Control of the causes of the damping off disease on pepper by some biological agents in Babylon province-Iraq

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ABSTRACT

The aim of the study was to isolate and diagnose the pathogens of the Damping off of pepper seedlings in Babylon province-Iraq and to evaluate the effectiveness of some biological control agents in disease control. 7 species of fungi associated with root rot and Damping off of pepper seedlings were diagnosed. *Fusariumsolani* was the most common fungus followed by *Macrophominaphaseolina* and *Rhizoctoniasolani*. The results showed that *Trichodermaharzianium* and *Pseudomonas fluorescens* have a high level of antagonistic contrast with pathogens. The results appeared the efficacy of the Black Cumin (*Nigella sativa*) extract in inhibiting the growth of pathogenic fungi. All the used treatments resulted in an increase in the percentage of pepper seed germination. A significant improvement in the biologic integration of *T. harzianum* and *P. fluorescens* and water extract of the Black Cumin increasing the percentage of germination of pepper seeds and provide good protection from the infection by pathogenic fungi causing Damping off disease, ranging between 62.5 - 87.5%. The conclusion of this study the effectiveness of the *T. harzianum*, *P. fluorescens* and the black cumin seed water extract in the protection of pepper seeds and seedlings from the infection by some pathogenic fungi

Keywords: pepper; damping off, Biological control, Plant Extracts, Black Cumin.

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INTRODUCTION

The pepper crop is *Capsicum annuum* L. of the world's important vegetable crops (Cheddar, 2003, Marin *et al*., 2004). The total cultivated area for 2017 in Iraq amounted to about 9885 dunums produced 13169 tons (Central Statistical Organization, 2017). Root rot is one of the most common diseases affecting pepper (Hyder *et al*, 2018; Koijamand Sinha, 2018; Majeed *et al*., 2019). Several strategies have been adopted to combat the root diseases caused by Fungi. However, these methods did not effectively combat these causes (EL-Mohamedy *et al*., 2011). The use of chemical pesticides in the control of plant diseases has negative effects on the environment, human health, non-target organisms, and the emergence of resistant strains of pesticides. Therefore, it is not a successful solution (Kotze *et al*., 1982). In recent decades, the biological control of pathogens has received widespread interest in the use of *Trichodermaharzianum*, which has been successful in vitro and field experiments (Zeilinger and Omann, 2007). *Pseudomonas fluorescens* have proven highly efficient against fungi that cause root rot (Showkat, 2012; Al-Fadhal *et al*, 2019). Plant extracts have also been used in the last two decades to be highly effective against many fungi and have desirable characteristics such as their rapid degradation (Lokendraand Sharma, 1978; Abdel-monaimand Ismail, 2010; Al-Tameemi and Matloob, 2019; Aftab*et al*., 2019). The study aims to determining the problem in some areas of Babylon and isolating and diagnosing the pathogen and evaluation of the effectiveness of some biological control agents in disease control under laboratory and nursery conditions.

METHODS AND MATERIALS

Field visits and sample collection

Field visits were conducted in six pepper growing nurseries in Babylon province for the period from 21/11/2018 to 14/12/2018 (Table 1). The seedling was collected in polythene bags and brought to the laboratory for isolation in the next day.

Table 1. Geographical and temporal distribution of pepper plants from which samples were collected.

No. sample	Site \ Babylon province	Date of visit in 2018
1	Nile	11/21
2	Imam	12/3
3	Anana	12/8
4	Alexandria	12/13
5	Al-Mussaib	12/13
6	Mashroa	12/14

Isolation and diagnosis of pathogens

The infected seedling was cut into pieces were then transferred to a 9 cm diameter Petri dish on a Potato Dextrose Agar medium (PDA), 4 pieces were used for each dish. The dishes were left in the incubator at 25 + 2 ° C for 5 days. The fungi were identified, depending on the taxonomic keys (Parmeter and Whitney, 1970; Ellis, 1971, Booth, 1977, Sneh*et al*., 1996).

Detect the pathogenicity of fungi associated with pepper roots using pepper seeds

The pathogenicity potential of the following fungal isolates (*F. solani, R. solani*, and *M. phaseolina*). This test was carried out according to method of Bolkan and Butler (1974). Use the pepper seeds were sown Rounded 1 cm from the edge and use 5 seeds / dish. Place the dishes in the incubator at 25 + 2 temperature for 7 days. The seed germination ratio was calculated.

