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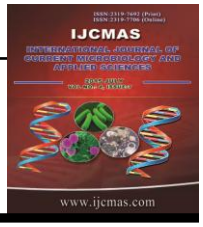

Original Research Article

In vitro and *In vivo* Efficiency of *Trichoderma harzianum* against *Phoma* and *Glocladium* Soft Rot Occurred on Tomato Fruits (*Lycopersicon esculentum*)

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Keywords

Phoma sp., soft rot, *Glocladium sp.*, *Trichoderma harzianum*, *Lycopersicon esculentum*, In vitro, In vivo, confrontation

A B S T R A C T

The present investigation aims to evaluate the *in vitro* and *in vivo* ability of *T.harzianum* to control the *Phoma* and *Glocladium* soft rot, that occurred on tomato fruits (*Lycopersicon esculentum*). *Phoma sp.* and *Glocladium sp.* were isolated from infected tomato fruits, which were brought from Oum-elbouaghi market, and identified in laboratory of microbiology, university of Oum-elbouaghi (Algeria).One isolate of *T.harzianum* / *Hypocrea lixii* was brought from the same laboratory. The results of direct confrontation (*in vitro*) of *T.harzianum* against *Phoma sp.* and *Glocladium sp.* on PDA medium, showed that a different inhibition in the mycelia growth of the tested fungus. That inhibition was equal in the fourth day of the experiment to 39.58 % and 25.92% in *Phoma sp.* and in *Glocladium sp.* respectively. The microscopic observations of mycelia showed that the mycelia of *T.harzianum* was capable of overgrowing and degrading mycelia and chlamydospores of *Phoma sp.*, coiled around the mycelia of *Phoma sp.* and *Glocladium sp*. However, it did not show any growth of the tested fungus when re-planting a disk from the interaction hyphal area between *T.harzianum* and *Phoma sp.* or *Glocladium sp.* from dual cultures, while *T.harzianum* grew alone in plates. *In vivo* screening and after 7 days of incubation *T.harzianum* showed an antagonistic activity against the tested fungus on tomato fruits, with inhibition equal 71.43% and 100%, in *Phoma sp.* and in *Glocladium s.p* respectively, compared with controls. Beside we found after cutting the superficial layer of the tested tomato fruits, that the treated fruits with *T.harzianum* stayed saints, compared with control, when *Glocladium* rot infected their deep tissues. This strain of *T.harzianum* may offer potential for biological control of tomato *Phoma* and *Glocladium* soft rot.

Introduction

Fungi of the genus *Phoma* are at present one of the more widespread ones in respect of their geography, and they consist of a large number of species, which one can find in

varied ecological niches. From among 3000 taxa described so far, 110 are pathogenic species often infecting those plant parts that are important from the economic point of view (Aveskamp *et al*. 2008). *Phoma* is a cosmopolitan genus of coelomycetous fungi. Many species have been reported from wide range of hosts, substrates, particularly as pathogens from plants, as well as soil-borne but predominantly saprophytic and opportunistic species have also been isolated (Irinyi *and al*., 2007). In India, average loss due to *Phoma parasitica* was reported to be 7-10% in winter season (Aulakh and Grover, 1969). Leorakkar *et al*. (1986) recorded tomato crop losses up to 100% due to *Phoma audina* in Columbia. Some species belonging to the *Hypocrea* and *Gliocladium* genera have been known as agents of green mold disease, which affects cultivated mushrooms such as *Agaricus bisporus*, *Lentinula edodes* and *Pleusotus ostreatus* (Savoie and Mata (2003) ; Park *and al*.(2005)).*Gliocladium viride* an anamorph of *Hypocrea lutea* as agent of green mold was isolated in Korea from oak log beds used for shiitake (*Lentinula edodes*) cultivation that were infested by mushroom flies(Jun *and al*., 2010).

The aim of the present investigation was to evaluate the *in vitro* and *in vivo* efficiency of *T.harzianum*, to control the *Phoma* and *Glocladium* soft rot occurred on tomato fruits (*L.esculentum*).

Materials and Methods

Fungal strains

Phoma sp. and *Glocladium sp.* were isolated from infected tomato fruits, which were brought from Oum-elbouaghi market, and identified based on the microscopic observations of their reproductive and colony characteristics in laboratory of microbiology, university of Oum-elbouaghi (Algeria) (Robert *and al.*, 1981; Botton *et al.*, 1990; Rémi, 1997). A local strain of *T.harzianum* / *Hypocrea lixii* , was identified in the same laboratory and verified in Walloon Center of Biology Industrial, University of Liege, Belgium.

