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Article

The effect of different concentrations of a leaky isolation fun- gus (Fusarium solani) and it is treated in seedlings Okra plants aged 30 days

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ABSTRACT

This study aimed at the investigation of root rot disease root to , deteriorating and death of okra plants;and the isolation of okra plants in Baghdad, Babel and Karbala provinces. Diagnosis and test the path- ogenicity. Fungi associated with the roots were isolated and identified. .The field survey results showed the dissemination of root rot disease and death of okra plants in the three provinces. The survey infection percent- age ranged from 50 to 100%,while the severity of infection was 18- 89%.Microscopic examination showed the existenceof six innate genera associated with the roots of thedeterioratingokraplants. ina varied replicater- atesas These fungi,namely Fusarium solani ,Rhizoctonia so lani, Macrophomina phaseolina ,Pythium sp., Mu- cor sp. and Aspergillus sp wereinvaried incidence rates. Thehighest incidencepercentagewas 58% for Fusarium solani..The Pathogenicity tests, using radish seed, f or of the 63 pathogenicfungi isolated fromthe- root-softheokra showed allisolateswere pathogenic.Seed germi nationpercentageranged from 084% , compared to 100% forcontroltreatment .The Pathogenicity test of ten F. solani isolates,showedthe germination percent ranged from 020percentcompared to100% control. Cultu re filtrate heat treated of F.solani(F.sH6) at 25, 50, 75 and 100% concentration affected the infection severity rate by 85and 91, 100and 100, 56 and, 80, 100 and 100%, respectively. while the infectionrate of untreated culture of F.solani (F.sH6). was 100%forall concen-trationsandthe infection sever ity percentageswere 88.50 and 90.10, 100 and 100%, respectively..

Keywords: Fusarium solani, Root rot disease ,Radish plant,Okra plant ,Heat

INTRODUCTION

Okra is one of the most important summer vegetable crops in Iraq. They are grown in all regions of the country [1]. Okra is opt to attack various fungal diseases Fusarium spp. Rhizoctonia solani and Macrophomina phaseolina, Aspergillus sulphorus.Chaetomium sp and Phytophthora spp.[2] Fusarium solani, a common soil fungus that colonises plant materials, has been linked to plant illness, as well as human and animal infection, including corneal infection. F. solani can infect soybeans, avocados, citrus, orchids, passion fruit, peas, peppers, potato, and squash during prehar- vest or postharvest storage, as we all know [3]. Long red shortness appears on Daybreak, turns taproot dark brown, and returns what cracked lengthwise happens to the root, and after the symptoms de- velop on the shoot of the most essential of wilting plants and reveal pale- colored leaves[4]. Fusarium produce four main types of toxins which Fumonisin, moniliformin, Zearalenone and beauvericin using TLC techniques HPLC. The role of these toxins in the disease to affect events in the cell membranes or inhibiting the action of enzymes and then block the enzymatic reac- tions responsible for oxidative phosphorylation or poison antidote works metabolically lead to a shortage of workers needed for plant growth factors [5].

MATERIALS AND METHODS

Field survey

Field survey of the root rot and death of okra plants have been carried out in the province of Baghdad, Babel, Karbala and included a survey 33 areas signed by the provinces as it has scanned the-infected plants, which developed symptoms of the disease in terms of degradation and yellowing and death, has been taking the test samples from the roots of the plants were placed in Polyethylene bags and then preserved in a refrigerator at a temperature 4 c and transferred the next day to the lab to be isolated fungi associated with whole root.

Fungi isolated from root system plant.

These samples were obtained from some provinces in the middle and south of Iraq. Samples were cut into 0.5-1cm and washed under running tap water for 30 minutes, then surface sterilized in 1% sodium hypochlorite for 2 minute and cultured on Potato dextrose agar (PDA) supplemented with 200 mg\l Tetracycline and incubated at 25±1C° for 7 days, single spore technique was made for each isolate. Isolates were identified to the species level according to their cultural and morphological characteristics The isolation frequency and relative density of genera and species were calculated according to [15] as follows:

Apperance (%) = No. of samples of occurrence Total No. of samples X100

Frequency (%) = No. of plant segments of species occurrence Total No. of segments used X100

