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Biological Control of Leaf Spot Disease Caused by *Alternaria alternata* on Eggplant

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I. Abstract

The study aimed to evaluate the effectiveness of combining two biological control agents the fungal suspensions of *Trichoderma hairzenium* and *Trichoderma viride*, and the aqueous extract of garlic (Allium sativum) in inhibiting the growth of the pathogenic fungus *Alternaria alternata*, which causes leaf spot disease in eggplant. The results showed that the fungal suspensions of *T. hairzenium* and *Trichoderma viride* inhibited the radial growth of the pathogenic fungus by 90%. Similarly, the aqueous extract of garlic and the fungicide Revus top demonstrated high efficacy in inhibiting the radial growth of the pathogenic fungus, reaching 90% for the combined treatment. The results of mixing the biological factors and their effect on the germination of eggplant seeds showed that the treatment of the fungus *T. hairzenium* + aqueous extract of garlic was superior, reaching 96%, followed by the treatment of the fungus *T. viride* and aqueous extract of garlic alone, reaching 90% and 80% respectively. The results also showed that the treatment with the fungus *T. hairzenium* was superior in increasing growth indicators, with the average seedling height reaching 17.6 cm. This was followed by the treatment with the fungal suspension *T. virid*, which increased growth indicators to 14.6 cm. The average fresh and dry weights of the shoot system were 12.22 g and 3.43 g, respectively, in the *T. hairzenium* suspension treatment. The average fresh and dry weights of the root system were 2 g and 1.42 g, respectively. The treatment with the aqueous garlic extract recorded averages of 2.65 g and 1.23 g for the dry and fresh weights of the shoot and root systems, respectively.

Keywords: *Alternaria alternata*, *T. harzianum*, *T. viride*, garlic extract, Revus Top

II. Introduction

Alternaria alternata is an important fungus that is widespread in nature. It lives saprophytically in most types of soils and plants. Its colonies are black or olive-black in color with long branching chains. It is considered a soil pathogen. It is transmitted from one field to another by wind. It causes many losses among agricultural crops, as it causes *Alternaria* spot disease on eggplant and tomato crops, as well as brown spot on citrus trees and fruit rot in apples (33,35). The fungus produces many toxic compounds that cause diseases in many plants, leading to their death, such as alternaric acid and alternariol (29). Several fungicides have been used against the fungus *A. alternate*. It was found (7) that the use of Rizolex showed 100% efficacy in inhibiting the radial growth of the pathogen. Similarly, among those expelled (25), the use of the insecticides Sword Endosulfan and Vertimit inhibited the germination of the spores of the pathogenic fungus *A. alternate* and reduced the severity of leaf spot disease in tomato plants. Recently, there has been an increased interest and trend towards using biological control agents as an alternative in combating plant diseases. Among the most important agents used are types of *Trichoderma* fungi, which are soil fungi that have been widely used in the field of biological control (34). *Trichoderma species* are among the most widely used fungi as important agents in biological control. Commercial preparations have been developed from them as biofungicides, biofertilizers, and soil amendments (31). Additionally, the biocontrol agent.*T. hairzenium* and an aqueous extract of garlic have been used to inhibit the pathogenic fungus A. alternate on eggplant and reduce the severity of the disease (22). Furthermore, the use of plant extracts is environmentally friendly and an alternative to harmful pesticides that cause diseases (18,24). Garlic (*Allium sativum*) is effective in

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inhibiting many plant pathogens. It has also been found that the aqueous extract of garlic has effective antifungal properties due to its volatile sulfur compounds, including alliin, S-alliin cysteine, and S-allyl mercaptocysteine, which have the ability to penetrate the fungal cell wall, leading to the release of cell contents, disruption of osmotic pressure, and inhibition of vital enzymes necessary for respiration and metabolism, as well as affecting DNA and RNA through their effect on proteins, thus preventing cell division (23 ,19).

III. Materials and Methods

1-Isolation and diagnosis of the pathogenic fungus

The fungus *A.lternata*.A was isolated from tomato plants infected with leaf spot in the greenhouses of the College of Agriculture, University of Basra. Infected leaves were taken and cut into small pieces (1-2 cm), then washed with running water to remove dirt. They were then left to dry briefly and sterilized with a 10% sodium hypochlorite solution of the commercial preparation for three minutes. After that, they were cultured in a sterile 9 cm diameter Petri dish containing sterile PDA nutrient medium, with three replicates per dish, and then incubated at a temperature of 25 ± 2°C for seven days. The fungus was identified based on its taxonomic characteristics by Dr. Yahya Saleh Ashour, College of Agriculture, University of Basra, as stated in (17).

