AGRICULTURE AND BIOLOGY JOURNAL OF NORTH AMERICA
ISSN Print: 2151-7517, ISSN Online: 2151-7525, doi:10.5251/abjna.2014.5.6.240.244

In vitro and *in vivo* efficiency *of Trichoderma harzianum* against *Rhizopus* soft rot occurred on tomato fruits (*Lycopersicon esculentum*).

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ABSTRACT

The present investigation aims to evaluate the *in vitro* and *in vivo* ability of *T.harzianum* to control the *Rhizopus* soft rot, that occurred on tomato fruits (*Lycopersicon esculentum*). *Rhizopus stolonifer* was isolated from infected tomato fruits, which were brought from Oum-elbouaghi market, and identified in laboratory of microbiology, university of Oum-elbouaghi (Algeria). *T.harzianum* / *Hypocrea lixii*) was brought from the same laboratory. The results of direct confrontation (*in vitro*) of *T.harzianum* against *R.stolonifer* on PDA medium, showed that an inhibition in the mycelia growth of *R.stolonifer*, it was equal in the fourth day of the experiment to 43.66 %. The microscopic observations of mycelia showed that the mycelia of *T.harzianum* was capable of overgrowing and degrading *R.stolonifer* sporangiophores and sporangiospores. Besides, it coiled around the sporangiophores of *R.stolonifer* with appressoria structure. However, it did not show any growth of *R.stolonifer* when re-planting a disk from the interaction hyphal area between *T.harzianum* and *R.stolonifer* from dual culture, while *T.harzianum* grew alone in the plate*. In vivo* screening of *T.harzianum* showed an antagonistic activity against *R.stolonifer* on tomato fruits with 82.86% inhibition after 7 days, however the tomato fruits stayed intact, compared with control, where *Rhizopus* soft rot destroyed the tomato fruits. This strain of *T.harzianum* may offer potential for biological control of tomato *Rhizopus* soft rot.
Key words: *Rhizopus* soft rot, *Trichoderma harzianum, Lycopersicon esculentum*, appressoria structure.

INTRODUCTION

More than 40 species of *Rhizopus* was identified, most usually met on the seeds was *R.*stolonifer on all varieties of seeds, *Rhizopus* are generally saprophytes which, under certain conditions of development, can invade tissues of the plants or the fruits (bean, tomato, strawberry,..), they cause, sometimes, catastrophic rots, in particular during the transport (Rémi, 1997). Rhizopusis omnipresent on stored organs of plants, when the epidermal cells are collapsed, the fungus emerges through the wounds and produces aerial sporangiophores, sporangia, stolons, and rhizoids, the latter capable of piercing the softened epidermis (Agrios, 1997). Head rot caused by *R.stolonifer* reduces sunflower seed yield and quality (Ismet *and al.*, 2010). *Rhizopus* soft rot is a one of the most costly postharvest diseases of sweet potatoes(Scot, 2009).*R.stolonifer* was isolated from pears in conservation in the cold room in Oulmès (Maroc) (Ilhame *and al,* 2008). In the spring of 2001, Jin-Hyeuk*and al*. found that a disease suspected as *Rhizopus* soft rot occurred on cherry tomato (*Lycopersicon esculentum*) in Jinju City Agricultural Products Wholesale Market, the infection rate of the disease in some containers reached to 6.7%, *Rhizopus* attacked only cracks of matured fruits of cherry tomato, but not young and immature ones.

The aim of the present study was to evaluate *in vitro* and in *vivo* ability of *T.harzianum* to control the *Rhizopus* soft rot occurred on tomato fruits (*Lycopersicon esculentum*).

