Effect of Some Biological Agents on Fungi Isolated from Roots and Soil around it of *Cupressus* spp. Trees

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Abstract

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Relative occurrence of fungi associated with six root and six soil samples from the rhizosphere of Cupressus/cypruss trees with root rot, leaf blight or wilt symptoms were investigated. Results of the root samples (average of six samples) showed that the relative occurrence of *Fusarium culmorum* was the highest (36.81%), followed by *F. solani* (29.7%), *Alternaria. alternate* (14.19%), *F. oxysporum* (10.67%), *R. solani* (5.51%), *Pythium* spp. (1.69%), *Helminthosporium* spp. (0.85%), *Bipolaris* spp. (0.24%) and *Stemphilium herbarum* (0.18%). However, the average occurrence in the six soil rhizosphere samples was the highest for *F. solani* (41.28%), followed by *R. solani* (19.76%), *F. culmorum* (16.5%), *F. oxysporum* (8.77%), *Bipolaris* spp. (6.17%), *A. alternata* (3.99%), *Pythium* spp. (2.70%), *Stemphylium herbarum* (0.60%), and *Helminthosporium* spp. (0.27%). Results also showed that *Trichoderma harzianum* had a high antagonistic efficiency against the three studied fungi isolated from cypress tree roots and the surrounding soil, namely *F. culmorum*, *F. solani* and *Helminthosporium* spp. Based on 1-5 scale, the inhibition level was 1.0 against each of the pathogenic fungi *F. culmorum*, *F. solani*, and 2.0 against *Helminthosporium* spp. The results also showed that the use of *B. subitlis* as bacterial bio-control agent led to a significant inhibition of the growth of the three isolated fungi, *F. culmorum*, *F. solani* and *Helminthosporium* spp. cultured on PDA medium.

Keywords: Bio-control agents, *F. culmorum*, *F. solani*, Cypress trees.

Introduction

The *Cupressus* (cypress) is a plant genus belonging to the family Cupressaceae, and the cypress is an evergreen tree that is cultivated to control soil erosion, and planted in public gardens for its beauty. This tree is abundant in temperate climates, including northern Iraq, and it is currently cultivated in all countries of the Mediterranean basin (Gerald *et al.*, 2013).

Fungi with other microorganisms such as bacteria, viruses, and some animals and plants play an important role in the fertility of the cypress soil. At the same time, soil-borne fungi can cause rotting of seeds and roots, damping-of seedlings and wilting of plants, causing great economic losses (Agrios, 2005). *Fusarium oxysporum*, *Pythium aphanidermtum* and *Rhizoctonia solani* are among the most important and most pathogenic causes that infect cypress trees, as they cause seed rot, seedling death and tree wilt (Marin-Felix *et al.*, 2017).

The leaves of the affected plant or the affected plant part lose their turgidity and become wilted, light green to greenish yellow in color, and the leaves finally wither, turn brown and die. If a cross-section is made in the affected stems and branches, brown colored areas appear in the form of a complete or an incomplete ring. Vascular tissue can be clogged with fungal mycelium and conidia or by polysaccharide compounds produced by the pathogenic fungi (Soltani & Moghaddam, 2015).

The objective of this study is to identify fungal pathogens associated with the roots and rhizosphere of *Cupressus* spp. trees infected with root rot and the effect of some biocontrol agents for their control.

Materials and Methods

Isolation of fungi

Six roots samples and another six samples from the soil rhizosphere of different cypress trees showing root rot, leaf blight or vascular wilt symptoms and planted in the gardens of Mosul University were collected. The isolation of fungi from soil rhizosphere samples was carried out by the direct method, taking 1 gm of soil and spreading it in sterile conditions on the surface of a Petri dish containing potato dextrose agar (PDA) medium containing the antibiotic chloramphenicol, with three replicates for each sample. The dishes were then incubated upside down in an incubator at 25 °C for one week.

Trees roots were washed with water to remove the suspended soil particles, then cut into pieces of 1 cm long and surface sterilized by dipping in a sodium hypochlorite (NaOCl) solution at a concentration of 1% for three minutes, and five pieces were placed in each dish containing PDA medium with the antibiotic chloramphenicol added at a concentration of 0.05 mg/L, in five replicates. The dishes were then incubated upside down in an incubator at a temperature of 25°C for a week.

