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INVESTIGATION OF THE CAUSES OF ROOT ROT AND DAMPING-OFF DISEASES OF OKRA AND TESTING THE EFFECTIVENESS OF *PSEUDOMONAS FLUORESCENS* IN DISEASE CONTROL

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Abstract

This study was conducted to detect the fungal causing root rot and damping-off diseases of okra and control them biologically using *Pseudomonas fluorescens* bacteria. The results showed the isolation of several pathogenic fungal, as well as fungi is also accompanying them, where the *Rhizoctonia solani* recorded the highest percentage of appearance and frequency which was (14.6, 16%) respectively, followed by *Fusarium solani* and *Macrophomina phaseolina*. Five isolates from *R. solani* and three isolates from *F. solani*, and two isolates from *M. phaseolina* fungal were isolated, and the results showed a significant variation for these isolates in the effecting on seed germination and damping-off seedling. *R.solani* 4 isolate was recorded a superiority in reducing cabbage seed germination and damping-off its seedling by (76.7, 62.9%), respectively. The results also showed a high antagonism ability of *P. fluorescens* against pathogenic fungal and caused the radial growth inhibition for *R. solani, F. solani* and *M. phaseolina* by (86.7, 64.7, 48.3%), respectively. Finally, pots results also showed that the bacterium *P. fluorescens* had a clear ability in increasing the germination percentage of okra seeds and reduction in damping-off its seedling, as well as it has the ability to reduce the ratio and severity of infection by pathogenic fungal individually or in combination and to varying percentages.

Key words : Okra, Rhizoctonia solani, Fusarium solani, Macrophomina phaseolina, Pseeudomonas fluorescens.

Introduction

Okra (*Abelmosechus eseulentus* L.) is an economic importance vegetable crop that belongs to the Malvaceae family, where Ethiopia its original country and spread widely around the world in the tropics, subtropical and warm region of the world. It considers as good source of proteins, carbohydrates, vitamins, calcium, enzymes and total minerals, as well as it has a therapeutic effects in the treatment of ulcer and relief of hemorrhoids, and as a substitute for blood plasma. Additionally is useful in the treatment of urinary and reproductive system disorders (Singh *et al.,* 2014). Furthermore, it can be beneficial from its fresh leaves, buds, flowers, horns, stems and seeds, and it is an immature fruit as vegetables. Its seeds are a source of oil, where the oil concentration range from 20% to 40% and contains tryptophan acid up to 47.4%, in the form of unsaturated fatty acid that necessary for human feed. Moreover, Okra helps to reduce cholesterol in blood, the other part of the okra is an insoluble fiber, which helps to maintain intestinal health, and it is rich in phenolic compounds with biological properties such as quartering and flavonol derivatives, catechin oligomers and hydroxycinnamic derivatives (Gemede *et al.,* 2015). Due to the importance of this crop, its cultivation has expanded considerably and many difficulties have accompanied it, including many diseases such as damping-off and root rot, which causes a significant loss of the crop, which lead to the re-patching of agricultural sites in vain. Diseases are a determining factor in the production of okra and most of the pathogens endemic in soils are difficult to control through traditional methods such as the use of resistant or synthetic varieties (Weller *et al.,* 2002). Therefore, there are many and varied ways to control these pathogens in order to avoid harming the crop cultivation. The most important is the use of microorganisms in the biological resistance for plant pathogens and endemic in the soil as an alternative or complementary method of chemical and agricultural resistance. Endophytic bacteria are bacteria that live in

plant tissues without causing harm, and are useful in protecting the plant and improving its growth, such as *Pseudomonas fluorescens* (Siddiqui and Ethteshamul-Haque, 2001). Finally, the research aims to investigate the fungal cause's okra root rot and evaluate the efficiency of *Pseudomonas fluorescens* in protecting the plant from these pathogens, in addition to its reflection on the growth and productivity indicators of the plant.

