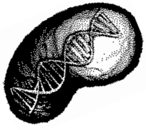

Journal of Microbiology,
Biotechnology and
Food Sciences

El-Katatny & Emam 2012 : 1 (6) 1505-1528

REGULAR ARTICLE

CONTROL OF POSTHARVEST TOMATO ROT BY SPORE SUSPENSION AND ANTIFUNGAL METABOLITES OF *TRICHODERMA HARZIANUM*

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ABSTRACT

Rot of cherry tomato (*Lycopersicon esculentum*) fruits caused by several fungal pathogens is a detrimental disease leading to substantial yield loses worldwide. *Alternaria* isolates were the most common fungal species isolated from healthy or rotten fruits. *Trichoderma harzianum* spore suspension and culture filtrate were tested for their antagonistic activity on controlling tomato fruit rot. *T. harzianum* isolates suppressed or interfered with the growth of different postharvest tomato fungal pathogens albeit at different degrees. Their culture filtrate inhibited pathogen spore germination possibly due to the released extracellular diffusible metabolite(s). Besides, aberrant morphology of conidia was observed with deformation of hyphal tips. Furthermore, the resulting mycelia appeared desiccated with coagulated protoplasm leading to complete collapse of protoplasm in presence of *T. harzianum* culture filtrate. Application of *T. harzianum* spores to tomato fruits decreased disease severity significantly with the most profound effect at higher spore concentrations (10^8 cells per ml). Similarly, culture filtrate of *T. harzianum* prevented pathogen spore germination on the surface of tomato fruits leading to decreased incidence of rot symptoms at high culture filtrate concentrations. This work provides strong evidence that *T. harzianum* is a competent antagonist and its spore suspension and culture filtrate can be used efficiently to control postharvest tomato rot.

Keywords: *Trichoderma harzianum*, Biological control, Postharvest, Tomato, Pathogens

INTRODUCTION

Microbial decay is one of the main factors that determine losses and compromises the quality of the fresh produce. The extent of postharvest losses varies depending on commodities and country. These loses range between 4–8% in countries where refrigeration facilities are well developed to 50% where these facilities are minimal **(Eckert and Ogawa, 1985).**

Cherry tomato (*Lycopersican esculentum*) represents a valuable source for improving the status of dietary antioxidants (lycopene, ascorbic acid and phenols) in our diet **(George *et al.,* 2004)**. There are numerous microorganisms that cause postharvest tomato decay and these are nearly ubiquitous in nature. Once harvested, fruits and vegetables have a limited postharvest life. Naturally occurring senescence leads to softening of tissues and often a loss of antioxidant and antimicrobial activities. As the fruit begins to senescence and proceed to an overripe stage, the pectin is converted to pectic acid by the enzyme pectinase. Pectic acid imparts the characteristic mushy texture to overripe fruit **(Whitaker, 1996)**. Delay in senescence of tomato could be achieved using a pre-determined hormic dose of UV-C radiation **(Maharaj *et al.,* 2010)**. It was proposed that ethylene is the plant hormone responsible for fruit ripening as well as senescence of vegetative tissues **(Crocker *et al.,* 1935)**.

Harvested tomato fruits carry heavy spore loads while in the field. As a result of poor packaging and improper management, the fruits get bruised and squeezed allowing different types of rots to develop when the favorable growth conditions are available. Tomato fruits are beset with problems of both field and storage rot. Therefore, handling methods that preserve the fresh-harvest quality of the product are also likely to minimize the development of decay **(Mahovic *et al.,* 2004)**.

Ramsey and Link (1932) identified 20 different fungi in postharvest decay of tomato. The main postharvest pathogens that have been reported for cherry tomato include *Alternaria alternata* **(Feng and Zheng, 2007)** and *Rhizopus stolonifer* **(Stevens *et al.,* 1997)**. *Alternaria alternata* is a common postharvest pathogen that causes fruit black rot at high frequency **(Feng and Zheng, 2007; Wang *et al.,* 2008)**.

Several kinds of synthetic fungicides have been successfully used to control the postharvest decay of fruits and vegetables **(Adaskaveg *et al.,* 2004; Kanetis *et al.,* 2007)**.

However, there are two major concerns: (a) the increasing consumer concern over pesticide residues on foods **(Wisniewski and Wilson, 1992)**; (b) the predominance of fungicide-resistant strains due to excessive use of fungicides **(Naseby *et al.,* 2000; Rosslenbroich and Stubler, 2000)**. Therefore, there is a need for new effective means of postharvest disease control that poses less risk to human health and the environment. Currently, several promising biological approaches that include microbial antagonists **(Schena *et al.,* 1999; Xi and Tian, 2005)** have been advanced as potential alternatives to synthetic fungicides to control postharvest decay of cherry tomato fruits.

The use of biological control requires further understanding of the mechanism(s) of action of microbial antagonists and natural products, innate and induced resistance in the host and the biology of decay pathogens. This will lead to new, innovative approaches for controlling of decay due to postharvest disease **(Droby, 2006)**. Biocontrol of postharvest diseases of different fruits using antagonistic microorganisms isolated from plant tissues has been successfully achieved **(Janisiewicz and Korsten, 2002; Zheng *et al.,* 2005; Zheng *et al.,* 2007)**. Some antagonists were successfully applied in biocontrol of postharvest diseases of cherry tomato fruit **(Schena *et al.,* 1999; Wang *et al.,* 2008)**.

Antagonistic *Trichoderma* species are considered as promising biological control agents against numerous phytopathogenic fungi **(Sarhan *et al.,* 1999; Mohamed and Haggag, 2006)**. *Trichoderma* spp. are among the most studied fungal BCAs and commercially marketed as biopesticides, biofertilizers and soil amendments **(Harman, 2000; Harman *et al.,* 2004; Lorito *et al.,* 2004)**.

Weinding and Emerson (1936) observed that some *Trichoderma* species excrete extracellular compound which was named gliotoxin. Many antibiotics and extracellular enzymes were also isolated and characterized later, and the biocontrol mechanisms became clearer **(Naseby *et al.,* 2000; Kubicek *et al.*, 2001; Harman *et al.*, 2004; Pal and McSpadden Gardener, 2006; Tariq *et al.,* 2010)**. It was suggested that the production of antifungal metabolites, extracellular enzymes, and antibiotics are responsible for the ability of *Trichoderma* to control the growth of pathogens **(El-Katatny *et al.,* 2001; El-Katatny *et al.,* 2006; Shoulkamy *et al.,* 2006; Montealegre *et al.,* 2010)**.

Two isolates of *T. harzianum* (T3 and T24) from the collection of our laboratory were previously tested for their potential role as biocontrol agents and their antagonistic action mechanisms against phytopathogens **(El-Katatny *et al.,* 2001; Shoulkamy *et al.,* 2006; El-Katatny *et al.,* 2006)**. The aim of this study was to investigate the efficiency of *T. harzianum*

in controlling the postharvest disease caused by some fungal pathogens in fresh cherry tomato fruit.

MATERIAL AND METHODS

Tomato fruits

Cherry tomato (*Lycopersicon esculentum*) fruits were purchased at various different local vegetable markets at El-Minia Governorate, Egypt, with a commercial level of maturity. Fruits were brought in clean polythene bags to the laboratory. Fruits were used immediately or stored at 4°C for no longer than 48h before use. Before treatments (for biocontrol assay or pathogenicity test), fruits were washed with tap water, surface-disinfected with 10% ethanol for 2 minutes, cleaned with distilled water and air dried prior to use.