2-Growth of the biological fungi used in the research

The fungi used (*Trichoderma hairzenium* and *Trichoderma virida*) were cultured and activated on sterile PDA culture medium. The plates were incubated at 25 ± 2°C for seven days. Subsequently, the spore suspension was prepared by scraping the fungal growth surface of each fungus several times with a sterile ring conveyor to obtain conidia. These were then placed in test tubes containing distilled water, and the concentration was adjusted to 1×10^{-8} spores/ml using a Haemocytometer (30) slide. The fungal isolate was obtained by Professor Dr. Abdul Nabi Saleh Matroud, College of Agriculture, University of Basra.

3-Preparation of the aqueous extract of garlic powder

(50) grams of garlic powder were obtained from local markets and an aqueous extract was prepared by steeping it in hot water for 12 hours to obtain the aqueous extract for use in subsequent laboratory experiments. (4 ,21)

4-Testing the pathogenicity of the fungus *Alternaria alternate*

In this experiment, I used 4-week-old eggplant seedlings. The plants were planted in 1 kg plastic pots containing a 3:1 mixture of soil and peat moss, sterilized with commercial formalin using a 1:50 formalin:water solution at a rate of 3 liters/m3. Two weeks after planting, the plants were individually inoculated with a fungal suspension at a concentration of 1 x 10^{-8} using a 1-liter hand sprayer. The concentration was controlled using a hemocytometer. The plants were covered with a plastic box to increase humidity during the first three days after inoculation and to ensure infection. After that, the plastic box was removed, and the pots were left inside the greenhouse for two weeks. The severity of infection was calculated using a five-point scale. The experiment was carried out with three replicates per dish using a completely randomized design (10).

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Grade	Number of Spots
0	None
1	1-3
2	4-6
3	7-9
4	Lower Leaf Death

The severity of the injury was calculated according to the McKinney 1923 equation, as follows:

$$\text{Severity of injury} = \frac{\text{Total (Number of spots} \times \text{Grade number)}}{\text{Total number of spots} \times \text{highest score}} \times 100$$

Laboratory experiments

1- The antagonism between biological control agents and the pathogenic fungus in the dishes

The ability of the biotic agents to antagonize the pathogenic fungus was tested. The pathogenic fungus was cultured in a Petri dish containing PDA medium as a single treatment. For antagonism testing, a double culture method was used. A Petri dish containing sterile PDA medium was divided into two equal sections. The center of the first section was inoculated with a 0.5 cm diameter disc of the colony of the biotic fungus *T. hairzenium*. The second section was inoculated with a similar disc of the colony of the pathogenic fungus *A. alternata*. The fungus *T. viride* was inoculated in the same way, with three replicates per dish. The dishes were then incubated at 25 ± 2°C. The degree of antagonism was calculated after the growth in the control treatment reached the edge of the dish according to a scale (12). The fungicide Revus Top, belonging to the 40-Carboxylic Acid Amides group and manufactured by Syngenta, was added to the PDA medium before it solidified, using the concentration recommended by the aforementioned company. A disc of the pathogenic fungus was then cultured in three plates, each plate being replicated three times. One gram of the aqueous extract of garlic powder was added to each plate and distributed around the center of the plate in five spots. A 0.5 cm disc of the pathogenic fungus *A. alternata* was then cultured in the center of the plate and incubated as described above.

2- Effect of biological control agents on eggplant seed germination in the laboratory
Eggplant seeds purchased from local markets were used to determine their germination rate and to demonstrate the effect of biological control agents on them, as follows:

A- Eggplant seeds were treated with the fungal suspension of *Trichoderma hairzenium*. They were soaked for 30 minutes, then dried and dusted with a 1:1 aqueous garlic extract. They were then planted in a sterile Petri dish on filter paper moistened with sterile distilled water, with 10 seeds per dish and three replicates. They were incubated at laboratory temperature for 72 hours, and the germination rate was calculated. Eggplant seeds were also treated in the same way with the fungal suspension of *Trichoderma viride.*

B- Dusting eggplant seeds with garlic extract: The seeds were moistened with sterile distilled water, then dusted with a 1:1 aqueous garlic extract and planted as above.

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C- . Comparison Treatment: The seeds were treated with sterile distilled water only and planted in the dishes as described above.

Field experiments

The experiment was conducted in one of the plastic fields at the College of Agriculture, University of Basra. Pots were placed inside the greenhouse and filled with soil at a rate of (1) kg per pot, sterilized with 6% commercial formalin. All pots were then contaminated with a fungal suspension of the pathogenic fungus *A. alternata* at a rate of 10 tablets per pot, mixed well with the soil. Eggplant seeds were then planted in them at a rate of 20 seeds per pot after being treated with the following procedures.

