*Biopestic. Int.*16(1):71-76 (2020) ISSN 0973-483X / e-ISSN 0976-9412

The Efficiency of Plant Extracts and Biological Control Agents on Some Pathogens Causing Damping-off and Root Rot Disease of Cucumber

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KEY WORDS

Biological control
Cucumber
Damping-off
Plant extracts
Plant pathogens
Root rot

ABSTRACT This study was conducted to evaluate the factors responsible for pathogenicity due to pathogenic fungi that cause seedling death and root rot of cucumber, *Cucumis sativus* plants. *Rhizoctonia solani*, *Fusarium solani*, *Macrophomina phaseolina*, *Aspergillus* spp., *Penicillium* spp., and *Rhizopus* spp. were isolated from the roots of the infected cucumber plants. Out of these *R. solani*, *F. solani*, and *M. phaseolina* affected the germination of cucumber seeds and showed severe infections in the range of 66.66–75%. The pathogenic fungi also significantly reduced the soft and dry weights of the vegetative parts and the roots. *Aspergillus niger* and *Pseudomonas fluorescens* were used to prevent the infection and high efficiency of inhibition of these pathogenic fungi was recorded. *P. fluorescens* showed high antagonistic ability in inhibiting the growth of *R. solani*, *F. solani*, and *M. phaseolina* by 93.1, 96.83, and 95.83%, respectively. The aqueous extract of plants, *Linum usitatissimum* seed, *Peganum harmala* seed, and *Boswellia carterii* latex was also evaluated at 50% concentration of the obtained extracts and a significant inhibition of these pathogenic fungi recorded was approximately in the range of 79–97% depending on the extract used.

INTRODUCTION

Cucumis sativus L. belongs to family Cucurbitaceae and is one of the most important and desirable economic crop in Iraq. Cucumber fruit contains 0.4% protein, 0.1% fat, 2.8% carbohydrates, and 0.3% mineral elements and little vitamins (Lefeber and Chakravarty, 1966). Cucumber crop in greenhouse and exposed agriculture is affected by many plant pathogens that cause severe damage, including damping-off, and root rot. These diseases are caused by *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* (Ali Dar *et al*., 2018). Several methods have been used to control damping-off and root diseases such as use of chemicals, agricultural cycles, hoeing, weeding, land flooding, resistant varieties, and plant extracts (Sulaiman and Abdulhafedh, 2013). The indiscriminate use of chemical pesticides has resulted in negative effects on the environment, human health, and non-target organisms (Won *et al*., 2018; Jasman *et al*., 2019). Therefore, alternative strategy has been the use of microorganisms such as pathogenic fungi, namely, *Aspergillus*, *Trichoderma*, and *Penicillium* and bacteria such as *Pseudomonas* and *Bacillus* (Nawrocki, 2007). Among

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these *Aspergillus* and *Pseudomonas* spp. are microorganisms widely used against root and leg rot diseases (Phillips *et al*., 2012). These programs also include the use of plant extracts, which differ in their chemical properties and, therefore, differ in their impact on pathogenic organisms such as fungi and bacteria (Smolińska, 2000). The important reason for using plant products has been the least possibility of environmental impact, rapid degradation, and less chances of insect resistance as they are usually evaluated as mixtures. Thus, the objective of the present study was to evaluate some phytopathogens and plant based extracts against the disease causing pathogens of cucumber under local conditions in Iraq.

MATERIALS AND METHODS

Isolation and Diagnosis

The samples were taken from the roots of planted cucumber plants inside the greenhouses from some areas in Babylon Governorate in 2018, which showed symptoms of infection such as yellowing of the leaves and ulceration or rot of the base of the stem and the rot of the roots, represented by brown color. The roots were brought to the laboratory in polyethylene bags and washed with running water for 15 min to remove the suspended soil. Roots were cut into small pieces (0.5–1 cm) and then sterilized in 1% sodium hypochlorite solution for 3 min. Subsequently, the chopped roots were washed with sterile distilled water for 2 min and transferred on sterile filter paper to remove excess of water. The root pieces were then transferred to Petri dishes of 9 cm diameter containing potato sucrose agar (PSA) medium (200 g potatoes, 10 g sucrose, 20 g agar, and 1 L of distilled water), supplemented with tetracycline (200 mg/L), sterilized at a temperature of 121° and pressure of 1.5 kg/cm² for 20 min in an autoclave. In each Petri dish, four pieces were used. The dishes were incubated in the incubator for 3 days at a temperature of 25 ± 2°C. The dishes were then opened, obtained fungi were purified and examined under a compound microscope and the genera and species were determined using approved classification keys (Reid *et al*., 1965).