Antagonistic test of the *Trichoderma harzianium* against fungi that causes the damping off disease of pepper seedlings

This test was conducted to evaluate the efficacy of *T. harzianum* (obtained from the Plant Pathology Laboratory at the Al-MussaibTechnical College) against the pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina*. The test was carried out using the dual culture technique. Degrees of antagonistic ability were estimated depend on scale which mentioned in Bell *et al*. (1982) of the five degrees of antagonism. The biological control agent was accepted if showing antagonistic 1 or 2 degree with pathogenic fungi.

Efficacy of *Pseudomonas fluorescens* against pathogenic fungi *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina*

P. fluorescens were obtained from the Plant Pathology Laboratory and tested the effectiveness against pathogenic fungi *F. solani, R. solani*, and *M. phaseolina* on the PDA medium by adding 1 ml of bacterial isolate grown to the nutrient broth medium for 2 days. The bacteria inoculums were added to the PDA dish with a moving to distribute the inoculums homogeneously before adding the pathogenic fungus in the center of the Petri dish. Incubated dishes (25 ± 1 ° C for 7 days) (Fatima *et al*., 2009). The growth rate of pathogenic fungi and the percentage of inhibition were calculated according to the formula:

% Inhibition = [(R – r)/ R] × 100.

Where,

r is the radius of the fungal colony with *P. fluorescens* and

R is the radius of the fungal colony without the *P. fluorescens*.

The effect of the water extract of the Black Cumin in the growth *Fusarium solani, Rhizoctonia solani and Macrophomina phasemolina* on the PD.

The method of Shekhawat and Prasada (1971) was followed to find out the growth of the fungi. The concentration (5, 10, 15%) of the extract was obtained and added to the PDA medium. After the hardening of the medium, the dishes were inoculated in the center with a disc diameter (0.5 cm) of the growth of pathogenic fungi *R. solani*, *F. solani* and *M. phaseolina* at 5 days old. The results were calculated the growth of each colony. The percentage of inhibition was calculated.

Evaluation of the efficacy of the *Trichoderma harzianium* and *Pseudomonas fluorescens* and black cumin extract in the protection of pepper seeds and seedlings from some of the causes of seedling disease

under nursery conditio. 217

This experiment was carried out in the Nursery, Al-Mussaib Technical College, Department of biological control Techniques (14/3/2019). The *F. solani*, *R. solani*, *M. phaseolina*, and *T. harzianium* were prepared as method mentioned by Dewan (1989) on millet seeds. The soil was sterilized under a temperature of 121 ° C and pressure of 1.5 kg / cm 2 for 30 minutes. The sterilized soil was distributed the following week (sterile soil + organic matter 1: 1) in the seedtray (19 x 11 cell), then the fungal inoculum was added to the pot by 1% (weight / weight). Each treatment included three replicates. The following treatments were included: 1). *R. solani* alone, 2). *R. solani* + *T. harzianium,* 3). *R. solani* + *P. flourescense,* 4) *R. solani* + black Cumin extract, 5). *R. solani* + *T. harzianium* + *P. flourescense,* 6). *R. solani* + *T. harzianium* + *P. flourescense* + black cumin extract. 7). *R. solani* + pesticide Beltanol (as control). The treatments were repeated using *F. solani* and *M. phaseolina,* 8). control treatment (adding only sterile millet seeds), 9. *T. harzianium,* 10). *P. flourescense*, 11). black cumin extract, 12). *T. harzianium* + *P. flourescense,* 13). *T. harzianium* + *P. flourescense* + black cumin extract. The pots were planted with local pepper seeds with 4 seeds / cell. The tray was irrigated with water every 3 days. When seedling was completed emergence in the control treatment, the percentage of seed germination was calculated according to the following equation:

% Germination =(Number of seeds germinated \ Total number of seeds)× 100.

T. harzianium inoculums was added by 1% (weight / weight), before adding the pathogenic fungus inoculum by three days. Treatment with bacterial suspension was performed at a rate of 5% (weight / weight) before 24 hours of planting. Beltanol was added at a concentration of 1 mL / L to the soil after 24 hours of the addition of the pathogenic fungus. The water extract of the black cumin plant was added after treatment with the fungus directly at a rate of 0.25 mL/ pot. The experiments were carried out according to the complete random design CRD.

