Impact of Acetic Acid on controlling Tomato Fruit Decay

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Abstract: Treatments of tomato fruits with different concentrations of glacial acetic acid wither liquid or as vapour significantly reduced the growth of *Alternaria alternata* and *Botrytis cinerea* in both (*in vitro* and *in vivo*). Also, submersed tomato fruits in different concentrations of acetic acid solution significantly reduced the severity of infection caused by *A. alternata* and *B. cinerea*. Infection increased in tomato fruits with increasing time of storage and decreased gradually with increasing acid concentration. *A. alternata* was more sensitive to acetic acid treatment than *B. cinerea*.Fumigation of infected tomato fruits with 40 ul/l acetic acid vapour showed greatly inhibition in fruit rots stored up to 16 days. The present data indicated that natural infection along the time of experiment was prevented completely by dipping or fumigating healthy non inoculated fruits by any concentration of acetic acid used.

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1. Introduction

Tomato (Lycospersicon esculentum Mill) is one of the most important and widely distributed horticultural vegetable crops in the world. It is the second leading vegetable crop worldwide with a production of 152.9 million ton with a value $74.1 billion (FAO. STAT Database, 2009). In addition, tomatoes are major contributors of the carotendiondes (especially lycopene), phenolics and vitamin C in daily diets (Causse *et al.,* 2003). Moreover, results from the epidemiological studies have shown that tomato and its products may have a positive effect against various forms of cancer, especially prostate cancer and cardiovascular diseases (Ellinger *et al.,* 2006). Worldwide post harvest tomato losses are as 30 to 40% (Kader, 1992) due to the use of improper handling procedures and lack of methods to prevent decay and senescence (Prigojin *et al.,* 2005). Due to increasing demand, tomato has a great potential for increased commercialization. More efficient tomato production requires better knowledge of its pathogens and control methods (Sanderson,2000). Currently, fungicides are used to control pre-harvest losses of tomato production (Baider and Cohen, 2003), but the use of prophylactic chemicals in these commodities (tomato fruits) is not allowed in different countries due to their environmental risks (Unnikrishnan and Nath, 2002). In recent years much researches have focused on developing alternative non chemical strategies against postharvest diseases trigger softening and dramtic reduction in fruit firmness (Droby *et al.,* 2009; Eshel *et al.,* 2009; Abd-Allahl *et al.,* 2011; Abd-Alla *et al.,* 2011; Tzortzakis *et al.,* 2011; Gharezi *et al.,* 2012). Acetic acid was commonly used by food manufactures as antimicrobial preservative or acidulates in a variety of food products and safe to environment (Davidson and Juneja, 1990; El-Katatny et al., 2012). The vapour of acetic acid was found extremely effective for killing spores of post harvest fungi, which cause decay to various fruits and cereal grains (Banwart, 1981; Sholberg and Gaunce, 1995 and 1996; Sholberg *et al.,* 1996 and 1998; Sholberg, 2009). Morsy *et al.* (1999 and 2000) and Abd-El-Kareem (2001) mentioned that acetic acid vapour at appropriate concentrations highly decreased or completely inhibited mycelial growth and spore germination of the common storage fungi, *i.e. Alternaria* spp., *Aspergillus flavus*, *A. niger*, *A. terreus*, *Botrytis cinerea*, *Fusarium moniliforme* and *Penicillium* spp. Also, fumigation with acetic acid vapour was done to control tomato fruit rots caused by *Alternaria alternata, A. niger* and *B. cinerea* (Sholberg *et al.,* 2000; Shehata, 2006).

The present study was undertaken to determine the ability of liquid and vapour glacial acetic acidCH_3COOH (Seastar Chemicals Inc, Sidney, Canada):

(i) On linear growth of black mold fungus *Alternaria alternata (Fr.) Keissler* and gray mold fungus *Botrytis cinerea Pers.ex. Pers.* under *In-vitro* conditions

(ii) On controlling postharvest decay of tomato fruits caused by *A. alternata* and *B. cinerea* under *In-vivo* conditions.

2. Materials And Methods

2.1. Materials:

2.1.1. Study sample:

Samples of this study was tomato fruits showing typical symptoms of decay. They were collected from local market located at Jazan, Saudi Arabia.

2.1.2. The causal fungi:-

Isolation and identification

Samples were surface sterilized by submerged in sodium hypochlorite (2%) for one minute then washed three times using sterilized distilled water. The fruits left to dry on filter paper (whatman,1). Surface sterilized small pieces of these decayed fruits were transferred onto potato dextrose agar (PDA**)** plates and incubated at 25 °C for 3-5 days. The emerged fungi were picked up, purified using hyphal tip technique (Dhingra and Sinclair., 1985) onto freshly PDA medium. The purified isolates were identified according to their morphological features using the Keys given by Ellis (1971). Barnett and Hunter (1972) and Jarvis (1977). Stock cultures of the obtained fungi were maintained onto PDA slants and stored in a refrigerator.