Identification of fungi

The growth of fungal cultures isolated from the soil surrounding the root zone of cypress trees was examined microscopically by taking part of the fungal growth by a sterile inoculation needle and placing it on a glass slide containing a drop of lactophenol solution and spreading the sample in the loading drop, then placing the slide cover and examining microscopically to identify the characteristics of

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mycelium and spores according to the approved taxonomic keys (Domach *et al*., 1980; Ellis, 1971; Leslie & Summerebll, 2006; Nelson *et al*., 1983). The slide culture technique was used to observe the mycelium and the arrangement of spore chains of fungal isolates using light microscopy (Harris, 1986).

Purification of fungi by single spore isolation
The studied fungi were purified using the single spore technique except for *R. solani* (Nelson *et al*., 1983).

Calculation of the fungal relative occurrence
The relative fungal occurrence (%) was calculated as follows:

$$\% \text{ Fungus occurrence} = \frac{\text{The number of fungus colonies in the samples}}{\text{Total number of colonies}} \times 100$$

Antagonistic ability test of fungal bio-control agent *Trichoderma harzianum* and bacterial bio-control agent *Bacillus subtilis* against *F. culmorum, F. solani* and *Helminthosporium* spp. in vitro
The antagonistic ability of *T. harzianum* was tested by double culture of the bio-control agent with any of the three isolates, *F. culmorum*, *F. solani* and *Helminthosporium* spp. in 9 cm petri dishes, containing PDA medium divided from its outer base by a wax pen into two halves. A 0.5 cm disk of a seven days of fungal bio-control colony was placed in the first half with a similar disk of the pathogenic fungus colony placed in the second half placed in an inverted manner so that the mycelium touches the surface of the medium, and the space between the two discs was 4 cm, with five replicates for each pathogenic fungus. Plates inoculated with each of the pathogenic fungi or the bio-control agent, served as controls. The results obtained were recorded one week after incubation at 28°C, and the degree of antagonism was determined according to Bell *et al.* (1982).

The antagonistic ability of *Bacillus subitlis* was tested based on the method of Al-Jubouri (2012), by inoculating Petri dishes containing PDA medium by making two parallel lines of *B. subitlis* suspension on both sides of the Petri dish at a concentration of 1×10^5, with a 5 cm distance between them using a sterile loop. A 0.5 cm disc taken from the edges of young cultures of *F. culmorum*, *F. solani* and *Helminthosporium* spp. was placed in the center of the dish with five replicates for each fungus, and the dishes were incubated at 28°C. The results obtained were recorded when the control dishes (without bacteria inoculation) were completely filled with fungal growth. Inhibition rate was calculated as follows:

$$\text{Inhibition rate (\%)} = \frac{\text{A-B}}{\text{A}} \times 100$$

Where: A= the mean diameter of the colony in the control treatment, B= the mean diameter of the colony in the different treatments

Results and Discussion

Isolation of fungi from the Rhizosphere of cypress trees
Table 1 summarizes the isolated fungi and their relative occurrence in the rhizosphere of cypress trees that showed symptoms of infection with vascular wilt caused by root rot fungi. The average occurrence in the six soil rhizosphere samples was the highest for *F. solani* was 41.28%, followed by *R. solani* 19.76%, *F. culmorum* 16.5%, *F. oxysporum* 8.77%, *Bipolaris* spp. 6.17%, *A. alternata* 3.99%, *Pythium* spp. 2.70%, *Stemphylium herbarum* 0.60%, and *Helminthosporium* spp. 0.27%. Relative occurrence in each soil sample is shown in Table 1.

Isolation of fungi from the roots of cypress trees
Table 1 summarizes the isolated fungi and their relative occurrence in the roots of cypress trees infected with root rot. Results obtained from the root samples (average of six samples) showed that the relative occurrence of *Fusarium culmorum* was the highest 36.81%, followed by *F. solani* 29.7%, *Alternaria. alternate* 14.19%, *F. oxysporum* 10.67%, *R. solani* 5.51%, *Pythium* spp. 1.69%, *Helminthosporium* spp. 0.85%, *Bipolaris* spp. 0.24% and *Stemphilium herbarum* 0.18%. Relative occurrence in each sample is shown in Table 1.

