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CONTROL OF POST HARVEST TOMATO FRUIT ROTS USING ACETIC ACID

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ABSTRACT

Acetic acid at a concentration of 1.75 ml/1 completely inhibit growth of black mold *Alternaria alternata* (Fr.) Keissler and gray mold *(Botrytis cinerea* Pers. Ex. Pers.). Also fumigating an inoculum (dishs, 3 mm-diam.) of both fungi in a closed glass container with continuous sterilized air circulation for 30 minutes using 8.0 µl/l acetic acid (v/v) and cultivating it in acetic acid free PDA medium was also sufficient to prevent fungal growth. On the other hand, dipping artificially inoculated tomato fruits in 4% acetic acid solution or fumigating them by 40 µl/l (v/v) for 30 min and storing both treatments at 13°C for 16 days inhibited tomato fruits rot caused by the two pathogens. Scanning electron micrographs for the infected areas obtained from both treated and untreated infected fruits showed that the mycelia of both pathogens appeared completely distorted, associated with malformation of conidiophores.

INTRODUCTION

Saudi Arabia is an arid country which is located at 16 °N and 32 °E. Climate is characterized by long, hot, dry summer and mild, cool and short winter. The agricultural lands of Saudi Arabia, which are coarse textured containing salts to varying degrees and mostly irrigated with saline groundwater, are not considered suitable for some of the commonly grown crops (AlJaloud *et al.,* 2001, Al Surhanee, 2009).

The chemical fungicides have been used as the main strategy for controlling many diseases and subsequently increased yield

production (Abdel-Moneim *et al.*, 1980; Keinath and DuBose, 2004; Wolf and Verreet, 2008). Unfortunately, the current and indiscriminate use of the fungicides posed a serious threat to human health, environment and production of fungicide resistant pathogen strains (McGrath, 1991; Garcia, 1993 and Durmusoglu *et al.*, 1997, Fernandez-Aparicio *et al.*, 2009 and Al Surhanee, 2009). The development of nontoxic alternative to chemical fungicides would be useful in reducing the undesirable effects of their uses.

Acetic acid (AA) was commonly used by food manufactures as antimicrobial preservative or acid lent in a variety of food products and save to environment (Davidson and Juneja, 1990). The vapors of acetic acid were found extremely effective for killing spores of post harvest fungi, which cause decay to various fruits and cereal grains (Sholberg and Gaunce, 1995 and 1996; Sholberg *et al.*, 1996 and 1998). Mosry *et al.* (1999 and 2000) and Abd-El-Kareem (2001) found that acetic acid vapor 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. The aim of this work was to investigate the ability of acetic acid in controlling post harvest tomato fruit rots caused by *A. alternata* and *B. cinerea.*

MATERIALS AND METHODS

Alternaria alternata (Fr.) Keissler and *Botrytis cinerea* Pers. Ex. Pers. were previously isolated from rotted tomato fruits, collected from agricultural lands of Saudi Arabia and they were found to be pathogenic fungi to healthy fruits (Fallik *et al.*, 1993).

Treatment with acetic acid *in vitro*

Different volumes of analytically pure acetic acid CH_3COOH (99.99%) were added individually to 250 ml Erlenmeyer flasks each containing sterilized PDA medium to obtain the following concentrations; 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 1.75, and 2.0 ml/l, then plates from each concentration were prepared. The plates were inoculated singly with disks (3 mm-diam.) of fungal growth taken from 10 days old culture of *A. alternata* and *B. cinerea.* Also, 10 days old cultures of both fungi were fumigated with acetic acid vapour at

concentrations, of 2, 4, 6, 8 and 10 µl/l (v/v) for 30 min. in container with enclosed current of air. Disks (3 mm-diam.) from fumigated plates were inoculated into new PDA plates, Inoculated plates by each of the two fungi without any treatment by acid solution or fumigation served as control. Three replicates were prepared for each concentration of both treatments and linear growth of fungi was measured when the control plates reached full growth at 20°C (Fallik *et al.*, 1993).