Materials and Methods

Field and laboratory experiment was conducted for the period from 20/3/2018 to 15/6/2018 in the field and laboratory of the Plant Protection Department, College of Agriculture, Maysan University, where the laboratory experiments included-

Isolation and diagnosis of fungi from the okra root

Samples were taken from okra plants, which a symptoms of root rot disease observed with it, represented by the coloring of the main and secondary root, and root hairs with dark brown color were brought from five fields in Maysan province to the laboratory. The root zone was separated from the rest of the plant parts, and the affected area was washed with running water for 30 minutes, and then left it a period to dry on Whatman- N0.4 filter paper to remove water from them, these parts were cut into small pieces by a length of (0.5-1cm). It sterilized with 10% of sodium hypochlorite solution for 2-3 minutes, then washed with sterile distilled water for a minute to remove the residue of the sterile solution, and dried over filter paper. Moreover, a 4 pieces were transferred to petri dishes containing the water agar prepared by dissolving 20 g of agar in 1 liter of water with an addition of the Chloromphenicol antibiotic at 250 mg /L. Finally, the dishes were incubated in the autoclave at 25 ± 2°C for three days, after which the fungal growths were transferred to a sterile petri dishes that containing sterile potato dextrose agar PDA after three days of incubation, by three dishes. Subsequently, the dishes were taken out and the growth fungal identified to the species depending on the taxonomic key (Watanaba, 2002).

Calculate the percentage of frequency and appearance of isolated fungal

The percentage (frequency and appearance) of the diagnosed isolated fungal was calculated according to the two equations:

% appearance = (Number of fungal appearance (genus or species) / total number of samples) × 100

% frequency = (number of fungal colonies to be calculated / total colonies of all fungal × 100

Pathogenicity test of fungal isolated from okra roots

The pathogenicity test of the isolated fungal from infected roots was conducted on the culture media (water agar) in petri dishes, where the center of each dish was inoculated with a 0.5 cm tablet from the edge of pure colonies at the age of 7 days by three replicates per isolates. All dishes were incubated at 25 ± 2°C for 48 hours, then it was planted with Seeds of cress sterilized by 2% sodium hypochlorite, then washed twice with sterile distilled water. These seeds planted circularly around the colony and about 1 cm from the edge of the dish, by 20 seeds per dish, and incubated at 25 ± 2°C. After 72 hours, the germination percentage was calculated and after two weeks, the percentage of healthy and infected seedlings were calculated according to the following equations:-

For germination% = (Number of germinating seeds / Total number of seeds) 100

For damping-off seedling = (Number of damping-off seedlings / Number of apparent seedlings) 100

Evaluation of the efficiency of *Pseudomonas fluorescens* in the radial growth inhibition of fungal isolated from okra

The bacteria isolates was obtained from the Plant Protection Department / College of Agriculture / University of Basra, where bacterial concentration was (5.5×10^7) colony forming unit / ml, which was adopted in subsequent studies. The efficacy of *P. fluorescens* in the radial growth inhibition of the fungal was evaluated using the mixing method with the culture media PDA of 1 ml bacteria / liter, then the beaker was shaken well, and the contents of the beaker were then poured into three dishes and the dishes were incubated at a temperature of 30°C for 24 hours. the three dishes were then inoculated by (5 mm) diameter tablets of fungal colonies and were grown in the culture media PDA at the rate of one tablet at the center of each dish, leaving a comparison treatment without adding bacteria, and then all the dishes were incubated in the incubator at a temperature (27°C) for a week. The percentage of inhibition was calculated according to the equation of (Abbot 1925).

Pots Experiment, the Pathogenicity test of fungal isolated from okra roots and evaluate the efficiency of *P. fluorescens* in protecting okra plant from infection

The vaccine of pathogenic fungal was prepared according to Dewan 1989 method. In this experiment, a loamy soil was used and sterilized using an autoclave, where a sterile soil was distributed with 5 kg plastic pots in equal quantities, then added the vaccine of pathogenic

fungal, which was loaded with local millet seeds by 2% w/w (*Jones et al.,* 1984). While the treatment of bacteria, okra seeds (Petra variety) were treated with bacterial suspension at a concentration of (5.5×10^7) colony forming unit / ml with an addition of a small amount of Arabic gum for 15 minutes. Furthermore, the seeds were left to dry on the filter paper for 6 hours, and then planted in the treatments of bacteria. The soil was moistened for 3 days, and then the seeds of okra (Petra variety) were planted by of 20 seeds per pot. A control treatment (untreated soil) was added, and all pots were watered with filtered water until the end of the experiment. The treatments were as follows: 1-.Control, *R.* 2- *solani,* 3- *F. solani,* 4- *M. phaseolina,* 5- *R. solani* + *F. solani,* 6- *R. solani* +*M. phaseolina,* 7- *Rsolani* +*F. solani*+ *M. phaseolina* 8- *R. solani* +*P. fluorescens,* 9- *F. solani* + *P. fluorescens* 10- *M. phaseolina* + *P. fluorescens* 11- *R. solani* + *F. solani*+ *P. fluorescens* 12- *R. solani* +*M.phaseolina* + *P. fluorescens* 13- *F. solani* + *M. phaseolina* + *P. fluorescens* 14- *R. solani* + *F. saolani* + *M. phaseolina* + *P. fluorescens*.