Trees in natural forests and those managed on plantations of native or non-native species are under increased threat from attack by fungal diseases (Ghelardini *et al*., 2017; Wingfield *et al*., 2015), The genus *Seiridium* includes several important plant pathogenic species that infect *Cupressus* spp., such as *S. cardinale*, *S. cupressi* and *S. unicorne* (Barnes *et al*., 2018; Bonthond *et al*., 2018; Marin-Felix *et al*. 2017), but none of these pathogens was detected in this study.

Reduced growth or death of cypress plants is considered a serious threat to cypress forests around the world (Crous *et al*., 2015). The main pathogens reported earlier to be associated with the roots of cypress trees are *F. oxysporum*, *Cylindrocarpon destructants*, *Phoma exigua*, *Gnomonia fructicola*, *Phytophthora cactorum*, *Pythium ultimum* and *Macrophomina phaseolina* (Xiao *et al.*, 2004). Association of plant pathogenic fungi with sample No. 3 collected from plants that did not show symptoms of vascular wilt disease could be due to either the infection was early, or the plant was tolerant to the invading fungi.

Assay of antagonistic ability of fungal bio-control agent *T. harzianum* against fungal pathogenic agents *F. culmorum, F. solani* and *Helminthosporium* spp. on PDA medium
Results obtained indicated that the fungal bio-control agent *T. harzianum* had a high antagonistic efficiency against the three studied fungi isolated from the roots of cypress trees and the surrounding soil, namely, *F. culmorum, F. solani* and *Helminthosporium* spp. Based on a 1-5 scale, the inhibition level was 1.0 against each of the pathogenic fungi *F. culmorum, F. solani,* and 2.0 against *Helminthosporium* spp.

Table 1. Fungi isolated from the roots of the studied samples of cypress trees and the soil surrounding the roots and their occurrence rate.

Symptoms appeared on the vegetative parts	Rhizosphere fungi		Root fungi	
	Isolated fungi	% occurrence	Isolated fungi	% occurrence
Sample No. 1				
Leaf bight and wilting of most branches	*F. solani*	70.14	*F. culmorum*	47.12
	R. solani	11.28	*F. solani*	45.03
	F. oxysporum	10.2	*F. oxysporum*	3.21
	A. alternata	6.32	*R. solani*	2.63
	Stemphylium herbarum	2.06	*Helminthosporium* spp.	2.01
Sample No. 2				
Leaf blight and wilting of most branches	*F. solani*	66.43	*F. solani*	54.30
	F. culmorum	12.71	*F. culmorum*	21.56
	R. solani	9.50	*F. oxysporum*	12.30
	A. alternata	10.0	*R. solani*	8.22
	Helminthosporium spp.	1.36	*Bipolaris* spp.	1.46
			A. alternata	1.11
			Stemphylium herbarum	1.05
Sample No. 3				
No vascular wilt symptoms appeared on the vegetative part	*R. solani*	59.87	*A. alternata*	85.14
	F. solani	23.91	*F. solani*	12.44
	Pythium spp.	16.22	*Pythium* spp.	2.42
Sample No. 4				
Leaf blight and wilting of some twigs	*F. solani*	55.71	*F. culmorum*	75.17
	Bipolaris spp.	37.02	*F. solani*	15.12
	R. solani	5.75	*Pythium* spp.	7.71
	St. herbarum	1.52	*Helminthosporium* spp.	2.0
Sample No. 5				
Leaf blight and wilting of some twigs	*F. culmorum*	40.41	*F. culmorum*	30.91
	R. solani	32.17	*F. oxysporum*	26.06
	F. oxysporum	19.78	*R. solani*	22.22
	A. alternate	7.64	*F. solani*	20.81
Sample No. 6				
Severe wilt of vegetative part of the trees + death of most branches and roots	*F. culmorum*	45.91	*F. culmorum*	46.12
	F. solani	31.51	*F. solani*	30.30
	F. oxysporum	22.58	*F. oxysporum*	22.47
			Helminthosporium spp.	1.11

The results obtained also showed that the use of *B. subtilis* as bacterial bio-control agent led to a significant inhibition of the growth of *F. culmorum*, *F. solani* and *Helminthosporium* spp. cultured on PDA medium.