MATERRIALS AND METHODS

Fungal strains: *R.stolonifer* was isolated from infected tomato fruits (fig.2.1), 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 *R.stolonifer*, on PDA medium (direct confrontation):** To study the confrontation between *T.harzianum* and *R.stolonifer* , two plugs of mycelium (8mm diameter) were cut from the margins of actively growing PDA cultures, one carrying the stock of *T.harzianum* and the other of *R.stolonifer* were then placed at the periphery of Petri plate (9cm in diameter) at the same distance on PDA medium. One plug of *R.stolonifer* was maintained as control (alone culture). Each replicates 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). The speed of fungal colony growth (V) was measured by using the formula given below: V = [(L2 -L1) + (L3 - L2) ... (Ln-Ln -1] / n -1, V = the speed of growth (mm / day), L =mycelia growth (mm), L1 = growth in the first day. Ln = growth in the last day . D = (D1 + D2) / 2. L = D - d / 2. L = the growth of the fungal mycelia (mm), D = diameter of the fungal colony (mm), d = diameter of the initial fungal disk(Rapilly, 1968).

Preparation of tomato fruits: Intact red tomatoes (*Lycopersicon 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, 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 *R.stolonifer* on tomato fruits:** Fresh cultures of *R.stolonifer* 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 growing PDA cultures, one carrying the stock of *T.harzianum* and the other of *R.stolonifer,* were then placed one beside of the other at the center of the rectangular area of the tomato fruit. As control, fruits were either inoculated with *R.stolonifer* alone. The fruits were then stored at 20°C for 7 days in autoclaved glass jars with hermetic covers. The percentage of disease reduction of *Rhizopus* soft 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 *R.stolonifer* alone, and B is the lesion diameter in infected *Rhizopus* soft rot tomato fruit treated with *T.harzianum*. All *in vivo* antagonism assays were made in triplicate (Imane *and al.*, 2012).

RESULTS

***In vitro*.Evaluation of the antagonistic capability of *T.harzianum* against: *R.stolonifer*on PDA medium (direct confrontation):** The results of the direct confrontation of *T.harzianum* against *R.stolonifer* on PDA medium, showed that when the mycelium of the both cultures came in contact with each other, the hyphal growth of *R.stolonifer* was found to be inhibited by hyphae of *T.harzianum*, that inhibition was equal in the fourth day of the experiment to 43.66%(table1). Microscopic observations showed that the mycelia of *T.harzianum* was capable of overgrowing and degrading *R.stolonifer* sporangiophores and sporangiospores (fig.1.a), coiling around the sporangiophores of *R.stolonifer* with appressoria structure (fig.1.b), compared with control (Fig.1.c). The present results did not show any growth of *R.stolonifer* when re-planting a disk from the interaction hyphal area between *T.harzianum* and *R.stolonifer* from dual cultures, while *T.harzianum* grew alone in the plate(fig.1.3).

Table 1: *In vitro*. Effect of *T.harzianum* on the mycelia growth of *R.stolonifer*, and speed of mycelia growth in dual, and alone cultures, on PDA medium.

		Radial growth rate (mm) after:					
	Fungus species	24 hour	48 hour	72 hour	96 hour	Percentage inhibition of mycelia growth	Speed of mycelia growth(mm/day)
Dual culture	*R.stolonifer*	10.5	40	36	20	43.66	3.16
	T.harzianum	5	20	30	40	/	11.66
Alone culture	*R.stolonifer*	10	39	65	75	/	21.66