2.2.Methods

2.2.1.Pathogenicity test:-

Apparently similar health colored tomato fruits were thoroughly washed under running tap water then surface sterilized with ethanol 70%, two superficial pores using cork poorer 4mm in diameter were made on the surface of the fruits, then infested with an equal disc taken from 5 days old culture of any of *A. alternata* and *B. cinerea*. Check treatments were apparently healthy fruits infested only by disks of PDA medium. Treatments were replicated three times (10 fruits/each). All treatments were incubated in a plastic moist chamber with 70-80% RH and 20-25 °C. After 4 days from infestation rotted area appeared on the surface of tomato fruits artificially infested by any of the fungi tested (Acedo 1997; El-Katatny et al., 2012).

2.2.2.In-vitro experiments:

a-Effect of different acetic acid concentrations on linear growth of *A. alternata* and *B. cinerea*

Eight concentrations 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75, and 2.0 ml of glacial acetic acid CH_3COOH (99.9%) each were added to 250 ml Erlenmeyer flasks containing sterilized PDA medium, and ten plates from each concentration were prepared. The plates were inoculated singly with one disk (3 mm diam.) of fungal growth taken from 7 days old culture of *A. alternata* and *B. cinerea*. Twenty plates inoculated with each of *A. alternata* and *B. cinerea* (ten/each fungus) and untreated with acetic acid served as check treatment.

b-Effect of fumigation with acetic acid vapour on linear growth of *A. alternata* and *B. cinerea*

Ten days old cultures of *A. alternata* and *B. cinerea* were fumigated with five concentrations 2, 4, 6, 8 and 10 ul/l (v/v) of acetic acid vapour for 30 min. in container fumigated *A. alternata* and *B. cinerea*. Inoculated plates of non-fumigated *A. alternata* or *B. cinerea* were served as check treatment. Each treatment was replicated ten times for each concentration. Linear growth (mm) of *A. alternata* and *B. cinerea* were measured when the control plates reached full growth at 20 °C (Fallik *et al.,* 1993).

2.2.3.In-vivo experiments:-

a-Effect of liquid and vapour acetic acid on post-harvest tomato fruit infected with *A. alternata* and *B. cinerea*

Apparently healthy Castle Rock tomato cultivar fruits at light red maturity stage were surface sterilized through immersion in 1% sodium hypochlorite for 2 minutes then washed several times with sterilized distilled water, left to dry on sterilized filter paper (Whatman, 1) at room temperature and inoculated separately by three disks (3 mm-diam.) of each of *A. alternata* and *B. cinerea* through small scratch in the middle surface of fruits. Fruits were divided into two groups each group subjected to one of the following treatments. Fruits for 1st group were emerged in 10, 20, 30, 40 and 50 ml of acetic acid solution for 3 min. then air dried in laminar-flow hood for 2 hrs.

Fruits for 2nd group were fumigated with 10, 20, 30, 40 and 50 ul/l (v/v) of acetic acid vapour in air closed glass container with continuous air circulation for 30 min. The treated fruits were packaged and put in perforated sterilized carton boxes (25x40cm). Check treatments containing non inoculated tomato fruits were divided into two groups, 1st group was emerged in acetic acid solution, whereas the 2nd group was fumigated with acetic acid vapour at the same concentrations mentioned before. All treatments were stored for 4, 8, 12 and 16 days at 13 ºC and 90-95% RH. Thirty tomato fruits were used/each treatment. The results were recorded as severity of infection which calculated as percentage of the external rotten area in proportional to the total area of the fruits (Morcos, 1984). Decay percentage was expressed as number of rotten fruits per total fruits x 100

2.2.4.Statistical analysis

The obtained data were statistically analyzed using the completely randomized blocks, the split plot and split split plot designs (Sendecor and Cochran, 1967). Averages were compared at the 0.05 level of probability using least significant difference (LSD) as suggested by Fisher (1958).

3.Results

3.1. Effect of different acetic acid concentration on linear growth of *A. alternata* and *B. cinerea*

Data presented in Table 1 showed that treatments with acetic acid concentrations significantly reduced the linear growth of *A. alternata* and *B. cinerea*. Reduction in linear growth was increased as the concentration increased and the fungal growth was completely inhibited by 1.7 ml/l of acetic acid compared to check treatment.