1- Soak eggplant seeds for 30 minutes in a suspension of the fungus *T. hairzenium*, then plant them in soil contaminated with a suspension of the pathogenic fungus *A. alternata*.
2- Soak eggplant seeds in a suspension of the fungus *T. hairzenium* for 30 minutes, then dust with the fungus Revus top and plant in soil contaminated with the pathogenic fungus *A. alternata.*
3- *Soak eggplant seeds in a suspension of the fungus T. hairzenium for 30 minutes, then dust with a 1:1 aqueous extract of garlic and plant in soil contaminated with the pathogenic fungus A. alternata*
4- . Dust eggplant seeds with a 1:1 aqueous garlic extract, then moisten with sterile distilled water, and then plant in soil contaminated with pathogenic fungi.
5- Eggplant seeds were treated with sterile distilled water only and planted in soil contaminated with the pathogenic fungus as a control treatment. The same experiments were then repeated using the biogenic fungus *T. viride*

Statistical analysis:

The results were analyzed according to a completely randomized design (CRD) and the means were compared at a probability level of 0.01 for laboratory experiments and 0.05 for field experiments (10).

Results and Discussion

First - Laboratory Experiments

1- Antagonism between the pathogenic fungus *A. alternata* and the resistance agents used in the experiment
A- Antagonism between the pathogenic fungus *A. alternata* and the fungus *T. hirzenium*
The results shown in Figure (1) indicate that the fungus *T. hairzenium* inhibited the growth of the pathogenic fungus *A. alterata* by 90% at a concentration of 1×10^{-8}. This result is consistent with what the researchers stated regarding the ability of the *T. hairzenium* to inhibit many plant pathogens (27).

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Image (1) The contrast between the biotic fungus and the pathogenic fungus

B- Antagonism between the pathogenic fungus *A. alternata* and the fungus *T. viride*

The results, as shown in Figure (2), indicate that the fungus *T. viride* inhibited the growth of the pathogenic fungus *A. alterata* by 90% at a concentration of 1×10^{-8}. This result is consistent with the researchers' findings regarding the ability of the biogenic fungus *T. viride* to inhibit several plant pathogens (1).

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Image (2): Antagonism between the pathogenic fungus *A. alterata* and the fungus *T. viride*

C-- Effect of aqueous garlic extract on the growth of the pathogenic fungus *A. alternata*

The results in Figure (3) show the ability of the aqueous extract of garlic to inhibit the growth of the pathogenic fungus *A. alternata* by 100%. This is attributed to the presence of several sulfur compounds, which is consistent with (16 ,6).

Garlic extract was used against a range of plant pathogens, demonstrating the effectiveness of allicin in reducing fungal spore germination in vitro, such as *Alternaria brassisicola* and *Botrytis cinerea*, whether through fumigation or direct application to the site of infection. This led to a decrease in disease incidence due to its direct effect against the pathogen. Garlic preparations can be used as an alternative to synthetic fungicides and in the production of organic food. (26)

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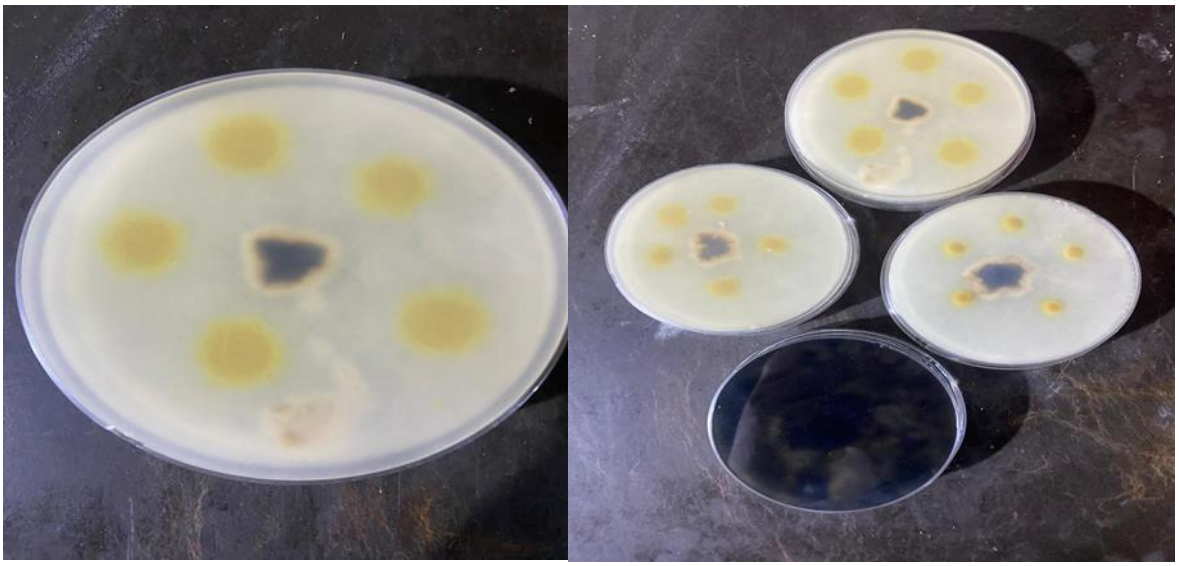

