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THE EFFECTIVENESS OF SOME PLANT EXTRACTS AND BIOLOGICAL CONTROL FUNGI *TRICHODERMA HARZIANUM* IN CONTROLLING CUCUMBER SEEDLING FALL DISEASE *PYTHIUM APHANIDERMATUM*

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Abstract:
This study was conducted in order to evaluate the effectiveness of some plant extracts, namely Eucalyptus camaldulensis leaf extract and Cinnamomumzy lanicum extract (cinnamon). At concentrations of (0.5, 1.0 and 1.5) % (w/v) and the filtrate of the bio-control fungus Trichoderma harzianum at concentrations of (50, 75 and 100) % (w/v) in controlling the cucumber dropping off disease caused by the fungus Pythium aphanidermatum under laboratory and greenhouse conditions. The results showed the effectiveness of the bio-control fungus T. harzianum filtrate in inhibiting the growth of the fungus p. aphanidermatum in vitro" by increasing the concentrations used where the 100% concentration treatment achieved the highest rate of inhibition of the growth of pathogenic fungi, where the percentage of inhibition was 98.75%, and the concentration treatment 1.5% achieved the highest rate of 89.21 and 58.39% for the extracts of eucalyptus leaves and the study plant in inhibiting the growth of the fungus p. aphanidermatumrespectively.The results of the study, under greenhouse conditions, showed the excelled of the treatment of the bio-control fungus T. harzianum infiltrate in reducing the infection rate of the pathogenic fungus P. aphanidermatum and the percentage of healthy plants growing in soil contaminated with pathogenic fungus and treated with fungus filtrate after 7 and 30 days exelled on the treatment of soil contaminated with pathogenic fungus and treatment with plant extracts (eucalyptus leaves and cinnamon plant extract) The following rates were achieved (77.97 and 74.57% for the fungus filtrate treatments, 66.33 and 63.53% for the eucalyptus leaf extract and 53.70 and 52.27% for the cultivar extract for the periods 7 and 30 days, respectively. These results indicate the possibility of using some plant extracts and control elements Bio control to chemical pesticides in the management of cucumber seedling dampping fall disease pathogens.

Keywords: cucumber seedlings, plant extracts, Trichoderma harzianum

Introduction:

damping off Seedling disease is one of the important diseases that determine the cultivation of vegetables in the open and protected cultivations. The disease is caused by a group of soil fungi, the two most common being Pythium aphanidermatum and Rhizoctonia solani, which attack seeds and seedlings before and after their emergence and lead to their fall and death (Agrios, 2005; Pscheidt and Ocamb, 2009).The cucumber crop, Cucumis sativus L., is considered one of the most important and desirable economic crops in Iraq, and recent years have witnessed an increase in the cultivated area,The area planted with all kinds of cucumber in the 2019 agricultural season was 69502 with a productivity of 149,302 tons, with an average productivity of 2152.3 kg/dunum

(Central Statistics Organization, 2019). The cucumber plant is affected by many diseases, the most important of which is the damping off Seedling disease. The severity of this infection is related to the increase and decrease in soil temperature and moisture and the delay in seed germination, and because the pathogen lives in the soil, it often kills the root system without feeling its presence (Al-Khafaji, 1985, Intana and Chamswarng, 2007). The modern trend in the control of various agricultural pests tends towards the use of integrated pest management, which means the use of various natural, mechanical, biological, chemical, legislative and subjective control methods in an optimal system aimed at reducing pest damage while preserving the integrity of the environment (Driesche et al., 2008). The use of plant extracts and biological control means of integrated control. (Gray, 2006). The natural extracts are effective in many fungal and insect diseases, because they are safe and environmentally friendly, as well as their ease of use and cheapness. From this section, this research was proposed using:

1. Eucalyptus camaldulensis leaf extract:

Al Dosh (2004) found that the extract of eucalyptus E. camaldulensis contains phenolic, peptide and alkaloid compounds.(Farrukh et al., 2001) found that extracts of plant oils Syzygium aromaticum and Eucalyptus glabules have antifungal activity Fusarium spp., Alternaria spp. and pythium spp. It was also found that the inhibition of fungal growth by plant extracts in a liquid environment is similar to the inhibition of fungal growth in a solid environment. (Ribau et al. 1995) found that the extract of volatile oils of Eucalyptus camaldulensis D. and extracts of other plant oils have antifungal activity against many fungi.

