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ROLE OF BIOTIC AND ABIOTIC AGENTS ON CONTROLLING ALTERNARIA FRUIT ROTS OF TOMATO AND PEPPER

BY

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ABSTRACT

Several fungal species belong to different genera were isolated from naturally infected tomato and pepper fruits collected from different local markets at El-Sharkia governorate. Among them *Alternaria alternata* (Fr.) Keissler was the most isolated one, therefore *A. alternata* was chosen for the further studies. Data indicated that, wounded tomato and pepper fruits at red stage were most susceptible than tomato at light red stage or pepper at mature green stage than unwounded ones. Tomato cv. orit and pepper cv. baladi fruits, at both tested stages were highly susceptible to be infected with *A. alternata* than tomato cv. Gs and pepper cv. California wonder.

In vitro study, revealed that, tested essential oils (craway oil followed by garlic) detergents (citrol and rabso) and tested bio-agents bacteria (*Bacillus subtilis* and *Psudomonas fluorescens*) and or fungi (*Trichoderma harzianum* and *T. viride*) were effective in inhibiting mycelial growth of *A. alternata*. The effect of oils and detergents was increased by increasing the concentrations compared to the control and tested fungicides. Detergents used completely inhibited the growth of the fungus compared to the fungicides. Scanning electron microscope (SEM) revealed that, *Trichoderma* spp. act as mycoparasite on mycelial of *A. alternata*.

In vitro treatment, testing bio-agent isolates revealed significant decrease in the percentage of infection and diameter of infected area when used immediately after inoculation. Caraway and garlic oils effectively reduced decay in tomato and pepper fruits caused by *A. alternata*. Washing water contain industrial detergent (citrol) reduced decay of naturally infected tomato and pepper fruits. In addition dipping of *A. alternata* inoculated tomato and pepper fruits in water contain citrol reduced the percentage of infection and diameter of infected area compared to untreated fruits.

Key words: Tomato, pepper, fruit rots, *Alternaria* spp. Bio-agents, plant oils, detergents.

INTRODUCTION

Decay of tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) fruits during storage, wholesale, retail markets and through consumption are mainly caused by different pathogenic fungi, i.e. *Alternaria* alternata, *A. solani, Alternaria* spp., *Botrytis cinerea, Fusarium solani, Rhizopus stolonifer, Geotrichum candidum, Penicillium* spp., *Phytophthora infestans* and *Aspergillus* spp. (Ragab Mona *et al.*, 2001; El-Essaway *et al.*, 2003). Resistance and susceptibility of tomato and pepper fruits to infect with pathogenic fungi are depends on: variety (Aly *et al.*, 1993), mature stage of fruits (Wall and Biles, 1993 and El-Essawy *et al.*, 2003), presence of wounds (Wall and Biles, 1993 and El-Essawy, *et al.*, 2003) and infection with insects (Bruton, *et al.* 1989).

Biological control of post-harvest diseases of fruits has greatly advanced during the past decade regarding human health and environmental risks associated with chemical residues in food that has been the main riving force of the search from new and safer control methods (Droby *et al.*, 1998).

Among the proposed alternatives, the use of naturally occurring antagonistic microorganisms has been the most extensively studied one. Some of these microorganisms has been patented and commercially produced (Abadias *et al.*, 2003). Several fungi and bacteria have been reported to have antagonistic effect against post-harvest pathogens on a variety of harvested commodities (Chand-Goyal and Spotts 1997; El-Ghaouth *et al.*, 1998, Singh, *et al.*, 2000, Sivakumar *et al.*, 2000; Jiang *et al.*, 2001; and El-Essaway *et al.*, 2003).

Moreover, there is an increasing demand among consumers and regulatory agencies about the health hazards of chemical residues (Beever *et al.*, 1989). Therefore, alternative control methods for post-harvest diseases are needed. During the recent years, the search of various naturally occurring compounds with antimicrobial activity has become quite intense. Various plant essential oils have been found to possess various degrees of antimicrobial activity against various plant pathogenic fungi (Srivastova *et al.*, 1995, Nath, *et al.*, 1994 and Ragab Mona *et al.*, 2001).

In addition, the anti-fungal activity of *Litsea cubeba* oil against several pathogens confirmed by Gegoi et al. 1997. Lemon grass oil was tested as anti-fungal against several plant pathogenic fungi, i.e. *A. alternata, Botrytis cinerea, Aspergillus* spp., *Rhizopus* spp, *F. solani, Penicillium* spp. (Inouye *et al.*, 1998 and Ragab Mona *et al.*, 2001).

The aim of this study is to investigate the role of tomato and pepper cvs., mature stage, wounds, biological control agents, plant oils and industrial detergents on the growth and infection decay with *A. alternata* the causal pathogen of tomato and pepper fruit rots.

MATERIALS AND METHODS

1- Isolation, and inoculum preparation of the pathogenic fungi:

Rotted tomato and pepper fruits collected from different local markets in Abou-Hammad and Zagazig provinces, El-Sharkia governorate, as well as from field growing tomatoes. Collected fruits were surface sterilized and cut into small pieces then transferred to water agar medium in 9 cm Petri dishes and incubated at 27 ± 2°C and observed daily. Colonies of developed fungi, were picked-up and transferred to potato dextrose agar (PDA) medium. The isolated fungi were purified using single spore and/or hyphal tip technique as described by Brown (1924) and Pathak (1984), then these isolates were identified according to the description of Barnett and Hunter (1998). The isolates were kept in the refrigerator at 5 ± 1°C as stock culture for the further studies. Inoculation of tomato and pepper fruits were done using fungal suspension (spore and mycelial fragments) obtained from 7days old culture of tested fungi grown on PDA medium at 27 ± 2°C in the dark/light conditions. Spore suspension was prepared by adding 5 ml of sterile distilled water containing 0.03 % tween 80 per Petri dish and gently scraping-off the spores using sterile spatula.