Finally, the following measurements were taken: Percentage of germination and damping-off seedling, and the Percentage of infection as follows = (the number of infected plants / total number of tested plants) × 100. Moreover, the severity of infection using the disease index contained from 5-degree: as follows, (0) intact roots, (1) the secondary roots coloring with. As well as, (2) the secondary roots and part of the main root coloring with brown, (3) the main root completely coloring, with present of sores near the base of the stem. Finally index (4), where the main root coloring, rot and wilting of plants According to the following equation:

% for the severity of infection = the total number of plants of the 0 × 0 degree +..... + the total number of plants of the 4 × 4 degree / the total number of tested plants × 100

Statistical analysis

Laboratory experiments were conducted according to the Complete Randomized Design (C.R.D), while the Complete Randomized Block Design (R.C.B.D) were conducted to the field experiment treatments, and the significant differences between the averages were compared with the least significant difference test L.S.D = 0.01 for laboratory experiments, and L.S.D = 0.05 for field experiments.

Results and Discussion

Isolation and diagnosis of fungal

Several genera of fungal were isolated and diagnosed from the roots and the bases of okra stems that exhibited symptoms of the disease, as shown in Fig. 1. The phenotypic and microscopic characteristics of each fungus were relied upon. *R. solani* was characterized by its white-brown colonies. The isolates of the fungus varied in the growth speed and the formation of dark-colored stone bodies and the mycelium branched in the form of right angles and had a stenosis at the branching and the presence of barriers in the branches near the emergence area. As for the fungus *F. solani* its colonies was characterized of white cotton texture with regular edges, and the presence of broad Macroconidia has a crescent shaped, and Microconidia oval shaped, and the squamous spores (Chlamydospores) spherical shaped, and foot cell rounded shaped, and the basal cell cylindrical shaped. Finally, the form of *M.phaseolina* its colonies were white color and then turned gray and then black because of the formation of stone bodies which seen under a light microscope.

Calculate the percentage of frequency and appearance of fungal:

Table 1 shows that the highest percentage of appearance and frequency of fungal were recorded by *R. Solani* amounted to (14.6, 16%), respectively, followed

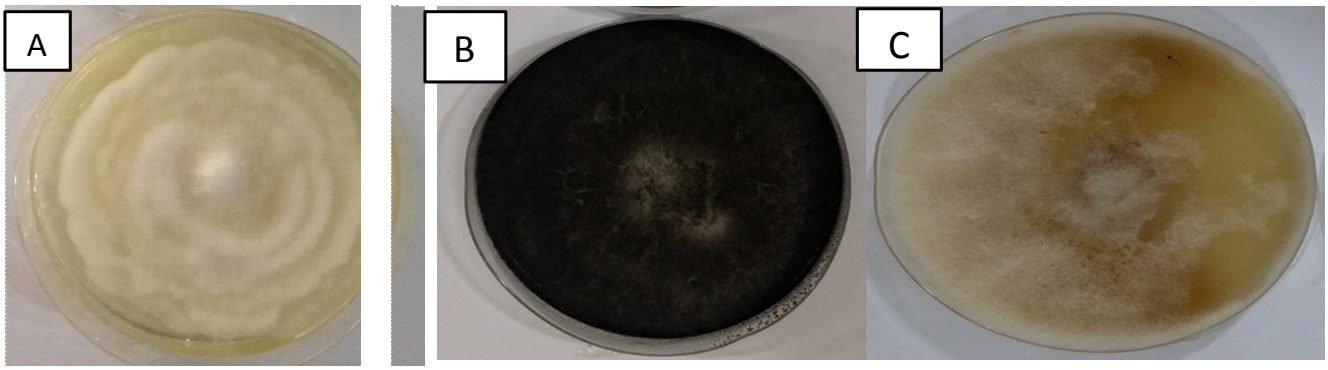

Fig. 1: Forms of some fungal colonies isolated from infected okra roots and planted on PDA A. *R.solani* B *F. solani* C. *M. phaseolina.*

by *F. solani* (13.1, 14%) respectively, and *M. phaseolina* recorded (11, 1.10%) respectively. (Thiong'o *et al.,* 2003) pointed out that the most frequent fungal and isolated from the bean plant's roots planted in Guinea were *R. solani, F. solani and M. phaseolina*. Other fungal isolates was observed, but their frequency was low.

