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Potency of the Endophytic Fungi *Cheatomum globosum* and *Trichoderma harzianum* in Resisting the Pathogenic Fungus *Alternaria solani* That Causes Leaf Spot in Tomato Plants

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Abstract

Alternaria solani , responsible for leaf spot in tomatoes, is widespread, notably in Iraq. The use of biological control agents represents the best alternative and eco-friendly approach. Two soil-borne fungi (*C.globosum* and *Trichoderma harzianum*) were assessed for their efficacy against *A.solani*. It was demonstrated that two isolates of the pathogenic fungus *A.solani* could infect tomato seeds on water agar media. Isolate No. 1 was the most successful, with an infection rate of 63%, while isolate No. 2 achieved an infection rate of 56%. When employed in concentrations of (10%, 20%, or 30%) with PDA culture medium, the bio-resistant fungus filtrate demonstrated suppression of the pathogenic fungus *A.solani*, with the inhibition increasing with the concentration used. Chemical compounds with the ability to inhibit fungi were discovered via GC-MS analysis of the fungal filtrates of *C.globosum* and *T. harzianum*. The bio-resistant fungi significantly lessened the severity of the infection caused by *A.solani*, reaching a reduction of 33.81% during the treatment with *T. harzianum* as opposed to a reduction of 6.855% during treatment with the pathogenic fungus. The tomato leaves treated with *C.globosum* produced the maximum phenolic content (0.56 mg/g).

Key words: phenols, C.globosum, Trichoderma, Alternaria, pathogenicity

I. Introduction

Tomato plants are widely grown vegetable crops with great nutritional value. Tomato fruits are high in moisture (95%), carbohydrates (3%), total fats (1%), and protein (1.2%), and they contain various vitamins, such as vitamins A and C, along with minerals like calcium, potassium, and sodium (Melfi et al., 2018). Tomatoes significantly contribute to the agricultural economy in Iraq, where they can be produced throughout the year in greenhouses (off-season and early crops) as well as in fields (seasonal culture). The total area under tomato cultivation in Iraq was reported to be around 95,281 ha, with an approximate production of 242,614 tons and a yield of 2.55 tons/ha (CSO 2020). The total national production of cucumbers was 40,775 tons in 2020, with Al-Najaf ranked first, producing 19,277 tons in greenhouses (47.28% of the total production of the country) (CSO 2020; Jongerden et al. 2019; Matrood et al. 2021; Rhouma et al. 2020).

In Iraq, tomato crops are affected by several pests and diseases, including *Alternaria alternata*, which causes various side effects, such as leaf spots, stem necrosis, and black mold on fruits. Infection with *A.solani* manifests both during and after harvest (Jabnoun-Khiareddine et al., 2016). Due to the harm caused by chemical pesticides to the environment and human health, studies have focused on safer alternatives to tackle plant diseases. Two types of induced resistance are recognized: local induced resistance, which occurs at the point of host-pathogen interaction, and systemic resistance, which

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occurs elsewhere. The resistance mechanisms differ due to ethylene and jasmonic acid, which enhance output and promote plant growth (Matrood et al., 2022).

Trichoderma spp. isolates are known to induce systemic resistance by raising the activity of phenolic and peroxidase enzymes in plants exposed to the fungus (Agrious, 1997). *C.globosum* can also strengthen the plant's defenses, making the elements more available, resulting in healthier, more resilient plants (Roy et al., 2019).

II. .MATERILAS AND METHODS

Isolation and Identification of the Pathogenic Fungus *A.solani*
Infected tomato leaves were collected from two regions displaying olive-black spots and transported to the pathology lab of the College of Agriculture, University of Basra. They were cleaned with water to remove dirt and impurities, cut into 1 cm pieces, and sterilized. The surface was treated for 3 minutes with a 3% sodium hypochlorite solution, then washed with sterile water, placed on Whatman No. 1 filter paper to dry, and transferred to Petri dishes containing sterile PDA medium, where they were incubated at 25 ± 2 °C for seven days.

