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CONTROLLING TOMATO FRUIT ROTS CAUSED BY *Alternaria tenuis* AND *Stemphylium botryosum* DURING STORAGE USING SOME PLANT OILS AND EXTRACTS.

Mostafa, M.A.; K.K. Sabet and M.H.Abou Hatem
Pl. Pathol. Dept. Fac. of Agric. Cairo Univ.

ABSTRACT

Isolation trials from rotted tomato fruits, collected from different markets in Giza, Qalubyia and Ismailia governorates yielded eleven fungal species belonging to ten fungal genera. *Alternaria tenuis* was the most prevailing fungus, followed by *Stemphylium botryosum*. The fungi isolated varied in their pathogenic capabilities to tomato fruits. *A. tenuis* and *S. botryosum* were the most destructive ones attacking advanced mature green tomatoes. Among 12 natural plant oils tested against the growth of the two tested fungi, cumin (*Cummin cyninun*) oil was the most effective one, completely inhibited the mycelial growth of *A. tenuis* and *S. botryosum* at concentration 0.5%, while almond (*Amygdalus communis*) and fenugreek (*Trigonella foenumgraecum* L.) oils at 3% occupied the second position in this regard. Treating tomato fruits by natural plant oils completely prevented fruits from infection by the two fungi tested when storage was carried out for 12 days at room temperature (20°C±2 °C) or at 10°C for 12 days. Between five plant leaf extracts tested, neem (*Azadirichta indica*) extract when used with 100% completely inhibited the growth of the two tested fungi. Treating tomato fruits with any of the five plant leaf extracts at high concentration (100%) significantly reduced the percentage of fruit rot caused by the two tested fungi when stored under room temperature (20±2 °C) or at 10°C. Under field conditions, the effectiveness of spraying fruited tomato plants (Castle Rock cv.) at advanced mature green stage with some natural plant oils and plant leaf extracts for controlling postharvest tomato fruit rots was also studied.

INTRODUCTION

Tomato plants grown under Egyptian conditions are severely attacked by many fungal diseases resulting in high yield losses (Abd El-Rahim, 1988; Hasan, 1995; Ouf *et al.*, 1998; Soltan, 1998; Ragab *et al.*, 2001; Abd-Elaziz,Shadia, 2003 and Abdel-Sayed, 2006).
Crop devastation due to *Alternaria tenuis* and *Stemphylium botryosum* has been reported in Bangladesh and S.E Asia, where this disease is a major constraint to tomato production (Baker and Umetsu, 2001 and Saha, *et al.* 2005).

The ability of *A. tenuis and S. botryosum* to infect tomato fruits depend on their adaptability to the environmental conditions prevalent in Egypt. Both fungi are affect on wide host range under different environments, suggesting their ability to adapt to varying climatic conditions. As with most host-pathogen interactions, temperature and moisture are the primary environmental factors affecting *A. tenuis and S. botryosum* conidial germination and disease development (Dodds *et al.* 1991; Patel and Patel. 1991; Patil *et al.*, 1995; Ruchi-Sood and Sharma, 2003; Tohamy *et al.*, 2004). However, few studies have been investigated the effects of temperature and moisture on conidial germination of *Alternaria* spp and *Stemphylium* spp.

Most of these studies have been on *Alternaria* spp., which is closely related to genus *Stemphylium*. According to these studies, conidial germination occurs over a wide range of temperature (5° to 30°C) and starts within 1 to 3 h of incubation at optimal temperatures (Jacobi, *et al.*, 2000). *Alternaria* spp. and *Stemphylium* spp., in general, require the presence of free water to germinate (Patel and Patel, 1991). *Alternaria tenuis* and *Stemphylium* spp. are able to penetrate the host directly through the epidermis or indirectly through the stomata after the formation of the appressoria (Abdel-Sayed, 2006).

The application of chemical treatments (fungicides, insecticides, acaricides and herbicides) resulted in harmful effects on the Egyptian environment including animals, plants, soil, water, air and human beings. Use of natural plant products has been emerged as a new strategy in disease management program (Adisa 1985, Akhatar, *et al.*, 1994; Ouf, *et al.*, 1998; Karthikeyan, *et al.*, 2002; Schilder, *et al.*, 2002; Abd-Elaziz,Shadia, 2003 and Saha, *et al.*, 2005). A large number of plant products derived from forage, pasture, aromatic, medicinal, wild plants etc., has been used successfully to diminish some plant pathogenic microorganisms. Some natural plant oils and plant extracts (Cumin, Fenugreek, Fennel, Marjoram, Almond, Caster, Anise, Eucalyptus, Parsley, Mint, Onion and Garlic) play important roles in inhibiting growth and sporulation of wide range of mold fungi (Singh and Gupta, 1992; Narasimhan *et al.*, 1993; Chattopadhyay, 1999; Mekuria *et al.*, 1999; Rushid, 2000; Abd-Elaziz,Shadia, 2003; Saha *et al.*,2005 and Marietta Petróczy *et al.*, 2006)

The objective of this study is to through some light on the importance of using some natural plant oils and plant leaf extracts on controlling tomato fruit rots caused by *Alternaria tenuis* and *Stemphylium botryosum* during storage.