2. Bio-agent isolates:

The bio-agent isolates were obtained from Agricultural Botany and Plant Pathology Department, Faculty of Agriculture, Zagazig University. The bacterial isolates (*Bacillus subtilis* and *Psudomonas fluorescens*) were grown in nutrient agar and /or King's B medium (King *et al.*, 1954) in Petri dish. While, the *Trichoderma harzianum* and *Trichoderm viride* isolates were grown on PDA and or glitoxin fermentation medium (Brain and Hemning, 1945).

3-Pathogenicity test:

The most isolated fungus was identified as *A. alternata* (Fr.) Keissler. It's pathogenic capabilities was tested on healthy tomato cv. orit and pepper cv. baladi fruits. Tomato and pepper fruits immediately transported from field to Laboratory of Plant Pathology, Faculty of Agriculture, Zagazig University. The selected healthy appearance fruits, were uniform in size and color as well as free from microbial infection and physical damage. Selected fruits were washed with tap water and surface disinfested by immersion in 2 % sodium hypocholorite for 2 min, then rinsed with sterile water and dried using filter papers. Disks 4 mm in diam of 7 days old culture *A. alternata* grown on PDA medium were transferred into a small jap (4 mm in diam.). In other treatment fruits were inoculated with 50µl fungal spore suspension into a small jap using micropipette. Three replicates were used with ten fruits per each. The inoculated fruits were incubated separately in sterilized boxes and kept at 27 ± 2°C, then observed daily. The percentage of infection and diameter of infected area were determined.

4-Reaction of some tomato and pepper cultivars at two ripening stages to infection with *Alternaria alternata*:

Tomato cvs. (orit and Gs) and pepper cvs. (baladi and California wonder) at two ripening stages (light red and red stages of tomato as well as mature green and red stages of pepper) were tested for their susceptibility to

infect with *A. alternata*. Fruits were selected and inoculated with disks 4 mm in diam as mentioned in pathogenicity test. Disease parameters was determined as mentioned before.

5. *In vitro* experiments

5.1. Effect of the bio-agents isolates on mycelial growth of *Alternaria alternata*:

The antagonistic interaction between different bacteria (*B. subtilis* and *Pseudomonas fluorescens*) isolates on the mycelial growth of *A. alternata*. were tested under laboratory conditions. Petri dishes (9 cm in diameter) containing PDA medium were inoculated in the center with a disk (5 mm∅) taken from 7 days old culture of *A. alternata*. Dishes were inoculated with the bacterial isolates, by streaking with a loop on the surface of the media beside the fungal growth (at the distance of 1.5 cm from the edge of the dishes) with the aid of dual culture method. However, in case of fungi (*T. harzianum* and *T. viride*) isolates dishes were inoculated with disks (5 mm∅) of the tested fungi at the distance of 1.5 cm from the edge of the dishes. Dishes inoculated with *A. alternata*. alone were used as a control. Three dishes were used for each treatment. Then dishes were incubated at 27 ± 2°C. When the dishes of the control were filled with the mycelial growth of *A. alternata* the mean diameter of the mycelial radical growth in different treatments was measured. Percentage of growth reduction were calculated from the following formula (Gang *et al.*, 1994).

The percentage of growth reduction = (1-T/C) x 100.

Whereas

T = mycelial growth in the treatment and C = mycelial growth in the control.

5.1.1. Scanning electron microscope (SEM):

The interaction zone between *A. alternata* and *T. harzianum* and *T. viride* was checked using scanning electron microscope. Agar blocks with 3x7 mm (in dimension) of the interaction zone were cut out and fixed in 6% aqueous glutaraldhyde over night at 4 °C, washed twice in 2-methoxy-ethanol for 20 minutes. Blocks were then washed twice in absolute acetone then dried in CO_2 in a critical point drying apparatus, mounted on stubs with double sided sticking tape and coated with gold in a polaron sputter coater (Harley and Fergusen, 1990). The specimens were examined and photographed through Jeol scanning electron microscope (Fac. Agric, Ain Shams Univ.).

5.2. Effect of different plant oil concentrations on mycelial growth of *Alternaria alternata*:

The crude oils were obtained from the local market. Different concentrations (0, 1.25, 2.50 and 5 %) of the crude oil (caraway, garlic, aloe and brassica) and or fungicides (benlate and zeinb) were directly added to PDA medium. The oil concentrations were solubilized in the PDA medium using 0.03 % tween 80. Twenty ml of the mixture (medium with the different oil concentrations) were poured into Petri dishes (9 cm in diameter). Dishes with the same percent using tween 80 in sterile distilled water served as a control. Dishes then inoculated at the center with a disk (5 mm∅) taken from 7 days old culture

of *A. alternata*. Dishes were incubated at 27 ± 2°C. Three dishes were used for each treatment. When the dishes of control were filled with the mycelial growth of *A. alternata* the mean diameter of the mycelial radical growth in different treatments was measured. The percentage of growth reduction was calculated as mentioned before.

5.3. Effect of different concentrations of industrial detergents on mycelial growth of *Alternaria alternata*:

Different concentrations (0, 1.25, 2.50 and 5 %) of industrial detergents (citrol and rabso) were directly added to PDA medium. Twenty ml of the mixture (medium with the different aforementioned concentrations) were poured into Petri dishes (9 cm in diameter). Dishes with only sterile distilled water were used as a control. Dishes were inoculated, incubated and results were taken as mentioned before. Three dishes were used for each particular treatment.

6. *In vivo* experiments

6.1.Biological control of *Alternaria alternata* fruit rot of tomato and pepper:

Fruits of tomato cv. orit at light red stage and pepper fruit at mature green and red stages, were selected and surface disinfested as mentioned before. Selected fruits were divided into several groups. The first group was inoculated with a mycelial disk of *A. alternata* as mentioned before, the second group was left without inoculation. The third group was inoculated, then was dipped for 5 min in prepared concentration of bio-agents (*B. subtilis* and *P. fluorescens*) isolates at 10^8 cfu and both of *T. harzianum* and *T. viride* at 10^6 cfu), control treatment has been carried out using sterile water. Treatments were then separately put in plastic boxes and incubated for ten days. Diseases parameters were determined as mentioned before.