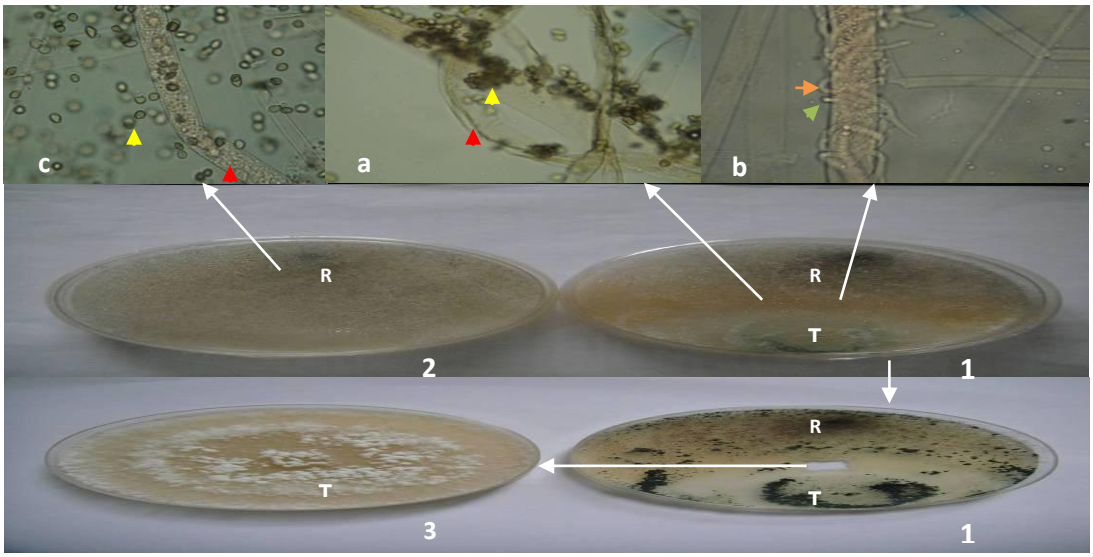

Figure 1. *In vitro* effect of *T.harzianum* against *R.stolonifer*. dual culture(1), control (2), re-planting plate (3). R= *Rhizopus*, T=*Trichoderma* . Microscopic observations (magnification: 10 × 40 observation), decomposition (lyses)phenomenon(a), mycoparasitism phenomenon(b), control(c). yellow arrow = sporangiospores, red arrow = sporangiophore, orange arrow = *Trichoderma* hyphal coiling around of *Rhizopus* sporangiophore , green arrow = appressoria structure of *Trichoderma*.

***In vivo.* Evaluation of the antagonistic capability of *T.harzianum* against *R.stolonifer* on tomato fruits.**

T.harzianum showed an antagonistic activity against *R.stolonifer* on tomato fruits with 82.86% inhibition after 7 days. However, tomato fruits stayed intact (fig. 2.4), compared with control, where *Rhizopus* soft rot destroyed tomato fruits (fig.2.3).