Antagonists

The two isolates of *Trichoderma harzianum* (T3 and T24) used in this investigation were obtained from Department of Botany and Microbiology, Minia University, Egypt. T3 and T24 strains were isolated by **Shaban (1986)** from soil samples collected from El-Minia Governorate, Egypt and were morphologically identified by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Isolation of pathogenic fungi from healthy fruits

Thirty tomato fruits (Group 1) were sorted with similar size, appearance and freedom from decay and injury, then were transferred immediately to the laboratory in a clean plastic bags. Aliquots of 100 ml sterilized distilled water each were added to the bags which were gently shaken by hand for 5 minutes. One ml of washing water was transferred aseptically to a sterilized Petri dish before pouring the potato dextrose agar (PDA; Sigma, St. Louis, MO) medium.

Sixty tomato fruits were sorted with similar size, appearance and freedom from decay and injury. One half of the individual samples (Group 2) were incubated in a clean sterilized plastic bags for a week at 28°C, whereas, the other half (Group 3) were incubated in a clean

sterilized plastic bags in a refrigerator (at 4°C) for 15 days. The growing moulds were transferred aseptically from rotten fruits to the Petri dishes containing PDA.

Five plates containing PDA were prepared for each sample. All plates were incubated at 28°C for 7 days. The resulting fungi were examined microscopically and identified.

Isolation of pathogenic fungi from diseased fruits

Fifty tomato fruits (Group 4) with fungal rot symptoms (Diseased) were collected, from local markets in Minia Governorate and individually placed in a clean plastic bag. The fungal fragments were picked up from the fungal mass formed on the fruit surface and transferred to Petri dishes containing PDA. Tomato tissues were picked onto sterile filter paper using a sterile forceps and then wrapped with filter paper for 3–5 minutes. The dried infected tissues were placed onto several prepared sterile plates of PDA and the plates were incubated at 28–30°C for 3–5 days. The different fungi that grew from infected tissues were sub-cultured on separate sterile PDA plates and the resulting fungi were microscopically examined and identified.

Fungi were identified morphologically at the mycology unit of the Department of Botany and Microbiology, Minia University, Egypt. All fungi isolates were maintained on PDA at 4°C.

Preparation of antagonist and pathogen inoculum

Conidial stocks for both the antagonistic and pathogenic fungi were prepared from fresh cultures grown on PDA. Plates were flooded with sterilized distilled water and the conidia were gently scraped with sterile inoculation needle. The conidial suspension was stirred for 10 minutes and the hyphal debris was removed by filtration through fine mesh sieve. The conidial concentration was determined by the dilution plate method according to **Chung and Hoitink (1990)**. Spore concentration was adjusted to $1x10^6$, $1x10^7$ and $1x10^8$ colony-forming units (CFU) ml^{-1} for the antagonists and $1x10^3$, $1x10^5$ for the pathogens.

Trichoderma spp. was grown on potato dextrose broth (PDB) at 28°C for 5 days. Fungal biomass was removed by dual filtration through filter paper and a 0.45 micrometer filter. Culture filtrates were used at concentrations of full strength (100%), 50% and 25% concentrations. Culture filtrates of the fungal strains were filter-sterilized by Millipore filter (0.22 micrometer).

Assay of the antimicrobial activity of *Trichoderma* culture filtrate

The effect of *T. harzianum* T3 and T24 (growing on potato dextrose broth, PDB) culture filtrate on spore germination of the pathogens was tested in two ways:

i) In the first method **(Droby *et al.*, 1997)** spores of the harvested pathogen were suspended in *T. harzianum* cell-free culture filtrate. Culture filtrate of biocontrol-inactive *T. harzianum* was used as control. The concentration of the pathogen spores was adjusted to 1×10^5 spore ml^{-1} and the suspension was incubated in test tubes at 30ºC for 5 days. Pathogen growth was compared visually.

ii) The second method was carried out according to **Shoulkamy *et al.* (2006)**. In this method 40 µl of the spore suspension of each of pathogen (1×10^5) was mixed with 40 µl of sterilized culture fluid of *T. harzianum* on a sterile glass slide placed in sterile Petri dish. Plates were lined with wetted filter paper to maintain moisture and were incubated at 28ºC. Slides were examined after 48 hours by light microscopy to observe spore germination. Mycelial growth was examined and growth abnormality was monitored and photographed. PDB in which a biocontrol-inactive *T. harzianum* strain had been grown was used for control.

Pathogenicity test on healthy tomato fruits

Fresh tomato fruits visibly free of any physical damage and disease symptoms were used in this study. Fruits were washed and surface-disinfected before treatment with pathogens. Fruits were dipped individually in spore suspension of each pathogen at different concentrations (10^3 and 10^5 $CFU.ml^{-1}$) for about 5 min with shaking. Control fruits were dipped in sterile water instead. The excess water and spore suspension was drained off over a sterile filter paper. Fruits were placed in clean and sterile polythene bags containing moistened wet filter paper to create a micro-humidity chamber and incubated at 28 ± 1°C for 7 days. The intensity of fruit rotting was evaluated visually and scored on a scale ranging from – for lack of rot up to ++++ for severe rot.

Biocontrol assay on tomato fruits:

i) Using *T. harzianum* (T3 and T24) spore suspension:

Fresh healthy fruits were washed, surface-disinfected and inoculated with 10^5 CFU.ml^{-1} of each pathogen. Fruits were then treated by dipping in different concentrations (10^6, 10^7 or 10^8 CFU.ml^{-1}) of antagonist spore suspension. Fruits were examined after 5 and 7 days and the intensity of fruit rots was evaluated as previously described. The percentage of infected fruits was recorded after 15 days to assess the effect of time on the antagonist activity.

ii) Using *T. harzianum* (T3 and T24) culture filtrate

Culture filtrates of *T. harzianum* cultures in a full strength (undiluted, 100%), 50% and 25% dilution of the filtrate were tested. Fruit were dipped in *T. harzianum* culture filtrate at different concentrations (25, 50 and 100%) for 5 minutes. Pre-treated fruits were then inoculated with spore pathogen suspension (10^5 CFU.ml^{-1}). Fruits were incubated for 5 or 7 days and examined to measure the fruit rot incidence. The percentage of rotten fruits was calculated after 15 days to determine the ability of culture filtrate to control postharvest tomato pathogen at prolonged time period.

Design of experiment and statistical analysis

Experiments were carried out in at least three replicates. Each treatment contained about 250 g (four fruits) for each test. Results were statistically analyzed using the SPSS for Windows (Release 10.0.1) computer package and mean comparison was by LSD (Least Squared Difference) Test. Mean values of all replicates and repetitions were compared at 0.05 level of significance.

RESULTS

Fourteen different fungi were isolated from healthy or rotten tomato fruits and were identified as *Alternaria* species (four isolates), *Aspergillus niger* (three isolates), *Aspergillus*

flavus (two isolates) and one isolate of each of *Geotricum candidum, Penicillium steckii, Rhizopus* sp., *Fusarium* sp., and *Aspergillus* sp.