MATERIALS AND METHODS

1. Isolation, purification and identification of the associated organisms of tomato fruit rots:

Isolation trials were carried out from rotted tomato fruits collected from wholesale and retail markets at Ismailia, Giza and Qalubyiah. The collected fruits were washed carefully with tap water and then surface sterilized by immersing in 70% ethyl alcohol and dried for one minute using sterile filter papers. Sterilized samples were cut into small pieces and separately transferred to sterilize Petri-dishes containing plan agar (PA) medium, and then incubated at 25°C for 7 days. Petri-dishes were daily observed and the emerged fungi were picked up and transferred on fresh potato dextrose agar (PDA) medium to obtain pure culture. The isolated fungi were purified using single spore technique or by hyphal tip transfers method mentioned by Dhingra and Sinclair (1985) then kept in refrigerator on PDA medium for further experiments.

The fungal colonies arising on the plates were identified after consulting to the following references:

Ellis (1971); O'Donnell (1979); Nelson *et. al.* (1983) and Raper and Fennell (1992)

2. Pathogenic capability of fungi isolated from rotted tomato fruits:

The isolated fungi were investigated for their pathogenicity on apparently healthy tomato fruits of Castle Rock cv. Healthy fruits at Light-red stage were carefully washed rinsed with sterile water then kept to dry at room temperature, surface disinfected in 70% ethyl alcohol for one minute. By the aid of a small scratch, wounds were made in each fruit and inoculated by each fungus separately using the equal mycelial disks (3 mm in diameter), taken from the margin of 7 days old PDA cultures and set one disk in each scratch. Chech treatments were similarly carried out using fungal free medium. Three replicates, each containing ten fruits, were used for each treatment. The inoculated fruits for each treatment were put in sterilized carton box with 5 kg capacity and kept at room temperature (20-25°C) for 12 days. The resulted rot was calculated using the decay index and severity of infection according to Chastanger and Ogawa (1979) based on visual inspection of each fruit infection. Infected fruits were placed in one of the following five categories:

0=superficial fleck (no soft decay)
1=1-24% of the surface decayed
2=25-49% of the surface decayed
3=50-74% of the surface decayed
4=75% or more of the surface decayed

The decay index (DI) for each treatment was obtained as follows:

DI=Sum. (Number of fruits per category × category number) ÷ Total number of fruits infected

% severity of infection = (DI/4) × 100

3. Effect of some natural plant oils and plant leaf extracts on the linear growth of *A.tenuis* and *S. botryosum*:

a. Effect of natural plant oils

Natural twelve plant oils viz; casterbean (*Ricinus communis*), almond (*Amygdalus communis*), fenugreek (*Trigonella foograecum* L.), eucalyptus (*Eucalyptus rostorata*), fennel (*Foeneculum vulgare*), parsley (*Petroselium sativum*), mint (*Minth viridis*), garlic (*Allium sativum*), anise (*Piminella anisum*), onion (*Allium cepa*), cumin (*Cummin cyninum*) and marjoram (*Origanum majorana*) were extracted using steam distillation method described by Guenther (1961). The extraction was done in laboratory of Medicinal and Aromatic Pl. Res. St. El- Quanater El- Khairiah, Qalubyiah governorate, Hort. Res. Inst., Agric. Res. Center (ARC), Giza, Egypt. These oils were used at different concentration 0.5,1, 2, 3, and 5% to determine their effects on the radial growth of the two tested fungi on PDA medium. The stock medium of PDA for each oil concentration was poured into Petri-dishes. After solidification, dishes were inoculated at the center with 5 mm mycelial disc cut from the periphery of 7 days old of any of the two tested fungi. The inoculated plates were incubated at the optimum temperature for each fungus (25°C.for *A. tenuis* and 20°C for *S. botryosum*) .Three replicates were prepared for each treatment. Plant oil free PDA medium was used as control. The diameter of developed colonies was measured when the mycelial growth of the fungus covered the plates of check treatment.

Most of these studies have been on *Alternaria* spp., which is closely related to genus *Stemphylium*. According to these studies, conidial germination occurs over a wide range of temperature (5° to 30°C) and starts within 1 to 3 h of incubation at optimal temperatures (Jacobi, *et al.*, 2000). *Alternaria* spp. and *Stemphylium* spp., in general, require the presence of free water to germinate (Patel and Patel, 1991). *Alternaria tenuis* and *Stemphylium* spp. are able to penetrate the host directly through the epidermis or indirectly through the stomata after the formation of the appressoria (Abdel-Sayed, 2006).

The application of chemical treatments (fungicides, insecticides, acaricides and herbicides) resulted in harmful effects on the Egyptian environment including animals, plants, soil, water, air and human beings. Use of natural plant products has been emerged as a new strategy in disease

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b. Effect of plant leaf extracts:

Preparation of plant leaf extracts:

The juice of 100 g fresh weight of leaves the certain plant species, *i.e.* oleander (*Nerium oleander*), eucalyptus (*Eucalyptus rostorata*), neem (*Azadirichta indica*), basil (*Ocimum basilicum*), and marjoram (*Origanum majorqana*) were obtained by processing them in a blender for 5 minutes, filtered through cheese cloth, centrifuged at 3000 rpm for 15 minutes, and sterilized through G5 filter. Crude extract was considered 100%.