6.2. Effect of essential plant oils on *Alternaria alternata* fruit rot of tomato and pepper:

Tomato and pepper fruits were selected as mentioned before, surface disinfested, inoculated with *A. alternata* then dipped in essential plant oils of caraway and garlic at 2.5% in addition to 0.3% tween 80%. Fruits were then packed in plastic boxes separately and incubated. Three replicates with ten fruits were used for each treatment. Then disease parameters were determined as mentioned before.

6.3. Effect of industrial detergent on *Alternaria alternata* fruit rots of tomato and pepper:

Tomato cv. orit and pepper cv. baladi fruits were selected as mentioned before, surface disinfested, inoculated, dipped in 2.5% of citrol for 15 minutes and water as control treatment, packed separately in plastic boxes and incubated. Three replicates with ten fruits were used for each treatment. Diseases parameters were determined as mentioned before.

Other experiments were carried out through dipping tomato and pepper fruits in citrol 2.5% and/or sterile water to serve as control, another group of fruits were left without treatment with neither of water nor detergent, packed in plastic

boxes then left for natural infection and incubated at 13 °C, stored for two weeks. the results were recorded as percentage of infected fruits.

7. Statistical analysis:

Data obtained were subjected to statistical analysis proposed by Gomez and Gomez (1984), and means were compared using LSD multiple range test according to Duncan (1954).

RESULTS AND DISCUSSION

1. Isolated fungi:

Several fungal species belonging to different fungal genera were isolated from naturally infected tomato and pepper collected from different local markets in Abou-Hammad and Zazgzig provinces, El-Sharkia governorate (Table, 1). *Alternaria alternata* (Fr.) Keissler. was the most isolated fungus followed by *Botrytis* spp. Therefore, *A. alternata* was chosen for the further studies. Other fungal genera such as *Rhizopus stolonifer*, *Penicillium* spp., *Rhizoctonia solani*, *Aspergillus niger*, *Cladosporum* spp., *Fusarium* spp., and *Geotrichum* sp. were isolated. Different fungal genera were previously isolated from infected tomato and pepper fruits (Aktar *et al.*, 1994; Ragab Mona *et al.*, 2001; El-Essaway *et al.*, 2003 and Tohamy *et al.*, 2004).

Table (1): Frequency of isolated fungi from rotted tomato and pepper fruits collected from different local markets at El-Sharkia governorate.

Isolated fungi	Frequencies	
	Tomato	Pepper
Alternaria alternata	45.00	53.00
Alternaria spp.	10.00	12.00
Botrytis cinerea	11.00	8.00
Botrytis spp.	4.00	6.00
Rhizopus stolonifer	10.00	7.00
Penicllium spp.	5.00	6.00
Rhizoctonia solani	5.00	2.00
Aspergillus spp.	2.00	1.00
Mucor spp.	3.00	1.00
Cladosporium spp	2.00	3.00
Fusarium spp.	2.00	1.00
Geotrichum sp.	1.00	-

2. Pathogenicity test of *A. alternata*:

Pathogenic capability of *A. alternata* to infect wounded and unwounded tomato cv. orit and pepper cv. baladi, using fungal suspension (mycelial fragments and spores) and or mycelial growth disks were tested. Data in Table (2) reveal that, pathogenic ability of *A. alternata* was higher in case of wounded fruits, which gave 100 % of infection, than of un-wounded tomato and pepper fruits which, gave 15 % in case of tomato, while, in case of pepper the fungus

failed to infect the unwounded pepper fruits. As well as, the diameter of infected area was higher in case of wounded (35 and 37 mm in case of tomato and 20 and 13 mm in case of pepper respectively) fruits than of un-wounded ones. These results are in agreement with the results optained by several investigators (Ragab Mona *et al.*, 2001 and El-Essaway *et al.*, 2003).

Table (2): Pathogenicity testes of *Alternaria alternata* on tomato and pepper fruits measured as percentage of infection and diameter of infected area (mm).

Treatments	Wounded fruits				Un-wounded fruits			
	Fungal suspension (cfu)		Fungal growth (Agar disk)		Fungal suspension (cfu)		Fungal growth (Agar disk)	
	Infection (%)	Diam. infected area (mm)	Infection (%)	Diam. infected area (mm)	Infection (%)	Diam. infected area (mm)	Infection (%)	Diam. infected area (mm)
Tomato cv. orit								
Inoculated	100.00	35.00	100.00	37.00	100.00	15.00	100.00	12.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD at 0.5	Sig.	0.214	Sig.		Sig.	0.214	Sig.	0.214
Pepper cv. baladi								
Inoculated	100.00	20.00	100.00	13.00	00.00	0.00	00.00	0.00
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LSD at 0.5	Sig.	0.214	Sig.	0.214				

3. Reaction of different tomato and pepper cultivars at two ripening stages to infection with *A. alternata*:

Data in Table (3) indicate that, fruits at red stage were most susceptible than in light tomato red stage and at mature green stage of pepper, that shows the highest percentage of infection and the widest diameter of infected area. Tomato cv. orit at both tested stages was highly susceptible to be infected with *A. alternata* compared with Gs one which shows the lowest percentage of infection and the narrowest diameter of infected area. In case of pepper fruits, baladi cv. was more susceptible to the infection with *A. alternata* than California one. Similar results were obtained by Aly *et al.*, (1993) and Reda *et al.*, (1988). Differences between tomato cvs. and or pepper cvs. to the infection with *A. alternata* might be due to morphological and or biochemical genetic resistance (Reda *et al.*, 1988). Fruit repining stages play an important role on the infection with *A. alternata*, whereas, the infection increased by increasing the maturing stage. Similar results were obtained by Wall and Biles (1993). Schlösser, 1977 found that, differences in susceptibility to the infection at maturity stages may be attributed to the antifungal material (tomatin) presents in green tomato fruits that decrease rapidly by fruit development and ripening, reaching traces in red maturing stage. In addition, Labavitch *et al.* (1998) mentioned that when ripening start, the natural defenses are not longer effective.