In vitro evaluation of the antagonistic capability of *T.harzianum* against *Phoma sp.* and *Glocladium sp.*, on PDA medium (direct confrontation)

To study the direct confrontation between *T.harzianum* and *Phoma sp.* or *Glocladium sp.* Two plugs of mycelium (8mm diameter) were cut from the margins of actively cultures growing on PDA medium, one carrying the stock of *T.harzianum* and the other of *Phoma sp.* or *Glocladium sp.* Then they placed at the periphery of Petri plates (9cm in diameter) at the same distance on PDA medium(dual cultures). One plug of *Phoma sp.* or *Glocladium sp.* were maintained as controls (alone cultures). Each replicate has three plates. Both the dual and alone cultures were incubated at 25°c for four days, and measurement of colony diameters (in millimeters) was taken every 24 hours. The percentage of inhibition growth (I) was calculated by using the formula given below : [I (%) = (1 –T /C) x 100]. Where: I=Percentage inhibition of pathogen growth by antagonists. C=Radial growth in control. T=Radial growth in the treatment (Fadwa *and al.*, 2009; Mokhtar and Aid, 2013).

Preparation of tomato fruits

Intact red tomatoes (*L.esculentum* Mill.), uniform in size and color, were obtained from the market of Oum-elbouaghi city. The fruits were surface-sterilized by soaking in 2% aqueous sodium hypochlorite for 5 min, they were thoroughly rinsed with sterile distilled water, dried using sterile filter

papers, and then wounded by removing a rectangular area at the equator of each fruit, (3cmx4cm) in diam. and 3 mm in depth, from the surface, using a sterile scalpel (Imane *and al.*, 2012).

In vivo evaluation of the antagonistic capability of _T.harzianum_ against _Phoma sp._ and _Glocladium sp._ on tomato fruits.

Fresh cultures of *Phoma sp.*, *Glocladium sp.* and *T.harzianum* were used for each experiment to evaluate the antagonistic activity. Two plugs of mycelium (8mm diameter) were cut from the margins of actively cultures growing on PDA medium, one carrying the stock of *T.harzianum* and the other of *Phoma sp.* or *Glocladium sp.*, were then placed one beside of the other at the center of the rectangular area of the tomato fruits. As control, fruits were either inoculated with *Phoma sp.* or *Glocladium sp.* alone.

The fruits were then stored at 20°C± 2.for 7 days in autoclaved glass jars with hermetic covers. The percentage of disease reduction of *Phoma* or *Glocladium* rot on tomato fruits, was calculated using the following formula: (%) = (A-B)/A×100, where A is the lesion diameter recorded in tomato fruit inoculated with the *Phoma sp* or *Glocladium sp* alone, and B is the lesion diameter recorded in infected tomato fruits treated with *T.harzianum*. All *in vivo* antagonism assays were made in triplicate (Imane *and al.*, 2012).

Results and Discussion

In vitro evaluation of the antagonistic capability of _T.harzianum_ against _Phoma sp._ and _Glocladium sp._ on PDA medium (direct confrontation).

The results of the direct confrontation of *T.harzianum* against *Phoma sp.* and *Glocladium sp.* on PDA medium, showed that when the mycelium of the both cultures came in contact with each other, the hyphal growth of *Phoma sp.* or *Glocladium sp.* were found to be inhibited by hyphae of *T.harzianum* fig.(1.1 and.2.1). That inhibition was equal in the fourth day of the experiment to 39.58 % and 25.9%, in *Phoma sp.* and in *Glocladium sp.* Respectively (table1).

Microscopic observations showed that the mycelia of *T.harzianum* was capable of overgrowing and coiling around the hyphae of *Phoma sp.* and *Glocladium sp.* and degrading them. fig(1.5 and 2.4), compared with controls. fig(1.7 and 2.5). Besides, did not show any growth of *Phoma sp.* or of *Glocladium sp.* when re-planting the disks from the interaction hyphal area between *T.harzianum* and *Phoma sp.* or *Glocladium sp.* from dual cultures, while *T.harzianum* grew alone in the plates. Fig(1.3 and fig.2.3).

In vivo evaluation of the antagonistic capability of _T.harzianum_ against _Phoma sp._ or _Glocladium sp._ on tomato fruits.