2. Cinnamomumzy lanicum extract (Cinnamon)

The learners contain many effective chemical compounds, including the volatile oil that contains cinnamaldehyde and polyphenols 4-10% such as (eugenol, methyl eugenol and safrole), hydrocarbons such as (pinene, phyllandrin and carbophylline), Esters such as (Cinnamyl acetate, Eugenol acetate, Phenylpropyl acetate and Benzyl benzoate), Cinnzeylanin, Cinnzeylanol, Ketones, Alcohols and also contains Starch fibers and Mucilage (Treese, 2003) and other compounds (2004) It was found (Al-Zubaidi, 2005) that the cold aqueous extract of the students contained both the active compounds glycosides, tannins, phenols, soaps and resins, and these active substances were characterized by their effect on fungal growths. -cinnamldehyde..

Bio-control:

The bio-control method is one of the oldest methods used in pest control and the interest in it has increased in recent years. The bio-control of plants has received a great deal of attention, making it of great importance in integrated control (Harman, 2000)Several studies have indicated that the fungus Trichoderma harzianum possesses a high antagonistic ability against various plant pathogenic fungi. Its antagonistic ability is due to its possession of several mechanisms to influence the formation of compounds with a toxic effect on pathogens, including proteins and enzymes that degrade the cell walls of the fungus (chitinase, gluconase and proteases). In addition to secondary metabolites, antibiotics (Vinale et al., 2008 and Al-Taweil et al., 2009) and other mechanisms that act alone or in combination to eliminate the pathogen (Al-Taweil, 2010) Given the importance of

the damping off seedlings disease on cucumbers and the need for more information necessary to control this disease and reduce its economic damage, this study aimed at the following:

1- Isolate and diagnose the pathogen and test its pathogenicity.

2- Evaluation of the effectiveness of integrated control agents using plant extracts (eucalyptus leaves and tulips) and the bio control agent Trichoderma harzianum in controlling the cause of damping off seedling of cucumber plants.

Materials and methods:

Isolation and diagnosis of fungi:

1. The fungus pythium aphanidermatum

Soil was brought from a plastic house in which a disease of death and drop of seedlings had been previously recorded. Half kg of soil was placed in sterile 100ml glass bekers moistened with sterile water. Superficially sterilize cucumber fruits with 70% ethyl alcohol. The fruits were cut into small pieces 3-4 cm long by a sterile knife, and planted in the moistened soil of the pickers to half their length and incubated at a temperature of 25 + 2 °C until the appearance of a white cottony fungus on the fruits. A small piece of fungal growth was taken and planted on solid media with sweet potato dextrose agar (PDA) and incubated at 25 + 2°C. Until the emergence of colonies, an isolation process of mushroom colonies was carried out on PDA culture medium to obtain pure colonies of the fungus, characterized according to the taxonomic traits mentioned in Waterhouse (1967).

2. Trichoderma harzianum:

The isolate of the fungus *T. harzianum* was obtained from the biocide, the challenge of production of the Agricultural and Biological Research Department - Baghdad (active substance, spores and mycelium of the fungus *Trichoderma harzianum*, the pesticide was used in a mixture with water at a ratio of 10 g / 100 ml and cultured by pouring into Petri dishes containing medium PDA was incubated at 25°C for five days and after the emergence of colonies, isolation of fungi colonies was conducted on PDA culture medium to obtain pure colonies of fungi , characterized based on taxonomic characteristics given in Kubicek and Harman (2002). To prepare the filtrate of the fungus T. harzianum, PDB liquid media was used, inoculated with three (5) mm discs with a cork punch from the edge of the purified fungal colonies in the PDA culture medium and diagnosed at the age of seven days. The flasks were incubated at a temperature of (25±2) C. Considering shaking the flasks every 3-4 days to distribute the fungal growth, and after 28 days filter the inoculum using Whatman No. filter paper. 1 With a Buchner funnel with the help of a Vacum pump, the filter was re-filtered using a Millpore microfilter. The filter was used in subsequent experiments.