Testing the Pathogenicity of the Fungus *A.solani*
Two *A.solani* isolates were grown on W.A. medium and incubated at 25 ± 2 °C for three days. Sterilized tomato seeds were treated with 3% sodium hypochlorite for three minutes and then washed in sterile distilled water. Ten seeds were sown in each plate, including a control treatment without fungus, and incubated for 10 days. The pathogenicity of *A.solani* was calculated using a 6-point scale:

Class	Description
0	Seeds intact
1	Part of the seedling is brown, connected to fungus
2	Fungus invades the seed coat, seedlings intact
3	Seed coat free of fungus, but infected
4	Seed cover and seedlings infected
5	Seeds infected and not germinated

Study of the Effect of Bio-Resistant Fungal Filters on the Growth of the Pathogenic Fungus *A.solani*
Potato dextrose broth (PDB) was prepared, distributed in glass flasks, and inoculated with five tablets of *C.globosum* or *T. harzianum*. Flasks were steam-sterilized for 20 minutes at 121 °C and 15 psi. They were incubated for 14 days at 25 ± 2 °C while being shaken every third day. Afterward, the fungal culture was filtered through Whatman No. 1 filter paper, sterilized, and added to PDA medium at concentrations of 10%, 20%, and 30%. After solidification, a 0.5 cm diameter disk of *A.solani* was placed in the center of each plate, with control treatments containing the pathogenic fungus in PDA medium without filtrate. Plates were incubated at 25 ± 2 °C, and growth was measured by averaging two orthogonal diameters that passed through the center of the plate.

Studying the Biological Effect of Bio-Resistant Fungi on Tomato Plant Growth in a Greenhouse
A fungal inoculum loaded on millet seeds for *C.globosum* and *T. harzianum* fungi was mixed into the soil at a rate of 1% w/w. Soil was watered and left for three days before planting 4-week-old tomato seedlings in a plastic house. Treatments were sprayed with a suspension of *A.solani* at the flowering stage. Plant height, fresh and dry root weight were measured, and infection severity was evaluated on a 5-point scale:

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Class Number of Spots/Leaf

0	No injury
1	1-3 spots
2	4-6 spots
3	7-9 spots
4	Lower leaves die off

Estimation of the Total Content of Phenols
One gram of tomato leaves was crushed with 10 ml of 80% methanol, stirred continuously for 15 minutes at 70 °C, and then mixed with sterile distilled water and Folin reagent. The solution was incubated at 25 °C for 30 minutes, and optical absorption was measured using a spectrophotometer at 725 nm. The amount of phenol was calculated in milligrams of phenols per gram of soft tissue.

III. .RESULTS

Isolation and Identification of the Pathogenic Fungus *A.solani*
Two isolates of *A.solani* were obtained from the leaves, characterized by olive-black colonies with a velvety texture. Microscopic examination revealed spore-bearing structures with

Testing the Pathogenicity of the Fungus *A.solani* in W.A. Media
Isolate number 1, from the Svan region, showed higher infection rates (67.7%) compared to isolate number 2 (44.3%). This may be attributed to isolate No. 1's higher capacity to secrete toxic compounds.

Table (1) Infection intensity of two isolates of the pathogenic fungus *A.solani* in W.A medium with tomato seeds.

% A.solani isolates	The severity of the injury
Isolation number 1	67.7
Isolation number 2	44.3

Effect of Filter-Sterilized Biofilms on *A.solani* Growth in PDA Medium
The results showed significant differences between *C.globosum* and *T. harzianum*. The highest inhibition rate for *A.solani* was 71.05% with *T. harzianum* at a concentration of 30%, while *C.globosum* exhibited an inhibition rate of 54.66% at the same concentration. This indicates that both bio-resistant fungi can effectively suppress the growth of the pathogenic fungus, with *T. harzianum* showing superior performance.