Table (3): Reaction of different tomato and pepper at two reipning stages to infection with *Alternaria alternata*

Treatments	Stage	Wounded fruits			
		Fungal suspension (cfu)		Fungal growth (Agar disk)	
		Infection (%)	Diam. infected area (mm)	Infection (%)	Diam. infected area (mm)
Tomato	Orit at red stage	100.00	35.00	98.00	35.00
	Orit at light red	80.00	25.50	78.00	16.50
	Gs at red	93.00	25.00	90.00	18.00
	Gs at light red	78.00	20.00	71.00	14.00
	LSD at 0.5	1.487	1.471	0.218	0.295
Pepper	Baladi at red	100.00	20.00	100.00	19.67
	Baladi at mature green stage	83.33	13.00	80.67	13.00
	Calivornia wounder at red	100.00	18.00	96.50	17.33
	Calivornia wounder at mature green stage	79.50	12.50	74.67	11.50
	LDS at 0.5	1.538	1.667	1.603	1.250

4. *In vitro* experiments

4.1. Effect of the bio-agent isolates on mycelial growth of *Alternaria alternata*

Bacillus subtilis, was the most effective bacterial isolates in reducing radial growth of *A. alternata*, followed by *P. fluorescens*. *T. viride* was the most effective fungal in reducing radial growth of *A. alternata*, followed by *T. harzianum* compared to the control treatment (Table, 4). Similar results were obtiand by El-Ghaouth *et al.*, (1998), Singh, *et al.*, (2000), Sivakumar *et al.*, (2000) Jiang *et al.*, (2001), El-Essaway *et al.*, (2003). The differences between the bioagents in reducing radial growth of *A. alternata* might by due to the differences between them in their mechanisms and production of anti fungal substances and their interaction on *A. alternata* (Singh, *et al.*, 2000; Sivakumar *et al.*, 2000; Jiang *et al.*, 2001 and El-Essaway *et al.*, (2003).

Table (4): Effect of different bio-agents on mycelial growth reduction percent of *Alternaria alternata*

Bioagents	Mycelial growth (%)	Mycelial growth reduction (%)
Trichoderma harzianum	34.74	65.26
Trichoderma viride	32.00	68.00
Bacillus subtilis	68.42	31.58
Pseudomonas fluorescens	47.37	52.63
Control	100.00	00
LSD at 0.5		0.287

Scanning electron microscope (SEM) micrograph gave an overview on the role of *Trichoderma* spp. on reducing mycelial growth of *A. alternata*. Fig.1

show that, the mycelium of *Trichoderma* spp. proved to grow around and over the mycelial growth of *A. alternata* and coiled around it. This phenomenon with mycoparasitsm. *Trichoderma* spp. can be penetrate and grow within mycelial of different fungal genera which eventually destroyed and inconsequence no more growth in addition to production of toxic substances (Chet (1984) found that, *Trichoderma* sp. apparently acts as mycoparasite which detects its host by sugerlectin linkage and begins to excrete exteracellular enzymes such as β-1,3-glucanase, chitinase, protease and or lipase.

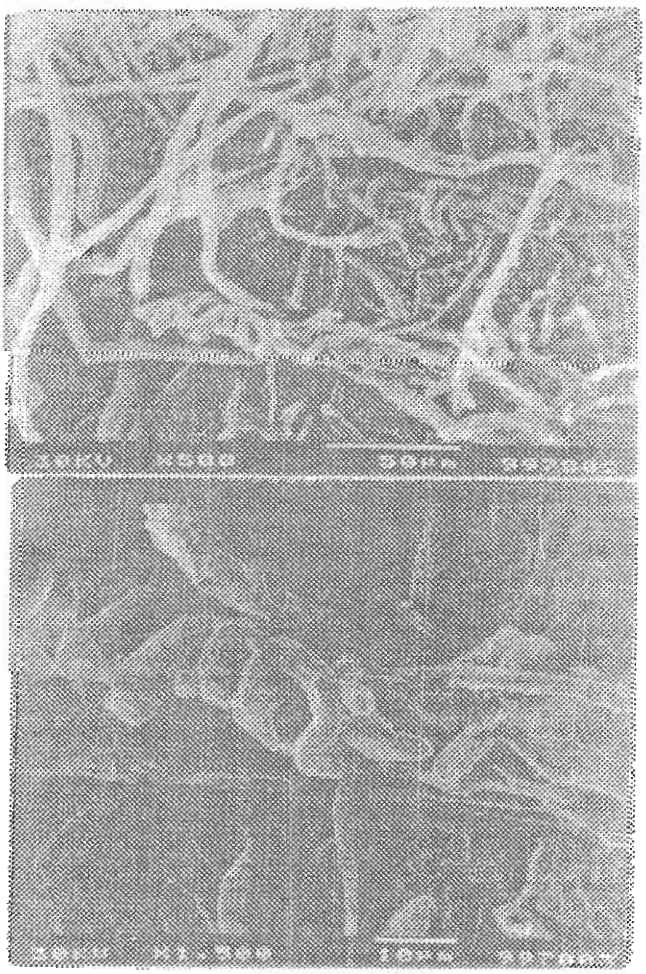

Fig. (1): Scanning electron microscope (SEM) micrographs showing the hyperparasitism (mycoparasitism) of *Trichoderma* spp. on the mycelium of *A. alternata* (notice, coiling of the mycelium of *Trichoderma* spp. around the mycelium of *A. alternata*.

4.2. Effect of different plant oil concentrations on mycelial growth of *Alternaria alternata*:

different essential plant oils were tested against fungal growth of *A. alternata iv vitro*. Caraway oil was the most effective one followed by garlic oil then brassic oil (Table, 5). The inhibitory effect of the tested oils increased by increasing the concentrations compared to the control and the fungicides used to control *A. alternata*. Similar results using plant oils were obtained by Srivastova *et al.*, (1995), Nath, *et al.*, (1994), Ragab Mona *et al.*, (2001) and Ibrahim *et al.*, 2003). The inhibitory effect of essential plant oils to pathogenic fungal growth may be regard to the presence of antifungal substances which may affect the synthesis of DNA and RNA (Xia *et al.*, 1995) and damaged the cell wall and cell membrane of the fungi i.e *A. alternata* (Gegoi *et al.*, 1997 and Ragab Mona *et al.*, 2001).