Collection and preparation of aqueous extracts of eucalyptus leaves and Cinnamon:

The leaves of Eucalyptus camaldulensis were collected, washed and dried by spreading them in thin layers over wide surfaces of the fabric and turning them constantly to prevent them from rotting and drying them quickly. As for the study plant (cinnamon) Cinnamu zeylanicicum .It was purchased from the local market. Then the plant parts of the samples, which are eucalyptus leaves and cinnamon, were crushed separately with an electric grinder, and then kept in clean, dry plastic

bags until the test was conducted .I followed the method (Parekh and Chanda, 2007). By taking (20) g of the vegetable powder in a glass beaker of 500 ml capacity, adding 200 ml of distilled water to it, and placing it in a vibrating incubator for 24 hours at a temperature of 37 °C. at 5000 rpm for 10 minutes, then filter the filtrate with 0.22 µm pore filter paper.Then the filtrate was evaporated using (Oven) at a temperature not exceeding 40°C to obtain a dry extract in the form of a powder. It was placed in a sealed and opaque tube and kept in the freezer until it was used. The process was repeated several times to obtain a sufficient amount of extract .To prepare the concentrations used in the experiment, (1) g of dry matter was taken for each extract separately and dissolved in (40) ml of distilled water, thus the concentration of the basic solution became (2.5)%, then concentrations were prepared from it (0.5, 1.0, 1.5) % (w/w). volume) according to the equation (V1 N1 = V2 N2), while the comparison treatment contained only distilled water.

Pathogenicity test of the pathogenic fungus Pythium aphanidermatum.

Sterilized mixed soil with an autoclave at a temperature of 121 °C and a pressure of 1.5 bar/cm2 for a period of 30 minutes and two consecutive days. Pots with a diameter of 13 cm were filled with sterilized soil and contaminated with a radius of 9 cm dish/pot with isolates of pathogenic fungi growing on PDA culture medium. johns fungal growth with 50 ml of sterile distilled water in an electric mixer and added to the potting soil with good mixing ,The soil was moistened with sterile water, then wrapped the pot with nylon bags for 48 hours and planted cucumber seeds (10 seeds/pot) of a selected variety superficially sterilized with a solution of sodium hypochlorite. The pot was placed in the greenhouse and irrigated with water as needed. The percentage of infection was calculated up to 15 days from germination .Fungi were re-isolated from infected seedlings according to Koch's hypothesis. As for the control pots (sterile soil only) they were planted with cucumber seeds only.

Testing the ability of Trichoderma harzianum to antagonize the pathogenic fungus Pythium aphanidermatum.

The sub culture method (sub Culture Technigues) was used to test the ability of the biological resistance fungus T. harzianum to antagonize the pathogenic fungus P. aphanidermatum in Petri dishes containing sterile PDA medium. The center of the first half of the dish was inoculated with a 1 cm diameter disc of T. harzianum The center of the other half of the dish was inoculated with a similar disc of the pathogenic fungus P. aphanidermatum growing on the PDA medium at the age of four days, A treatment was conducted compared to inoculating the center of one half of the plate with the pathogenic fungus only. The occlusion was incubated for seven days at a temperature of 25 ± 2 °C. The diameters of the colonies were measured after the incubation period and the degree of antagonism was estimated according to the Bell (1982) scale of five degrees.