The number of pieces in which the fungus appeared in dishes pathogenicity test pathogenicity tests on the radish seeds fungal isolates pathogenic associated with the roots of okra by using local radish seeds were tested estimated pathogenicity of 63 isolates of fungi that have been obtained from the acetate insulation operations, it has followed a method developed by Bolkan and Butler (1974) as it was prepared petri dishes diameter 9 cm container on the center and water (water Agar) record to add 20 gm Walker to liter sterile water (Autoclave) under a temperature of 121 m º pressure of 1.5 kg / cm² for 20 minutes and added the antibiotic Tetracycline and after the center-hardening were inoculated dishes in its disk diameter 5 mm from the fungus and the age of 7 days and the edges of the and then incubated dishes at a temperature of 25 ± 2 for three days and was then cultivate local radish seeds and sterile surface with a solution of sodium a concentration of 2% and in a circular, near the edges of the dish and a rate of 25 seeds each dish used four dishes for each isolation addition to treatment without comparison fungi dishes and incubated the same thermal grade and took the results after 7 days of Agriculture calculates the percentage of germination by the following equation:

Number of germinated seeds the percentage of germination = × 100 The total number of seeds

Pathogenicity the seeds of okra.

This experiment carried out using some isolates of F. solani fungus disease which F.sB2, F.sB6, FsB8, FsH3, FsH6, FsH11, FsK2, FsK3, FsK4, FsK6 which proved high pathogenesis ability of the test has been isolates nurse the seeds of millet Development after he was creating flasks glass capacity of 500 Sm³ and put in each and every one of them 100 grams of seeds and add to it 300 Sm³ water and soak for 6 hours and then poured the surplus water and sterilized Palmasd under temperature 121 m º and pressure of 1.5 kg / cm² for 20 minutes and then I left the next day and then was sterilized under the same conditions and having cooled vaccinated each flask seeds of millet have been vaccinated fungus pathogen by 3 tablets a diameter of 5 mm was taken from near the edges of the isolates Almnmah farms on the central Zorai PDA age of 7 days then incubated flasks under a temperature of 25 ± 14:00 º for 14 days with shaking every 2-3 days for a period of 10 minutes to ensure ventilation and distribution of vaccine fungi seeds [6]. Experiment carried out according to complete random design (Completety Randomized Design (CRD four replications using potted sterile plastic diameter of 12 cm and a height of 12 cm and a capacity of 1.5 kg soil Mazijah com- posed of two sand: 1 Pettmos since been added isolates pathogenic fungus into the soil by 1% w / w A week after the passage of okra seeds are sown at 10 seeds per Sindanh comparison with treat- ment without mushroom nurse and then irrigated pots and underwent follow-up and after 10 days was

germinated seeds account according to the following equation.Number of germinated seeds The percentage of germination = × 100The total number of seeds.The effect of isolates pathogenic fungus Fusarium solani in infected okra plants. This experiment carried out using some isolates of F. solani fungus disease which F. sB6, F. sB8, F. sH6, F. sH11, F. sK2 which high pathogenicity in the previous test has been the development of pathogenic isolates the seeds of local millet in the previous test. The experiment carried out in (CRD) with four replications using potted sterile plastic diameter of (12 cm and a height of 12 cm) and a capacity of 1.5 kg soil composed of two sands: 1 Pettmos since been added isolates pathogenic fungus into the soil by 1% w / w. Planted the seeds of okra at 5 seeds per pots with the comparison treatment without fungi and then irrigated pots and underwent follow-up and after 45 days and until the onset of symptoms of the disease have been uprooting see dlings and proportion and the severity of the incidence was estimated by [7].

RESULTS

Results showed that the deterioration and death of okra plants in all the provinces covered by the field survey as it has been cleared 10 areas of Baghdad province and 15 area of the province of Babylon, and 8 regions of the province of Karbala as more areas surveyed interested in the cultivation of the crop okra ranging ratio infection between 50% - 100%.Also that all fungal isolates tested led to a significant reduction in the percentage of germination of radish seeds as a record ratio between 0-84% ranged treatment comparison of 100% as the percentage of germination reached 0.00%. Isolates pathogenic fungus R. sB1 and R. sB5 and R. sK1 as she was high pathogenesis ability.

Frequency of fungus

Results showed that (table -1) Fusarium solani the highest frequency as 58%, as found in all samples except three samples followed by the Rhizoctonia solani

Table 1. Fungi associated with the roots of infected plants okra.