Effect of different concentrations of plant leaf extracts on the linear growth of the tested fungi:

Five different concentrations (0, 25, 50, 75 and 100%) of plant leaf extracts of the previously mentioned plants were used. To obtain any concentration mentioned before crude extract was separately mixed with PDA medium before solidification, and then poured into sterilized Petri-dishes. Three replicate plates for each concentration were inoculated at the center with equal discs, 5 mm in diameter, taken from 7 days old culture of the two pathogenic fungi. The plates were incubated at the optimum temperature for each. Linear growth, in mm, of the tested fungi was measured after the diameter of the growth in control treatment reached to 90 mm.

4. Effect of plant oils *in vivo:*

In vitro studies indicated that cumin; marjoram; mint, eucalyptus and fenugreek were the most effective oils in reducing growth of the two tested fungi especially at 5% concentration, hence, these oils were used for controlling tomato fruit rots caused by *A. tenuis* and *S. botryosum in vivo*. Surface sterilized light-red tomato fruits of Castle Rock variety uniform in size and colour were divided into two parts. First part, stander amount of inoculum discs (5 mm diameter) of each fungus was introduced through a very small scratch in the middle surface of fruit as previously described. Second part, tomato fruits were left without inoculation. One group of both inoculated and non inoculated tomato fruits were dipped for one minute in any concentration mentioned before of one of the tested oils, and the other group immersed in sterile water as check. Each treatment was also divided into two groups, the first was stored under room temperature (20°C±2°C.), and the second was stored at 10°C. Three replicates were prepared and twenty fruits for each replicate. The results obtained after 12 days were expressed as percentages of fruit rot.

5. Effect of plant leaf extracts *in vivo:*

In vitro studied revealed that leaf extract of oleander, eucalyptus, neem, basil, and marjoram, each at high concentration (100%) gave noticeable reduction or completely inhibited mycelial growth of the two tested fungi. These plant extracts at 100% were also tested for controlling the disease *in vivo*. Sterilized light-red tomato fruits of Castle Rock cv. (uniform in size and colour) were grouped into two groups, the first was artificially inoculated with stander amount of inoculum discs (3 mm diameter) of any of the tested fungi fungus introduced via a very small scratch in the middle surface of the fruit. Meanwhile, the second group contained tomato fruits left without inoculation. One group of both inoculated and non inoculated tomato

fruits were dipped for one minute in the extraction of any of the tested leaf extracts, and the other group was immersed in sterile water as check. Each treatment was divided into two groups, the first was stored under room temperature (20°C±2°C.), and the second was stored at 10°C. For each treatment, three replicates were used and twenty fruits per replicate. Percentages of fruit rot were assessed 12 days after inoculation.

6. Effect of spraying tomato plants with some natural plant oils and extracts under field conditions, on the occurrence of fruit rots during storage:

This experiment was carried out to study the effect of spraying tomato fruits on advanced green stage under field conditions with different natural plant oils and extracts on the percentages of fruit rots after harvesting and storing at room temperature (20°±2)°C or at 10 °C, for 12 days.

Tomato plants (Castle Rock cv.) received all the routine treatments were sprayed at advanced green stage under field condition in Qalubyia governorate (Experimental station of Vegetable Research Center, El-Dokki) during November, 2005 with plant oils (5%) and plant leaf extracts (100%).This experiment was repeated again on the same field during November 2006.

Thirteen tomato plants (Castle Rock cv.), were sprayed with any of plant oils (5%) and plant leaf extracts (100%) as mentioned before. Fruits at light red stage were harvested and transferred in perforated carton boxes (capacity 5 Kg), and kept in a refrigerator (10 °C) during transportation. In lab tomato fruits were grouped into two groups, the first was stored under room temperature (20°C±2), and the second was stored at 10 °C in a refrigerator for 12 days. The results were expressed as percentages of fruit rots.

Statistical analysis:

Most of the obtained data were statistically analyzed using randomized complete block and the split plot designs according to Snedecor and Cochran (1967). Averages were compared at 0.05% level of probability using Least Significant Difference (LSD) as mentioned by Fisher (1958).

RESULTS AND DISCUSSION

Tomato fruits are subjected during storage and marketing, to infection by various pathogens that reduce its quality and quantity. Fungal fruit rots are considered the most important factor, which limits the exportation of the crop.

For all these reasons, the objectives of this study were aimed to determine this problem and to reach safety control measures for fruit rots of tomatoes.

Isolation and identification of the associated organisms of tomato fruit rots:

In the present study (Table,1), *Alternaria tenuis* , *Stemphylium botryosm*, *Aspergillus niger*, *A. flavus*, *Botrytis cineria*, *Fusarium solani*, *Mucor* sp., *Rhizopus stolonifer*, *Helminthosporium* sp., *Rhizoctonia solani* and *Penicillium* sp. were the main fungi associated with rotted tomato fruit, collected from different markes located in Giza, Qalubyia and Ismailia governorates .

Data also revealed that the most prevailing fungus was *A. tenuis* which presents in high frequency, followed by *S.botryosum*. These results are in accordance with those reported by several investigators (Ruchi-Sood and Sharma, 2000; Farrag, 2001; Tietjen, *et al.* 2001; Amadioha and Uchendu, 2003; Rosa-Marquez and Fornaris-Ruilan, 2003; Ruchi-Sood and Sharma, 2003; Patel, *et al.* 2005 and Shehata, 2006).

Pathogenic capability of fungi isolated from rotted tomato fruits:

The isolated fungi were varied in their pathogenic capabilities to tomato fruits (Table, 2). *A. tenuis and S. botryosum* were the most destructive pathogens attacking advanced mature green tomatoes.