.1Anti-fungus covers the whole plate
.2Anti-fungus covers 3/4 of the plate area
.3Antifungal and pathogenic fungi each cover half the area of the plate
.4The pathogenic fungus covers 3/4 of the plate area
.5The pathogenic fungus covers the entire plate<

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Testing the efficacy of plant extracts and filtrate of the biological resistance fungus Trichoderma harzianum on the growth of the fungus Pythium aphanidermatum, the pathogen of Cucumber damping off seedling Disease, in vitro:

Dextro Agre Potato was prepared using 200 gm of potatoes, 20 gm of dextrose and 20 gm of agar in a liter of water and distributed in 250 ml flasks .Then it was placed in the autoclave at a temperature of 121 °C and a pressure of 1.5 bar/cm2 for 30 minutes, after the sterilization period ended and the temperature of the medium was reduced to before solidification, the antibiotic Chloramphenicol was added to it at a concentration of 250 mg/L .Then aqueous extracts of eucalyptus and thyme leaves were added at concentrations (0.5, 1.0, 1.5)% and the fungus filtrate T. harzianum and at concentrations (50, 75, 100) % (weight/volume) leaving a nutrient medium without adding any extract as a control treatment,

The flasks containing the food medium were agitated and the extracts were added to it for the purpose of homogeneity. The media were poured into sterile 9 cm petri dishes and inoculated after hardening with a 1 cm diameter disc of the fungus culture p. aphanidermatum and with four replicates for each treatment. The plates were incubated at a temperature of 25 ± 2 °C. The diagonal growth of the developing fungus was measured by taking the average of two perpendicular diameters from the back of the colony of the fungus passing through the center of the disc every 24 hours until the growth in the control treatment reached the edge of the plate, and the percentage of fungal growth inhibition was calculated according to the following equation (Shaaban and Al-Mallah, 1993):

$$\text{inhibition of fungal growth\%}=\frac{fungus\ growt\quad rate\ in\ control-fungus\ growt\quad rate\ in\ treatment}{ungus\ gro\qquad rate\ in\ control}\times 100$$

Testing the efficacy of plant extracts and filtrate of the biological resistance fungus Trichoderma harzianum on the growth of the fungus Pythium aphanidermatum, the pathogen of cucumber damping off disease, under greenhouse conditions:

Plastic pots with a diameter of 12 cm were filled with sterilized soil. The pots were divided into five groups in the greenhouse. The soil of the first and second groups was added to the eucalyptus leaf extract and the plant extract at concentrations (0.5, 1.0, 1.5)% at a rate of 50 ml/kg soil, respectively, left for two days, and then contaminated with the pathogenic fungus at the rate of a quarter plate/kg soil from a mushroom farm on the PDA culture medium at the age of 3 days. .As for the third group, the fungus T filter was added to it. T.harzianum at concentrations (50, 75, 100) % (weight/volume) and was contaminated with the pathogenic fungus at the same average as the previous two days after adding it,As for the fourth group, it was only contaminated with the pathogenic fungus with the same amount as before, and the fifth group was left without contamination and without treatment for control. The potting soil was planted with cucumber seeds of the chosen cultivars at average of 10 seeds/pot and with four replicates for each treatment. The percentage of healthy plants was estimated after 7 days and after 30 days, and the treatments were as follows:

- Sterile soil contaminated with pathogenic fungi, to which the extract of eucalyptus leaves was added, in three concentrations, at an average of 50 ml/kg soil. Spray the plants after germination with the extract.
- Sterilized soil contaminated with pathogenic fungi, adding to it the extract of the plant of the study, in three concentrations, at an average of 50 ml / kg of soil. Spray the plants after germination with the extract.
- Sterile soil contaminated with pathogenic fungi, to which the bio-resistance fungus filtrate was added, in three concentrations, at a rate of 50 ml/kg soil. Spray the plants after germination with the filtrate.

Sterilized soil contaminated with the pathogenic fungus Pythium aphanidermatum

- Sterile, untreated and uncontaminated soil / control.

statistical analysis:

The results of the study were analyzed according to the factorial experiment model and randomized complete block design of greenhouse experiments. The Least significant difference (L.S.D) test under the 0.05 probability level was used to test the significance of the results (Al-Sahoki and Wahib 1990). The data of the experiment were statistically analyzed using Statistical Analysis System SAS (2012).