Fungi associated with healthy and diseased tomatoes

The data presented in figure 1 show the percentage frequency of fungal species isolated from both healthy and diseased tomato fruits. Mycological analysis of 50 samples of rotten tomatoes yielded three fungal species belonging to three genera. *Alternaria* sp. (4) caused rotting in 68% of diseased tomato fruits examined. *Geotricum candidum* came next with 22% of the samples. *Fusarium* sp. occupied the third position and caused rotting in 10% of the tested diseased fruits.

From healthy tomatoes of Group (1) *Alternaria* sp. (1) showed high frequency of occurrence representing (50%). Whereas, in healthy tomatoes of Group (2), *Alternaria* sp. (2) was shown in a high frequency of occurrence (70%) followed by *Aspergillus niger* (2) (40%). Finally, healthy tomatoes of Group (3) that were incubated for 15 days in a refrigerator, *Alternaria* sp. (3) reported high frequency of occurrence and presenting (40%) followed by *Rhizopus* sp. (35%) (Figure 1).

Effect of *T. harzianum* culture filtrate on pathogen spore germination

Spore germination in test tubes of the tested pathogens was greatly inhibited by full strength culture filtrate of *T. harzianum*. The results indicate that *T. harzianum* grown on PDB released extracellular diffusible metabolite(s) that inhibited spore germination of the tested tomato postharvest pathogens.

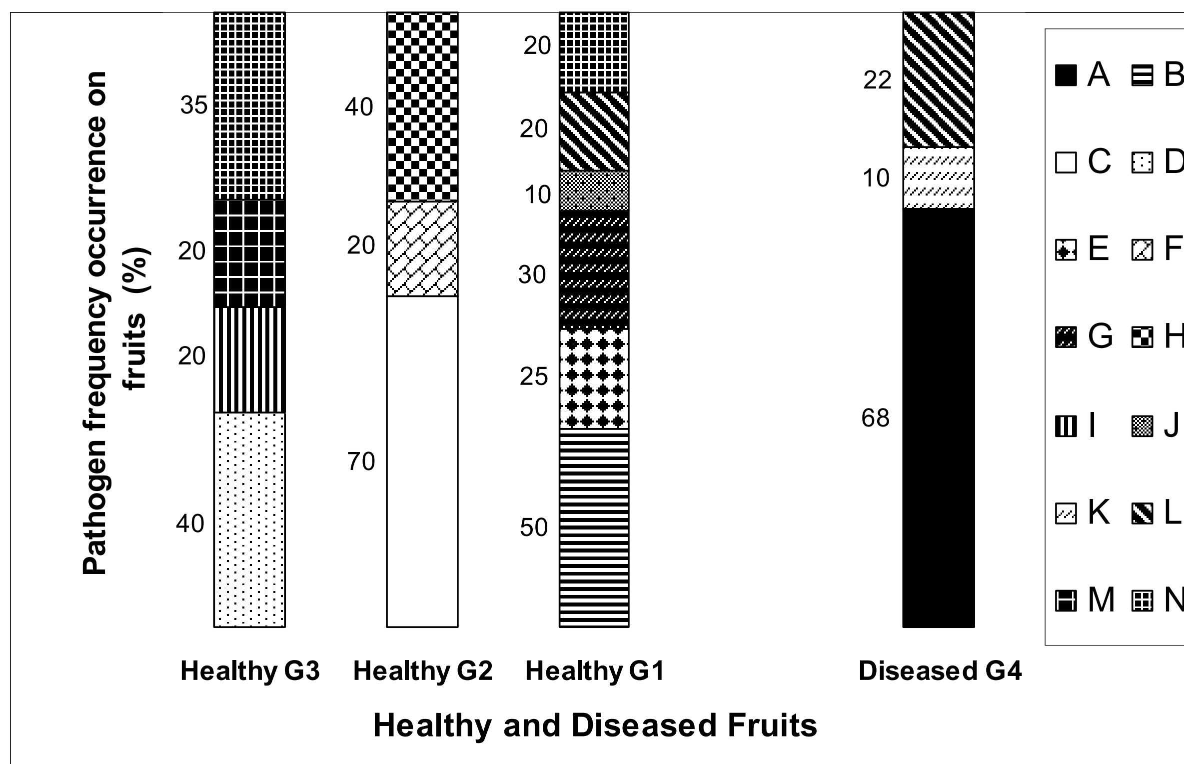

Figure l Frequency of fungi occurrence on both healthy and diseased tomato fruits. *A = Alternaria* sp. (4)*; B = Alternaria* sp. (1)*; C = Alternaria* sp. (2)*; D = Alternaria* sp. (3)*; E = A. flavus* (1)*; F = A. flavus* (2)*; G = A. niger* (1)*; H = A. niger* (2)*; I = A. niger* (3); *J = Aspergillus* sp.; *K = Fusrium* sp.; *L = Geotricum candidum; M = Penicillium steckii; N = Rhizopus* sp.

Similarly, metabolites in culture filtrate of *T. harzianum* on glass slides greatly inhibited spores germination of all the tested pathogenic fungi compared with fresh PDB of control. Non-germinated pathogen conidial spores in the presence of culture filtrate produced short, swollen germ tubes of uneven diameter. Moreover, aberrant morphology of conidia was observed with deformation of hyphal tips with slower growth when compared with control. For example, addition of T3 or T24 culture filtrate with conidia of *Alternaria* sp. (1), the resulting mycelia (after about 48 hours) showed occurrence of bubbles, vacuoles, swelling hyphae and swollen germ tube (Figure 2). Moreover, hyphae of the most tested pathogens appeared desiccated with coagulated protoplasm and swollen short hyphae followed by the complete destruction and protoplasm collapse. In contrast, in absence of culture filtrate, vegetative hyphae were morphologically normal, long, and smooth-walled with no swelling or vacuolization (Figure 2).