The antagonistic ability of *T. harzianum* is likely due to the secretion of antibiotics or degrading enzymes which inhibited the growth of the three soil-borne fungi (Ibrahim, 2009). It has been reported that this fungus produces many antibiotics such as Trichodermin, Trichodermol, Harzianum A and Harzianolide that control cotton wilt caused by *F. oxysporum* f. sp. *vasinfectum* (EL-Bondkly & Talkhan, 2007). The inhibitory effect may also be due to the production of volatile compounds such as esters and alcohols that can inhibit many phytopathogenic fungi (EL-Bondkly & Talkhan, 2007). The results obtained in this study are consistent with the findings of Al-Dujaili (2008) that the isolates of *T. harzianum* have a high antagonistic efficiency against *F. oxysporum.* It can be concluded from this study that the bio-control agents *T. harizanum* and *B. subtilis* have good potential to control cypress root rot. However, more field evaluation is still required to confirm the practical value of such treatments.

الملخص

العامري، هديل أحمد. 2024. تأثير بعض المكافحات الحيوية في الفطريات المعزولة من جذور أشجار السرو (*Cupressus* spp.) والتربة المحيطة بها. مجلة وقاية النبات العربية، 42(2): 224–228. https://doi.org/10.22268/AJPP-001233

تمّ التحري عن الفطور المصاحبة لـ 6 عينات من الجذور و 6 عينات من التربة المحيطة بمنطقة جذور أشجار السرو (Cypruss) المصابة بتعفن الجذور، لفحة الأوراق أو أعراض الذبول. أظهرت النتائج أنه في عينات الجذور (متوسط 6 عينات) كانت أعلى نسبة وجود للفطر *Fusarium culmorum* (36.81%) يليه

الفطر *F. solani* (29.7%)، ثم الفطر *Alternaria. alternata* (14.19%) والفطر *F. oxysporum* (10.67%)، والفطر *R. solani* (5.51%)، والفطر *Pythium* spp. (1.69%)، والفطر *Helminthosporium* spp. (0.85%)، *Bipolaris* spp. (0.24%) و *Stemphilium herbarum* (0.18%). وكان أعلى متوسط للظهور في عينات التربة الستة هو للفطر *F. solani* حيث وصلت إلى 41.28%، يليه *R. solani* (19.76%)، *F. culmorum* (16.5%)، *F. oxysporum* (8.77%)، *Bipolaris* spp. (6.17%)، *A. alternata* (3.99%)، *Pythium* spp. (2.70%)، *Stemphylium herbarum* (0.60%)، *Helminthosporium* spp. (0.27%). كما أوضحت النتائج أن عامل المكافحةالحيوية *Trichoderma harzianum* ذو كفاءة تضادية عالية ضدّ الفطور الثلاثة المدروسة المعزولة من جذور أشجار السرو والتربة المحيطة بها، وهي: *F. culmorum* و *F. solani* و spp *Helminthosporium*. عند اعتماد مقياس 1–5، كانت درجة التثبيط 1.0 ضدّ كلٍ من الفطور *F. culmorum* و *F. solani*، وبلغت 2.0 ضدّ *Helminthosporium* spp. كما أظهرت النتائج أن استخدام البكتيريا *B. subitlis* كعامل مكافحة حيوية أدى إلى تثبيط نمو الفطور المعزولة الثلاثة *F. culmorum* و *F. solani* و *Helminthosporium* spp. التي زرعت على الوسط الغذائي PDA.

كلمات مفتاحية: عوامل مكافحة حيوية، *F. culmorum*، *F. solani*، شجر سرو

عناوين الباحثين: هديل أحمد العامري، قسم البيولوجيا، كلية العلوم، جامعة الموصل، الموصل، العراق. البريد الالكتروني للباحث المراسل: Hadsbio34@uomosul.edu.iq

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