Fungi	Isolates	higher%perce ntage	Low % perce ntage
F.solani	all isolates	100%	58%
R.solani	10,14,29	100%	10,14,29
M.phas	1,2,3,4,8,10,12,15,19,	100%	34%

Pathogenicity test

results (Table – 2) showed that all fungal isolates ested led to a significant reduction in the percentage of germination of radish seeds as a cord between 0-84% ranged treatment compared with control of 100% as the percentage of germination. These results are consistent with what was found12-13. when they studied the pathogenicity of fungal isolates isolated from okra roots. Infected. Since all the fungal isolates were pathogenic, there is a difference in the percentage of their effect on germination, and the reason for this may be attributed to the toxic substances secreted by the fungal isolates, the difference in the quantity and quality of these toxic substances, and the difference in their ability to secrete pectin-degrading enzymes, as they have the ability to secrete the enzyme polygalacturonase. While non-pathogenic fungal isolates do not have the ability to secrete this enzyme, or they have low effectiveness in producing this enzyme14. In addition to its ability to secrete enzymes that degrade kinin present in the host cell wall, such as peroxidase and ligninase, which is of importance in causing infection and spreading fungus toxins and enzymes in those cells15-16

Table 2. Test the pathogenicity of the fungal isolates associated with the roots of the infected Okra plants using radish seeds.

Isolat ed code	% Germi-nati on	Isolat ed code	% Ger-minati on
F.sB1	31.25	F.sH4	16.00
R.sB1	o.oo	R.SH2	14.00
M.pB1	24.00	F.sH5	23.00
F.sB2	12,.50	F.sH6	7.00

R.sB2	10.00	F.sH7	13.00
M.pB2	35.00	F.sH8	14.00
F.sB3	20.50	R.SH3	8.00
R.sB3	18.00	F.sH9	11.00
As.B1	84.00	Mu.H1	25.00
F.sB4	14.00	F.sH10	16.00
R.sB4	41.00	P.H1	9.00
P.B1	20	F.sH11	21.00
F.sB5	48.00	F.sH12	18.00
13.00	9.25	R.sH4	13.00
Mu.B1	25.00	M.pH4	7.00
F.sB6	32.00	F.sH13	31.00
M.pB3	20.00	F.sH14	22.00
F.sB7	18.00	R.sH5	13.00
M.pB4	0.00	F.sK1	12.00
F.sB8	10.00	R.sK1	0.00
R.sB5	19.25	F.sK2	9.00
M.pB5	8.00	R.sK2	16.00
F.sB9	63.00	M.pK1	19.00
R.sB6	39.00	F.sK3	11.00
As.B2	17.00	M.pK2	3.00
Mu.B2	11.00	F.sk4	8.00
F.sH1	13.00	F.sK5	21.00
M.pH1	20.00	R.sK3	0.00
F.sH2	10.00	F.sK6	6.00
R.sH1	10.00	F.sK7	19.00
F.sH3	30.00	Contro	100.00
M.pH3	42.00	R.sH4	13.00
M.pH3	31.25		

Results showed that (table 3) Fusarium solani isolates reduction of germination percentage range 0-20%. However, there are differences in pathogenicity, as these isolates cause seeds to rot before they germinate or seedlings to rot. The cause of pathogenicity is due to the difference in the quantity and quality of the toxic substance and enzymes secreted by these isolates17

Table 3. Test the pathogenicity of some isolates of Fusarium solani on Okra seeds

CODE	Seed growth%	Death seedling %	Seed growth%
F.sB2	20.00	10.00	20.00
F.sB6	10.00	10.00	10.00
F.sB8	10.00	20.00	10.00
F.sH3	20.00	10.00	20.00
F.sH6	00.00	10.00	00.00
F. sH11	10.00	20.00	10.00
F.sK2	10.00	00.00	10.00
F.sK3	20.00	10.00	20.00
F.sK4	20.00	00.00	20.00
F.sK6	20.00	20.00	20.00
Control	100.00	00.00	100.00

Results revealed that (table -4) Fusarium solani isolates effected different significant to dis- ease sverity,all isolates showed that incidence 100% compared with control 0.00, disease sverity range 73-89% compare to control 0.00.