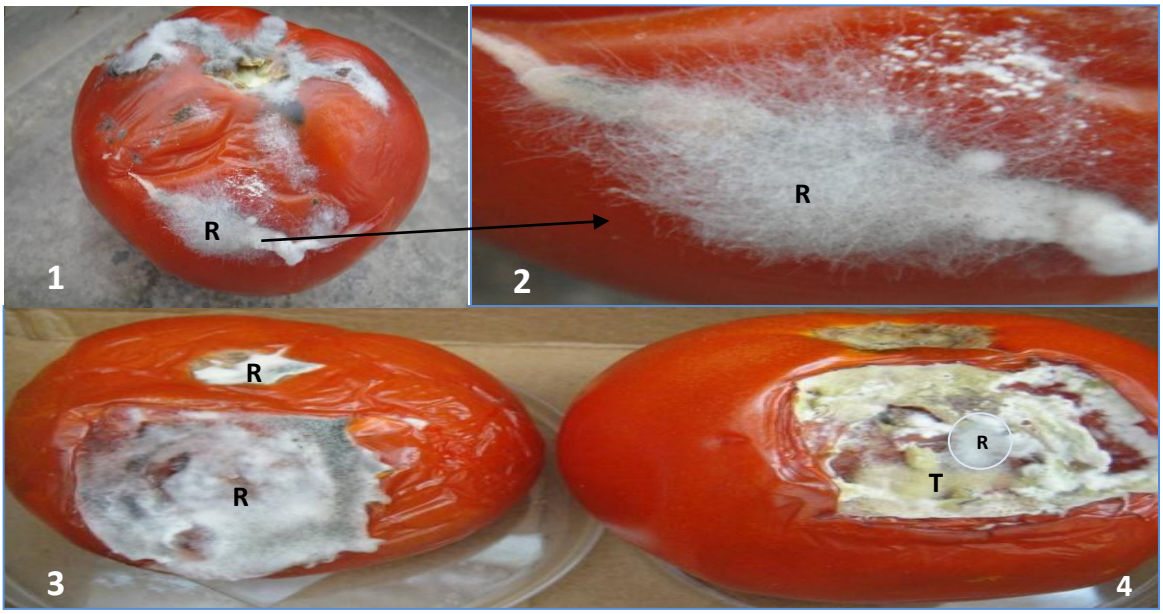

Fig. 2. *In vivo* effect of *T.harzianum* against *R.stolonifer.* Infected tomato fruit which was brought from Oum-elbouaghi market(1); infected lesion with *Rhizopus* soft rot (2). *In vivo* test. Control(3), total inhibition by *T.harzianum* (4). R=*Rhizopus*. T=*Trichoderma*.

DISCUSSION

In this investigation *T. harzianum* showed a high activity both *in vitro* and *in vivo* confrontation against *R.stolonifer*, this results confirm by observations of Fadwa *and al* .(2009) when found that the *T.harzianum* and *T.viride* inhibited the growth of six isolates of *Bipolaris* with a different ratios, however they inhibited the spore's formation, with recording a different degrees of parasitism. *T.harzianum* inhibited *F.oxysporium* growth with a ratio more than 65% , compared with control (Hibar *and al*., 2005).Besides, it showed a different effect on 3 isolates of *R.solani* Kuhn, which affected the mycelia growth with a different degrees (Comporota, 1985).The results of the *in vitro* study of the antagonistic ability of *T.harzianum,* against *Alternaria alternata, A.infectoria, Stemphylium botryosum, Botrytis cinerea, Cladosporium sp*, indicated the inhibition of mycelium growth to varied degrees, and microscopic observations showed that *T.harzianum* induced cell lyses, destroyed mycelia and spores of the tested isolates, and produced haustoria on mycelia of tested isolates through mycoparasitism (Mokhtar and Aid, 2012; 2013).Larrade *and al*. (2008) chose 9fungal isolates of *Trichoderma* : 2 of *T.atroviride*, 2 of *T.longibrachiatum* and 1 of each *T.reesi* and*T.koningiopsis* and *T.citrinoviride* and 2 did nospecific type from 30 isolates of *Trichoderma*, where they inhibited the growth of *Macrophomina phaseolina* with proportions higher than 50% during the antagonism study, the microscopic observations in the hyphal interaction showed that the antagonistic fungus had an ability to analyze the hyphae and sclerotes of the pathogenic fungus , the analysis of the metabolic substances of these antagonistic fungi in laboratory revealed that there is a positive correlation between the strength of inhibition of these fungi with the high quantity of enzymatic production of B-1, 3glucanase and N-acetylhexosaminade. Azza and Allam (2004) discovered that the *Trichoderma* isolates have a strong antagonism against wilt diseases caused by *Fusarium sp*, when

its growth decreased in PDA medium with the following proportions: 88%, 86% and 80% for *T.harzianum*, *T.hamatum* and *T. viride* respectively. Interactions between *T.harzianum* strains and some soil borne plant pathogens *G.graminis* var. tritici, *F.culmorum* and *F.moniliforme* were studied on PDA medium, and was found that the all tested *T.harzianum* strains produced a metabolite inhibited the growth of plant pathogenic fungi on PDA medium. When grown in liquid cultures containing laminarin, chitin or fungal cell walls as sole carbon sources, 2 strains of *T.harzianum* produced 1 , 3- b- glucanase and chitinase in the medium, higher levels of these enzymes were induced by *T.harzianum* T15 (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* was significantly reduced on treated foliar discs with this strain, compared with the non –treated(control)(Yacoub, 1999). This strain of *T.harzianum* may offer potential for biological control of tomato *Rhizopus* soft rot.

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