Treatment with acetic acid *in vivo*

Healthy Castle Rock tomato fruits at light red maturity stage were sterilized through immersion in 70% ethanol for one minute, left to dry at room temperature and inoculated separately by disks (3 mm-diam.) of each of the two pathogenic fungi through small scratch in the middle surface of fruits. Fruits were divided into two groups each group subjected to one of the following treatments:

1. Dipped in 10, 20, 30, 40 and 50 ml/l acetic acid solutions for 3 min. and air dried in laminar-flow hood for 2 hr. or,
2. Fumigated with acetic acid vapor 10, 20, 30, 40 and 50 µl/l (v/v) in air closed glass container with continuous air circulation for 30 min. The treated fruits were packaged in plastic net bags, put in perforated sterilized carton boxes, another two groups of noninoculated tomato fruits; one group was dipped-in acetic acid, and the other was fumigated with acetic acid concentrations as mentioned above and all of them were stored for 4, 8, 12 and 16 days at 30°C and RH 90-95%. For each particular treatment three replicates were used, each replicate containing 10 fruits. The results were recoded as severity of infection, which calculated as percentage of the external rotten area in proportional to the total area of the fruits (Morcos Jeanette, 1984). Decay percentage was expressed in number of rotten fruits per total fruits x 100.

Statistical analysis:

The obtained data were statistically analyzed using the completely randomized blocks, the split plot and 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).

Scanning electron microscopy (SEM) preparation:

Specimens of inoculated tomato fruits either by *A. alternata* or *B. cinerea* were taken after 4% acetic acid treatment for 3 minutes *in vivo* with its control and stored at 30°C for 16 days were prepared according to Harley and Fergusen (1990), examined and photographed in Joel T330 A SEM.

RESULTS AND DISCUSSION

Results

I. Effect of acetic acid (*in vitro*) on growth of *A. alternata* and *B. cinerea*:

1) Effect of acetic acid solution

Acetic acid concentrations significantly reduced the linear growth of the two fungi tested (Table, 1). Reduction in linear growth was increased as the concentration increased and the fungal growth was completely inhibited by 1.75 ml/l of acetic acid.

Table (1). Effect of different concentrations of acetic acid on linear growth of *A. alternata* and *B. cinerea in vitro.*

Acetic acid concentrations (ml/l)	Linear growth (mm)		Mean
	A. alternata	*B. cinerea*	
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
Control (without treatment)	90.0	90.0	90.0
Mean	26.4	33.9	

L.S.D. at 0.05 level for:
Concentrations (C) = 2.7
Fungi (F) = 1.3
C x F = 3.8

2) Effect of acetic acid fumigation

Data presented in Table (2) indicated that the mycelium growth of both fungi 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 µl/l acid concentration. Generally *A. alternata* was more sensitive to acetic acid treatment than *B. cinerea.*

Table (2). Effect of different concentrations of acetic acid fumigation on linear growth of *A. alternata* and *R. cinerea in vitro.*

Acetic acid concentrations (µl /l)	Linear growth (mm)		Mean
	A. alternata	*B. cinerea*	
2	42.0	66.0	54.0
4	30.0	54.0	42.0
6	22.0	30.0	26.0
8	0.0	0.0	0.0
10	0.0	0.0	0.0
Control (without treatment)	90.0	90.0	90.0
Mean	30.7	40.0	

L.S.D. at 0.05 level for:
Concentrations (C) = 0.9
Fungi (F) = 0.5
C x F = 1.2

II. Effect of acetic acid (*in vivo*) on tomato fruits rot:

1) Effect of acetic acid solution

Dipping tomato fruits in different concentrations of acetic acid significantly reduced the severity of infection caused by *A. alternata* and *B. cinerea* (Table, 3). Infection increased in tomato fruits with increasing time of storage up to 16 days and decreased gradually with increasing acid concentration. Complete inhibition of rot was noticed 4 days after fruits treated by 40 ml/l and this effect clearly appeared from scanning electron micrographs (Fig., 1). Acetic acid (40 ml/l)

treatment of inoculated fruits caused distortion of mycelia and deformation of conidiophores of the two pathogens within the treated fruits as compared with the normal growth, conidiophores and conidial formation of both fungi within untreated tomato fruits (control, Fig. 2).

2) Effect of acetic acid fumigation:

It is worthy to mention that, fumigating tomato fruits, infected by *A. alternata* and *B. cinerea*, by 40 µl/l greatly inhibited fruits rot stored up to 16 days as shown in Table (4). Other concentrations lower than 40 µl/l significantly reduced severity of infection, which increased by increasing storage period up to 16 days. Data in Tables (3 and 4) also indicated that natural infection along the time of experiment was prevented completely by dipping or fumigating healthy noninoculated fruits by any concentration of acetic acid used.

