*Egypt. J. Phytopathol.*, Vol. 32, No. 1-2, pp. 129-138 (2004)

# Control of Postharvest Tomato Fruit Rots: II. Using Heat Treatments

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Effectiveness of heat treatments on controlling tomato black mould caused by *Alternaria alternata* (Fr.) Keissler and grey mould caused by *Botrytis cinerea* Pers. ex Pers. was investigated. *In vitro*, spore suspension of *B. cinerea* exposed to hot water treatment at 50°C for 7 min., failed to germinate, whereas that for *A. alternata* failed to germinate when exposed to hot water at 55°C, for 7 min. Exposing discs bearing growth of any of the two tested fungi to hot air at 40°C for 72hr caused suppression of growth. *In vivo*, dipping tomato fruits in hot water at 55°C for 7 min. or keeping in hot air for 72hr at 38°C prevented decay development in uninoculated or artificially inoculated fruits with *A. alternata* and *B. cinerea* up to 15 days when stored at 20°C. Scanning electron micrographs of infected tissue, in treated and untreated fruits, were used and the mode of action of heat treatment was discussed.

**Key words** *Alternaria alternata*, black mould, *Botrytis cinerea*, grey mould, heat treatments, postharvest and tomato.

Postharvest decay is the major limiting extension of shelf life in tomato fruits (*Lycopersicon esculentum* Mill.). *Alternaria alternata* and *Botrytis cinerea* causing black and grey moulds are the two main fungi responsible for storage decay in Egypt (El-Essawy *et al.*, 2003). Heat treatments as hot water dips, vapour heat and hot dry air have been reported by several workers for their ability to inhibit spore germination and mycelial growth of many postharvest fungi which damaged fruits and vegetables (Couey *et al.*, 1984; Spalding and Reeder, 1986; Teitel *et al.*, 1991; Fallik *et al.*, 1996; Nguyen *et al.*, 1998 and Satvinder and Kaur, 2000). Fallik *et al.* (1993) found that holding inoculated mature green and pink tomato fruits for 3 days at 38°C completely inhibited decay, caused by *Botrytis cinerea*. Nafaa (2001) found that dipping cantaloupe fruits, inoculated with *Fusarium simetectum, Cladosporium herbarum* and *Alternaria alternata*, in hot water at 50°C for 2.5, 5 and 10 min. has inhibited decay by these fungi. Jacobi *et al.* (2000) emphasized that "Kensington" mango fruits were more resistant to postharvest diseases by treatment with hot air from 22°C to 42°C for 16 to 4hr and/or hot water at 45°C for 30 min. or 47°C for 15 min, respectively.

Thus, the aim of the present work was to evaluate the effect of dipping tomato fruits in hot water or exposing to hot air, on reducing rot decay during marketing and storage.

## Materials and Methods

*Alternaria alternata* (Fr.) Keissler and *Botrytis cinerea* Pers. ex Pers. were isolated from different rotted tomato fruits collected from different markets in El-Sharkia and Giza governorates, Egypt. These isolates were found to be pathogenic (El-Essawy *et. al.*, 2003) and identified according to Ellis (1971) and Samson *et al.* (1995).

Both fungi were grown separately on potato dextrose agar medium (PDA) for 7 days at 25°C for *A. alternata* and 10 days at 20°C for *B. cinerea*. The obtained growth was used for preparing uniform discs (3-mm-diam.) and spore suspensions ($10^6$ spore/ml) in sterile water supplemented with 0.03% Tween–20 to maintain a uniform spore distribution.

*In vitro heat treatments:*

For hot water treatment, test tubes each containing 4 ml sterilized water & Tween–20 were separately supplemented by one ml of the spore suspension. The tubes were heated in a water bath at 45°C, 50°C and 55°C for 1, 3, 5 and 7 min. for each temperature degree then cooled under running tap water. Unheated spore suspensions served as check. Sterilized filter paper discs (3-mm-diam.) were saturated with the tested spore suspension (0.1 ml/disc) and individually placed at the centre of 9-cm PDA plates. On the other hand, for hot air treatment, inoculated PDA plates by any of the two tested fungi, were incubated at 36°C, 38°C and 40°C for 72hr. All plates of both treatments were then incubated at 20°C. Three plates were used as replicates for each treatment. The diameter of the developed colonies was measured when the mycelial growth covered the plate in check treatment (Fallik *et al.*, 1993).