After 7 days of incubation, *T.harzianum* showed an antagonistic activity against *Phoma sp.* and *Glocladium sp.* on tomato fruits, with inhibition equal 71.43% and 100%, in *Phoma sp.* (fig. 3.2), and in *Glocladium sp.* (fig.4.2) respectively compared with controls Fig (3.1 and 4.1).

Beside we found after cutting the superficial layer of the tomato fruits under the tested rectangular area(fig.4.4 and 4.6), that the treated fruits with *T.harzianum* stayed saints(fig. 4.5), compared with control, when *Glocladium* rot infected their deep tissues(fig.4.3).

Table.1 in vitro. Effect of *T.harzianum* on the mycelia growth of *Phoma* sp. and *Glocladium* sp., on PDA medium

		Radial growth rate (mm) after:				
	Fungus species	24 hour	48 hour	72 hour	96 hour	**Percentage inhibition of mycelia growth**
Dual culture	***Phoma sp.***	2	16	20	20	39.58
	T.harzianum	10	60	90	90	/
Alone culture	***Phoma sp.***	4	20	32	40	/
Dual culture	***Glocldium sp.***	3	17	20	20	25.92
	T.harzianum	12	49	90	90	/
Alone culture	*Glocldium sp.*	3	18	24	36	/

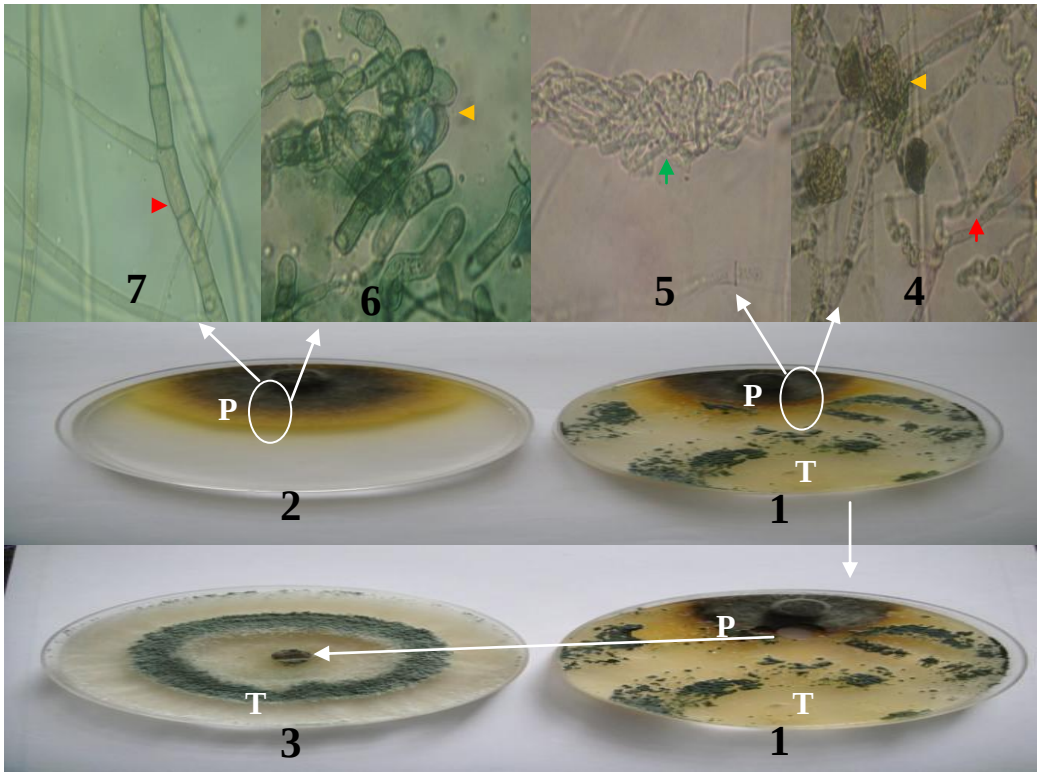

Figure.1 *In vitro* effect of *T.harzianum* against *Phoma sp*. dual culture(1), control (2), re-planting plate (3). Microscopic observations (magnification: 10 × 40 observation); decomposition (lyses) phenomenon(4); mycoparasitism phenomenon(5); Contol (6 and 7). red arrows= *Phoma* hyphal. Orange arrows = *Phoma chlamydospores.* green arrow = *Trichoderma* hyphal coiling around of *Phoma* hyphal. P= *Phoma*, T=*Trichoderma*