Table (5): Effect of different oil concentrations on the reduction of *A. alternate* mycelia growth compared with fungicides

Treatments	Concentrations (%)	Mycelial growth (%)	Mycelial growth reduction (%)
Aloe	0.00	100.00	0.00
	1.25	100.00	0.00
	2.50	65.67	34.33
	5.00	56.88	43.12
Mean		**80.64**	**19.36**
Garlic	0	100.00	0.00
	1.25	61.50	38.50
	2.50	55.00	45.00
	5.00	50.00	50.00
Mean		**66.63**	**33.37**
Caraway	0.00	100.00	0.00
	1.25	80.00	20.00
	2.50	45.67	51.33
	5.00	32.33	63.67
		64.5	**35.5**
Brassic	0.00	100.00	0.00
	1.25	85.00	15.00
	2.50	65.00	35.00
	5.00	58.45	41.55
Mean		**77.11**	**22.89**
Benlate	0.00	100.00	0.00
	125	66.20	33.80
	500.00	34.70	65.30
	1000.00	28.00	72.00
Mean		**57.23**	**42.77**
Zeinb	0.00	100.00	0.00
	125	89.98	10.02
	500.00	40.07	59.93
	1000.00	39.01	60.99
Mean		67.27	**32.73**
LSD at 0.5		Treatments (T) = 0.127 Concentrations (C) = 0.163 T x C = 0.399	

4.3. Effect of different concentrations of industrial detergents on mycelial growth of *Alternaria alternata*

The antifungal effect of industrial detergents against the growth of *A. alternata* was determined *iv vitro*. The different concentrations of two tested detergents completely inhibited the growth of *A. alternata* compared to the check (control) and the fungicides used to control *A. alternata* (Table, 6). Similar results were observed (El-Sharouny, 1988, Shkaraba *et al.*, 1991; Stanghellini and Miller, 1997 and Al-Dahmashi, 2004), who obtained the same effects with different detergents on different fungal genera.

Table (6): Effect of different concentrations of industerial detergents on mycelial growth reduction of *Alternaria alternata.*

Treatments	Concentrations (%)	Mycelial growth (%)	Mycelial growth reduction (%)
Citrol (liquid)	0.00	100.00	0.00
	1.25	0.00	100.00
	2.50	0.00	100.00
	5.00	0.00	100.00
Mean		**25.00**	**75.00**
Rabso (powder)	0.00	100.00	0.00
	1.25	0.00	100.00
	2.50	0.00	100.00
	5.00	0.00	100.00
Mean		**25.00**	**75.00**
Benlate	0.00	100.00	0.00
	125	66.20	33.80
	500.00	34.70	65.30
	1000.00	28.00	72.00
Mean		**57.23**	**42.77**
Zeinb	0.00	100.00	0.00
	125	89.98	11.02
	500.00	40.07	59.93
	1000.00	39.01	60.99
Mean		**67.27**	**32.73**
LSD at 0.5		Treatments (T) = 0.127 Concentrations (C) = 0.163 T x C = 0.399	

5. *In vivo*

5.1. Biological control of *Alternaria alternata* fruit rot of tomato and pepper:

Bacillus subtilis treatments of *A. alternata* inoculated tomato and pepper fruits showed the lowest percentage of infection (20 and 18.67 % of tomato and pepper, respectively), followed by *P. fluorescens* (22 and 19.5 % for tomato and pepper, respectively). *T. viride* was also effective in controlling fruits rot of tomato and pepper (25 and 22.67 %, respectively) than *T. harzianum* being (30 and 27.15%, respectively) as shown in Table (7). Obtained results were highly effective if compared with the tested fungicides.

Table (7): Effect of different bio-agents on tomato and pepper fruit rots caused by *Alternaria alternata*.

Host	Treatments	Infection		Disease severity	
		Infected (%)	Reduction (%)	Infected diam (cm)	Reduction (%)
Tomato	*T.harzianum*	30.00	70.00	1.36	65.39
	T.viride	25.00	75.00	1.23	68.70
	Bacillus subtilis	20.00	80.00	1.70	56.74
	Pseudomonas fluorescens	22.00	78.00	1.58	59.80
	Control	100.00	0.00	3.93	0.00
LSD at 0.5		1.314	1.667	1.052	1.091
Pepper	*T.harzianum*	27.15	72.85	1.25	44.20
	T.viride	22.67	77.33	1.18	47.33
	Bacillus subtilis	18.67	81.33	1.44	35.72
	Pseudomonas fluorescens	19.50	80.50	1.38	38.40
	Control	100.00	0.00	2.24	0.00
LSD at 0.5		1.800	0.233	0.622	1.030

T. viride was the most effective bio-agents in reducing the diameter of infected area of tomato and pepper being (1.23 and 1.18 cm for tomato and pepper respectively) followed by *T. harzianum* (1.36 and 1.25 cm, respectively). However, *Bacillus subtilis* was the lowest one. Similar results, were obtained by Tohamy, *et al.*, (1994); Chand-Goyal and Spotts (1997); El-Ghaouth *et al.*, (1998); Sivakumar *et al.*, (2000); Jiang *et al.*, (2001) and El-Essaway *et al.*, (2003).

Trichoderma spp. proved to be active against plant pathogenic fungi through, production of metabolites in the medium such as gliotoxin, which has a fungi-static effect (Brain and Hemning, 1945). Production of certain antibiotic i.e tricholin, which inhibit the mycelial growth when spread in the medium(Lin *et al.*, 1994) proved to producers for lytic enzymes, which degrade fungal cell wall (Sivan, & Chet, 1989 and Benhamou & Chet, 1993). In plant disease control, application of bacteria for controlling the diseases through producing salicylic acid (Chen *et al.*, 1999), bacterial metabolites (Steiner and Schönbeek, 1995), induction of laytic enzymes i.e chitinase and ß,1-3,gluconase (Chen *et al.*,1999; Esh & El-Kholi, 2005 and Kishore *et al.*,2005).