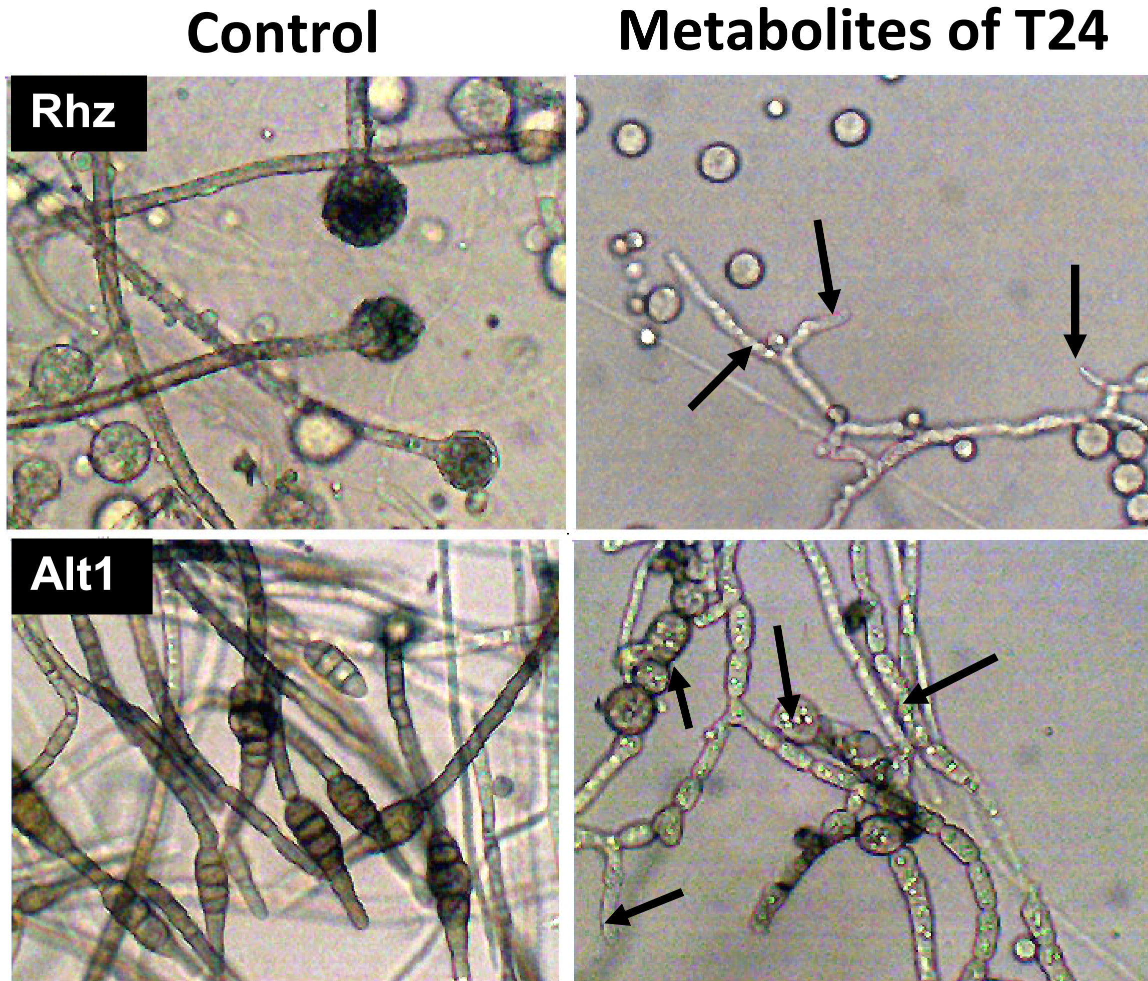

Figure 2 Effect of crude culture filtrate from *T. harzianum* (T24) on conidial germination inhibition of Rhz, *Rhizopus* sp. and Alt1, *Alternaria* sp. (1), after incubation for 48 hours, at 28ºC. Controls showing normal germinated spores and healthy hyphal growth, and the treated with metabolites of T24 showed the occurrence of bubbles, vacuoles, swelling hyphae, in addition to deformation of hyphal tips with slower growth (arrows showing the antifungal effect) (320 x)

Pathogenicity test on healthy tomato fruits

All the isolated fungi were virulent, however, *Rhizopus* sp. was the most virulent pathogen with the highest rot index. In contrast, *A. flavus* (1) showed no rotting at the lower spore concentration (10^3 CFU.ml^{-1}) and exhibited moderate rot (++) at 10^5 CFU.ml^{-1} after 7-days incubation period (Table 1).

Table 1 Pathogenicity* of fourteen different fungal isolates from healthy or rotten tomato. Isolates were tested at two different concentrations (10^3 and 10^5 $CFU.ml^{-1}$). Shown results were detected 7 days after inoculation

Pathogen	**$CFU.ml^{-1}$**	
	10^3	**10^5**
Alternaria sp. (1)	-	++++
Alternaria sp. (2)	+	++++
Alternaria sp. (3)	+	++++
Alternaria sp. (4)	+	++++
A. flavus (1)	-	++
A. flavus (2)	+	++++
A. niger (1)	+	+++
A. niger (2)	+	++
A. niger (3)	+	++++
Aspergillus sp	+	+++
Fusarium sp.	+	++++
Geotricum candidum	+	++++
Penicillium steckii	+	++++
Rhizopus sp.	+++	++++

(The numbers [1-4] between the parentheses after the species name referred to the group number)

* Pathogenicity: The intensity of fruit rots for each isolated fungi were evaluated as the following: - = no rot (no symptom), + = low rot (rot symptoms less than 10%), ++ = middle rot (rot symptoms less than 50), +++ = high rot (rot symptoms less than 100%), ++++ = severe rot (no healthy tissue visible)

Biocontrol assay on tomato fruits

Biocontrol of postharvest tomato pathogens by *T. harzianum* spore suspension

The effect of the treatment with *Trichoderma* spore suspension of T3 and T24 strains on control of rots in cherry tomato fruit caused by the fourteen fungal pathogens is shown in Table 2. In absence of spore suspension, fruits exhibited middle to severe rotting intensity after 7 days. In contrast, treatment with *Trichoderma* spore suspension (10^6, 10^7 and 10^8 cells per ml) increased the resistance against rotting except in the case of *Rhizopus* sp. Furthermore, the disease incidence was significantly lowered at high spore concentration (10^8 cells per ml). The efficiency of spore suspension of the two isolates of *T. harzianum* (T3 and T24) were comparable efficient. Treatment of fruits inoculated with *A. niger* (1, 2, and 3) with *T. harzianum* suspension reduced rot intensity at low concentration (10^6 $CFU.ml^{-1}$) and abolished it completely at higher concentrations (10^7 and 10^8 $CFU.ml^{-1}$). On the other hand, treatment with T3 or T24 spores failed to protect fruits against *Rhizopus* sp. (Table 2).

The percentage of infected fruits after 15 days is presented in Table 3. The results show that treatment with *T. harzianum* spores drastically reduced the percentage of infection, and this reduction was correlated to the increase in spore concentration (Table 3). However, *Rhizopus* sp. was the most virulent and caused a severe rot for tomato fruits (81.25-93.75%), moreover, increasing spore concentration of *T. harzianum* did not decrease the percentage of infection. Fresh and healthy tomato fruits did not develop rot symptoms when treated with spore suspension of *T. harzianum* only (data not shown).

Biocontrol of postharvest tomato pathogens by *T. harzianum* culture filterate

Tables 4 and 5 show the effect of different concentrations of culture filtrate of *T. harzianum* (T3 and T24) on the growth of the various pathogens causing tomato rot. The undiluted culture filtrate (100%) of *T. harzianum* completely inhibited the spore germination of all the tested fungi except in the case of *Rhizopus* sp.. Similar inhibition was observed even when culture filtrate was diluted.