*In vivo heat treatments:*

For hot water treatment, healthy Castle Rock tomato fruits, of uniform size at light red stage (Anonymous, 1976) harvested from a commercial field, were divided into four groups. Two groups were sterilized by dipping in 70% ethanol for one minute, air dried and inoculated with mycelial discs (3-mm-diam.) of any of the two tested fungi through very small scratch in the middle surface of each fruit. The other two groups of fruits were left without inoculation. One group, of both uninoculated and inoculated fruits, was dipped in hot water at 45°C, 50°C and 55°C for 3, 5 and 7 min. per each degree. Similarly, a group of each uninoculated and inoculated fruits were immersed in sterile water at room temperature and served as check. On the other hand, two groups of tomato fruits one uninoculated and the other inoculated by any of the two tested fungi were exposed to 36°C, 38°C and 40°C for 72hr., while check treatments were directly exposed to 20°C. Three replicates, containing 10 fruits were used as replicates for each particular treatment. Tested tomato fruits were placed into plastic net bags. The bags were put in sterilized perforated carton boxes and stored at 20°C for 15 days. Severity of infection was estimated as percentage of the external rotten area in proportion to the total area of the fruit (Morcos, 1984). Decay percentage was expressed as number of rotten fruits per total fruits.

*Scanning electron microscopy (SEM) preparation:*

At the end of incubation period (15 days), specimens of tomato fruits inoculated by *A. alternata* or *B. cinerea* were taken after heat treatment either by hot water at 55°C for 3 min. or by hot air at 38°C for 72hr. each with its check. The specimens were prepared, examined and photographed in a Jeol T 330 A SEM according to Harley and Fergusen (1990).

*Statistical analysis:*

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

## Results

*A) In vitro effect of heat treatments:*

*1- Hot water treatment:*

Linear growth of the two tested fungi, obtained from standardized number of spores pretreated by 45°C, 50°C and 55°C for different periods, was inversely proportional to temperature treatments and time of dipping (Table 1). Mycelial growth of *A. alternata* was significantly decreased from 90 mm in check plates to 35 mm when its spore suspension was exposed to 55°C for 7 min. However, mycelial growth of *B. cinerea* was completely inhibited when its spore suspension was previously exposed to 50°C for 7 min. Generally, linear growth of both fungi was significantly decreased as the temperature raised and dipping time increased. It is also clear that *B. cinerea* was more sensitive to hot water treatment than *A. alternata.*

*2- Hot air treatment:*

Data presented in Table (2) show the effect of hot air treatment, at 36°C, 38°C and 40°C for 72hr., on mycelial growth of the two pathogens. Generally, no fungal growth of both tested fungi was detected after 72hr. of hot air treatment. Meanwhile, the average of linear growth increased significantly as the incubation period increased. Inocula of both fungi pretreated by 36°C, began to grow after 3 days and their growth increased gradually up to 15 days after incubation, but those pretreated at 38°C started their growth after 11 days. Moreover, no growth was detected during different experimental periods for those pretreated at 40°C.

*B) In vivo effect of heat treatments:*

*1 - Hot water treatment:*

Dipping of uninoculated tomato fruits in 50°C hot water for 7 min. was sufficient to prevent fruit decay during storage at 20°C for 15 days as indicated in Table (3). Severity of infection by *A. alternata* or *B. cinerea* was gradually reduced as water temperature raised from 45°C, 50°C to 55°C and dipping time increased from 3 to 5 and 7 min. before storage, while decay percentage of tomato fruits was completely suppressed up to 15 days when treated by hot water at 50°C for 7 min.

**Table 1.** ***In vitro*** **effect of hot water treatment on mycelial growth of** ***A. alternata*** **and** ***B. cinerea***

| Hot water (°C) | Dipping time (min.) | Linear growth (mm) | | Mean |
|---|---|---|---|---|
| | | *A. alternata* | *B. cinerea* | |
| 45 | 1 | 75.0 | 70.0 | 72.5 |
| | 3 | 70.0 | 58.0 | 64.0 |
| | 5 | 68.0 | 45.5 | 56.8 |
| | 7 | 60.0 | 34.5 | 47.3 |
| Mean | | 68.3 | 52.0 | 60.2 |
| 50 | 1 | 70.0 | 40.0 | 55.0 |
| | 3 | 62.0 | 26.0 | 44.0 |
| | 5 | 60.0 | 15.0 | 37.0 |
| | 7 | 50.0 | 0.0 | 25.0 |
| Mean | | 60.5 | 20.3 | 40.3 |
| 55 | 1 | 65.0 | 0.0 | 32.5 |
| | 3 | 60.0 | 0.0 | 30.0 |
| | 5 | 50.0 | 0.0 | 25.0 |
| | 7 | 35.0 | 0.0 | 17.5 |
| Mean | | 52.5 | 0.0 | 26.3 |
| Check | | 90.0 | 90.0 | 90.0 |
| Grand mean | | 67.81 | 40.6 | |

LSD at 0.05 for: Treatment (T)= 1.2; Dipping time (D)= 1.2; Fungi (F)= 0.8
T x D= 2.3; T x F= 1.7; D x F= 1.7; T x D x F= 3.3.