Table 1: Percentage of frequency and appearance of fungal isolated from Okra roots.

Fungal isolated from Okra roots	% frequency	% appearance
Aspergillus flavus	3	4.8
Aspergillus fumigatus	0.2	3.7
Cladosporium oxysporum	5	7
Chaetomium Convolotum	2	4.8
Phoma sp	2	2.4
Rhizoctonia solani	16	14.6
Macrophomina phaseolina	10	11.1
Fusarium solani	14	13.1

Pathogenicity test of fungal isolated from okra roots

The results showed a significant variation in the isolates effect of the fungal *R. solani, F.solani and M.phaseolina* on the cabbage seeds germination and damping-off its seedlings as shown in (Table 2). Where the *R.solani* showed a clear superiority in reducing cabbage seeds germination and increasing the damping-off, as the isolates of 4 *R.solani* recorded (76.7% and 62.9%) respectively, which was not significantly different from isolates of 1 *F.solani*. While in the isolation of *F. solani* it was observed that there were a significant differences between these isolates in the effect on cabbage seed germination and damping-off, and the isolates of 2 *F.solani* recorded (60.2, 62.9%), respectively. There was a significant differences for the *M.phaseolina* between the two isolates, where the second isolates achieved (56.4% and 66.4%), respectively. This difference between the isolates in their effect on the percentage of cabbage seeds germination and damping-off, which may be due to the genetic difference between the isolates of fungal. Alternatively, the isolates differed in their ability to secrete enzymes that dissolving pectolytic and cellulytic in the early stages of infection, which play an important role in the impact on seeds or secretion of toxins that cause the killing of seed embryos. The most pathogenic isolates of the three genera were selected for use in subsequent experiments.

Effect of *P. fluorescens* bacteria on radial growth inhibition *of R.solani, F. solani and M. phasaeolina*

The test results showed a high antagonism ability of *P. fluorescens* against the pathogenic fungal, it has caused inhibition of the radial growth for *R.solani* fungus by (86.7%), as fungal of *F. solani and M.phasaeolina* was

Table 2: Effect of fungal isolates *R. solani, F.solani and M.phaseolina* in the percentage of seeds germination and damping-off.

Isolates	% seeds Germination	% Damping-off
R.solani 1	76	31.3
R.solani 2	70.6	42.6
R.solani 3	73.3	36.6
R.solani 4	76.7	62.9
R.solani 5	67.5	58.8
F.solani 1	55.5	58.8
F.solani 2	60.2	62.9
F.solani 3	43.2	49.4
M.phaseolina 1	47.2	52.3
M.phaseolina 2	56.4	66.4
Control	100	0
L.S.D at significant level 0.01	2.4	2.5

* Each number represents the rate of three replicates.

recorded (64.7,48.3%) respectively, compared with the control treatment as shown in Fig. 2. The high antagonism ability of *P. fluorescens* may be due to its produce of different types of antibiotics such as Phenzain -1-caroxylate or to produce enzymes that decompose the cell walls of fungal such as Chitinolytic enzyme, Catalase, HCN, Chitinaseylytic, Protease and b-1.3-glucanase (Zidean *et al.,* 2005).