Table (1): Fungi isolated from rotted tomato fruits and their frequencies.

Fungi	Giza		Qalubyiah		Ismalia	
	*No.	%	*No.	%	*No.	%
Alternaria tenuis	25	32.1	39	50.0	34	36.2
Stemphylium botryosum	8	10.3	12	15.4	11	11.7
Botrytis cinerea	7	9.0	0	0.0	5	5.3
Fusarium oxysporum	8	10.3	0	0.0	4	4.3
Aspregillus niger	9	11.5	6	7.7	7	7.4
A. flavus	5	6.4	0	0.0	5	5.3
Penicillium sp.	8	10.3	7	8.9	6	6.4
Rhizopus stolonifer	9	11.5	8	10.3	9	9.6
Rhizoctonia solani	0	0.0	0	0.0	6	6.4
Mucor sp	0	0.0	0	0.0	2	2.1
Helminthosporium sp.	0	0.0	6	7.7	5	5.3
Total	7.18	79.0	7.09	78.0	8.00	94.0

***No. Number of fungal colonies**

These two fungi are well known as producer of pectinolytic enzymes which play a role in plant cell wall degradation during their invasion (Deuel and Stutz, 1958). Therefore, they were chosen for further studies in this work. Tomato fruits infected by *A. tenuis* and *S. botryosum* contain high amounts of polygaiacturonase (PG) and pectinmethylesterase (PME) than those produced from healthy (non inoculated) fruits (Chandra and Tandon, 1964). On the other hand, *A. tenuis* was reported as a producer of PG and PME higher than *S. botryosum* in inoculated fruits (Garibaldi and Bateman (1971). Similar results were also reported by Patil *et al.* (1995); Farrag-Eman (2001) and Ragab *et al.* (2003). While Ruchi-Sood and Sharma (2003) found that *Alternaria* spp viz; *A. alternata, A. solani,* and *A. tomato* are causing postharvest losses to tomato fruits in India.

Effect of some natural plant oils and plant leaf extracts on the linear growth of the tested fungi.

Data obtained (Tables 3 and 4) point out that all the tested natural oils have positive effects in reducing mycelial growth of the two tested pathogenic fungi. As the concentration of each oil increase, the linear growth of both fungi decreased, and this effect was differed according to the oil type.

Table (2): Decay index and severity of infection (%) of fungi isolated from rotted tomato fruits on Castle Rock tomato cv., 12 days after inoculation at room temperature (20°-25°C).

Fungi	Decay index	% severity of infection
Alternaria tenuis	3.00	75.00
Stemphylium botryosum	2.40	60.00
Botrytis cineria	2.20	37.50
Fusarium oxysporum	2.20	55.00
Aspregillus niger	1.50	37.50
A. flavus	2.00	50.00
Penicillium sp.	1.00	25.00
Rhizopus stolonifer	1.65	41.25
Rhizoctonia solani	1.00	25.00
Mucor sp	1.00	25.00
Helminthosporium sp.	1.00	25.00
Mean	1.72	41.48

Cumin oil was the most effective one, which completely inhibited the mycelial growth of *A. tenuis* and *S. botryosum* at 0.5% followed by almond, mint and garlic in this regard (Table, 3). The respective averages of the linear growth were 26.0, 31.6 and 22.6 mm, respectively. On the other hand, caster bean oil followed by onion oil were least effective ones, being 44.6 and 44.4 mm, respectively. Mint, almond and Fenugreek oils at 3% decreased the mycelial growth of *S. botryosum* from 90 mm in the absence of oil (control) to 5, 11, 5 mm, respectively (Table, 4).

Table (3): Percentage of reduction in the mycelial growth of *A. tenuis* as a result of incorporating PDA medium with different plant oils.

Oils tested	Linear growth in mm at % concentration of natural plant oils 0.5%	%Red.	1%	%Red.	2%	%Red.	3%	%Red.	5% %Red.		Mean
Almond	68.0	34.4	32.0	64.4	25.0	72.2	5.0	94.4	0.0	100.0	26.0
Anise	67.0	25.6	49.0	45.6	52.0	42.2	33.0	63.3	22.0	75.6	44.6
Caster	68.0	24.4	56.0	37.8	46.0	48.9	40.0	55.6	22.0	75.6	46.4
Cumin	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
Fennel	58.0	35.6	55.0	38.9	46.0	48.9	20.0	77.8	15.0	83.3	38.8
Fenugreek	57.0	36.7	50.0	44.4	44.0	51.1	20.0	77.8	15.0	83.3	37.2
Eucalyptus	55.0	38.9	47.0	47.7	38.0	57.8	40.0	55.6	0.0	100.0	36.0
Garlic	37.0	58.9	35.0	61.1	20.0	77.8	36.0	60.0	35.0	61.1	32.6
Marjoram	81.0	10.0	45.0	50.0	39.0	57.0	42.0	53.3	0.0	100.0	41.0
Mint	50.0	44.4	48.0	46.7	40.0	55.6	20.0	77.8	0.0	100.0	31.6
Onion	54.0	40.0	56.0	37.8	50.0	44.4	36.0	60.0	27.0	70.0	44.6
Parsley	58.0	35.6	41.0	54.4	30.0	66.7	23.0	74.4	11.0	87.8	32.6
(Check)	90										
Mean	54.4	40.4	42.8	54.4	40.4	60.2	54.4	40.4	12.3	54.4	40.4