**Table 2.** ***In vitro*** **effect of hot air treatment on mycelial growth of** ***A. alternata*** **and** ***B. cinerea***

| Fungus | Hot air (°C)* | Linear growth (mm) after (days) | | | | Mean |
|---|---|---|---|---|---|---|
| | | 3 | 7 | 11 | 15 | |
| *A. alternata* | 36 | 0.0 | 30.0 | 70.0 | 90.0 | 47.5 |
| | 38 | 0.0 | 0.0 | 0.0 | 50.0 | 12.5 |
| | 40 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 20** | 22.5 | 42.5 | 85 | 90.0 | 60.0 |
| Mean | | 5.63 | 18.13 | 38.75 | 57 | 30.0 |
| *B. cinerea* | 36 | 0.0 | 26.3 | 75 | 90.0 | 47.8 |
| | 38 | 0.0 | 0.0 | 0.0 | 26.0 | 6.5 |
| | 40 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 20** | 30.0 | 68.0 | 90.0 | 90.0 | 69.5 |
| Mean | | 7.5 | 23.6 | 41.3 | 51.5 | 31.0 |
| Grand mean | | 6.56 | 20.9 | 40 | 54.5 | |

LSD at 0.05 for: Fungi (F)= 0.3; Hot air (H)= 0.5; Days (D)= 0.5
F x H= 0.7; H x D= 1.0; F x D= 0.7; F x H x D= 1.4.

* Hot air exposure for 72 hours.
** Check treatment.

**Table 3. Effect of hot water treatment on tomato fruits infected by *A. alternata* and *B. cinerea* and stored for 15 days at 20°C**

| Hot water (°C) | Dipping time (min.) | Infection severity (%) in artificially inoculated fruits with | | Mean | Decay (%) in naturally infected tomato fruits |
|---|---|---|---|---|---|
| | | *A. alternata* | *B. cinerea* | | |
| 45 | 3 | 17.9 | 25.3 | 21.6 | 6.8 |
| | 5 | 15.3 | 20.3 | 17.8 | 4.2 |
| | 7 | 13.9 | 17.5 | 15.7 | 2.3 |
| Mean | | 15.7 | 21.0 | 18.4 | 4.4 |
| 50 | 3 | 11.1 | 22.6 | 16.9 | 4.2 |
| | 5 | 8.6 | 18.6 | 13.6 | 1.4 |
| | 7 | 7.5 | 11.2 | 9.3 | 0.0 |
| Mean | | 9.1 | 17.5 | 13.3 | 1.9 |
| 55 | 3 | 7.3 | 12.7 | 10.0 | 0.0 |
| | 5 | 7.0 | 10.8 | 8.9 | 0.0 |
| | 7 | 6.2 | 9.0 | 7.6 | 0.0 |
| Mean | | 6.9 | 10.8 | 8.8 | 0.0 |
| Check | | 45.0 | 50.0 | 47.5 | 35.0 |
| Grand mean | | 19.2 | 24.8 | | 10.3 |
| LSD at 0.05 for: Treatment (T)= 1.2; Dipping time (D)= 1.0; Fungi (F)= 0.9; T x D= 2.2; T x F= 1.8; D x F= 1.5; T x D x F= 3.0. | | | | | T = 1.0<br>D = 1.2<br>T x D= 1.7 |

*2. Hot air treatment:*

It is obvious from the data presented in Table (4) that exposing inoculated or uninoculated tomato fruits by the tested fungi to hot air at 36°C for 3days has completely prevented fruits from decaying during storage period extending up to 15 days at 20°C. Meanwhile, elongating exposure time of inoculated tomato fruits with *B. cinerea* to hot air at 36°C for 7 days raised severity of infection to 5.6% during the storage period. The same trend was also recorded when tomato fruits, artificially infected by *A. alternata*, were exposed to hot air at 36° C for 11 days. Raising temperature of hot air to 38°C, completely prevented infection by the two pathogens as compared with the check treatment. It is worthy to mention that exposing tomato fruits to hot air at 40°C for 3, 7, 11 or 15 days caused softness and shrinkage of fruits.