Table 2 Effect of different concentrations (10^6, 10^7, 10^8 CFU.ml^{-1}) of spore suspensions of *T. harzianum* isolates (T3 and T24) on the relative amount of tomato fruit rot caused by 14 fungi isolates

Pathogen	Control		T3						T24					
			10^6		10^7		10^8		10^6		10^7		10^8	
	5-d	7-d	5-d	7-d	5-d	7-d	5-d	7-d	5-d	7-d	5-d	7-d	5-d	7-d
Alternaria sp. (1)	++	++++	-	-	-	-	-	-	-	+	-	-	-	-
Alternaria sp. (2)	+++	++++	-	+	-	-	-	-	-	-	-	-	-	-
Alternaria sp. (3)	++	++++	-	+	-	-	-	-	-	+	-	-	-	-
Alternaria sp. (4)	++	++++	-	+	-	-	-	-	-	-	-	-	-	-
A. flavus (1)	++	++++	-	+	-	+	-	-	-	+	-	-	-	-
A. flavus (2)	+++	++++	-	++	-	+	-	-	-	+	-	+	-	-
A. niger (1)	++	+++	+	++	-	+	-	-	+	++	-	++	-	-
A. niger (2)	++	+++	+	++	-	+	-	+	+	++	-	++	-	-
A. niger (3)	+++	++++	+	++	-	+	-	-	+	+++	-	++	-	++
Aspergillus sp.	++	+++	-	+	-	-	-	-	-	++	-	-	-	-
Fusarium sp.	+++	++++	-	+	-	-	-	-	-	+	-	-	-	-
Geotricum candidum	++	++++	-	+++	-	++	-	-	-	+++	-	++	-	-
Penicillium steckii	++	++++	-	+	-	+	-	-	-	+	-	-	-	-
Rhizopus sp.	++++	++++	+++	++++	++	+++	+	++	+++	++++	++	+++	+	++

Legend: (The numbers [1-4] between the parentheses after the species name referred to the group number) The intensity of fruit rots for each fungal isolated were evaluated as the following: - = no rot (no symptom), + = low rot (rot symptoms less than 10%), ++ = middle rot (rot symptoms less than 50), +++ = high rot (rot symptoms less than 100%), ++++ = severe rot (no healthy tissue visible)

Table 3 Percentage of rot incidence of tomato fruits treated with *T. harzianum* (T3 and T24) spore suspension of different concentrations (10^6, 10^7, 10^8 CFU.ml^{-1}) and inoculated with postharvest pathogens after a period of 15 days

Pathogen	**Control**	**Antagonist**					
		T3			**T24**		
		10^6 *	**10^7 ***	**10^8 ***	**10^6 ***	**10^7 ***	**10^8 ***
Alternaria sp. (1)	100	81.25	50.00	12.50	87.50	50.00	12.50
Alternaria sp. (2)	100	81.25	50.00	12.50	87.50	50.00	12.50
Alternaria sp. (3)	100	87.50	50.00	12.50	87.50	50.00	12.50
Alternaria sp. (4)	100	87.50	50.00	12.50	87.50	50.00	12.50
A. flavus (1)	100	87.50	43.75	18.75	87.50	50.00	18.75
A. flavus (2)	100	93.75	50.00	43.75	93.75	50.00	18.75
A. niger (1)	100	93.75	50.00	43.75	93.75	50.00	43.75
A. niger (2)	100	93.75	50.00	43.75	93.75	50.00	43.75
A. niger (3)	100	93.75	50.00	43.75	93.75	50.00	43.75
Aspergillus sp.	100	81.25	43.75	12.50	87.50	43.75	12.50
Fusarium sp.	100	87.50	50.00	12.50	87.50	50.00	12.50
Geotricum candidum	100	87.50	50.00	12.50	93.75	75.00	12.50
Penicillium steckii	100	81.25	43.75	6.25	81.25	43.75	6.25
Rhizopus sp.	100	93.75	81.25	81.25	93.75	87.50	81.25

Legend: (The numbers [1-4] between the parentheses after the species name referred to the group number)

* Values are significantly different at $P \leq 0.05$

Treatment of *A. niger* (1 and 2) or *A. flavus* (1 and 2) infected fruits with *T. harzianum* spore suspension did not have high inhibitory effect leading to rot incidence ranging from low to high level. Interestingly, extending the incubation period from 5 to 7 days showed an increase in rot incidence at 25% concentration of culture filtrate. Moreover, percentage of fruits infected after 15 days decreased with increasing culture filtrate concentration (Table 5).

Data show that *Rhizopus* sp. is the most virulent fungus causing severe rot of tomato fruit (100%) even when treated with the diluted culture filtrate. The infection rate decreased only to 93.75% when infected fruits where treated with concentrated culture filtrate.

DISCUSSION

Applications of fungicides and fumigants can have drastic effects on the environment and consumer, and are often applied in greater quantities than herbicides and insecticides in agricultural production **(Vinale *et al.,* 2008)**. Therefore, several physical and biological means have been evaluated as safer alternatives for the use of chemical fungicides. Alternative methods include: (a) biological control agents (BCAs) **(Schena *et al.,* 1999; Xi and Tian, 2005)**, (b) plant bioactive compounds **(Guillen *et al.,* 2007; Liu *et al.,* 2007)** and (c) physico-chemical methods **(Mari *et al.,* 2009)**. The use of microbial antagonists for control of postharvest diseases received special interest, and has been extensively investigated **(Schena *et al.,* 1999; Droby, 2006; Xi and Tian, 2005)**.

There are a number of mechanisms whereby fungi act as biocontrol agents. Several *Trichoderma* spp. have been used to protect commercially important fruits and vegetables such as banana, apple, strawberries, mango, potato, and tomato during postharvest storage **(Verma *et al.,* 2007)**. *Trichoderma* based biofungicide, TRICHODEX (Makhteshim Chemical Works Ltd., Beer Sheva, Israel) is now commercially available for the control of *Botrytis cinerea* **(Elad, 2000a; 2000b)**.

Table 4 Effect of different concentrations (25, 50 and 100%) of culture filtrate of *T. harzianum* isolates (T3 and T24) on the relative amount of tomato fruit rot caused by 14 fungi isolates

Pathogen	**control**		**T3**						**T24**					
			25%		**50%**		**100%**		**25%**		**50%**		**100%**	
	5-d	**7-d**	**5-d**	**7-d**	**5-d**	**7-d**	**5-d**	**7-d**	**5-d**	**7-d**	**5-d**	**7-d**	**5-d**	**7-d**
Alternaria sp. (1)	++	++++	-	+	-	-	-	-	-	+	-	-	-	-
Alternaria sp. (2)	+++	++++	-	++	-	-	-	-	-	+	-	-	-	-
Alternaria sp. (3)	++	++++	-	+	-	-	-	-	-	+	-	-	-	-
Alternaria sp. (4)	++	++++	-	+	-	-	-	-	-	-	-	-	-	-
A. flavus (1)	++	++++	++	+++	++	+++	-	+	+	++	-	++	-	+
A. flavus (2)	+++	++++	++	+++	+	++	-	+	++	+++	-	++	-	+
A. niger (1)	++	+++	+	++	-	+	-	-	+	++	+	++	-	-
A. niger (2)	++	++	+	+++	-	++	-	-	+	+	-	-	-	-
A. niger (3)	+++	++++	+	+	-	-	-	-	+	+++	-	-	-	-
Aspergillus sp.	++	+++	-	+	-	-	-	-	-	+	-	-	-	-
Fusarium sp.	+++	++++	-	++	-	-	-	-	-	++	-	-	-	-
Geotricum candidum	++	++++	+	++	-	+	-	+	+	+++	-	++	-	-
Penicillium steckii	++	++++	-	+	-	-	-	-	-	+	-	-	-	-
Rhizopus sp.	++++	++++	+++	++++	++	+++	+	+++	+++	++++	++	++++	++	+++

Legend: (The numbers [1-4] between the parentheses after the species name referred to the group number) The intensity of fruit rots for each fungal isolated were evaluated as the following: - = no rot (no symptom), + = low rot (rot symptoms less than 10%), ++ = middle rot (rot symptoms less than 50), +++ = high rot (rot symptoms less than 100%), ++++ = severe rot (no healthy tissue visible)