5.2. Effect of essential plant oils on *Alternaria alternata* fruit rot of tomato and pepper:

Caraway and garlic oils at a concentration 2.5% effectively controlled tomato and pepper fruit rots incited by *A. alternata*. Caraway oil was higher than garlic in reducing the percentage of the disease. However garlic was higher than caraway in reducing the diameter of infected area of both tomato and pepper. The effect of tested oils on disease percentage was similar to the effect of fungicides tested. While the opposite was true in case of the diameter of infected area (Table, 8 and 9). Similar results with essential oils were obtained by Inouye *et al.*, (1998);

Ragab Mona *et al.*, (2001) and Ibrahim *et al.*, (2003). The effect of plant oils on disease reduction might be regard to the presence of antifungal substances which may affect the synthesis of fungal DNA and RNA (Xia *et al.*, 1995) and damaged the cell wall and cell membrane of the fungi i.e *A. alternata* (Gegoi *et al.*, 1997; Wilson, *et al.*, 1997 and Ragab Mona *et al.*, 2001).

Table (8): Effect of caraway oil on tomato and pepper fruit rots caused by *Alternaria alternata*.

Host	Treatments	Infection		Disease severity	
		Infected (%)	Reduction (%)	Infected dim (cm)	Reduction (%)
Tomato	At light red stage	15.00	85.00	1.13	48.40
	Control	100.00	0.00	2.19	0.00
	At red stage	23.85	76.15	1.29	62.20
	At red stage benlate	13.33	86.67	2.33	31.87
	At red stage zeinb	16.53	83.47	2.73	20.17
	Control	100.00	0.00	3.42	0.00
LSD at 0.5			0.881		1.439
Pepper	At mature green stage	14.00	86.00	1.30	41.17
	Control	90.00	10.00	2.17	0.00
	At red stage	23.33	76.67	1.40	35.48
	At red stage benlate	11.67	88.33	0.90	59.27
	At red stage zeinb	12.50	87.50	1.3	41.17
	Control	100.00	0.00	2.21	0.00
LSD at 0.5			1.477		1.358

Table (9): Effect of garlic oil on tomato and pepper fruit rots caused by *Alternaria alternata*.

Host	Treatments	Infection		Disease severity	
		Infected (%)	Reduction (%)	Infected dim (cm)	Reduction (%)
Tomato	At light red stage	18.00	82.00	0.96	56.16
	Control	100.00	0.00	2.19	0.00
	At red stage	21.85	78.15	1.10	67.83
	Control	100.00	0.00	3.42	0.00
LSD at 0.5			0.840		0.982
Pepper	At mature green stage	11.00	87.78	0.85	60.82
	Control	90.00	0.00	2.17	0.00
	At red stage	18.33	81.67	0.60	72.85
	Control	100.00	0.00	2.21	0.00
LSD at 0.5			1.125		1.643

5.3. Effect of industrial detergent on *Alternaria alternata* fruit rot of tomato and pepper:

Industrial detergent (citrol at 2.5 %) reduced decay development in tomato and pepper caused by *A. alternata* after dipping the inoculated fruits for 15 minutes compared to the un-treated one (Table, 10). In addition washing tomato and pepper fruits in citrol 2.5 % significantly reduced the natural decay of tomato and pepper fruit rot compared to water washed fruits and or un-treated one (Table, 11). The effect of detergents in reducing tomato and pepper fruits may be due to, the anti-fungal effect of detergents on fungal growth (El-Sharouny, 1988, Shkaraba *et al.*, 1991; Stanghellini and Miller, 1997; and Al-Dahmashi, 2004).

Table (10): Effect of dipping infected tomato and pepper in detergent (citrol) at 2.5 % for 15 min on the rot infection caused by *A. alternata*.

Host	Treatments	Infection		Disease severity	
		Infected (%)	Reduction (%)	Infected dim (cm)	Reduction (%)
Tomato	At light red stage	23.33	76.76	1.25	42.92
	Control	100.00	0.00	2.19	0.00
	At red stage	29.96	70.04	1.45	57.60
	Control	100.00	0.00	3.42	0.00
LSD at 0.5			1.299		1.167
Pepper	At mature green stage	15.00	85.00	1.20	44.70
	Control	90.00	0.00	2.17	0.00
	At red stage	24.33	75.67	1.35	38.91
	Control	100.00	0.00	2.21	0.00
LSD at 0.5			0.966		1.226

Table (11): Effect of washing tomato and pepper fruits in liquid detergent (citrol) at 2.5% on the rots of tomato and pepper under naturally infection

Host	Treatments	Infection	
		Infected (%)	Reduction(%)
Tomato	Inoculated at light red stage	10.00	90.00
	Control (water)	35.67	64.33
	Control (untreated)	63.00	37.00
	Inoculated at red stage	13.50	86.5
	Control (water)	40.00	60.00
	Control (untreated)	67.00	33.00
LSD at 0.5		0.485	0.549
Pepper	Inoculated at green stage	0.00	100.00
	Control (water)	33.33	66.67
	Control (untreated)	40.00	60.00
	Inoculated at red stage	20.00	80.00
	Control (water)	35.50	64.50
	Control (untreated)	50.00	50.00
LSD at 0.5		0.315	0.128

REFERENCES

Abadias, M.; Teixido, N.; Usall, J., Benabarre, A. and Vinas, I. (2003): Viability, efficacy and storage stability of freeze-dried biocontrol agent Candida sake using different protective and dehydration media. J. Food Protection, 64, 856-861.

Aktar, K.B.; Matin, M.; Mirza, J.H.; shakir, A.S. and Rafique, M. (1994): Some studies on post-harvest diseases of tomato fruits and their chemical control. Pakistan J. Phytopathology, 6:125-129.

Al-Dahmashi, M.S.H. (2004): Effect of detergent polluted water on tomato, cucumber and some soil microorganisms and its chemo-remediation. Ph.D thesis, Fac. Agric., Alexandria Univ., pp 211.

Aly, A.Z.; Korim, A.M.A.; Kheder, M. A. and Shalaby, M.S.A. (1993): Studies on fruit rot of tomato. Zagazig J. Agric. Res., Vol. 20 (4) : 1255 –1270.

Barnett, H.L. and Hunter, (1998): Illustrated genera of the imperfect fungi. Burgess Publishing Company, Minneapolis, Minn. USA, 218 pp., Fourth Ed.