LSD at level 0.05% for
Plant oil treatment (O) 2.34
Concentreation (C) 1.66 O×C 5.74

Table (4) shows that the fungus *A. tenuis* was greatly affected by the tested plant oils than the fungus *S. botryosum* and cumin followed by almond still the most effective ones, being 0.0 and 13.6 mm, on the average,

respectively. Meanwhile, eucalyptus oil followed by parsley oil were the lowest effective ones, being 42.2 and 40.0 mm, on the average respectively. Natural plant essential oils have long been recognized as good fungitoxic compounds but they have not been developed into products for postharvest treatments since industry finds it easier to potent and protect newly synthesized compounds than natural plant products (Singh, 1980). Many essential oils exhibited inhibitory properties in challenge tests against microorganisms (Beuchat, 1994). These oils, however, contained specific components that can inhibit the growth of certain microorganisms (Lawless, 1995)

Table (4): Percentage of reduction in the mycelial growth of *S. botryosum* as a result of incorporating PDA medium with different plant oils.

Oils tested	Linear growth in mm at % concentration of natural plant oils 0.5%	%Red.	1%	%Red.	2%	%Red.	3%	%Red.	5%	%Red.	Mean
Almond	23.00	74.4	19.00	78.9	15.00	83.3	11.00	87.8	0.00	100.0	13.6
Anise	65.00	27.8	41.00	54.4	29.00	67.8	23.00	74.4	15.00	83.3	34.6
Caster	80.00	11.1	61.00	32.2	39.00	56.6	20.00	77.8	0.00	100.0	40.0
Cumin	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.0
Eucalyptus	30.00	66.7	27.00	70.0	26.00	71.1	20.00	77.8	0.00	100.0	20.6
Fennel	25.00	72.2	55.00	38.9	30.00	66.7	26.00	71.1	15.00	83.3	30.2
Fenugreek	17.00	81.1	17.00	81.1	5.00	94.4	5.00	94.4	0.00	100.0	8.8
Garlic	54.00	40.0	53.00	41.1	37.00	58.9	32.00	64.4	20.00	77.8	39.2
Marjoram	50.00	44.4	31.00	65.6	25.00	72.2	20	77.8	15.00	83.3	28.2
Mint	60.00	33.3	56.00	38.8	45.00	50.0	0.00	100.0	0.00	100.0	32.2
Onion	53.00	41.1	51.00	43.3	47.00	47.8	40.00	55.6	35.00	61.1	42.2
Parsley	20.00	77.8	36.00	60.0	29.00	67.8	15.00	83.3	10.00	88.9	22.0
(Check)	90										
Mean	39.75	49.38	37.25	58.69	27.25	73.7	17.67	86.35	9.17	89.80	

LSD at 0.05 for

Plant oil treatment (O)	2.34		
Concentreation (C)	1.75	O×C	6.07

Effect of some plant extracts on the linear growth of the two tested fungi:

Regarding to the effect of some plant extracts against the linear growth of the two pathogenic fungi, data obtained (Table, 5) clearly showed that all extracts at any concentration significantly reduced the mycelial growth of both *A. tenuis* and *S. botryosum* in comparison with check treatment. It was also noticed from data that, the increase in concentration of each plant leaf extract tested caused noticeable decrease in the linear growth. This effect was greatly differed according to the type of the plant extract tested. Neem extract completely inhibited the growth of the two fungi tested at 100%. Meanwhile, marjoram and basil leaf extract gave the lowest effect on the linear growth of both *A. tenuis* and *S. botryosum*.

The effect of twelve plant oils, *i.e.* caster bean , almond, fenugreek, eucalyptus, fennel, parsley, mint, garlic, anise , onion , cumin and marjoram on the infection by the two pathogenic fungi was tested for their effectiveness to control tomato fruit rots during storage at room temperature (20°±2°C.) and at 10°C. Results obtained (Table, 6) demonstrate that the percentages of

fruit rot infection significantly decreased as the results of using any of the tested oils. Tomato fruits either artificially inoculated with *A. tenuis* or not and treated with garlic and cumin, completely prevented the fruits from rot when storage carried out under room temperature (20°±2 °C) or at 10°C.

Table(5): Effect of incorporating PDA medium with different plant leaf extracts at five different concentrations (0, 5, 25, 50, 75 and 100%) on the linear growth of *A. tenuis* and *S. botryosum* after 8 days of incubation .

Plant leaf Extract	Concentration%	Linear growth (mm) of *A. tenuis*	Linear growth (mm) of *S.botryosum*	Mean
Basil	5.0	87.0	85.0	86.0
	25.0	80.0	80.0	80.0
	50.0	77.0	79.0	78.0
	75.0	86.0	51.0	68.5
	100.0	55.0	40.0	47.5
(Check)	0.0	90.0	90.0	90.0
Eucalypt	5.0	67.0	79.5	73.0
	25.0	38.0	60.0	49.0
	50.0	47.0	51.0	49.0
	75.0	31.0	40.0	35.5
	100.0	19.0	16.0	17.0
(Check)	0.0	90.0	90.0	90.0
Marjoram	5.0	84.0	87.0	85.5
	25.0	75.0	84.0	79.5
	50.0	74.0	73.0	73.5
	75.0	72.0	65.0	68.5
	100.0	60.0	53.0	56.5
(Check)	0.0	90.0	90.00	90.0
Neem	5.0	83.3	70.0	76.6
	25.0	66.0	59.0	62.5
	50.0	50.0	45.0	47.5
	75.0	33.0	23.0	28.0
	100.0	0.0	0.0	0.0
(Check)	0.0	90.0	90.0	90.0
Oleander	5.0	90.0	84.0	87.0
	25.0	64.0	71.5	67.5
	50.0	41.0	35.0	38.0
	75.0	36.0	20.0	28.0
	100.0	10.0	0.0	6.0
(Check)	0.0	90.0	90.0	90.0
Mean		60.10	54.87	-----