Table .4 Effect of some isolates of Fusarium solani in the infection of okra plants

Cod E	Inside Nce %	Severiy%	Fresh Weight %	Dry Weig Ht
F.sB6	100.00	73.000	1.46	.49
F.sB8	100.00	80.00	1.28	.42
F.sH6	100.00	89.00	.95	.31
F.sH1 1	100.00	85.00	1.15	.39
F.sK2	100.00	79.00	1.32	.45
Control	00.00	00.00	4,92	9.62

This is due to the high pathogenicity of the FSH6 isolate, as it is characteristic of some isolates of the fungus F solani. By secreting a wide range of enzymes that degrade the host cell walls that help penetrate the host, including chitinase, cellulose, protease, and polygalcturine, which degrade the middle sheet of the cell wall, as this group of enzymes has a major role in parasitizing living cells18, in addition to the fungus F. solani producing a number of metabolic compounds and toxins that have a major role in causing plant injury, such as phytotoxin, such as Fusaric acid Jaranicin, anhydrofusarubin, and polypeptide toxin, which has a major role in killing plant tissue. And then an invasion through necrotrophic intrusion19 -20 Results presented (table 5, table 6) Fusarium solani leaky thermally (25,50,75) effected different in disease severity and incidence percentage. The effectiveness of the filtrate of the pathogenic fungus isolate (F.solani FSH6) is attributed to a number of metabolic compounds, toxins and enzymes that degrade cell walls, which have an important and fundamental role in causing the disease, as the toxins affect the permeability of the cell membranes of the infected plants and act As chelating compounds for some mineral elements such as iron and copper, the filtrate of the pathogenic fungus F. solani (FSH6) that is not treated with heat contains enzymes as well as toxins and metabolic compounds.

Table 5. Effect of Different Concentrations heat treated of Fusarium solani Insecticide for 30 days

concentrate	Incidenc%	Severity%	Weig ht of plant
25%	8.10	8.10	8.10
50%	6.85	6.85	6.85
75%	100.00	100.00	5.51
100%	100.00	100.00	4.90
Control	00.00	00.00	13.20

Table 6.Effect of different concentrations of unheated-treated fungal leachate for the isolation of Fusarium solani on 30-day papaya seedlings

concentrate	Incidenc%	Severity%	Weig ht of plant
25%	100.00	88.00	6.71
50%	100.00	90.10	6.01
75%	100.00	100.00	5.40
100%	100.00	100.00	5.24
Control	00.00	00.00	13.20

DISCUSSION

The highest germination rate of 84% in isolation fungal AspB1 as it is one of the saprophytic fungus pathogenicity low-lying. While the percentage of germination in isolates of F. solani fungus

pathogen did not exceed 32% of the percentage of germination of radish seeds and germination percentage isolation F. sH6 7%, the lowest percentage of germination compared Bazlat path- ogenic fungus F. solani [8] . These results agreed with what a number of researchers found that the fungus F.solani is one of the main causes of cucumber root rot disease[9]. These results agreed with10 as 35 isolates of the fungus that causes cucumber root rot were collected from infected cucumber plants from the fields of Riyadh / Kingdom of Saudi Arabia, and the F. solani isolates were efficient in increasing the infection. This also agreed 70 The results are also consistent with what was recorded[10], where the fungus F. solani was isolated and diagnosed from the roots of cucumber plants from several fields in Najaf Governorate. A number of studies have indicated that the fungus F. solani is one of the main causes of root rot disease for a number of field crops, including sprouts, okra, and beans[11] was able to obtain seventy-seven isolates. From the fungus F. solani from the fields of Salah al-Din and Diyala governorates. Fungi isolates associated with the roots of infected plants okra. results showed (Table – 1) that fungi associated with the root systems of infected plants okra were six types of fungal a Fusarium. These results agree with what was reported[12] as each of them isolated the fungus F.solani.. R. solani M. phaseolin Pythium and Mucor and Aspergillus flavus [13.]

The most important of these enzymes is the Polygalacturonase (PG) enzyme, which has a major role in attacking the pectin compounds in the walls of the transport vessels. And bronchioles, which leads to Analy sis of the middle lamina of wood parenchyma, as well as the enzyme Pectinmethylestrase (PME), which plays a role in causing plant infection [14-15]. This test showed that the filtrate of the pathogenic fung us isolate (F. solani (FSH6) that is not sterile is more effective than Sterile leachate on 30-day-old okra seedlings [16]-17. The reason for this is that the sterile leachate lost wall-degrading fungal enzymes during the heating process [18-19]. These results are consistent with what Qasim[16] found regarding the effect of fungal leachate on plants [20].

CONCLUSION

The results showed the presence of six fungal genera that cause okra root rot, and there is no effect of heat treatment on the effectiveness of the F. solani fungus, as heat treatment and non-treatment led to an increase in the severity of the infection and okra root rot

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