Beever, R.E.; Laracy, E.P. and Pak, H.A. (1989): Strains of *Botrytis cinerea* resistance to dicarboximide and benzimidazole fungicides in New Zealand vineyard. Pl. pathology, 38: 427-437.

Benhamou, N. and Chet, I. (1993): Hyphal interaction between *Trichoderma harzianum* and *Rhizoctonia solani* ulterastructure and gold cytochemistry of the mycoparasitic process. Phytopathology, 83: 1062-1071.

Brain, P.W. and Hemning, H.G. (1945): Gliotoxin a fungistatic metabolite produce of *Trichoderma viride*. Ann. Appl. Biol, 32: 214-220.

Brown, N. (1924): Two mycological methods. II A method of isolated single strain of fungi by cutting a hyphal tip. Ann. Bot; 38: 402-404.

Bruton, B.D.; Chondler L.D. and Miller, M.E. (1989): Relationships between pepper weevil and internal mold of sweet pepper. Rev. Pl. Path: 68 (3): 2955.

Chand-Goyal, T. and Spotts, R.A. (1997): Biological control of post-harvest diseases of apple and pear under semi-commercial and commercial conditions using three saprophytic yeasts. Biol. Control, 10:199-206.

Chen, C; Belanger, R.; Benhamou, N. and Paulituz, T. (1999): Role of SA in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber. Eur. J. Pl. Path. 105: 477-486.

Chet, I. (1984): Application of *Trichoderma* as biocontrol agent. Proc. 6th cong. Mediterr. Phytopathology, Univ. cairo, Egypt, 110-111.

Droby, S.; Cohen, L.; Daus, A.; Weiss, B.; Horev, B.; Chalutz, E.; Katz, H.; Keren-Tzur, M. and Shachnai, R. (1998): Commercial testing of Aspire: A yeast preparation for the biological control of post-harvest decay of citrus. Biol., control 12:97-101.

Duncan, D.B. (1954): Multiple range and multiple F test. Biometrics, 11: 1-42.

Esh, A.M.H and El-Kholi, M.M.A. (2005): Effect of *Pseudomonas fluorescens* extracellular enzymes and secondary metabolites on *Rhizoctonia solani*, the causal of sugar beet damping-off disease. Zagazig J. Agric Res., 32 (5) 1537-1557.

El-Essawy A. A; Brahim, K. I.; Hellal, G. A. and Abd-El-Aziz, Shadia A. (2003): Control of post-harvest tomato fruit rots (1) Biological Control. Tenth Congress of Phytopatholoyy, Giza, December, 2003:106-117.

El-Ghaouth, A.; Wilson, C. and Wisniewski, M. (1998): Ulterastructural and cytological aspect of the biocontrol activity of *Candida saitoana* in apple fruit, Phytopathology, 88:282-291.

El-Sharouny, H.M.M. (1988): Effect of detergents on cellulose decomposition in Egyptian soils. Egyptian J. Bot. 31 (1-3): 217-227.

Gang, S.W.; Chin, L.I.; and Shih, Y.C. (1994): Antifungal activity of chitosan and its preservative effect on low-sugar *Candida kumquant*. J. Food Protection, 56: 136-140.

Gegio, P.; Baruah, P. and Nakth, S. C. (1997): Antifungal activity of the essential oil of *Litsea cubeba* Pers. J. Essential oil Res., 9: 213-215.

Gomez, Kwanchai A. and Gomez A.A. (1984): Statistical Procedures for Agricultural Research, 2nd Ed. John Wiley & Sons Ltd., New York, 680 pp.

Harley, M.M. and Fergusen, I.K. (1990): The role of SEM in pollen morphology and plant systematic. pp 45-68. in: Scanning electron microscope in taxonomy and functional morphology. Claugher, D. (ed.). Systemic Association special Vol. 41, Larendon Press, Oxford.

Ibrahim, K.I; El-Essawy, A.A.; Tohamy, M.R.A. and Abd-El-Aziz, Shadia A. (2003): Control of post-harvest tomato fruit rots III using chemical treatment. Tenth Congress of Phytopathology, Gize, Egypt, December: 119-129.

Inouye, S.; Watanabe, M.; Nishigama, Y.; Takeo, K.; Akao, M. and Yamagachi, H. (1998): Antisporulating and respiration-inhibitory effects of essential oils on filamentous fungi. Mycoses, 41: 403-410.

Jiang, Y.M; Zhu, X.R. and Li, Y.B. (2001): Post-harvest control of litchi fruit rot by *Bacillus subtilis*. Lebensmittel Wissenschaft and Technologie, 34: 430-436.

King, E.O.; Ward, M.K. and Raney, D.E. (1954): Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44: 301-307.

Kishore, G.K.; Pande, S. and Podile, A. R. (2005): Biological control of late leaf spot of peanut (*Arachis hypogaea*) with chitinlytic bacteria. Phytopathology 95: 1157-1165.

Labavich, J.M.; Johnson, C.I.; Havley, E. and Joyce, D.C. (1998): Fruit ripening and defence against pathogens loss of resistance or gain of susceptibility. ACIAR. Proc. Series, 80: 53-59.

Lin, A.; Lee, T.M. and Rern, J.C. (1994): Tricholin a new antifungal agent from *Trichoderma viride* and its action in biological control of *Rhizoctonia solani*. J. Antibiotics, 47 (7): 799-805.

Nath, S.C.; Hazarika, H.; Singh, S. and Ghosh, A.C. (1994): *Litsea cubeba* Pers. A promising source of citral from Northeast India. Indian Perfum., 38: 26-28.

Pathak, V.N. (1984): Laboratory manual of plant pathology. 2nd ed Oxford & IBH Pub. Co., New Delhi, pp 23-25.

Ragab, Mona, M.; Ragab, M.; El-Naggar, M.A. and Farrag Eman S. (2001): Effect of lemon grass oil on post-harvest decay of tomato fruits. Egypt .J. Phytopathology., 61-68.