Effect of Pathogens

Effect of pathogenic fungi *R. solani*, *F. solani*, and *M. phaseolina* on cucumber seed germination was determined. This experiment was carried out according to the complete random design (CRD) using plastic pots of 1 kg soil capacity under nursery conditions. The pots were filled with sterile soil and kept for sterilization at 121°C and a pressure of 1.5 kg/cm² for 1 h. Sterilization was repeated the next day for 1 h, left for 7 days and then the soil contamination process was carried out with the pathogenic fungi. The fungus vaccine was added at 1% (weight/weight) loaded on the seeds of local millet thrice, leaving three iterations by adding sterile millet seeds only for comparison. Pots were moisten with water and after 3 days soil contamination with the fungal vaccine were achieved. Pots were planted with cucumber seeds, at the rate of three seeds/pot. After the seedlings appeared, the pots were watered. The percentage of infection severity was scored as under after 30 days of cultivation using the following pathological evidence:

1. White root system with healthy root hairs.
2. Light brown color of the roots 1–25%.
3. Dark brown color of the roots 25–50%.
4. Dark brown color of the roots 50–75% with the fall of the lower leaves.
5. Dark brown color of the roots 75–100% and plant death.
6. The severity of the injury was calculated by following formula (McKinney, 1923).

$$I = \frac{\Sigma(n \times k)}{N \times K} \times 100$$

Where

n – number of plants by categories

k – the number of categories

N – number of all investigated plants

K – number of adopted categories

Antagonist Ability Test of *Aspergillus niger*

The antagonist ability test of *A. niger* against pathogenic fungi *R. solani*, *F. solani*, and *M. phaseolina* on the PAS culture medium was done using *A. niger* double implantation technique. A 9 cm diameter Petri dish containing PSA was divided into two equal parts. The first part of the dish was vaccinated with the 7-day-old pathogenic fungus (0.5 cm disk) and the other part of the dish was inoculated with a 0.5 cm disk. The experiment was carried out with three replicates. The plates were placed in the incubator at a temperature of 25 ± 2°C for a week. The contrast ability was estimated on five basic parameters (Bell *et al*., 1982). Fungus cover in the Petri dishes was categorized as under:

1. Anti-fungus cover was two-thirds of the Petri dish.
2. Counter-fungus covered the rest of the Petri dish. A counter fungus was considered effective if the degree of contrast was between 1 and 2.
3. Pathogenic fungus covered two-thirds of the dish.
4. Pathogenic fungus covered Petri dish completely.

Antagonist Ability Test of *Pseudomonas fluorescens*

The antibacterial ability was tested against all the three pathogenic fungi using PSA culture media spread homogeneously in a Petri dish. Each dish was vaccinated with *P. fluorescens* bacteria (1 mL) grown on the nutrient

broth. Each dish was provided with a 0.5 cm disk from the cultivated pathogenic fungi colony. The experiment was done in three replicates along with controls, i.e., three Petri dishes without adding bacteria. The Petri dishes were incubated at a temperature of 25 ± 2°C for 1 week. Inhibition was calculated on the basis of the growth of the fungus colony in bacteria treated dishes versus untreated ones using following formula (Montealegre *et al.*, 2003):

$$\text{Inhibition}(\%) = 1 - \left(\frac{\text{Mean fungal growth in the bacteria treated ones}}{\text{Mean fungal growth in the untretaed ones}} \right) \times 100$$

Plant Extract

Three plant products, *Linum usitatissimum* seed; *Peganum harmala* seed, and *Bosellia carerii* latex, were used for the study. Aqueous extracts were prepared using the procedure of Khaleel *et al.* (2016). In case of *L. usitatissimum* and *P. harmala*, seeds were powdered and 20 g of each soaked in 400 mL of distilled water in a 1000 mL capacity glass beaker. The extraction was allowed for 72 h with continuous shaking on a shaker. Subsequently, the mixture was filtered through 0.22 mesh Millipore filler. The filtrate was kept in sealed containers in the refrigerator at 4°C until further use (Altaee *et al*., 2018).

Plant Extract Bioassay

The method of Khaleel *et al*. (2016) was followed by mixing each aqueous extract of the selected plants with PSA nutritional medium after sterilization and cooling. Three concentrations of 10, 20, and 50% were used in three replicates for each extract. After the solidification of the food medium, the dishes were vaccinated in its center with a disk of 0.5 cm diameter from the edge of a growing fungal colony on the PSA medium for each of the pathogenic fungus *R. solani, F. solani, and M. phaseolina.* The dishes were incubated at a temperature of 25 ± 2°C. For controls, dishes were without extract treatment. The results were calculated as an average of two orthogonal drops from the growth of a fungal colony. The percentage inhibition was calculated using the following equation:

$$\text{Inhibition}(\%) \left(\frac{\text{fungus growth in the treatment} - \text{fungus growth in the controls}}{\text{growth of the fungus in controls}} \right) \times 100$$

Statistical Analysis

The results were statistically analyzed using CRD. Fisher's least significant difference test at $P \leq 0.05$ was used to determine the significance of the differences between the different treatments.