*Ultra structure examination of heat-treated infected tomato fruits:*

It was very clear from the above results that hot water and hot air treatments significantly reduced tomato fruit rots caused by *A. alternata* and *B. cinerea*. This was also confirmed by examination of scanning electron micrographs of untreated (Figs. 1A and B), hot water treated (Figs. 2A and B) and hot air treated (Figs. 3A and B) tomato fruits.

**Table 4. Effect of hot air treatment on tomato fruits infected by *A. alternata* and *B. cinerea* and stored for 15 days at 20°C**

| Hot air (°C) | Exposure time (days) | Infection severity (%) in artificially inoculated fruits with | | Mean | Decay (%) in naturally infected tomato fruits |
|---|---|---|---|---|---|
| | | *A. alternata* | *B. cinerea* | | |
| 36 | 3 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 7 | 0.0 | 5.6 | 2.8 | 0.0 |
| | 11 | 5.0 | 8.0 | 6.5 | 0.0 |
| | 15 | 8.0 | 12.4 | 10.2 | 0.0 |
| Mean | | 3.3 | 6.5 | 4.9 | 0.0 |
| 38 | 3 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 11 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 15 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mean | | 0.0 | 0.0 | 0.0 | 0.0 |
| 40 | 3 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 11 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 15 | 0.0 | 0.0 | 0.0 | 0.0 |
| Mean | | 0.0 | 0.0 | 0.0 | 0.0 |
| 20 (check) | 3 | 20.0 | 25.0 | 22.5 | 0.0 |
| | 7 | 38.0 | 40.0 | 39.0 | 35.0 |
| | 11 | 45.0 | 65.0 | 55.0 | 50.0 |
| | 15 | 68.9 | 95.0 | 81.9 | 65.0 |
| Mean | | 43.0 | 56.3 | 49.6 | 37.5 |
| Grand mean | | 11.6 | 15.7 | | 9.4 |
| LSD at 0.05 for: Treatment (T)= 1.0; Days (D)= 1.0; Fungi (F)= 0.7; T x D= 1.9; T x F= 1.4; D x F= 1.4; T x D x F= 1.8. | | | | | T = 1.5<br>D = 1.5<br>T x D= 1.3 |

Figures (1A and B) show the condensed mycelia and abundant number of highly branched conidiophores and conidia of *A. alternata* (A) and *B. cinerea* (B) in the untreated fruits (check). Figs. (2A and B) show that hot water treatment at 55°C for 3 min. caused nearly complete inhibition of spore formation of both fungi while, the mycelia of them appeared lush. On the other hand, in hot air treatment (38°C for 72hr) the mycelia of *A. alternata* (Fig. 3A) highly distorted and irregularly branched when compared with check (Fig. 1A) while, the growth of *B. cinerea* was completely inhibited by the same treatment (Fig. 3B). It is worth to note that the disappearance of some morphological properties of the two causal pathogens might be due to the presence of tomato juice within the infected fruits.


**Fig. 1. (A & B): Scanning electron micrographs of untreated tomato fruits infected by *Alternaria alternata* (A) and *Botrytis cinerea* (B):**
**A) Normal structure and branched mycelia bearing conidiospores and bearing conidia of *A. alternata* (arrow).**
**B) Condensed mycelium bearing conidiospores and abundant number of conidia of *B. cinerea* (arrow).**


**Fig. 2. (A & B): Scanning electron micrographs of infected tomato fruits treated with hot water at 55°C for 3 min. showing the mycelium mat was lush (arrow) associated with a complete inhibition of conidial formation of both *A. alternata* (A) and *B. cinerea* (B).**

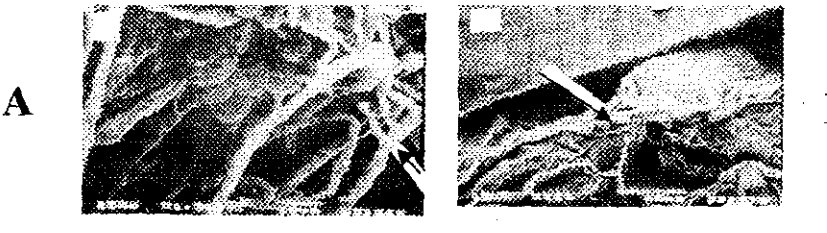


**Fig. 3. (A & B): Scanning electron micrographs of tomato fruits infected by *A. alternata* and *B. cinerea* treated with hot air at 38°C for 72hr and stored at 20°C for 15 days.**
**A) The mycelia of *A. alternata* distorted and irregularly branched with highly decrease in conidiophores and conidia.**
**B) Complete inhibition of *B. cinerea*. Notice that none of mycelium or conidiospores and conidia could be detected in tomato fruits**