RESULTS

Isolation and diagnosis

A microscopic check of the fungal growths which isolated from the infected pepper roots showed that identified 7 species of fungi associated with root rot and damping off disease of pepper seedlings (Table 2). The most common fungus was *Fusarium solani*. It was found in most samples with a recurrence rate of 27.03%.

Table 2. The fungi associated with infected peppers, their location and frequency in samples

Fungi	No. sample*	Frequencies of fungi in samples (%)**	
		Highest ratio	Average %
Alternaria alternate (Fres.)Keissler	1,2	04	2.5
*Aspergillusniger*VanTieghem	1,5	17.0	11.6
Fusariumsolani(Mart.) Sacc.	1-6	42.8	27.03
Penicilliumspp.	4,6	7.1	5.3
RhizoctoniasolaniKuhn	1-3,5	33.5	16.2
Rhizopusstolonifer(Ehrenb. Ex Link) Lind.	3,6	7.1	6.6
Macrophominaphaseolina(Tassi) Goid.	1, 3-6	38.3	22.5

*Number represent sample collection district (Table 1).**Repeat the fungus in the sample (%)= (Number of pepper seedling in which the fungus appeared in the dishes\ Number of total seedling used in the sample) x 100.

The highest incidence of fungus in the area was 42.8%. Followed by the fungus *Macrophomina phaseolina*, which appeared in all regions except the area of the Imam district to the highest rates in the samples of Alexandria 22.5%.In addition, *Rhizoctonia solani* isolates from the samples of the Nile, Imam, Anana and Al-Mussaib were repeated 16.2% and the highest rate of appearance was in the samples of the Imam district to reach 33.5% respectively.The results of the isolation showed the presence of many fungi associated with root rot and damping- off of pepper seedlings with lower recurrence rates such as *Alternaria alternata* and *Penicillium* spp. *Rhizopus stolonifer* and *Aspergillus niger*.

Detection of pathogenic fungi associated with roots using pepper seeds.

Table 3 shows that all tested fungal isolates achieved a significant decrease in the percentage of pepper seed germination compared to the control treatment with a germination rate of 100.00%. The most isolating effect on pepper seed germination was *R. solani* isolate Rs5 as it prevented the germination of all seeds. Fs1 isolate of *F. solani* was significantly reduced the germination of pepper seeds by 1.3%. The isolate of *M. phaseolina* (Mp3) isolate the Anana distract which was 9.3%.

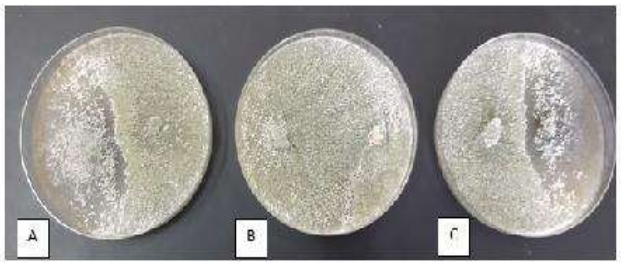
Table 3. Detection of pathogenic fungi associated with root rot and damping off on pepper seeds

Treatments	Isolate	Seed germination (%)
Fusarium solani	Fs1	1.3
Fusarium solani	Fs2	20.0
Fusarium solani	Fs3	9.3
Ma. phaseolina	Mp1	20.0
Ma. phaseolina	Mp3	9.3
Ma. phaseolina	Mp6	25.3
Rhizoctonia solani	Rs1	13.3
Rhizoctonia solani	Rs3	2.6
Rhizoctonia solani	Rs5	0.0
Control	-	100.0
L.S.D(p 0.05)	-	3.23

The efficiency of *Tr. harzianium* against fungi causes the pepper damping off disease

The results showed that *Trichoderma* had high antagonistic ability based on the Bell *et al*. (1982), with the 1 degree of contrast with *F. solani* and *M. phaseolina* (Figure1). The biological control agent controlled most of the area of the dish to prevent the growth of the pathogen, while the level of contrast with the *R. solani* was 2. The variation in effectiveness may be due to the variability of fungal species.

Figure 1. Antifungal ability of *Trichodermaharzianum* against fungi which causes the damping off disease of pepper on the PDA medium. A-against *Rhizoctoni asolani*. B-



against *Fusarium solani*. C-against *Macrophomina phaseolina*.