Results and discussion:

Pathogenicity of the fungus Pythium aphanidermatum:

The results in Table (1) showed that the tested isolate of the fungus caused an infection of the cucumber seedlings and led to their damping after emergence, The percentage of germination after 7 and 15 days was 45.33 and 40.33%, respectively, compared to "94.67%" after 7 and 15 days in the control treatment. The reason for this may be due to the fact that the fungus P. aphanidermatum attacks the seeds and penetrates them through the cracks of the seed coat during germination to the embryo.It also attacks the fresh tissues of the embryonic peduncle and secretes enzymes that degrade cell walls and the median plate and cause their decomposition. The fungus also attacks the roots and penetrates them directly or through wounds and causes them to atrophy and rot. (Al-Khafaji, 1985 and Al-Maliki, 2002 and Moses, 2006).

Table (1) The effect of the fungus Pythium aphanidermatum on the germination average of cucumber seeds (the pathogenicity of the fungus)

%of plants grown after 15 days	%for germination after 7 days	Treatments
40.23	45.33	Isolation of the pathogen pythium aphanidermatum
94.67	94.67	control
15.33	13.75	L.S.D ($p \leq 0.05$)

The ability of the fungus Trichoderma harzianum to antagonize the pathogenic fungus Pythium aphanidermatum

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The results of the antagonism test mediated by sub-culture suggested by (Bell, 1982) showed a high antagonistic ability between the bio- control fungi and the pathogenic fungus, as the fungus achieved a high antagonism degree of 1.3 after seven days of inoculation, This is consistent with what was mentioned before (Al-Mousawi, 2003). The ability of T. harzianum to eradicate the fungus p. aphanidermatum is embodied through many activities, most notably the mechanisms of competition and parasitism (Al-Ta'i, 2010).

Effectiveness of plant extracts and filtrate of the bio-control fungus Trichoderma harzianum on the growth of the fungus Pythium aphanidermatum, the pathogen of Cucumber damping off disease in vitro:

The results in Table (2) show the effectiveness of different concentrations of eucalyptus leaf extract and dracaena plant in inhibiting the growth of the fungus p. aphanidermatum. The inhibitory ratio increased with the increase in the concentrations used, whereby the 1.5% concentration treatment achieved its highest rate of 89.21 and 58.39% for the two extracts, respectively, compared to using the 0.5% concentration for the two extracts, which amounted to 33.60 and 29.17%, respectively. The difference in the effect of the concentrations of the same extract may be due to the difference in the concentration of the extracted active substance, and the difference between plant extracts may be due to the type of extracted substance.The results of this study are in agreement with several studies in which it was mentioned the effect of the extract of eucalyptus on the growth of fungi due to the fact that it contains resins, tannins, glycosides, saponins, phenols and flavonoids (Al-Nuaimi et al., 2008).It is also noted from the results in Table (2) the effectiveness of the filtrate of the biological resistance fungus T. harzianum in inhibiting the growth of the fungus p. aphanidermatum by increasing the concentrations used, as the 100% concentration treatment achieved the highest rate of inhibiting the growth of pathogenic fungi, as the percentage of inhibition reached 98.75%, while the percentage of inhibition reached 40.00 and 62.35% for the concentrations 50 and 75%, respectively. The inhibitory effect of T. harzianum. It may be due to its production of toxic compounds such as Trichothecin, Gliotoxin, Pyrones and other substances (Chisalberti et al., 1990) It may be due to the presence of many degrading enzymes such as Protase, Chitinase, B, 1-4 glucanase and cellulase. (Hamid, 2002)

Table (2) The effect of the effectiveness of plant extracts and filtrate of the bio-control fungus Trichoderma harzianum on the growth of the fungus Pythium aphanidermatum that causes the disease of cucumber seedlings:

Inhibitory percentage of diagonal growth of the fungus Pythium aphanidermatum	**Concentration % (w/v)**	**treatments**	**No.**
33.60	**0.5**	**eucalyptus leaf extract**	**1**
57.11	**1.0**		
89.21	**1.5**		
56.64	**average**		
29.17	**0.5**	**Cinnamon extract**	**2**