Figure (3): Shows the effect of watery garlic extract on the growth of the pathogenic fungus *Alternaria alternate*

C- Effect of the fungicide Revus top on the growth of the pathogenic fungus *A. alternata*

The results showed that Revus top pesticide was able to inhibit the growth of the pathogenic fungus A. alterata by 70% (4). This is due to the pesticide's effect on the chitin in the fungal cell walls, causing their breakdown, or the pesticide's interaction with biological enzymes and inhibition of them, or on

oxidation-reduction reactions, which affects energy production (13).

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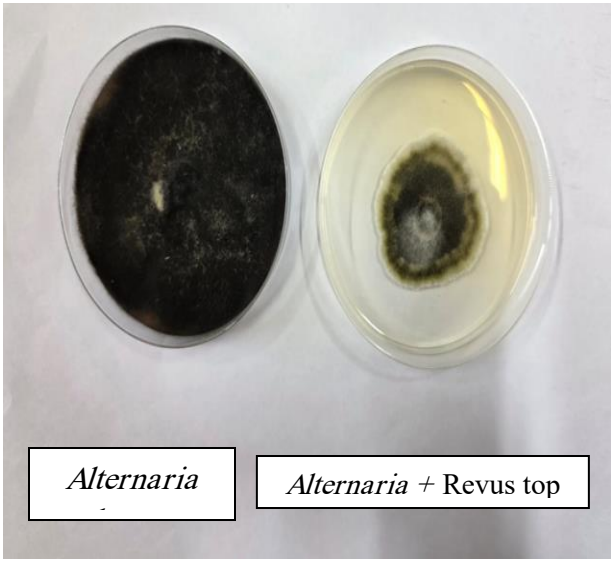

2- Effect of biological treatments and aqueous extract of garlic on the percentage of germination of eggplant seeds in the laboratory

The results in Table (1) show that dusting eggplant seeds with an aqueous garlic extract did not affect the germination rate, which reached 80%, significantly different from the control treatment which reached 90%. These results are consistent with (9) in evaluating the efficacy of plant extracts against pathogenic fungi, as garlic powder did not affect eggplant seed germination. Furthermore, the germination rate in the treatment with the fungal suspension of *T. hairzenium* + aqueous garlic extract reached 96%. With significant differences from the comparison treatment, the result agrees with (28 ,3) in the use of the fungal filtrate *T. hairzenium*, which led to an increase in the percentage and stimulation of seed germination due to the secretion of organic acids by the Biological Fungi which encourage seed germination. Treating eggplant seeds with the fungal suspension led to an increase in the percentage of seed germination, which reached 64%. The results also indicated that the treatment with the fungal suspension *T. viride* + garlic powder did not differ from the control treatment, as it reached 90%, and this is consistent with what was stated in (14).

Table (1) Effect of biological treatments and aqueous garlic extract on the percentage of eggplant seed germination in vitro

Treatment	Germination rate
Fungal Suspension *T. hirzenium* + aqueous extract of garlic	**a 96**
***Fungal Suspension T. viride* + aqueous extract of garlic**	**b 90**
aqueous extract of garlic	**C 80**

University of Thi-Qar Journal of agricultural research

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Comparative treatment	**b 90**
R.L.S.D	**2.77**

Second - Field Experiments:

1- Effect of using the fungal suspension of *T. hairzenium* and control agents on some growth indicators of eggplant seedlings.