Table 1. Effect of liquid acetic acid concentrations on linear growth of *A. alternata* and *B. cinerea* under *in vitro* conditions

Acetic acid concentrations (ml/l)	Linear growth (mm)		Mean
	A. alternata	*B. cinere*	
0.25	40.0	75.0	57.5
0.50	35.0	40.0	37.5
0.75	28.0	35.0	31.5
1.00	20.0	30.0	25.0
1.25	15.0	20.0	17.5
1.50	10.0	15.0	12.5
1.75	0.0	0.0	0.0
2.00	0.0	0.0	0.0
Check*	90.0	90.0	90.0
Mean	26.4	33.9	

* = Control (without treatment). L.S.D at 0.05 level for: Concentrations (C) = 2.7; Fungi (F) = 1.3 C X F = 3.8

Table 2. Effect of fumigation with acetic acid vapour on linear growth of *A. alternata* and *B. cinerea* under *in vitro* conditions

Acetic acid concentrations (ul/l)	Linear growth (mm)		Mean
	A. alternata	*B. cinerea*	
2	40.0	56.0	48.0
4	30.0	42.0	36.0
6	18.0	27.0	22.5
8	0.0	0.0	0.0
10	0.0	0.0	0.0
Check*	90.0	90.0	90.0
Mean	29.7	35.8	

* = Control (without treatment). L.S.D at 0.05 level for: Concentrations (C) = 0.9 Fungi (F) =0.5. C X F =1.23.

Table 3. Effect of liquid and vapour acetic acid on postharvest tomato fruit infected with *A. alternata* and *B. cinerea* under *in vivo* conditions

Acetic acid concentrations ml/l	Storage periods (days)	% Severity of infection under artificially inoculation with			% Decay (un-inoculated tomato fruits)
		A. alternata	*B. cinerea*	Mean	
10	4	4.0	5.0	4.5	0.0
	8	6.7	12.0	9.4	0.0
	12	10.3	18.4	14.3	0.0
	16	13.9	30.0	21.9	0.0
	Mean	8.7	16.4	12.5	
20	4	2.3	4.0	3.1	0.0
	8	5.0	10.0	7.5	0.0
	12	8.8	14.5	11.6	0.0
	16	10.5	27.0	18.8	0.0
	Mean	6.6	13.9	10.3	
30	4	1.9	3.5	2.7	0.0
	8	4.4	7.9	6.1	0.0
	12	5.9	12.2	9.0	0.0
	16	8.1	20.0	14.1	0.0
	Mean	5.1	10.9	8.0	
40	4	0.0	0.0	0.0	0.0
	8	3.1	6.6	4.8	0.0
	12	4.0	6.7	5.4	0.0
	16	6.3	10.7	8.4	0.0
	Mean	3.3	6.0	4.7	
50	4	0.0	0.0	0.0	0.0
	8	2.7	2.3	2.5	0.0
	12	3.5	3.1	3.3	0.0
	16	5.1	8.5	6.8	0.0
	Mean	2.8	3.5	3.2	
Check*	4	10.0	12.0	11.0	15.0
	8	20.0	25.80	22.9	18.0
	12	35.0	45.00	40.0	25.0
	16	50.0	76.50	63.3	36.0
	Mean	28.8	39.50	34.1	-
Mean	4	3.0	4.08	3.6	-
	8	7.0	10.76	8.9	-
	12	11.2	16.65	14.0	-
	16	15.7	28.78	22.2	-

*= Control (without treatment). L.S.D at 0.05 level for: Concentrations (C) =0.7 C X D = 1.4 Days (D) = 0.6. CX F = 0.8 Fungi (F) = 0.4 D X F = 1.0 C X D X F = 1.9

3.4.Effect of vapour acetic acid fumigation on postharvest tomato fruit infected with *A. alternata* and *B. cinerea*

It is worthy to mention that fumigation of infected tomato fruits by *A. alternata* and *B. cinerea*, with 40 ul/l acetic acid greatly inhibited fruit rots stored up to 16 days as shown in Table (4). Other concentrations lower than 40 ul/l significantly reduced severity of infection which increased by increasing storage period up to 16 days.

Data in Tables (3 & 4) also indicate that natural infection along the time of experiment was prevented completely by dipping or fumigating healthy non inoculated fruits by any concentration of acetic acid used.

3.2.Effect of fumigation with acetic acid vapour on linear growth of *A. alternata* and *B. cinerea*

Data presented in Table (2) revealed that the mycelium growth of *A. alternata* and *B. cinerea* significantly decreased as the concentration of the acid fumigation increased. Complete inhibition occurred when each of the two fungi were exposed to fumes of 8.0 ul/l acid concentration. Generally, *A. alternata* was more sensitive to acetic acid treatment than *B. cinerea*.