RESULTS AND DISCUSSION

Isolation and Diagnosis

Microscopic examination of the fungal growths confirmed the presence of three species of fungi accompanying the roots of cucumber seedlings. They were *R. solani*, *F. solani*, and *M. phaseolina.* Even minor traces of other fungi such as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Alternaria* were also seen. However, major infection determined was the three species mentioned above and, therefore, isolated further. These species have been reported as ecologically important as they cause various symptoms of infection such as root rot and damping-off diseases to many plant families (Sumner, 1990; Campbell *et al*., 2013; Porch *et al*., 2014).

Pathogenicity Test

The incidence of *R. solani*, *F. solani*, and *M. phaseolina* was significantly higher compared to controls (Table 1) and ranged between 66.7% and 75% causing severe injury to the cucumber seedlings. Mohsen *et al*. (2014) have reported that *R. solani* impacts the secretion of enzymes that causes decomposition of host cells. Release of toxic metabolites also leads to germination failure (Baker, 1981; Nelson *et al*., 1997) and toxins identified have been fusarubin, javanicin, Anhydro-fusarubin, proteinaceous, and polypeptides which play an important role in the pathogenicity of *F. solani* or enzymatic activity of the fungus (Moreira *et al*., 2016). *M. phaseolina* has also been reported to cause charcoal rot disease, seed rot, and damping-off in beans (Ebenezar and Wesely, 2000).

The results obtained in the present study have shown significant decrease in the growth of roots and shoots of cucumber due to these pathogenic fungi (Table 2). Compared to controls, decrease in shoot system was 5–7-fold on wet weight basis and 10–25-fold on dry weight basis. Almost

Table 1. The incidence of pathogenic fungi *R. solani, F. solani,* and *M. phaseolina* and their intensity on cucumber seeds

Pathogen used	Incidence of pathogen (%)	The Intensity of pathogen (%)
R. solani	100	75
F. solani	100	66.7
M. phaseolina	100	75
Control	0	0
LSD		63.7

LSD: Least significant difference, *R. solani: Rhizoctonia solani, F. solani: Fusarium solani, M. phaseolina: Macrophomina phaseolina*

Table 2. The amount of dry and wet weight of both root and shoot systems under greenhouse conditions

Treatment	Wet weight (g)		Dry weight (g)	
	Shoot system	Root system	Shoot system	Root system
R. solani	0.45	0.33	0.04	0.01
F. solani	0.59	0.15	0.05	0.03
M. phaseolina	0.36	0.9	0.02	0.005
Control	2.64	1.13	0.5	0.83
LSD	1.21	0.31	0.16	0.05

LSD: Least significant difference, *R. solani: Rhizoctonia solani, F. solani: Fusarium solani, M. phaseolina: Macrophomina phaseolina*

similar trend was observed in case of roots as well (2–7-fold on wet weight basis) and significant reduction in the dry weight of the root system due to pathogenic fungi under greenhouse conditions was also recorded (Table 2).

Contrast Ability Test

Anti-fungal ability test of A. niger

The results of the contrast experiment between *A. niger* and the isolated pathogenic fungi *R. solani*, *F. solani*, and *M. phaseolina* showed that *A. niger* was highly antagonistic and achieved the antigenic potential of 1.33, 1, and 1, respectively. According to the scale set by Vyas *et al*., 2015, the reason for the inhibition was that *A. niger* produced many secondary metabolites such as Flavicin, Griseofulvin, Fungal, Jawaheren, and Aspergillin (Nielsen *et al.*, 2009; Mikušová *et al*., 2014). These compounds may influence the vital activities of pathogenic fungi and then affect the radial growth as well as their production of wall-degrading enzymes such as chitinase, cellulase, lipase, and pectinase (Idan *et al*., 2017).