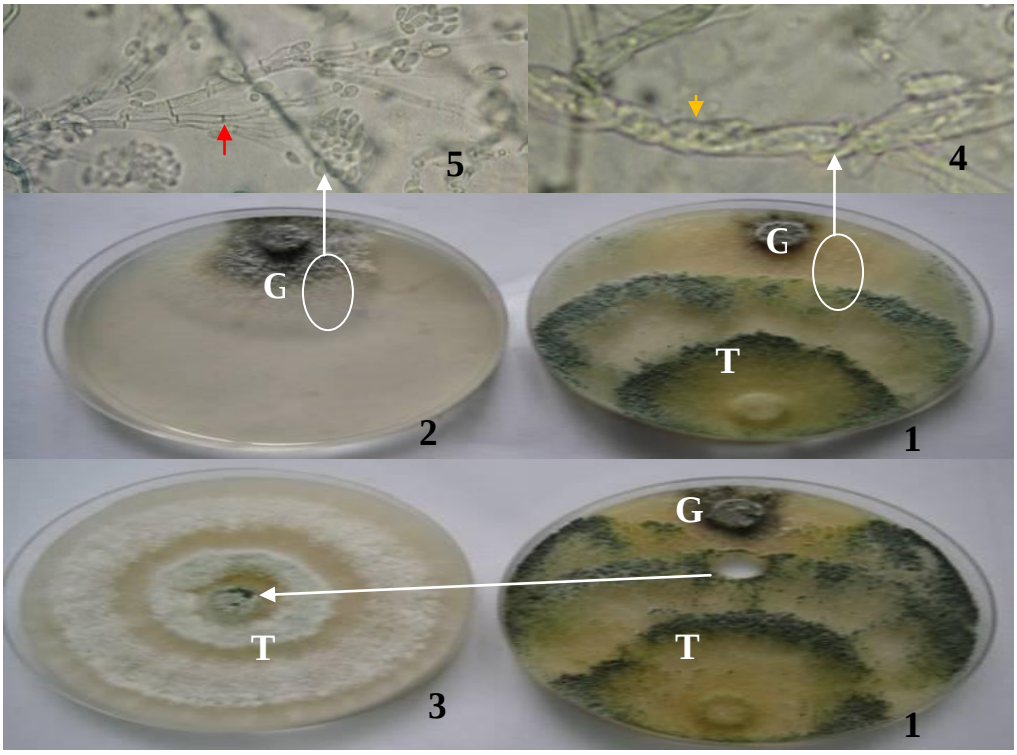

Figure.2 *In vitro* effect of *T.harzianum* against *Glocladium sp*. dual culture(1), control (2), re-planting plate (3). Microscopic observations (magnification: 10 × 40 observation); mycoparasitism phenomenon(4); Contol (5). red arrows= *Phoma* conidiophore with spores.. Orange arrows = *Trichoderma* hyphal coiling around of *Phoma* hyphal. G= *Glocldium*, T=*Trichoderma*

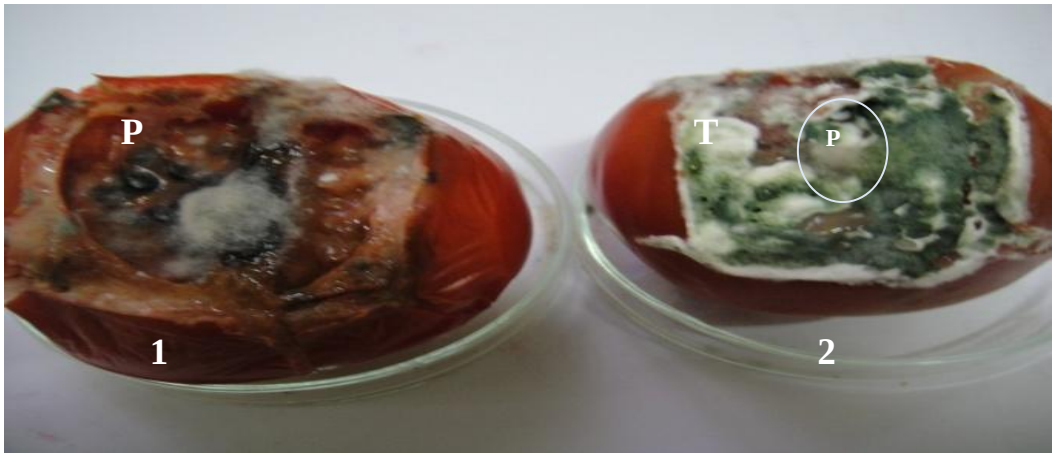

Figure.3 *In vivo* effect of *T.harzianum* against *Phoma sp*. control(1), dual culture(2). P=*Phoma*. T=*Trichoderma*