Table (3). Effect of acetic acid concentration on post harvest tomato fruit rots caused by *A. alternata* and *B. cinerea in vivo*.

Acetic acid concentrations (ml/l)	Storage periods (days)	% Severity of infection under artificially inoculation with			% decay (uninoculation 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	20.0	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	14.0	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	33	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	
Control (without Treatment)	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	30	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	-

L.S.D. at 0.05 level for:
Concentrations (C) = 0.7
Days (D) = 0.6
Fungi (F) = 0.4

C x D = 1.4
C x F = 0.8
D x F = 1.0
C D x F = 1.9

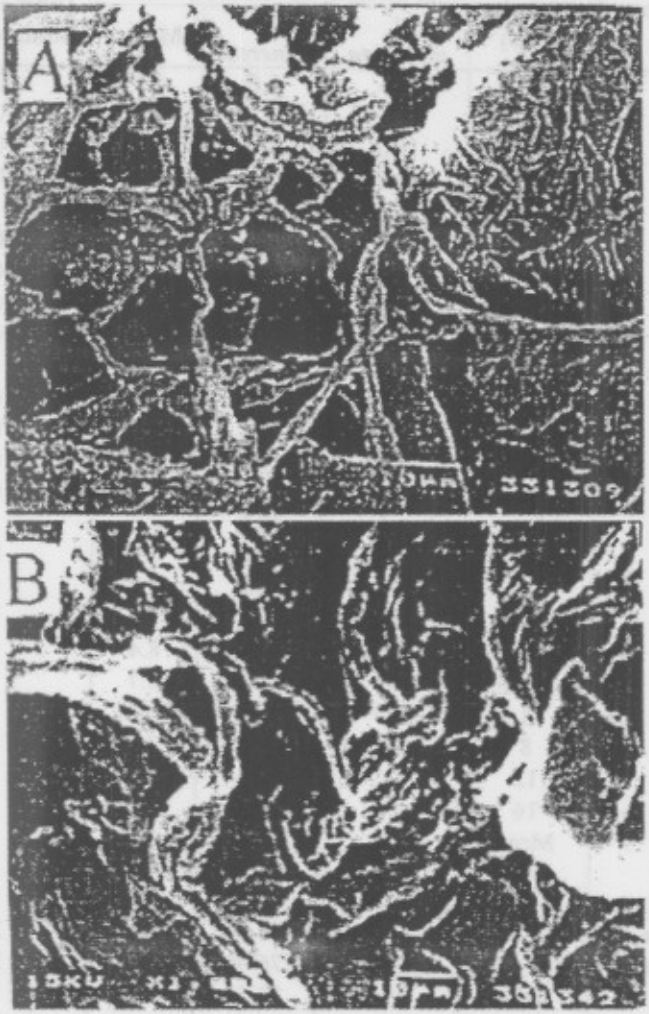

Fig. (1): Scanning electron micrographs of tomato fruits infected by *A. alternata* (A) and *B. cinerea* (B) treated with acetic acid at concentration 4% caused distortion of the mycelium associated with malformation of conidiospores of the two tested pathogens.