Table 5 Percentage of rot incidence of tomato fruits treated with *T. harzianum* (T3 and T24) culture filtrate of different concentrations (25, 50 and 100%) and inoculated with postharvest pathogens after a period of 15 days

Pathogen	**Control**	**Antagonist**					
		T3			**T24**		
		25% *	**50% ***	**100% ***	**25% ***	**50% ***	**100% ***
Alternaria sp. (1)	100	93.75	81.25	43.75	93.75	81.25	43.75
Alternaria sp. (2)	100	93.75	81.25	43.75	93.75	81.25	43.75
Alternaria sp. (3)	100	93.75	81.25	43.75	93.75	81.25	43.75
Alternaria sp. (4)	100	93.75	81.25	43.75	93.75	81.25	43.75
A. flavus (1)	100	93.75	87.50	50.00	93.75	81.25	50.00
A. flavus (2)	100	93.75	87.50	50.00	93.75	81.25	50.00
A. niger (1)	100	93.75	87.50	50.00	93.75	81.25	50.00
A. niger (2)	100	93.75	87.50	50.00	93.75	81.25	50.00
A. niger (3)	100	93.75	81.25	50.00	93.75	81.25	50.00
Aspergillus sp.	100	87.50	75.00	37.50	87.75	75.00	43.75
Fusarium sp.	100	93.75	75.00	37.50	87.75	81.25	37.50
Geotricum candidum	100	87.50	81.25	43.75	87.75	81.25	37.50
Penicillium steckii	100	87.50	75.00	12.50	87.75	75.00	12.50
Rhizopus sp.	100	100	100	93.75	100	100	93.75

Legend: (The numbers [1-4] between the parentheses after the species name referred to the group number)

* Values are significantly different at $P \leqq 0.05$

The present study addresses the antagonistic activity of *T. harzianum* against some of postharvest tomato pathogens. The results indicate that *T. harzianum* had inhibitory effect against the postharvest pathogens which suggests that it produces extracellular metabolites that have potential in control of tomato fruit rot pathogens. Postharvest decay of tomatoes can be caused by several molds, however, *Alternaria, Botrytis, Cladosporium, Fusarium, Rhizoctonia,* and *Rhizopus* species are most commonly involved **(ICMSF, 1998; Snowdon, 1991; Sommer *et al.,* 1992)**. Our results of postharvest pathogens isolated from tomato fruits are consistent with previous reports **(Ajayi *et al.,* 2007; Ajayi and Olasehinde, 2009; Feng and Zheng, 2007)**.

Abdel-Mallek *et al.* (1995) reported that *Alternaria* was one of the most common and isolated fungus from healthy or diseased tomato which is in accordance with our presented results, where *Alternaria* isolates were the most common fungal species isolated from healthy or rotten fruits and gave the highest frequency of occurrence (40-70%). This result is consistent with **Feng and Zheng (2007)** and **Singh *et al.* (1988)** reports.

Our results show that *Rhizopus* sp. was the most virulent pathogen and that rot intensity increased with the increase in pathogen spore concentration. *Rhizopus* is well known for causing soft rots of fruits, vegetables, and root crops, especially in postharvest storage situations **(Agrios, 2005; Harveson, 2000)**. In addition, *Rhizopus* grows intensively even on refrigerated fruits **(Mahovic *et al.,* 2004)**.

The effect of *T. harzianum* culture filtrate on spore germination was evaluated. Our results show that the culture filtrate of *T. harzianum* T3 or T24 greatly inhibited spores germination of the tested postharvest pathogenic fungi. These results indicate that *T. harzianum* grown on PDB might release extracellular diffusible metabolite(s) that inhibited spore germination of tomato postharvest pathogens. **Odebode (2006)** reported that culture filtrate from *T. harzianum* Rifai and *T. pseudokoningii* Rifai strains inhibited the growth of postharvest pathogens of some fruits. Random mutagenesis has been applied to improve production of antifungal metabolites and antagonistic potential of biocontrol agents (i.e. *Trichoderma* spp. and *Gliocladium* spp.) to control a broad spectrum of phytopathogens **(Haggag and Mohamed, 2007)**.

Light microscopy investigation revealed that the extracellular metabolites in the *T. harzianum* culture filtrate caused morphological changes including hyphal swelling, vaculation, distortion and cytoplasm aggregation. These findings are in accordance with the previous observations of multiple structural abnormalities in the antagonized conidia and

hyphae when treated with either fungal **(Haggag and Mohamed, 2007)** or bacterial **(Chaurasia *et al.,* 2005; Rahman *et al.,* 2009)** biocontrol agents.

Biocontrol test on tomato fruits showed lower rot intensity and decreased rate of infection with the increase in spore concentration of the antagonist. Moreover, treatment with *T. harzianum* (T3 and T24) culture filtrate repressed pathogen growth on fruits leading to complete inhibition in the case of the undiluted filtrate. These results are in agreement with the previously reported inhibition of yams rot sprayed with *T. viride* **(Okigbo and Ikediugwu, 2001)**. In contrast to other pathogens, the results show that the suppression of tomato rotting by *Rhizopus* spp. was not possible when fruits were treated with *T. harzianum* conidia.

An important attribute of successful biocontrol agent of postharvest pathogens of fruits is its efficiency at relatively low concentration **(Wisniewski and Wilson, 1992)**. Both *T. harzianum* strains were generally effective at low concentration of 10^6-10^8 conidia per ml. These concentrations are even lower than the recommended concentrations of other biocontrol agents **(Janisiewicz, 1988; Wang *et al.,* 2008)** thus considered suitable for commercial use.

CONCLUSION

In conclusion, our data show that the *T. harzianum* T3 and T24 strains have potential biocontrol activity against postharvest rot caused by different fungal pathogens in cherry tomato fruit. Therefore, the use of these isolates offer a promising, safe and effective alternative to fungicides in treatment of postharvest fungal diseases of tomato fruits. However, further studies are required to render these isolates technically and economically for efficient use as biocontrol agents on agronomic scale.

REFERENCES

ABDEL-MALLEK, A. Y. – HEMIDA S. K. – BAGY, M. M. K. 1995. Studies on fungi associated with tomato fruits and effectiveness of some commercial fungicides against three pathogens. In *Mycopathologia,* vol. 130, 1995, p. 109-116.

ADASKAVEG, J. E. – KANETIS, L. – SOTO-ESTRADA, A. – FÖRSTER, H. 2004. A new era of postharvest decay control in citrus with the simultaneous introduction of three new “reduced-risk” fungicides. In: *Proc. Int. Soc. Citricult*, p. 2004, p. 999-1004.

AGRIOS, G. N. 2005. *Plant Pathology*. Elsevier Academic Press, 2005, USA.

AJAYI, A. A. – ADEJUWON, A.O. – OLUTIOLA, P. O. 2007. Partial Purification of Polygalacturonase from Tomato Fruits infected by *Rhizopus arrhizus* Fisher. In *J. Plant Sci.,* vol. 2 no. 2, 2007, p. 216-221.

