samples were collected. The leading cause of cowpea root rot disease is several species belonging to the genus Fusarium and the fungus *Rhizoctonia solani*—the difference in the pathogenicity of fungal isolates in causing cowpea root rot disease.

Sample number	Location	Code	Fungi	The collection date
1	Anbar / Khalidiya	Fu1	Fusarium spp	13/6
2	Anbar / Amriya	Fu2	Fusarium spp	14/6
3	Wasit / Essaouira	Fu3	Fusarium spp	21/6
4	Baghdad / Radwaniyah	Fu4	Fusarium spp	23/6
5	Al-Anbar / Al-Bodhiab	Rh5	Rhizoctonia spp	13/7
6	Salah Al-Din / Sheikh Ahmed	Fu6	Fusarium spp	4/9

Table 2. The associated fungi with cowpea root rot disease were isolated and diagnosed phenotypically to the genus level.

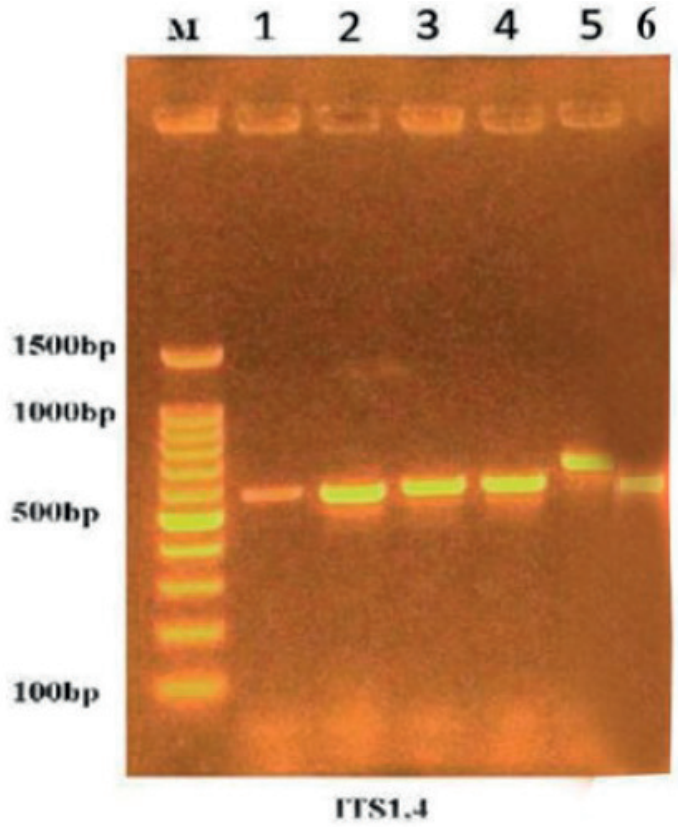

Figure 2. Amplification of ITS gene for unknown fungal species on agarose gel.

Sample number	Fungi	Accession
1	*Fusarium nygamai*	OK036871.1
2	*Fusarium nygamai*	MK752419.1
3	*Fusarium solani*	MT529726.1
4	*Fusarium solani*	MN817707.1
5	*Rhizoctonia solani*	AJ318420.1
6	*Fusarium solani*	MH612968.1

Table 3. The associated fungi with cowpea root rot were diagnosed by using the PCR technique.

Isolation name	Isolation code	Infection rate %
Anbar / Khalidiya Island	Fu1	86.70
Anbar / Amriya	Fu2	80.00
Wasit / Essaouira	Fu3	76.70
Baghdad / Radwaniyah	Fu4	90.00
Al-Anbar / Al-Bodhiab	Rh5	80.00
Salah Al-Din / Sheikh Ahmed	Fu6	66.70
Control	Co	0.00
LSD 0.05		13.24

Table 4. Effect of some isolated fungi from cowpea roots on the germination of radish seeds on water agar medium.

Figure 3. Effect of fungi isolates on the germination of radish seeds.

Isolation name	Isolation code	Infection ratio%	Infection severity %
Anbar / Khalidiya	Fu1	66.67	75.00
Anbar / Amriya	Fu2	33.33	66.66
Radwaniyah	Fu3	66.67	66.66
Essaouira	Fu4	55.56	70.00
Al-Bodhiab Island	Rh5	66.67	68.25
Control	Co	0.0	0.00
LSD 0.05		15.66	3.564

Table 5. Effect of some isolated fungi from cowpea roots on infecting the seeds and seedlings of cowpea under field conditions.

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