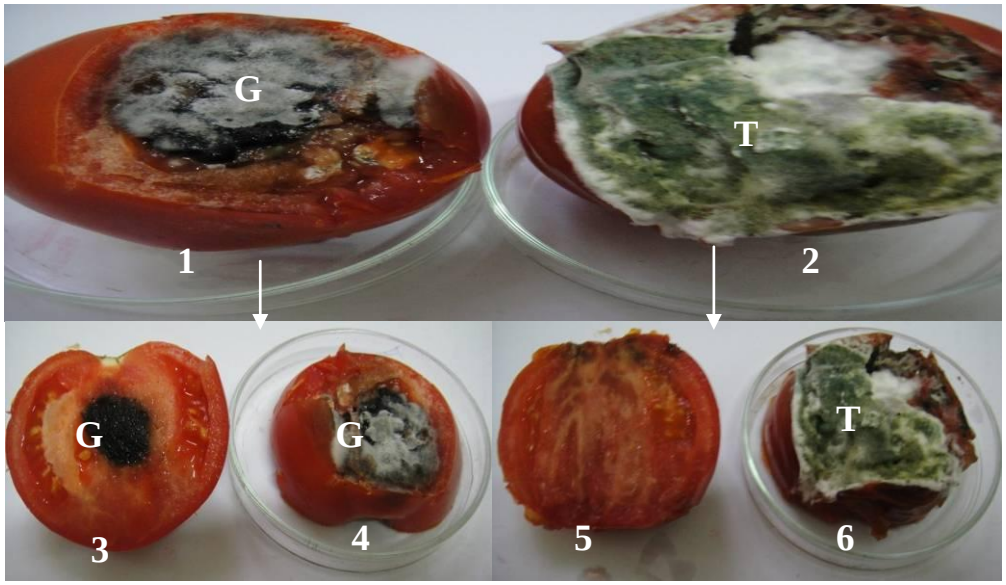

Figure.4 *In vivo* effect of *T.harzianum* against *Glocladium sp*. control(1), dual culture(2). Control after cutting the superficial layer(3). The superficial layer of control(4). Dual culture after cutting the superficial layer(5). The superficial layer of dual culture(6). G= *Glocldium*, T=*Trichoderma*

In this investigation, this local strain of *T. harzianum* showed a high efficiency both *in vitro* and *in vivo* against *Phoma sp.* and *Glocladium sp.* This results confirm by many of the published studies, when found that the *T.harzianum* inhibited the growth of *Bipolaris sp., F.oxysporium* , *Fusarium sp.* and *R.solani* with a different ratios, and inhibited there spore's formation, with recording a different degrees of parasitism (Fadwa *and al* .(2009) ; Hibar *and al*. (2005); Comporota, (1985); Azza and Allam (2004)). Beside the microscopic observations showed that the *T.harzianum* destroyed mycelia and spores of *Alternaria alternata, A.infectoria, Stemphylium botryosum, Botrytis cinerea, Cladosporium sp.*, and produced haustoria on mycelia of this tested isolates through mycoparasitism (Mokhtar and Aid, 2012; 2013). *T.harzianum* strains produced a metabolites inhibited the growth of *G.graminis* var. *tritici*, *F.culmorum* and *F.moniliforme* on PDA medium, when grown in liquid cultures containing laminarin, chitin or fungal cell walls as sole carbon sources, these metabolites were 1 , 3- b- glucanase and chitinase (Cigdem and Merih, 2004).*T.harzianum* reduced disease incidence significantly against *P.ultimum* and *R.solani* on both cucumber and tomato on greenhouse (Johanne *et al.*, 2002). Biological efficacy of *Trichoderma sp* against *B.cinerea* was assessed using foliar discs of strawberry, lesion development and number of conidiophores due to *Botrytis sp* was significantly reduced on treated foliar discs with this strain, compared with the non –treated(control)(Yacoub, 1999). This local strain of *T.harzianum* may offer potential for biological control of tomato *Phoma* and *Glocladium* soft rot.

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