AJAYI, A. A. – OLASEHINDE, G. I. 2009. Studies on the pH and Protein content of tomato (*Lycopersicon esculentum* Mill) fruits deteriorated by *Aspergillus niger.* In *Sci. Res. Essay.,* vol. 4 no. 3, 2009, p. 185-187.

CHAURASIA, B. – PANDEY, A. – PALNI, L. M. S. – TRIVEDI, P. – KUMAR, B. – COLVIN, N. 2005. Diffusible and volatile compounds produced by an antagonistic *Bacillus subtilis* cause structural deformations in pathogenic fungi *in vitro*. In *Microbiol. Res.* vol. 160, 2005, p. 75–81.

CHUNG, Y. – HOITINK, H. A. J. 1990. Interaction between thermophilic fungi and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark composted-amended container medium. In *Phytopathol.*, vol. 80, 1990, p. 73-77.

CROCKER, W. – HITCHCOCK, A. E. – ZIMMERMAN, P. W. 1935. Similarities in the effects of ethlyene and the plant auxins. In *Contrib. Boyce Thompson Inst.,* vol. 7, 1935, p. 231-248.

DROBY, S. 2006. Biological control of postharvest diseases of fruits and vegetables: difficulties and challenges. In *Phytopathol. Pol.,* vol. 39, 2006, p. 105-117.

DROBY, S. – WISNIEWSKI, M. E. – COHEN, L. – WEISS, B. – TOUITOU, D. – EILAM, Y. – CHALUTZ, E. 1997. Influence of $CaCl_2$ on *Penicillium digitatum*, grapefruit peel tissue and biocontrol activity of *Pichia guilliermondii*. In *Phytopathol.,* vol. 87, 1997, p. 310-315.

ECKERT, J. W. – OGAWA, J. M. (1985) The chemical control of postharvest diseases: subtropical and tropical fruits. In *Annual Review of Phytopathol.,* vol. 23, p. 421–454.

ELAD, Y. 2000a. *Trichoderma harzianum* T39 preparation for biocontrol of plant diseases-control of *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Cladosporium fulvum*. In *Biocont. Sci. and Tech.,* vol. 2000, 10, p. 499-507.

ELAD, Y. 2000b. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. In *Crop Protect.,* vol. 19, 2000, p. 709-714.

EL-KATATNY, M. H. – ABDELZAHER, H. M. – SHOULKAMY, M. A. 2006. Antagonistic actions of *Pythium oligandrum* and *Trichoderma harzianum* against phytopathogenic fungi (*Fusarium oxysporum* and *Pythium ultimum* var. *ultimum*). In *Arch. Phytopathol. Plant Protect.,* vol. 39 no. 4, 2006, p. 289-301.

EL-KATATNY, M. H. – GUDELJ, M. – ROBRA, K.- H. – ELNAGHY, M. A. – GÜBITZ, G. M. 2001. Characterization of a chitinase and an endo-β-1,3-glucanase from *Trichoderma*

harzianum Rifai T24 involved in control of the phytopathogen *Sclerotium rolfsii*. In *Appl. Microbiol. Biotechnol.*, vol. 26, 2001, p. 137-143.

FENG, W. – ZHENG, X. D. 2007. Essential oils to control *Alternaria alternate in vitro* and *in vivo*. In *Food cont.*, vol. 18, 2007, p. 1126-1130.

GEORGE, B. – KAURA, C. – KHURDIYA, D. S. – KAPOOR, H. C. 2004. Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. In *Food Chem.*, vol. 84, 2004, p. 45–51.

GUILLEN, F. – CASTILLO, S. – ZAPATA, P. J. – MARTINEZ-ROMERO, D. – SERRANO, M. – VALERO, D. 2007. Efficacy of 1-MCP treatment in tomato fruit: 1. Duration and concentration of 1-MCP treatment to gain an effective delay of postharvest ripening. In *Postharvest Biol. Technol.*, vol. 3, 2007, p. 23–27.

HAGGAG, W. M. – MOHAMED, H. A. 2007. Biotechnological aspects of microorganisms used in plant biological control. In *American-Eurasian J. Sust. Agric.,* vol. 1 no. 1, 2007, p. 7-12.

HARMAN, G. E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. In *Plant Dis.,* vol. 84, 2000, p. 377–393.

HARMAN, G. E. – HOWELL, C. R. – VITERBO, A. – CHET, I. – LORITO, M. 2004. *Trichoderma* species: opportunistic, avirulent plant symbionts. In *Nature Reviews Microbiol.*, vol. 2 no.1, 2004, p. 43-56.

HARVESON, M. R. 2000. *Rhizopus head rot of sunflower*. Nebraska Cooperative Extension, 2000, NF00-426.

International Commission on Microbiological Specifications for Foods, Fruits and fruit products 1998. In: *Microorganisms in FoodsMicrobial Ecology of Commodities*, *Vol. 6*, *Chapter 6*, Blackie and Academic Professional, London, UK, 1998, p. 252–273.

JANISIEWICZ, W. J. 1988. Biocontrol of postharvest diseases of apples with antagonist mixtures. In *Phytopathol.,* vol. 78, 1988, p. 194-198.

JANISIEWICZ, W. J. – KORSTEN, L. 2002. Biological control of postharvest diseases of fruits. In *Annual Review of Phytopathol.,* vol. 40, 2002, p. 411-441.

KANETIS, L. – FÖRSTER, H. – ADASKAVEG, J. E. 2007. Comparative efficacy of the new postharvest fungicides azoxystrobin, fludioxonil, and pyrimethanil for managing citrus green mold. In *Plant Dis.,* vol. 91, 2007, p. 1502-1511.

KUBICEK, C. P. – MACH, R. L. – PETERBAUER, C. K. – LORITO, M. 2001. *Trichoderma*: From genes to biocontrol. In *J. Plant Pathol.,* vol. 83 no. 2, 2001, p. 11-23.

LIU, J. – TIAN, S. – MENG, X. – YONG, X. 2007. Effect of chitosan on control postharvest diseases and physiological responses of tomato fruit. In *Postharvest Biol. Technol.*, vol. 44, 2007, p. 300–306.

LORITO, M. – WOO, S. L. – SCALA, F. 2004. Le biotecnologie utili alla difesa sostenibile delle piante: i funghi. In *Agroindust.*, vol. 3, 2004, p. 181-195.

MAHARAJ, R. – ARUL, J. – NADEAU, P. 2010. UV-C irradiation of tomato and its effects on color and pigments. In *Advan. Environment. Biol.,* vol. 4 no. 2, 2010, p. 308-315.

MAHOVIC, M. – SARGENT, S. A. – BARTZ, J. A. 2004. Identifying and controlling postharvest tomato diseases in Florida. University of Florida, 2004, (http://edis.ifas.ufl.edu/HS131)

MARI, M. – NERI, F. – BERTOLINI, P. 2009. New Approaches for Postharvest Disease Control in Europe. In: *Postharvest Pathology*, Plant Pathology in the 21st Century. *Chapter 9, Vol. 2*, D. Prusky and M.L. Gullino (eds.). DOI 10.1007/978-1-4020-8930-5_9, 2009, pages 119-135, © Springer Science+ Business Media B.V.

MOHAMED, H. A. – HAGGAG, W. M. 2006. Biocontrol potential of salinity tolerant mutants of *Trichoderma harzianum against Fusarium oxysporum*. In *Braz. J. Microbiol.,* vol. 37 no. 2, 2006, p. 181-191.