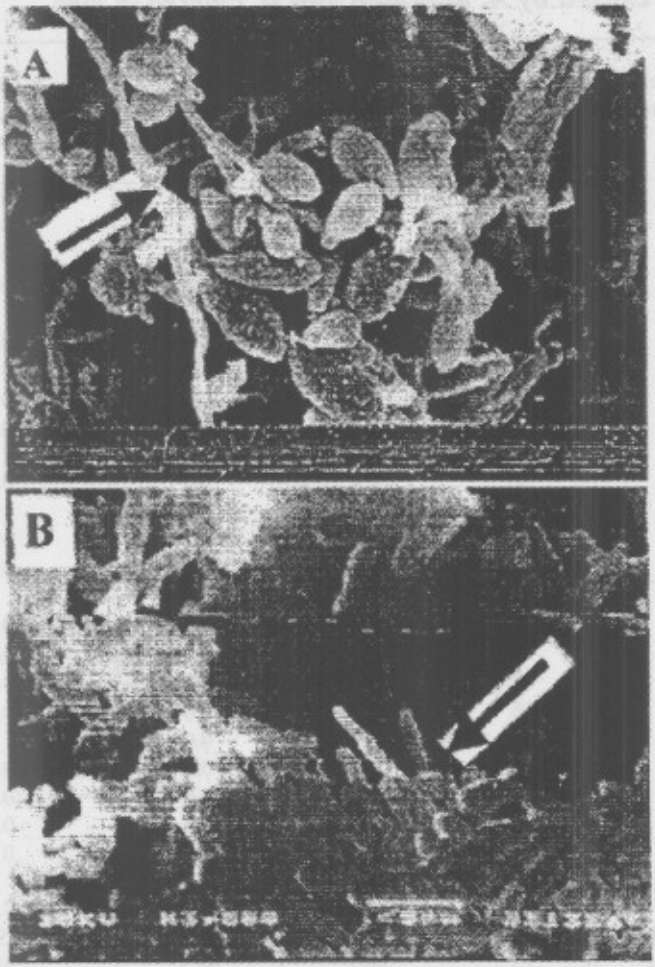

Fig.2(A&B): Scanning electron micrographs of untreated tomato fruits infected by *A. alternata* (A) and *B. cinerea* (B):

A) Normal structure and branched mycelia bearing conidiospores with conidia of *A. alternata* (as shown by arrow).

B) Condensed mycelium bearing conidiospores and abundant number of *B. cinerea* conidia: (as shown by arrow).

Table (4). Effect of acetic acid fumigation on post harvest tomato fruit rots caused by *A. alternata* and *B. cinerea in vivo*.

Acetic acid concentrations (µl /l)	Storage periods (days)	% Severity of infection under artificially inoculation with		% decay (uninoculation tomato fruits)
		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	
Control (without Treatment)	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	

L.S.D. at 0.05 level for:

Concentrations (C) = 0.7
Days (D) = 0.6
Fungi (F) = 0.4

C x D = 1.4
C x F = 1.0
D x F = 0.8
C D x F = 2.0

Discussion

Acetic acid was found to be more effective in controlling post harvest decay of tomato fruits caused by *A. alternata* and *B. cinerea* than other chemical treatments such as plant oils, organic acids and their salts or salts of inorganic acids as shown from our previous studies (Tohamy *et al.*, 2003 and Ibrahim *et al.*, 2003). Sholberg *et al.* (1998) emphasized that acetic acid was effective against a wide range of post harvest fungi. 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 non 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) 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.

Acetic acid vapor with concentrations 8.0 and 40 µl/l is more effective than its solution 1.75 and 40 ml/l in controlling mycelial growth and post harvest 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 vapor state. One of the most important results obtained during this work is that acetic acid treatment both in liquid or vapor state at any concentration prevent completely natural infection of healthy noninoculated tomato fruits during the time of experiment (16 days) and more, these data strongly agree with Sholberg *et al.* (1996 and 1998). Morsy *et al.* (1999 and 2000) and Abd-El-Kareem (2001) in recommending acetic acid treatment for controlling post harvest decay of fruits.

From these results, its clear that acetic acid which safety used long ago as food additive, can prevent completely rots when tomato fruits dipped in 4% acetic acid solution or exposed to its vapor at 40 µl/l, the method which can be easily and inexpensively used to preserve tomato fruits for long periods without any side effects, in refrigerators of the home, market, storage and exportation level.

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مقاومة أعفان ثمار الطماطم ما بعد الحصاد بإستخدام حمض الخليك

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إن إضافة حمض الخليك عند تركيز 1.75 ملل /لتر الى الوسط الغذائى منعت تماما نمو خيوط فطرة الترناريا الترناتا المسبب للعفن الأسود وفطرة بوتريتس سيناريا المسبب للعفن البنى فى ثمار الطماطم كما منعت أيضا معاملة الأقراص الكاملة لنمو الفطرتين ببخار الحمض بتركيز 8.0 ميكروليتر / لتر بإستخدام وعاء زجاجى مغلق مع الدوران المستمر للهواء من الإستمرار فى النمو. من ناحية أخرى أدى غمر ثمار الطماطم المعداه صناعيا فى تركيز 4% من محلول حمض الخليك أو تبخيرها بتركيز 40 ميكروليتر/ لتر لمدة 30 دقيقة ثم تخزينها تحت درجة 13°م لمدة 16 يوما الى منع إصابة الثمار بالعفن المتسبب عن أى من الفطرتين. وقد أظهرت الصور المأخوذه بالميكروسكوب الالكترونى الماسح للثمار المعداه بأى من الفطرتين والمعاملة بالحمض عند تركيز 4% أن الخيوط الفطرية داخلها مشوهة تماما ولم تنتج أى وحدات كونيدية.