The results in Table (2) show the superiority of the *T. hairzenium* fungal suspension treatment in its effect on seedling growth indicators, as the average seedling height reached 17.6 cm, a highly significant difference compared to the other treatments. The *T. hairzenium* fungal suspension treatment also recorded the highest increase in fresh and dry weight of the shoot system, reaching 12.22 and 3.42 g, respectively. Likewise, the same treatment was superior in increasing fresh and dry weight of the root system, reaching 2 and 1.42 g, respectively. The results are consistent with many studies showing that treating seeds with the fungal suspension stimulates systemic plant resistance, leading to the production of metabolic compounds beneficial to the plant and harmful to the pathogenic fungus (5)

The results also show that the treatment with the aqueous garlic extract was superior in increasing seedling growth indicators, with the average seedling height reaching 13.3 cm, significantly higher than the control treatment's 9.3 cm. The average fresh and dry weights of the shoots were 7 and 2.65 g, respectively, while the average fresh and dry weights of the roots were 1.51 and 1.23 g, respectively. This is significantly higher than the control treatment, where the fresh and dry weights of the shoots were 3.25 and 1.27 g, respectively, and the average fresh and dry weights of the roots were 1.19 and 1.02 g, respectively.

Table (2) Effect of using the Biological Fungi *T. hairzenium*, the fungicide Revus top, and the aqueous extract of garlic on some growth indicators of eggplant

Treatment	Plant height (cm)	The fresh weight of the vegetative parts (gm)	Dry weight of the foliage (gm)	soft weight of the roo (gm)	dry weight of the root total (gm)
Fungal Suspension *of T. hirzenium* + aqueous extract of garlic + pathogenic fungus	11.6	4.18	1.68	1.14	1.03
Fungal Suspension of T. hirzenium + fungicidal agent (Revus top) + pathogenic fungus	13.3	4.72	1.36	1.28	1.03

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pathogenic fungus+ aqueous extract of garlic	13.3	7	2.65	1.51	1.23
Fungal Suspension of *T. hirzenium* + pathogenic fungus	17.6	12.22	3.43	2	1.42
Comparative treatment	9.3	3.25	1.27	1.19	1.02
R.L.S.D	4.62	2.88	1.33	1.15	1.12

2- Effect of using theBiological Fungi *T. virid*, the fungicide Revus top, and the aqueous extract of garlic as control agents on some growth indicators of eggplant

The results in Table 3 show that the *T. virid* suspension treatment was superior in increasing seedling growth indicators, with the average seedling height reaching 3.12 cm, a significant difference from the control treatment of 8.3 cm. This was followed by the treatment with the aqueous garlic extract, where the seedling height reached 12.3 cm. The *T. virid* suspension treatment also led to an increase in the fresh and dry weight of the shoot system to 8.47 g and 1.17 g, respectively, while the control treatment increased by 0.19 g and 0.02 g, respectively. The average fresh and dry weight of the root system was 0.52 g and 0.09 g, respectively. While the control treatment reached 0.19 and 0.02 g respectively, these results are consistent with (15), which showed that the role of the biogenic fungi *Trichoderma spp*. led to an increase in the fresh weight of the shoot and root system of the tomato plant, reaching 6.39 and 5.29 g respectively, compared to the pathogenic fungus *F. oxysporum f.sp. lycopersici*, which reached 2.25 and 0.82 g respectively. The results in the same table showed that treatment with the aqueous garlic extract showed good indicators in raising and increasing the fresh and dry weight of the shoot system, reaching 6 and 1.65 respectively. The average fresh and dry weight of the root system reached 0.51 and 0.23 respectively. The results (20) are consistent with the use of the aqueous garlic extract on agricultural crops such as eggplant, cucumber, and pepper through foliar spraying or hydroponics as a biostimulant for plants, improving quality and increasing growth indicators.

The use of the fungicide Revus top has proven effective in inhibiting the growth of the pathogenic fungus, but the biological agents and the aqueous extract of garlic worked primarily to increase plant growth and stimulate systemic resistance.

Table (3) Effect of the Biological Fung *T. viride* and the insecticide Revus top (aqueous extract of garlic) on some growth indicators of eggplant.

Treatment	Plant height (cm)	The fresh weight of the vegetative parts (gm)	Dry weight of the foliage (gm)	soft weight of the roo (gm)	dry weight of the root total (gm)
Fungal Suspension *T. viride* + aqueous extract of garlic + pathogenic fungus	**12**	**3.1**	**0.35**	**0.23**	**0.04**

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Fungal Suspension *T. viride* + (Revus top) + pathogenic fungus	**9**	**2.8**	**0.38**	**0.21**	**0.03**
aqueous extract of garlic + pathogenic fungus	**12.3**	**6**	**1.65**	**0.51**	**0.23**
Fungal Suspension *T. viride* + pathogenic fungus	**14.6**	**8.47**	**1.17**	**0.52**	**0.09**
Comparative treatment	**8.3**	**2.25**	**0.27**	**0.19**	**0.02**
R.L.S.D	**3.62**	**1.88**	**0.33**	**0.15**	**0.12**

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