46.45	**1.0**		
58.39	**1.5**		
44.67	**average**		
40.00	**50**	**Fungal filtrate (*Trichoderma harzianum*)**	**3**
62.35	**75**		
98.75	**100**		
67.03	**average**		
98.75		**control**	**4**
11.650		**L.S.D) p≤ 0.05(**	

Effectiveness of plant extracts and filtrate of the bio-control fungus Trichoderma harzianum on the growth of the fungus Pythium aphanidermatum, the pathogenic causative agent of cucumber seedling dampping off , under greenhouse conditions:

The results in Table (3) showed that the filtrate of the bio-control fungus T. harzianum had a high efficiency in reducing the infection rate of the pathogenic fungus P. aphanidermatum The percentage of healthy plants growing in soil contaminated with pathogenic fungi and treated with bio-resistant fungus filtrate at a concentration of 100% (weight/volume) was 95.6 and 92.4% after 7 and 30 days, respectively. It did not differ significantly from the control treatment 96.4%, but it differed significantly from Treatment of soil contaminated with pathogenic fungi, in which the percentage of healthy plants after 7 days and 30 days reached 40.5 and 35.3%, respectively, for the previous two periods**.** The effectiveness of the bio-control fungus T. harzianum in resisting the pathogen in cucumber plantations may be due to the ability of the bio-control fungus to induce systemic control acquired in the host plant against the pathogenic fungus, and this is consistent with previous studies (Nederhoff 2001 and Ranasingh et al., 2006 and Altaie, 2010).Which showed the ability of T. harzianum isolates to induce systemic resistance in host plants against the disease through bio-control, competition and direct parasitism. Plants after germination significantly increased healthy plants compared to soil contaminated with pathogenic fungi**.** The average of healthy plants for the concentrations used after 7 and 30 days was 66.33, 63.53%, 53.7 and 52.27% for eucalyptus leaf extracts and study plants, respectively, compared to the treatment of soil contaminated with pathogenic fungi, which reached 40.5 and 35.3% after 7 and 30 days. The inhibitory effect of plant extracts (eucalyptus leaves and dacin) and on the growth of pathogenic fungi may be due to their containing effective compounds, including alkaloids, flavonoids, phenols, resins, and bacilli, which have the ability to inhibit the growth of some fungi. Dosh, 2004 and Al-Zubaidi, 2005 and Al-Sayed, 2012).The results of increasing the percentage of fungal growth inhibition by increasing the concentration of the plant extract were in agreement with what Al-Otaibi (2007) mentioned, which indicated that there was an inverse relationship between the concentrations of plant extracts, including Ziziphus extract, and the number of spores sprouting for some fungi. The superiority of eucalyptus leaf extract over citrus extract may be due to the ability of eucalyptus volatile oils to inhibit radioactive growth of fungi (Farrukh et al., 2001). The results reached in this study indicate the possibility of using integrated control of plant extracts

and bio-control elements for pathogens inherent in the soil as an alternative to chemical pesticides and may represent a great hope for resistance to plant pathogens.

Table (3) Effect of the effectiveness of plant extracts and the filtrate of the bio-control fungus Trichoderma harzianum on the growth of the fungus Pythium aphanidermatum, the pathogenic causative agent of cucumber seedlings, under greenhouse conditions:

%for healthy plants		Concentration % (w/v)	treatments	No.
after 30 days"	7days later			
50.7	53.4	0.5	Soil contaminated with pathogenic fungus + eucalyptus leaf extract	1
60.5	62.8	1.0		
79.4	82.8	1.5		
63.53	66.33	average		
37.6	39.0	0.5	Soil contaminated with pathogenic fungus + cinnamon plant extract	2
48.9	50.3	1.0		
70.3	71.8	1.5		
52.27	53.7	average		
55.6	58.2	50	Soil contaminated with pathogenic fungus + fungus filter Trichoderma harzianum	3
75.7	80.1	75		
92.4	95.6	100		
74.57	77.97	average		
35.3	40.5		Soil contaminated with the pathogen pythium aphanidermatum	4
96.4	96.4		control	5
12.25	12.16		L.S.D) p≤ 0.05(	

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