3.3.Effect of liquid acetic acid on postharvest tomato fruit infected with *A. alternata* and *B. cinerea*

Data obtained in Table (3) indicated that submerged tomato fruits in different concentrations of acetic acid solution significantly reduced the severity of infection caused by *A. alternata* and *B. cinerea*. Infection increased in tomato fruits with increasing time of storage up to 16 days and decreased gradually with increasing acid concentration. Complete decay inhibition was noticed 4 days after fruits treated with acetic acid at 40 ml/l (Table 3).

Table 4. Effect of vapour acetic acid fumigation on postharvest tomato fruit infected with *A. alternata* and *B. cinerea* under *in vivo* conditions

Acetic acid concentrations ul/l	Storage periods (days)	% Severity of infection under artificially inoculation with		% Decay (Check treatment)
		A. alternata	B. cinerea	
10	4	7.7	14.1	0.0
	8	11.8	18.3	0.0
	12	16.2	25.8	0.0
	16	20.0	32.0	0.0
	Mean	13.9	22.6	
20	4	6.7	11.8	0.0
	8	8.3	16.2	0.0
	12	12.5	21.8	0.0
	16	17.0	28.0	0.0
	Mean	11.1	19.5	
30	4	4.4	5.3	0.0
	8	7.8	8.9	0.0
	12	10.4	11.7	0.0
	16	14.0	20.0	0.0
	Mean	9.2	11.5	
40	4	0.0	0.0	0.0
	8	0.0	0.0	0.0
	12	0.0	0.0	0.0
	16	0.0	0.0	0.0
	Mean	0.0	0.0	
50	4	0.0	0.0	0.0
	8	0.0	0.0	0.0
	12	0.0	0.0	0.0
	16	0.0	0.0	0.0
	Mean	0.0	0.0	
Check*	4	12.5	18.9	0.0
	8	23.3	45.8	15.0
	12	40.0	65.3	20.0
	16	50.0	75.0	25.0
	Mean	31.5	51.2	
Mean	4	5.2	8.3	
	8	8.5	14.9	
	12	13.2	20.8	
	16	16.8	25.8	

*= Control (without treatment). L.S.D at 0.05 level for: Concentrations (C) =0.7 C X D = 1.4
Days (D) = 0.6 C X F = 1.0; Fungi (F) = 0.4 D X F = 0.8 C X D X F=2.0

4. Discussion

The present efforts indicated that different concentrations of acetic acid wither liquid or as vapour were significantly reduced the growth of *A. alternata* and *B. cinerea*. Also, submersed tomato fruits in different concentrations of acetic acid solution significantly reduced the severity of *A. alternata* and *B. cinerea* infection. These results are in harmony with those of (Causse *et al.,* 2003; Prigojin *et al.,* 2005; Simonne *et al.,* 2006; Abd-Alla *et al.,* 2011; Tzortzakis *et al.,* 2011). The inhibitory effect of acetic acid not related to pH alone but carbon chain length and inherent susceptibility of the microorganisms were also important. Also, the un-dissociated part from the acid was primarily responsible for its antimicrobial activity where it can penetrate the microbial cell and exert its toxic effect (Banwart, 1981). Sholberg *et al.* (1998) and Sholberg, (2009) mentioned that the mechanism of acetic acid effect on inhibiting microorganisms is apparently due to its effect on the cell membrane through the interfering with transport of metabolites and maintenance of membrane potential. Also, Shehata (2006) reported that all the tested acetic acid concentrations, *i.e.* 5, 10, 15, 20 and 25%, when applied for 1 h at 13°C, significantly reduced the percentage of infected areas in fruits compared with the control.

Acetic acid vapors with concentrations 8.0 and 40 ul/l is more effective than its solution 1.7 and 40 ml/l in inhibiting mycelial growth and controlling postharvest disease of tomato fruits by the two fungi tested. Increasing penetration ability of acetic acid than that in the liquid state through the fungal cell might be attributed to the vapour state. One of the most important results obtained during this work is that acetic acid treatments either as liquid or vapour state at any concentration prevent completely natural infection of healthy non inoculated tomato fruits (check treatments) during the time of experiment (16 days). Data also in the harmony of those obtained by Sholberg *et al.* (1996 and 1998). Many other researchers recommended acetic acid treatment for controlling postharvest decay of fruits (Morsy *et al.*, 1999 and 2000; Abd- El-Kareem, 2001; El-Mougy and El-Gamal, 2003; Sholberg *et al.*, 2006; El-Katatny et al., 2012).

In conclusion the present data clearly showed that acetic acid which has long been used been safely used long as food additive, can completely prevent decay of tomato fruits when dipped in 4% acetic acid solution or exposed to its vapour at 40 µl/l. This procedure can be easily applied and inexpensively to preserve tomato fruits for long periods without any side effects, in refrigerators at home, market, and storage and exportation level.

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