LSD at 5% for:
Plant leaf extracts (P) 7.44
Concentrations (C) 8.147 P×C 18.22

Effect of natural plant oils treatment *in vivo*:

Treatment with mint, anise, eucalyptus, fennel and marjoram oils prevented also infection by rot in fruits either crushed and inoculated with the tested fungus or non crushed ones when the fruits were stored at 10°C. On the other hand, storing tomato fruits treated by the previously mentioned treatments under room temperature (20°±2 °C), significantly decreased the incidence of fruit rot caused by the tested fungus.Data also revealed that treating tomato fruits either crushed or non with almond, eucalyptus, garlic,

anise and cumin oils, completely prevented fruits from rot caused by S. *botryosum* when storage carried out under room temperature (20 ±2 °C) or at 10°C .In case of storing under room temperature, it was noticed that all the tested oils significantly decreased fruit rot infection by the tested fungus (Table,7).

Table (6): Effect of treating tomato fruits with some plant oils then stored at room temperature (20°±2°C) and at 10°C. for 12 days on the occurrence of fruit rots caused by *A. tenuis*.

Plant oils	%Fruit rot on fruits stored at 10°C		Room temperature (20°±2°C)		Mean Crushed fruits	Mean Non	Over all Mean
	Crushed	Non	Crushed	Non			
Almond	5.0	0.0	10.0	3.7	7.5	1.9	3.8
Anise	0.0	0.0	10.0	2.5	5.0	1.3	2.5
Caster	5.0	0.0	10.0	3.7	7.5	1.9	3.8
Cumin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eucalyptus	0.0	0.0	5.0	1.2	2.5	0.6	1.3
Fennel	0.0	0.0	10.0	2.5	5.0	1.3	2.5
Fenugreek	5.0	0.0	10.0	3.7	7.5	1.9	3.8
Garlic	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Marjoram	0.0	0.0	10.0	2.5	5.0	1.3	2.5
Mint	0.0	0.0	5.0	1.2	2.5	0.6	1.3
Onion	5.0	0.0	10.0	5.0	7.5	2.5	5.0
Parsley	5.0	0.0	10.0	3.7	7.5	1.9	3.8
(Check)	45.0	20.0	100.0	56.3	27.5	38.1	56.3
Mean	5.39	1.54	14.61		-----	-----	-----
Over all mean	3.47		9.81				

LSD at 5% for:

Plant oil extracts (O)	**4.01**				
Temperature (T)	**1.57**	**O×T**	**5.67**	**T×F**	**2.22**
Fruit (F)	**1.57**	**O×F**	**5.67**	**O×T×F**	**8.02**

It was also obvious from data that cumin and garlic treatments completely prevent tomato fruits from infection by the two tested fungi either when these treatments carried out under room temperature (20 ±2 °C.) or at 10°C. Data also indicated that the percentages of fruit rot caused by any of the two tested fungi were generally lower in fruits stored at 10°C than that stored at room temperature (20°C±2 °C). Similar results were also obtained by Ryu and Hot (1993) in their experiments on apple fruits as they were proved that cumin oil (0.1%) decreased spoilage of *Penicillium expansum* on apple fruits. Shukla *et al.* (2002) found that the essential oil of *Eucalyptus pauciflora* leaves appeared to be strongest toxicant against dominant tomato storage fungi, *i.e. Aspergillus flavus, Penicillium italicum* and *Alternaria alternata*. They added that the oil at 5% killed the tested fungi within 4-6 h only when used as a contact toxicant. Our results are in accordance with those reported by Twari *et.al.* (1983), Singh and Gupata (1992), Wilson *et.al.* (1997), Rushid (2000) and Shukla *et al.* (2002).

Effect of plant leaf extracts *in vivo* :

Table (8 and 9) show the effects leaf extract of oleander, eucalyptus, neem, basil, and marjoram, each at the high concentration, (100%) on controlling tomato fruit rots caused by *A. tenuis* and *S. botryosum*. Treating tomato fruits with any of the previously mentioned plant leaf extracts

significantly reduced the percentage of fruit rots by any of the two tested fungi, either the fruits stored under room temperature (20±2 °C) or at 10°C. Moreover, treating tomato fruits with oleander completely prevented fruits either crushed or non from the infection by *A. tenuis*. Meanwhile, oleander and eucalyptus completely prevented the fruits from infection by *S. botryosum*, under the previously mentioned circumstances .It is worth to mention that storing the treated tomato fruits with the tested plant extracts at 10°C gave good results more than storing under room temperature (20±2°C.) in controlling the infection caused by the two tested fungi.

Table (7): Effect of treating tomato fruits with some plant oils then stored at room temperature (20°±2°C) and at 10°C. on the occurrence of fruit rot caused by *S. botryosum* .