MONTEALEGRE, J. – VALDERRAMA, L. – SANCHEZ, S. – HERRERA, R. – BESOAIN, X. – PEREZ, L. M. 2010. Biological control of *Rhizoctonia solani* in tomatoes with *Trichoderma harzianum* mutants. In *Elect. J. Biotechnol.,* vol. 13, no. 2, 2010, p. 1-11.

NASEBY, D. C. – PASCUAL, J. A. – LYNCH, J. M. 2000. Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* population, soil microbial communities and soil enzyme activities. In *J. Appl. Microbiol.,* vol. 88, 2000, p. 161–169.

ODEBODE, A. C. 2006. Control of postharvest pathogens of fruits by culture filtrate from antagonistic fungi. In *Journal of Plant Protec. Res.,* vol. 46, no.1, 2006, p. 1-6.

OKIGBO, R. N. – IKEDIUGWU, F. E. O. 2001. Biological control of tuber surface mycoflora of yam (*Dioscorea rotundata*). In *Trop. Sci.,* vol. 41, no.2, 2001, p. 85-89.

Pal, K. K. - McSpadden Gardener, B. 2006. Gardener, *Biological Control of Plant Pathogens*. The Plant Health Instructor, 2006. doi: 10.1094/PHI-A-2006-1117-02.

RAHMAN, M. A. – KHADIR, J. – MAHMUD, T. M. M. – ABDUL RAHMAN, R. – BEGUM, M. M. 2009. Screening of antagonistic bacteria for biocontrol activities on *Colletotrichum gloeosporioides* in Papaya. In *Asian J. Plant Sci.*, vol. 6, no. 1, 2009, p. 12-20.

RAMSEY, G. B. – LINK, G. K. K. 1932. Market diseases of fruits and vegetables: Tomatoes, peppers, eggplants. In *Misc. Publ. US Department Agric.,* vol. 121, 1932, p. 1-44.

ROSSLENBROICH, H. J. – STUEBLER, D. Q. 2000. *Botrytis cinerea* – History of chemical control and novol fungicides for its management. *Crop Protec.,* vol. 19, 2000, p. 557-561.

SARHAN, M. M. – EZZAT, S. M. – AL-TOHAMY, M. R. 1999. Application of *Trichoderma hamatum* as a biocontroller against tomato wilts disease caused by *Fusarium oxysporum* f. lycopersici. In *Egyptian J. Microbiol.,* vol. 34, 1999, p. 347-376.

SCHENA, L. – IPPOLITO, A. – ZAHAVI, T. – COHEN, L. – NIGRO, F. – DROBY, S. 1999. Genetic diversity and biocontrol activity of *Aueobasidium pullulans* isolates against postharvest rots. In *Postharvest Biologic. Technol.,* vol. 17, 1999, P. 189-199.

SHABAN, G. M. 1986. Physiological and Ecological Studies on the Genus *Trichoderma* in Egyptian Soils, *Ph.D. Thesis*, Faculty of Science, El-Minia University, El-Minia, Egypt, 1986.

SHOULKAMY, M. A. – GUEBITZ, G. M. – EL-KATATNY, M. H. – SHABAN, G. M. – ABDELZAHER, H.A. 2006. Antagonism of *Trichoderma* or *Gliocladium* species on two phytopathogenic species of *Fusarium.* In *J. Nat. Fibers,* vol. 3 no. 2/3, 2006, p. 1-17.

SINGH, J. P. – BHATNGAR D. K. – LAL, S. 1988. Effect of different fungicides on microbial decay and keeping quality of tomato fruits during storage. In Haryana Journal of Horticultural Science, vol. 17, 1988, p. 69-73.

SNOWDON, A. L. 1991. Solanaceous fruit vegetables. In: A.L. Snowdon, Editor, *A Colour Atlas of Postharvest Diseases and Vegetables*, *Vol. 2*, *Chapter 2*, In Wolfe Publishing, London, UK, 1991, p. p. 53-93.

SOMMER, N. F. – FORTLAGE, R. J. – EDWARDS, D. C. 1992. Postharvest diseases of selected commodities. In: A.A. Kader, Editor, *Postharvest Technology of Horticultural Crops*, *Chapter 15*, In University of California, Oakland, CA, 1992, p. 117–160.

STEVENS, C. – KHAN, V. A. – LU, J. Y. – WILSON, C. L. – PUSEY, P. L. – IGWEGBE, C. K. – KABWE, K. – MAFOLO, Y. – LIU, J. – CHALUTZ, E. – DROBY, S. 1997. Integration of ultraviolet (UV-C) light with yeast treatment for control of postharvest storage rots of fruits and vegetables. In *Biologic. Cont.,* vol. 10, 1997, p. 98-103.

TARIQ, M. – YASMIN, S. – HAFEEZ, F. Y. 2010. Biological control of potato black scurf by rhizosphere associated bacteria. In *Braz. J. Microbiol.,* vol. 41, 2010, p. 439-451.

VERMA, M. – BRAR, S. K. – TYAGI, R. D. – SURAMPALLI, R. Y. – VALÉRO, J. R. 2007. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control-A Review. In *Biochemic. Eng. J.,* vol. 37, 2007, p. 1-20.

VINALE, F. – SIVASITHAMPARAM, K. – GHISALBERTI, E. L. – MARRA, R. – WOO, S. L. – LORITO, M. 2008. *Trichoderma*–plant–pathogen interactions. In *Soil Biol. Biochem.,* vol. 40, 2008, p. 1–10.

WANG, Y. – BAO, Y. – SHEN, D. – FENG, W. – YU, T. – ZHANG, J. – ZHENG, X. D. 2008. Biocontrol of *Alternaria alternata* on cherry tomato fruit by use of marine yeast *Rhodosporidium paludigenum* Fell & Tallman. In *Int. J. Food Microbiol.,* vol. 123, 2008, p. 234-239.

WEINDING, R. – EMERSON, H. 1936. The isolation of toxic substance from the culture filtrates of *Trichoderma*. In *Phytopathol.,* vol. 26, 1936, p. 1068-1070.

WHITAKER, J. R. 1996. Enzymes: *In Food Chemistry.* (3rd ed.) O.R. Fennema (Ed.), In Marcel Dekker, New York, 1996, p. 431-530.

WISNIEWSKI, M. E. – WILSON, C. L. 1992. Biological control of postharvest diseases of fruits and vegetables: recent advances. In *HortScience*, vol. 27, 1992, p. 94-98.

XI, I. – TIAN, S. P. 2005. Control of postharvest diseases of tomato fruits by combining antagonistic yeast with sodium bicarbonate. In *Scientia Agricultura Sinica*, vol. 38, 2005, p. 950-955.

ZHENG, X. D. – YU, T. – CHEN, R. L. – HUANG, B. – WU, V. C. 2007. Inhibiting *Penicillium expansum* infection on pear fruit by *Cryptococcus laurentii* and cytokinin. In *Postharv. Biol. Technol.,* vol. 45, 2007, p. 221-227.

ZHENG, X. D. – ZHANG, H. Y. – SUN, P. 2005. Biological control of postharvest green mold decay of oranges by *Rhodotorula glutinis*. In *Eur. Food Res. Technol.,* vol. 220, 2005, p. 353-357.