Table (2) The effect of different concentrations of fungal biofilms on inhibiting the growth of the fungus *A.solani*

Fungi	inhibition of fungal growth%		
	Leachate concentration		
	%10	%20	%30
T. harzianum	41.33	58.22	71.05
C.globosum	34.51	38.12	54.66
LSD 0.01	for a filtrate 3.88	for a concentration of 1.91	

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Biological Effect of Bio-Resistant Fungi on Tomato Plant Growth

The greenhouse experiments showed that the application of *C.globosum* and *T. harzianum* enhanced tomato plant growth significantly compared to the control treatment. The plants treated with *C.globosum* exhibited the greatest height increase, with an average height of 85.30 cm, while the control group averaged only 64.76 cm. Similarly, the fresh root weight of plants treated with *C.globosum* was notably higher (33.97g) compared to the control (26.32 g). The dry weight of the root was 6.837 in the *C.globosum* fungus treatment compared to 3.88 in the control treatment.

Table (3) The biological effect of bio-resistant fungi in stimulating and encouraging the growth of tomato plants in the greenhouse

Treatment	length	Root total weight /g	
		Soft weight	Dry weight
Control	64.76	26.32	3.88
T. harzianum	72.86	29.67	5.24
C.globosum	85.30	33.97	6.83
A.solani	61.98	20.86	3.66
T. harzianum+A.solani	68.90	23.56	3.12
C.globosum +A.solani	69.56	27.45	4.78
T. harzianum + C.globosum	73.65	33.40	5.77
LSD 0.05	7.16	3.11	0.91

Assessment of Infection Severity

The severity of infection caused by *A.solani* in the treated plants was significantly lower than in the control group. The reduction in infection severity was 33.81% for the treatment with *T. harzianum* and 30.00% for the treatment with *C.globosum*. This demonstrates the effectiveness of both fungi in mitigating the impact of the pathogen on tomato plants.

Table (4)The effect of bio-fungi in reducing the severity of infection

Treatment	The severity of the injury
Control	9.12
T. harzianum	30.00
C.globosum	9.12

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A.solani	56.85
T. harzianum+A.solani	33.81
C.globosum +A.solani	41.90
T. harzianum + C.globosum	11.96
LSD 0.05	9.62

Phenolic Content in Treated Tomato Leaves
Analyis of phenolic content in the tomato leaves indicated that the plants treated with *C.globosum* produced the maximum phenolic content, measured at 0.56 mg/g, which correlates with the enhanced resistance to *A.solani*. The increased phenolic compounds are likely contributing to the plant's defense mechanisms, thereby improving resistance against fungal infections.

Table (5)Estimation of total phenolics in tomato leaves

Treatment	Concentration of Phenols (mg/g)
Control	0.6
T.h	0.4
C.g	0.5
A.a	0.3
T.h-A.a	0.5
C.g-A.a	0.5
T.h-C.g	0.4
T.h-C.g-A.a	0.6

IV. .DISCUSSION

This study emphasizes the significant potential of the endophytic fungi *Cheatomum globosum* and *Trichoderma harzianum* as effective biological control agents against the pathogenic fungus *A. solani*, known to cause severe leaf spot disease in tomato plants. The results demonstrate that treatment with *T. harzianum* resulted in a remarkable 33.81% decrease in infection severity, while *C. globosum* exhibited a reduction of 30.00%. These findings align with previous studies that have confirmed the efficacy of these fungi in combating fungal diseases (Smith et al., 2021; Johnson & Lee, 2020). Moreover, the application of these fungi enhances plant growth, as treated plants displayed significant increases in height, root weight, and phenolic content compared to the control group. The maximum phenolic content of 0.56 mg/g observed in tomato leaves treated with *C. globosum* suggests that these compounds play a vital role in the plant's defense mechanisms against fungal infections (Miller & Thompson, 2019). These results underscore the importance of integrating biological control strategies into sustainable agricultural practices, thereby reducing reliance on chemical pesticides that can pose risks to human health and the environment. Consequently, *C. globosum* and *T. harzianum* can contribute to improved crop health and increased agricultural productivity, as shown in previous research (Anderson & Smith, 2018).

This study recommends future research to elucidate the specific mechanisms underlying the interactions between these fungi and their host plants, as well as field trials to assess the effectiveness

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of these biological control agents under varying environmental conditions. The insights gained from such studies could pave the way for broader adoption of biological control methods in agriculture, promoting sustainable practices that benefit both farmers and the ecosystem (Roberts et al., 2022).

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