## Discussion

Heat treatment of postharvest products is one of the most available techniques to extend the storability and shelf life of fruits and vegetables such as; papaya (Couey *et al.*, 1984), melon (Teitel *et al.*, 1991), sweet red pepper (Fallik *et al.*, 1996) and cantaloupe (Nafaa, 2001). In this investigation, *in vitro* heat treatment for both spores and mycelia of postharvest pathogens, *i.e. A. alternata* and *B. cinerea*, and *in vivo* for both uninoculated and artificially inoculated tomato fruits indicated that hot water dipping at 50°C and 55°C for 7 min., or hot air exposure at 38°C for 72hr. were sufficient to inhibit growth of both fungi and decreased severity of infection. Similarly, Barkai-Golan (1973) reported that hot water dips at 39°C-52°C for 10-2 min., *in vitro* and *in vivo* inhibited spore germination of postharvest fungi and decay development in tomato fruits. On the same trend, Nguyen *et al.* (1998) reported that water treatment of Buoi mangoes at 52°C for 5 min. has a potential as a strategy for reducing the development of postharvest diseases and disorders with minimal fruit loss and shriveling while 52°C for 10 min. induced higher shrivel incidence and increased mass loss. Also, hot water dip at 52°C for 2 min. controlled the development of postharvest pathogens which cause spoilage of Orobanco fruits (*Citrus grandis* L.xc. paradisi) (Rodov *et al.*, 2000). Reduction in fungal growth or decay incidence might be attributed to the direct effect of heat on the spore germination as well as mycelial growth resulting in slowing growth rate of the fungus on the infected fruits (Couey, 1984; Fallik *et al.*, 1993 and Lopez-Carbera *et al.*, 1998). It is worthy to mention that hot air treatment at 40°C showed damage as softness and shrinkage on the surface of tomato fruits. Fallik *et al.* (1995) reported that holding apple fruits inoculated with *Penicillium expansum* for longer times at 42°C or 46°C probably inhibited disease development, but at the risk of heat damage to the fruits. The mode of action of heat treatment appears to be *via* direct interaction with the fungus itself or *via* physiological response of the fruit tissues.

Scanning electron micrographs obtained showed the effectiveness of hot air treatment at 38°C for 72hr in decreasing mycelial growth and sporulation of *A. alternata* and inhibiting growth of *B. cinerea* as compared by condensed growth and sporulation of both pathogens in the infected tissues of untreated tomato fruits. Fallik *et al.* (1995) used scanning electron microscopy to examine inoculated apple fruits treated with hot air at 38°C for 4 days and stored up to 14 days at 20°C, found few spores have germinated giving very thin hyphae with markedly reduced branching. In contrast, the mycelial mat in untreated fruits after 4 days at 20°C was thick and lush. This confirm the importance of hot air treatment in controlling postharvest decay of tomato fruits during storage and marketing.

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*(Received 13/09/2004;*
*in revised form 20/11/2004)*

## مقاومة اعفان ثمار الطماطم ما بعد الحصاد:
## ٢- باستخدام المعاملات الحرارية

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تأثير المعاملات الحرارية على مقاومة العفن الأسود المتسبب عن فطر الترناريا الترناتا والعفن الرمادى المتسبب عن فطر بوتريتس سينيريا تم بحثها حيث وجد أن الانبات الجرثومى لفطر بوتريتس سينيريا يتم تثبيطه تماما عند تعريض معلق جراثيم الفطر لدرجة حرارة ٥٠ °م لمدة ٧ دقائق بينما يثبط فطر الترناريا الترناتا عند درجة حرارة ٥٥ °م لمدة ٧ دقائق وقد وجد أن تعريض اقراص حاملة لنمو أى من الفطرين المختبرين للهواء الساخن على درجة ٤٠ °م لمدة ٧٢ ساعة يؤدى إلى تثبيط النمو.

كما وجد أن غمر ثمار الطماطم فى الماء الساخن عند درجة حرارة ٥٥ °م لمدة ٧ دقائق أو تعريضها لهواء ساخن درجة حرارته ٣٨ °م لمدة ٧٢ ساعة تمنع تقدم العفن حتى ١٥ يوما اذا ما خزنت على درجة ٢٠ م° وذلك فى الثمار الغير معداة والمعداة صناعيا بفطرى الترناريا الترناتا وبوتريتس سينيريا وقد تم أيضا دراسة ومناقشة تأثير المعاملة بالحرارة والعدوى بالفطريات تحت الدراسة على التركيب الداخلى بالفحص بالميكروسكوب الالكترونى الماسح.