Plant oils	%Fruit rot on fruits stored at 10°C		Room temperature(20°±2°C)		Mean Crushed fruits	Mean Non	Over all Mean
	Crushed	Non	Crushed	Non			
Almond	0.0	0.0	0.0	0.0	0.00	0.00	0.00
Anise	0.0	0.0	0.0	0.0	0.00	0.00	0.00
Caster	5.0	0.0	10.0	0.0	7.50	0.00	3.75
Cumin	0.0	0.0	0.0	0.0	0.00	0.00	0.00
Eucalyptus	0.0	0.0	0.0	0.0	0.00	0.00	0.00
Fennel	5.0	0.0	15.0	0.0	10.00	0.00	5.00
Fenugreek	10.0	0.0	10.0	0.0	10.00	0.00	5.00
Garlic	0.0	0.0	0.0	0.0	0.00	0.00	0.00
Marjoram	0.0	0.0	5.0	0.0	2.50	0.00	1.25
Mint	0.0	0.0	5.0	0.0	2.50	0.00	1.25
Onion	10.0	5.0	15.0	5.0	12.50	8.75	8.75
Parsley	5.0	0.0	10.0	0.0	7.50	0.00	3.75
(Check)	30.0	10.0	80.0	30.0	55.00	20.00	37.5
Mean	5.0	1.2	11.5	2.7	------	-----	-----
Over all mean	3.1		7.1				

LSD at 5% for:

Plant oil extracts (O)	**4.169**				
Temperature (T)	**1.635**	**O×T**	**5.895**		
Fruit (F)	**1.635**	**O×F**	**5.895**	**T×F**	**2.312**

Effect of spraying tomato plants in field with some natural plant oils and plant leaf extracts on the occurrence of tomato fruit rots during storage:

The effectiveness of spraying fruit tomato plants (Castle Rock cv.) under field condition, at advanced mature green stage with some natural plant oils and plant leaf extracts on controlling postharvest tomato fruit rots was studied (Tables, 10 and 11). The obtained data show that all the tested plant oils significantly reduced the incidence of fruit rot disease. Spraying fruit tomato plants with cumin oil completely prevented tomato fruits from rots during storage period extended to 12 days either stored at 10 °C or at room temperature [20°±2°C]. Spraying fruited tomato plants with garlic and anise oils protected tomato fruits from rot infection when the fruits were stored at 10 °C. On the other hand, this treatment caused significant reduction in the percentages of fruit rot infection. Treatment with eucalyptus and mint oils caused also noticeable decreases in the percentages of fruit rot incidence during storage under the previously mentioned conditions.

Table (8): Effect of treating tomato fruits with some plant leaf extracts then stored at room temperature (20°±2°C) and 10°C. on the occurrence of fruit rot caused by *A. tenuis* .

Plant oils	%Fruit rot on fruits stored at 10°C (20°±2°C)		Room temperature Crushed	Non	Mean Crushed fruits	Mean Non	Over all Mean
			Non	Crushed			
Basil	10.0	0.0	25.0	0.0	17.50	0.00	8.75
Eucalyptus	0.0	0.0	5.0	0.0	2.50	0.00	1.25
Marjoram	5.0	0.0	15.0	0.0	10.00	0.00	5.00
Neem	0.0	0.0	5.0	0.0	2.50	0.00	1.25
Oleander	0.0	0.0	0.0	0.0	0.00	0.00	0.00
(Check)	25.0	10.0	100.0	65.0	62.50	37.50	50.00
Mean	6.67	1.67	20.83	10.83	-----	-----	-----
General mean	4.17		15.83				

LSD at 5% for:

Plant leaf extracts (P)	3.974	P×T	5.621	P×T×F	7.945
Température (T)	2.295	P×F	5.621		
Fruit (F)	2.295	T×F	3.245		

Table (9): Effect of treating tomato fruits with some plant leaf extracts then stored at room temperature (20°±2°C) and 10°C. on the occurrence of fruit rot caused by *S. botryosum* .

Plant oils	%Fruit rot on fruits stored at 10°C Room temperature (20°±2°C)				Mean Crushed fruits	Mean Non	Over all Mean
	Crushed	Non	Crushed	Non			
Basil	5.0	0.0	20.0	5.0	12.5	2.5	7.5
Eucalyptus	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Marjoram	10.0	0.0	15.0	5.0	12.5	2.5	7.5
Neem	10.0	0.0	15.0	0.0	12.5	0.0	6.3
Oleander	0.0	0.0	0.0	0.0	0.00	0.0	0.0
(Check)	40.0	20.0	100.0	60.0	70.0	40.0	0.0
Mean	10.8	3.3	25.0	11.67	-----	-----	55.0
General mean	8.50		22.00				

LSD at 5% for:

Plant leaf extract (P)	5.44	P×T	7.687	P×T×F	10.87
Température (T)	3.14	P×F	7.687		

Data also revealed that the treatments with plant oils gave their positive actions in controlling tomato fruit rots if the fruits were stored at 10 °C. or at room temperature. Several investigators (Twari *et al.*, 1983; Singh and Gupata, 1992; Wilson *et al.*, 1997; Rushid, 2000 and Abd El-Latif Shadia, 2003) pointed out that cumin, garlic, eucalyptus and anise oils at 5% were the most effective plant oils in reducing tomato fruits caused by *A. tenuis* and *S. botryosum.* On the other hand, the previously mentioned plant oils were not effective against *A. tenuis* and *S. botryosum* when applied at 0.5, 1, 2, and 3% concentrations. Data (Table, 11) also revealed that all of the tested plant leaf extracts significantly reduced the incidence of fruit rot disease during storage either under room temperature or in the refrigerator at 10 °C. Spraying fruit tomato plants with eucalyptus and oleander reduced sharply the incidence of tomato fruit rots during storage at the previously mentioned conditions in comparison with the other tested plant extracts or check

treatment. Treatment with the tested plant leaf extracts gave best results in controlling tomato fruit rots if the fruits were stored at 10 °C. Sinha and Saxena (1990) and Purnima and Saxena (1990) showed that flower extract of *Lantana camara* reduced fruit rot of tomato caused by *Aspergillus niger* in storage. Prasad and Naik (2003) mentioned that extracts of garlic and onion were effective in controlling blight of pea (*Phytophthora derchsleri*). Chattopadhyay (1999) indicated that neem leaf extract (1%) and garlic bulb extract reduced of severity sunflower Alternaria blight disease caused by *Alternaria alternata* and increased yield over the control. Amadioha and Uchendu (2003) indicated that *Fusarium solani* is an important rot-causing organism of tomato fruit during storage.

Table (10): Effect of spraying fruit tomato plants under field conditions at advanced green mature stage with some natural plant oils on the occurrence of tomato fruit rots after storage for 12 days at room temperature (20°±2 °C.) and at 10 °C.

Plant oils	% Fruit rot under 10 °C	Room temperature(20°±2°C.)	Mean
Almond	10.0	20.0	15.0
Anise	0.0	5.0	2.5
Caster	15.0	25.0	20.0
Cumin	0.0	0.0	0.0
Eucalyptus	5.0	10.0	7.5
Fennel	10.0	25.0	17.5
Fenugreek	20.0	35.0	27.5
Garlic	0.0	5.0	2.5
Marjoram	10.0	15.0	12.5
Mint	5.0	15.0	10.0
Onion	15.0	30.0	22.5
Parsley	25.0	45.0	35.0
(Check)	40.0	80.0	60.0
Mean	7.3	23.9	

LSD at 5% for:
Plant oil extracts (O) 5.578
Temperature (T) 2.188 **O×T** 7.88

Table(11): Effect of spraying fruited tomato plants under field conditions, at advanced mature green stage with some plant leaf extracts on the occurrence of tomato fruit rots after storage for 12 days at room temperature (20°±2 °C) and at 10 °C .

Plant leaf extract	% Fruit rot after storage at 10 °C	Room temperature (20°±2 °C)	Mean
Basil	25.00	25.00	25.00
Eucalyptus	10.00	15.00	12.50
Marjoram	25.00	50.00	37.00
Neem	15.00	45.00	30.00
Oleander	10.00	20.00	15.00
(Check)	45.00	85.00	65.00
Mean	21.67	40.00	

LSD at 5% for:
Plant leaf extracts (P) 8.12
Temperature (T) 4.69 **P×T** 11.48

They added that alcohol and water of neem extracts, especially the bark, could be used by farmers to control the rot of tomato fruit caused *by F. solani* in storage.

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استخدام بعض الزيوت والمستخلصات النباتية لمقاومة اعفان ثمار الطماطم المتسببة عن الفطرين *Alternaria tenuis* و *Stemphylium botryosm* اثناء التخزين.

محسن احمد مصطفى ، كامل كمال ثابت و معمر حاتم ابو حاتم
قسم امراض النبات – كلية الزراعة – جامعة القاهرة

اظهرت عمليات العزل من ثمار طماطم لمصابة بالعفن جمعت من ثلاثة محافظات مختلفة هي الجيزة و القليوبية و الاسماعيلية ، تواجد احدى عشرة نوع فطريا كانت تنتمي الى عشرة اجناس فطرية . كان اكثر الفطريات تكرارا هو الفطر الترناريا تينوس و يلية الفطر ستيمفليوم بوتريوسوم . اختلفت الفطريات المعزولة في شدتها الممرضة حيث كان الفطرين السابق الاشارة اليهما اشدها عدوانية. ومن بين اثنا عشر زيت لنباتات طبية و عطرية ظهر ان الزيت المستخلص من الكمون عند تركيز ٠,٠٥% كان كافيا لتثبيط النمو لكلا من الفطرين المختبرين ، بينما احتلت الزيوت المستخلصة من الحلبة و زيت اللوز المر المرتبة الثانية في هذا المضمار عندما استخدمت بتركيز ٣% و قد نتج عن معاملة ثمار الطماطم بالزيوت السابق السابق الاشارة اليها الحصول على حماية كاملة ضد الاصابة بالعفن الناتج عن الاصابة باي من الفطرين عند التخزين على درجة حرارة الغرفة (٢٠-٢٢م) او في الثلاجة على درجة ١٠م لمدة ١٢ يوما. كما تبين ايضا انه من بين خمسة مستخلصات نباتية استخدمت ، كان مستخلص اوراق نبات النيم بتركيز ١٠٠% مثبطا تماما لنمو كلا الفطرين ، كما ادت معاملة ثمار الطماطم بنفس هذا المستخلص بنفس التركيز الحصول على حماية تامة للثمار عند التخزين تحت نفس الظروف السابق الاشارة اليها . كما تم تحت ظروف الحقل معاملة نباتات طماطم مثمرة في الطور الاخضر المتقدم بنفس الزيوت و المستخلصات النباتية المشار اليها سابقا .