Reda F; Mouse, O. M.; Sejing, M.J. and Nawar, L.S. (1988): Responses of tomato cultivars to infection with *Alternraia solani* in relation to growth and chemed composition of their fruits. Rev. Pl. Path, 67: 956.

Schlösser, E. (1977): Role of saponin in anti-fungal resistance. IV. Tomatin-dependent development of species *Alternaria* on tomato fruits. Acta Phytopathology. 11: 77-78.

Shkaraba, E.M.; Perevedentseva, L.G. and Mekhonoshin, L.E. (1991): Consortium between forest plants and fungi under conditions of industrial pollution. Ekologiya Sverdi 6:12-17.

Singh, R.S.; Puneet, S.; Jaspal, K.; Singh, P. and Kaur, J. (2000): *Trichoderma* based bio-fungicide: progress and prospects. Pl. Disease Research, 15:155-161.

Sivakumar, D.; Wijeratnam, R.S.; Wijesundera, R.L., Marikar, F.M., and Abeyesekere, M. (2000): Antagonistic effect of *Trichoderma harzianum* on post-harvest pathogen of rambutan (*Nephelium lappaceum*). Phytoparasitica, 28:240-247.

Sivan, A. and Chet, I. (1989): Degradation of fungal cell wall by lytic enzymes of *Trichoderma harzianum*. J. Genetic Microbial, 135:675-682.

Srivastova, S; Naik, S.N. and Maheshwari, R.C. (1995): *In vitro* studies on antifungal activity of palmorosa and eucalyptus oils. Indian Perfum., 34: 277-279.

Stanghellini, M.E. and Miller, R.M. (1997): Biosurfactants their identity and potential efficacy in the biological control of zoosporic plant pathogens. Pl. Dis. 81: 4-12.

Steiner, U. and Schönbeek, F. (1995): Induced resistance against biotrophic fungi. Modern fungicides and antifungal compounds, 11th International Symposium, May 14 th –20th, 1995 Germany.

Tohamy, M.R.; Aly, A.Z.; Abu-Zaid, M.I. and Shalaby M.S. (1994): Chemical and biological control of tomato and pepper fruit disease caused by *Alternaria* spp. Fifth Arab Congress of Plant Protection, Fez, Morocco, 27 Nov. 2 Dec., (Abst. No 94).

Tohamy, M.R.; Helal, G.A.; Ibrahim, K.I. and Abd-El-Aziz, Shadia, A. (2004): Control of post-harvest tomato fruit rots: II. Using heat treatments. Egyptian J. Phytopathology, 32: 129-138.

Wall, M.M. and Biles, C. L. (1993): Alternaria fruit rot of ripening chile peppers. Phytopathology, 83 (3): 324-328.

Wilson, C.L; Solar, J.M.; El-Ghoouth, A. and Wisniewski, M. E. (1997): Rapid evaluation of extracts and oils for antifungal activity against *Botrytis cinerea*. Pl. Dis., 81: 204-210.

Xia, Z.G.; Yang, J.X. and Li, P.T. (1995): Study on antifungal mechanism of *Litsia cubiba* oil in *Candida albicans*. Bull. Human Med. Univ., 20:107-108.

دور بعض العوامل الحيوية وغير الحيوية في مقاومة العفن الألترناري لثمار الطماطم والفلفل

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** قسم أمراض المحاصيل السكرية- مركز البحوث الزراعية

تم عزل العديد من الأنواع التابعة لأجناس فطرية مختلفة وذلك من ثمار الطماطم والفلفل المصابة طبيعيا والتي تم جمعها من أسواق مختلفة من محافظة الشرقية. وقد كان الفطر الترناريا الترناتا أكثر تلك الفطريات تكرارا في العزل، ولذلك تم اختياره لإكمال بعض الدراسات عليه.

أوضحت النتائج المتحصل عليها أن عدوى ثمار الطماطم والفلفل في طور النضج الأحمر والمجروحة أدي إلي زيادة حساسيتها للمرض، حيث زادت بها نسبة الإصابة وشدة الإصابة (قطر المنطقة المصابة) وذلك مقارنة مع ثمار الطماطم في طور النضج الأحمر الفاتح وثمار الفلفل في طور النضج الأخضر الناضج وأيضا الثمار غير المجروحة. وكانت ثمار صنف الطماطم أوريت أكثر حساسية من الصنف جي-إس وكذا ثمار صنف الفلفل بلدي أكثر حساسية من ثمار الصنف كاليفورنيا ووندر فى كل من طوري النضج تحت الاختبار.

أوضحت الدراسات المعملية أن زيت الكراوية وزيت الثوم وكذا المنظفات الصناعية (سترول ورابسو) وكائنات المقاومة الحيوية مثل البكتريا باسيلس ستلس والبكتريا سيدوموناس فلورسنت والفطر تريكودرما هارزيانم والفطر ترايكودرما فيردي كانت فعالة في تثبيط النموالخطي للفطر الترناريا الترناتا. وقد زاد تأثير الزيوت والمنظفات الصناعية بزيادة التركيز، حيث ثبطت المنظفات نموالفطر تماما، إذا ما قورنت بفعل مبيدي الزينب والبنلت. أوضح الفحص بالميكروسكوب الضوئي الماسح أن أنواع الفطر تريكودرما تحت الاختبار لها القدرة علي التطفل الفوقي علي ميسليوم الفطر الترناريا الترناتا.

خفضت معاملة نقع الثمار في معلقات كائنات المقاومة الحيوية نسبة الإصابة وشدتها عند تطبيقها بعد العدوي مباشرة. وقد خفض زيت الكراوية وزيت الثوم نسبة الإصابة وقطر المنطقة المصابة. كما أدي غسل ثمار الطماطم والفلفل في محلول ٢,٥ % من المنظف الصناعي سترول إلي خفض نسبة الإصابة الطبيعية في تلك الثمار مقارنة بالثمار المغسولة بالماء أوالثمار غير المعاملة تماما. بالإضافة إلي أن نقع ثمار الطماطم والفلفل والتي تم عدواها بالفطر الترناريا الترناتا أدي إلي خفض نسبة الإصابة وقطر المنطقة المصابة مقارنة بالثمار غير المعاملة.