Efficiency of *Pseudomonas fluorescens* against pathogenic fungi *F. solani, R. solani* and *M. phaseolina.*

The results of this test (Table 4) showed that the use of *P. fluorescens* as an biocontrol agent inhibited the growth of *R. solani* and *F. solani* fungi in the PDA medium by 100.0% compared to the control treatment without bacterium was 0.0%. While the rate of inhibition of *M. phaseiolina* was 88.8%.

Effect of water extract of black cumin in the growth of *Fu. solani, Rh. solani* and *Ma.phaseolina* under laboratory conditions.

The results (Table 5) showed the efficacy of the black cumin seed extract in inhibiting the growth of *F. solani*, *R. solani* and *M. phaseolina* in the PDA which was very high inhibition rates in all concentrations used and prevent the growth of pathogenic fungi into100% inhibition.

Evaluation of the effectiveness of the biological control agent *Tr. harzianum* and *Ps. fluorescens* and the water extract of the Black cumin in the protection of seeds and seedlings of pepper from the infection of some pathogenic fungi causes of damping off disease.

The results of this study (Table 6) showed that all treatments were increase the percentage of Peper seed germination and to reduce the percentage of losses with varying degrees compared to soil treated with pathogenic fungi alone. The significant improvement in the biological integration of *T. harzianum* and *P. fluorescens* and water extract of the black cumin on all biological control factors in increasing the percentage of germination of pepper seeds and provide good protection from the infection of pathogenic fungi causing seed rot and seedling death, the germination rates was ranging between 62.5 - 87.5%.

Table 4. Efficiency of *Pseudomonas fluorescens* against the pathogenic fungi.

Treatments	Growth rate (cm)			Inhibition (%)		
	R. solani	***F. solani***	***M. phaseolina***	***R. solani***	***F. solani***	***M. phaseolina***
Pseudomonas fluorescens	0	0	1	100	100.0	88.8
Control	9	9	9	0	0	0
L.S.D.(p.0.05)		1.3			3.56	

Each number in the table represents a rate of 4 replicates

Table 5. Effect of water extract of black cumin, *Nigella sativa* in the growth of pathogenic fungi causing the damping off disease of peppers seedlings on PDA.

Treatments*	**Concentrations (%)**	**Growth rate (cm)**	**Inhibition (%)**
BE+ *F. solani*	5	0.00	100.0
	10	0.00	100.0
	15	0.00	100.0
BE+ *R. solani*	5	0.00	100.0
	10	0.00	100.0
	15	0.00	100.0
BE+ *M. phaseolina*	5	0.00	100.0
	10	0.00	100.0
	15	0.00	100.0
F. solani	-	9.00	0.00
R. solani	-	9.00	0.00
M. phasiolina	-	9.00	0.00
L.S.D.(*p*.0.05)	–	0.2	2.6

*Each number represents the rate of three replicates., BE= water extract of black cumin.

The use of *T. harzianum* also increased the percentage of seed germination in the treatment with *F. solani*, *R. solani* and *M. phaseolina* 31.2, 25.0 and 50.0%, respectively, compared to germination in soil treated with pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina*, which were 6.5, 0.0 and 12.0% respectively. The use of *P. fluorescens* significantly increased germination seed rates of 25.0% to 50.0%. The treatment of adding black cumin seed extract to the soil of agriculture has also improved the rate of germination with the presence of root rot agents, with ratios ranging from 31.2 to 37.5%.

Table 6. Effectiveness of the biological control agents and the water extract of the black cumin in the protection of pepper seeds from the infection of some pathogenic fungi under the nursery conditions.

Treatments *	Germination (%)	Treatments	Germination (%)
Fs-1 alone	6.5	Mp-3alone	12.5
Th +Fs-1	31.2	Th +Mp-3	50.0
Ps+Fs-1	50.0	Ps+Mp-3	37.5
N+Fs-1	37.5	N+Mp-3	37.5
Ps+Th+Fs-1	68.8	Ps+Th+Mp-3	62.5
N+Th+Fs-1	81.25	Ps+Mp-3+N	50.0
N+Ps +Fs-1	75.0	N+Ps+Th+Mp-3	73.5
N+Ps+Th+Fs-1	87.5	Bel+Mp-3	87.5
Bel+Fs-1	75.0	Control	93.75
Rs-5 alone	0.0	Th	100.0
Th +Rs-5	25.0	Ps	100.0
Ps+Rs-5	25.0	N	100.0
N+Rs-5	31.2	Ps+Th	100.0
Ps+Th+Rs-5	43.8	N+Th	100.0
N+Th+Rs-5	50.0	Ps+Th	100.0
N+Ps +Rs-5	50.0	N+Ps	100.0
N+Ps+Th+Rs-5	62.5	N+Ps+Th	100.0
Bel+Rs-5	68.8	L.S.D.	3.86