Pathogenicity test of fungal isolated from okra roots and evaluating the efficiency of *P. fluorescens* in protecting okra from infection

The results of table 3 showed that the pathogenic *fungal R.solani, F. solani and M.phasaeolina* had a clear ability to reduce the percentage of germination and damping-off and ranged between (38.00, 8.16%) for *R.solani* respectively. Furthermore, the *F. solani* recorded (24.66 and 21.21%) respectively, while *M. phasaeolina* percentage of germination and damping-off in the inward treatments was (34.33, 13.20%) compared to the control treatment which were (100, 00%) respectively. The experiment results showed the ability of *P. fluorescens* to increasing the percentage of germination and decreasing damping-off. This may be due to the ability of these bacteria to infect the plant roots, and because of its properties such as competition, antagonism and parasitism. As well as, it stimulates systemic resistance or its produced of many antibiotics that would inhibit the growth of pathogenic fungal, or its presence directly on the root surface area, which makes it a good competitor for the place and materials that secretion from the roots. In addition to its ability to stimulate the plant to produce growth regulators that have

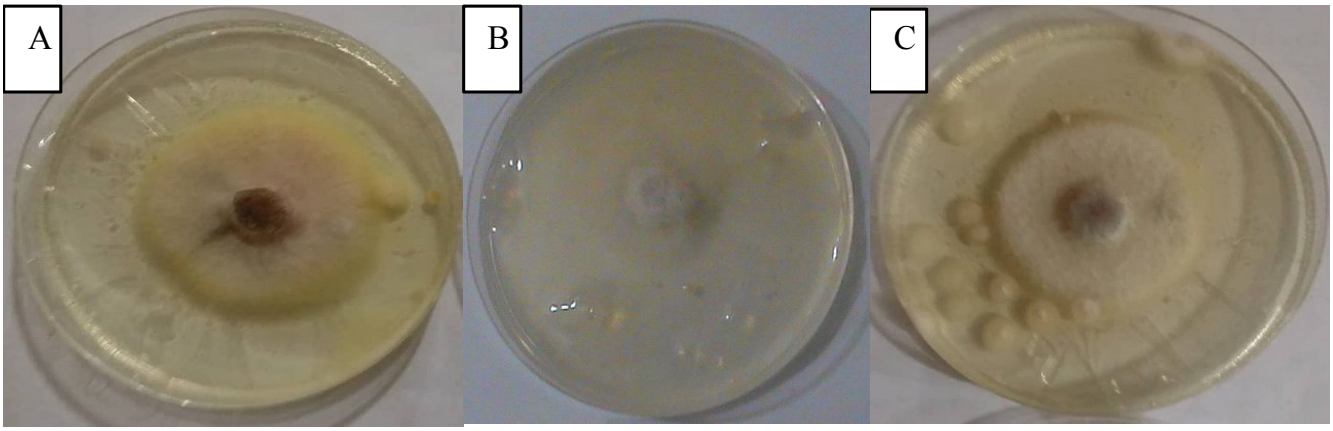

Fig. 2: Antagonism ability of P.*fluorescens against A. R.solani B. F. solani C. M. Phaseolina.*

Table 3: Effect of *P. fluorescens* on percentage of okra seeds germination, damping-off, percentage of infection and its severity in pots.

Treatments	% Seeds germination	% damping -off	severity of infection %	% infection
Control	100	0	0	0
R.solani	24.66	12.10	10.0	11
F.solani	34.33	21.21	14.6	10
M.phasaeolina	38.00	14.26	9.2	8
R.solani+F.solani	30.2	10.1	14.2	40
R.solani+ M.phaseolina	33.8	12.0		20
R.solani+F.solani+M .phaseolina	25.7	8.16	7.1	33
R.solani + P. fluorescens	51.66	10.20	12.9	26.2
M.phaseolina+ P. fluorescens	65.00	14.86	11.0	23.6
F.solani + P. fluorescens	52.66	25.13	12.9	14.3
R.solani +F.solani+ P. fluorescens	53.33	15.13	16.0	8.7
R.solani +M.phaseolina+ P. fluorescens	35.66	8.16	14.1	10.9
F.solani +Mphaseolina + P. fluorescens	38.00	8.2	12.0	38
R.solani+F.solani+M.phaseolina+P.fluorescens	29.2	3.23	7.9	14.8
L.S.D 0.05	11.45	3.23	1.4	7.4

* Each number represents the rate of three replicates.

a major role in accelerating the plant growth which treated with it. The results also showed that the use of *P. fluorescens* has reduced the percentage and infection severity of pathogenicity compared to the pathogenic fungal treatments individually or in combination and to varying percentages. The reason for this is that the bacteria *P. fluorescens* have the ability to produce the pathogenic antifungal compounds of the plant, which includes various types of compounds such as hydrogen cyanide and Siderophore.

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