Anti-pathogenic activity of P. fluorescens

P. fluorescens showed a high inhibitory activity against pathogenic fungi on the PSA medium. The inhibition of *R. solani*, *F. solani*, and *M. phaseolina* was 93.1, 96.8, and 95.8%, respectively. On the other hand, in controls no inhibition was recorded (Table 3). The inhibitory action of *P. fluorescens* may be due to the enzyme β-1,3-glucanase inhibiting many fungi and producing chelating iron-siderophores and then competing with the fungi (Menon and Sperling, 1988; Kostadinova, 1997; Park *et al*., 2011). These results are consistent with the ability of *P. fluorescens* to inhibit the growth of many pathogenic fungi in food crops (Chandekar and Bhaje, 2013). There is a strong antibiosis action of *P. fluorescens* against *R. solani* and *F. solani* that also causes tomato root rot. This inhibition has been reported to be caused by the production of several antibiotics such as 4-diacetyphloro glutinous 2, pyrrolnitrin, and pyoluteorin (Rezzonico *et al*., 2005). Akter *et al.* (2016) also confirmed the ability of *P. fluorescens* to inhibit the growth of pathogenic fungi *M. phaseolina* by 100% over the PSA medium.

Table 3. The impact of *P. fluorescens* on growth rate and inhibition of pathogenic fungi

Treatment	Growth (cm)	Inhibition (%)
R. solani + *P. fluorescens*	0.55	93.1
F. solani + *P. fluorescens*	0.25	96.8
M. phaseolina + *P. fluorescens*	0.33	95.83
R. solani	9	0
F. solani	9	0
M. phaseolina	9	0
LSD 0.05	0.44	5.6039

LSD: Least significant difference, *R. solani: Rhizoctonia solani, F. solani: Fusarium solani, M. phaseolina: Macrophomina phaseolina, P. fluorescens: Pseudomonas fluorescens*

Effect of Aqueous Extracts on Pathogenic Fungi

The efficacy of the aquatic extract of *P. harmala* seed, *L. usitatissimum seed*, and *B. carterii* latex was extract specific as well as the concentration dependent (Table 4). The three aquatic extracts of the plants used at 50% concentration of the extract obtained caused the highest inhibition of growth of *R. solani* (97, 90.6, and 79.9%, respectively). The efficacy against *F. solani* (97, 91.6, and 96.6%, respectively) and *M. phaseo*lina (97, 89.5, and 93.3%, respectively) was also significantly similar. At the lower concentrations of 20%, the inhibition caused was the least (Table 4). There was no difference in the efficacy at 10% concentration and controls because no inhibition was recorded in both the cases.

Eshraghi *et al.* (2009) used *P. harmala* extracts and recorded similar inhibition of some pathogenic fungi including *R. solani*, *F. solani*, *and M. phaseolina*. *P. harmala* aqueous extract could also inhibit the growth of *F. oxysporum* on PDA medium. (Vimal *et al*., 2005; Anon, 2011); however, use of high concentrations was required to achieve the required inhibition. The physiological action of aqueous plant

Table 4. The inhibition of pathogenic fungi caused by aquatic extracts of *P. harmala* seed, *L. usitatissimum* seed, and *B. carterii* latex

Treatment	*R. solani* inhibition (%)				Average	*F. solani* inhibition (%)				Average	*M. phaseolina* inhibition (%)				Average
	Concentration (%)					Concentration (%)					Concentration (%)				
	0	10	20	50		0	10	20	50		0	10	20	50	
P. harmala	0	0	8.33	96.83	26.29	0	0	0	96.23	24.05	0	0	4.56	96.63	25.28
L. usitatissimum	0	0	6.23	90.60	24.70	0	0	2.06	91.63	23.42	0	0	0	89.56	22.39
B. carterii	0	0	9.56	79.96	22.38	0	0	15.00	96.63	27.90	0	0	4.16	93.30	24.36
Plant extract LSD	7.7993														

LSD: Least significant difference, *P. harmala: Peganum harmala, L. usitatissimum: Linum usitatissimum, B. carerii: Boswellia carterii*

extracts may be an effect due to the secondary metabolites that can inhibit the growth of fungal pathogens and other microorganisms (Talibaev *et al*., 1992). Experiments and scientific research have proven that the alkaloids present in *P. harmala* seeds are highly toxic to microorganism (Farouk *et al*., 2007). Similalrly, *L. usitatissimum* seeds have been reported to have antifungal potency due to the abundance of linoleic and α-linolenic acids, which appeared promising to treat fungal infections (Abdelillah *et al*., 2013); however, these seeds have high antioxidant properties as well due to phenolic compounds (Alachaher *et al*., 2018). The extract of *B. carterii* containing boswellic acid has been reported as antifungal too, specifically inhibitory to the plant pathogens (Krohn *et al*., 2001; Culioli *et al*., 2003). In conclusion, it is obvious that both microorganisms and plant extracts can be used to control pathogenic fungi of crops, specifically the cucumber crop in the present case, using simpler techniques to isolate effective strains of fungi and plant extracts that can be processed by marginal farmers easily for use under field conditions.

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Received: 15 Mar 2020; Accepted: 04 Jun 2020