DISCUSSION

Many fungi wereisolated from the infected pepper roots. The presence of these fungal species may be attributed to the growth and penetration of fungus in the tissues of decomposing cells that have been previously infected with the fungus causing this condition, which has protected them from the action of the surface sterilizer or may include species of parasitic ability on pepper plants.The difference in fungi in their effect on the germination of pepper seeds may be attributed to the difference in their ability to produce metabolic materials such as toxins and enzymes or the difference in methods of penetration of the host or the difference in the speed of growth. The high-isolating isolates have a higher amount of these metabolic substances than the weak-pathogenic isolates (Wyllie, 1962).*Fusarium*has high ability to produce enzymes which caused rot such as Cutinase, Cellulocytic enzymes, Pectolytic, Protase and toxins such as Fusarubin, Javanicin, Protenoneous and Polypeptide (Nelson *et al*., 1997).The results were consistent with that found by Matloob (2012)

the *T. harzianum* (Th) was effective against on *F. solani*, *F. sulphureum*, *M. phaseolina* with100% inhibition ratio and 75.6 % In the growth of *R. solani*. The effect of *P. fluorescens* in inhibiting the growth of pathogenic fungi is due to the ability of these bacteria to secrete a group of antibiotics (Bakker *et al*., 2007) .Black cumin seed extract in inhibiting the growth of *F. solani*, *R. solani* and *M. phaseolina*. The reasons for the inhibition is that the black cumin plant contains high concentrations ofClotrimazole, quinones, phenols, alkaloids, acids, fats, glycosides, tannins, vitamins and many other active compounds that are effective against many pathogenic fungi (Aftab *et al*., 2019). The results of this study showed that all treatments were increase the percentage of pepper seed germination and to reduce the percentage of losses with varying degrees compared to soil treated with pathogenic fungi alone. This finding is consistent with Indar *et al*. (1994), who point to the ability of *Trichoderma* species to protect seeds from soil borne fungi contamination and its ability to induce resistance in plants against pathogens. Also, The results was agreement with Majeed *et al* (2019) that all treatments were found to be significantly effective in reducing the damping-off in chilli over control with the combination of treatment *viz., T. viride +T. harzianum +P. fluorescens* + *Basilllussubtilis* recorded minimum pre- and post- emergence damping off disease of 13.33 percent and 15.36 percent respectively. The efficacy of *P. Fluorescens*in control of pathogens is due to the ability to produce different types of antibiotics such as Oomycin, Pyrroles, Phloroglucinal and Pyrolnitrin against pathogenic fungi (Voisard*et al*., 1994). Bacteria also stimulate systemic resistance In plants leading to the production of compounds such as Phytoalexin (Bakker *et al*., 2007). The results was agreement with Islam and Faruq (2012) which appeared in their study The seed germination of three vegetable seedlings was also increased after sowing plant extracts treated seed in the pot. The effect of the *T. harzianum* of many pathogenic fungi depends on the Mycoparasitism properties (Harman, 2000). The parasitism is accompanied by the secretion of enzymes, such as B-1,3-glucanase, These enzymes inhibit the formation of polysaccharide, Chitin, B-glucanase, glucanase, and Proteases which are responsible for the hardening of the fungal wall of the pathogen causing the cell wall to be destroyed (Howell, 2006 and Hasan*et al*., 2014). Another mechanism of *T. harzianum* is produce antibiotic such as: Trichodermin, Steroids, Alamethacine, Suznkacillin Alkylpyrones, Isonitriles, Polyketides, Peptaibds, DiketoPiperzin which inhibit pathogenic fungi (Haran *et al*., 1996; Harman, 2000).

The main causes of the damping off disease of pepper seedlings in Babylon province are *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. The control of *Trichodermaharzianum* and *Pseudomonas fluorescens* is highly antagonistic to pathogenic fungi. Effectiveness of the *T. harzianum*, *P. fluorescens* and the black cumin seed water extract in the protection of pepper seeds and seedlings from the infection of pathogenic fungi under nursery conditions. This study was showed for the first time the effect of the black cumin extract which inhibit the growth of pathogenic fungi causes the damping off disease of pepper.

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