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Effect of Acetic Acid Fumigation and Waxing on Decay, Storability and Marketability of Mandarin Fruits

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ABSTRACT

Decay caused by Penicillium spp is a major problem for production storage marketing, storage and export of mandarin in Egypt. Acetic acid (AA) vapor and acetic acid (AA) vapor with wax could be an alternative to the use of postharvest fungicides for disease control. The Fumigation using acetic acid at concentration of 0.1 ml/L air either alone or with carnauba wax spray was applied to the naturally and artificially inoculated mandarin fruits with Penicillium digitatum or Penicillium italicum before storage at 5°C and relative humidity (RH) 90-95% for 45 days then at room temperature for 5 days and 75% RH as a simulation marketing life. In vitro results showed that treatment of acetic acid at 0.1ml/L air vapor with wax had significant impacts in reducing the extent of decay and growth of Penicillium spp. Also, the physicochemical properties of the natural and artificially inoculated mandarin fruits maintain the quality as delayed the changes in firmness, weight loss, titratable acidity, soluble solids content, ascorbic acid, juice ratio and respiration rate and increase marketability of the naturally fruits compared with control ones. Wax combined with acetic acid in the vapor phase could offer promise for controlling postharvest pathogens and maintain fruit quality during cold storage and increase marketability of mandarin fruits.

Key words: mandarin; citrus; Penicillium digitatum; Penicillium italicum; wax, acetic acid, vapor, quality and postharvest decay

Introduction

Clementine mandarin is the most economically important citrus, which is primarily exported to European markets. Green mold, caused by P. digitatum, and blue mold, caused by P. italicum are the most economically important postharvest diseases of clementine's. Currently, both diseases are primarily controlled by application of synthetic fungicides. Alternative methods are needed because of concerns about environmental contamination and human health risks associated with fungicide residues and because the widespread use of these chemicals in commercial packinghouses has led to the proliferation of resistant strains of the pathogens.

Acetic acid is a proven antimicrobial agent and a natural and safe food ingredient (Radi et al., 2010), also it is a valid candidate and effective in preventing postharvest fruit decay caused by P. digitatum and P. italicum. Studies have been conducted on citrus fruit such as oranges, lemons and grapefruit (Sholberg and Gaunce; 1995, Sholberg, 1998). Acetic acid or vinegar vapor was effective in preventing germination of conidia of brown rot, grey mould and blue mould and subsequent decay of stone fruit, strawberries and apples (Sholberg et al., 2000).

At present, wax has been used as an effective technology to increase the quality of postharvest fruits and vegetables (Tietel et al., 2010). Coatings could effectively retard the loss of the water, titratable acidity and ascorbic acid of sweet cherries (Dang et al., 2010). Waxing could improve firmness, titratable acidity, ascorbic acidity and the water content for citrus (Murcott tangor) stored at 15°C for 56 days (Chien et al., 2007). Also, using wax as surface coating material could be more effective maintaining the quality, taste and increases the storage capacity in mandarin (Porat et al., 2005). Waxes also provide a modified atmosphere within the fruit which decreases respiration and delays ripening (Bayindirli et al., 1995). The beneficial role of carnauba wax is well known for enhancing shelf life and maintaining postharvest quality of several fruits such as mango (Khuyen et al., 2008). Application of shellac-based waxes reduces internal O₂ levels, and increases internal CO₂ (Petracek et al., 1998). Waxing delays fruit ripening, maintens quality and extends shelf life of orange fruit (Martinez et al. 1991) and of pineapple fruits (Hu et al., 2011). Waxing of citrus fruits reduces mold caused by P. digitatum and P. italicum during cold storage (Waks et al., 1985).

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The aim of this research was to investigate effects of wax, acetic acid vapor alone or in combination against green and blue rots disease and the efficacy of these treatments on decay, physicochemical properties and market life to maintain the quality of stored clementine mandarin fruits.

Materials And Methods

Sources of fruit:

Fortune mandarin (Citrus reticulata Blanco), a hybrid of the 'Clementine' (mandarin group) and 'Dancy' tangerine, grown in sand-loam soil and in private orchard (Eva Group Company) in Wady Alnatroon, Giza Governorate. Fruits were received common horticulture practices and harvested at optimal harvest time in the last week of December 2010 and 2011 with the full color stage and average weight of 190 gm. The fruits were delivered on the same day to the laboratory and the physicochemical properties evaluated at harvest time as average values of the two seasons. Soluble Solids Content (SSC) [11.4%]; firmness [10 (lb/in²)]; Juice ratio [32.0%]; titratable acidity (TA) [1.02%]; Ascorbic acid (V.C.) [35.2 mg/100mL juice] and Respiration rate [10.5 ml CO₂ kg⁻¹ h⁻¹]. Non-defective 'Fortune' mandarins were selected, washed with fresh water, air-dried, and used in the experiments before postharvest treatments were applied.

Fungal inoculums in vitro:

Isolates P. digitatum (green mold) and P. italicum (blue mold) were taken from decayed mandarin fruit from Postharvest Diseases Department, Plant Pathology Research Institute-Agriculture Research Center (ARC) Giza, Egypt, and maintained on potato dextrose agar medium(PDA). Seven day-old cultures were used to obtain a spore suspension in sterile distilled water containing a drop of a wetting agent (Tween 80) per liter. Spore suspensions were adjusted to 5 x 10⁶ spores/ml and the plates were incubated overnight at 25±1°C under 85-90%.

Fumigation with Acetic acid (AA) in vivo:

All fungi were cultured on potato dextrose agar medium (PDA). Linear growth of P. digitatum and P. italicum as affected by acetic acid (AA) at different concentrations of 0.25, 0.50, 1, and 2ml/10L air was studied. Five replicate plates were placed into 10L glass jars, acetic acid vapor was introduced through pipeline in and out (Fig .1) and each jar along was sealed with plastic lid. Plates in a jar without acetic acid vapor were used as control. Acetic acid concentrations were used fumigation as vapor utilizing a breathing pump (Emed) (Model: A1000230 v- 50 Hz 90va Manufacturer: Elettroplastica spa via delay commercio 1 travagliato (BS) Italy).

Inoculation Fruit with Pencillium spp:

Fruits were sterilized with 70% ethanol, and then artificially wounded at the flavedo of each fruit once on the equator with a steel rod (2mm deep by 0.5 mm wide). Fruits were divided into 3 groups prior to treatments, each group containing 240 fruits. The first group as naturally infected fruits, the second group inoculated with P. digitatum and the third group inoculated with P. italicum (artificially infection).The inoculations was carried out by spraying the surface of fruits with spore suspensions previously isolated from diseased mandarin fruits. Inoculated fruit were kept at room temperature and allowed to air dry for 24 hours, before fumigation treatments were applied.

Treatments of Mandarin fruits with Acetic acid (AA) and/ or wax in vivo:

After 24h inoculation, fruits under sub each group (naturally infected fruits (N.I), inoculated fruits with P. digitatum (P.D) and inoculated fruits with P. italicum (P. l)) and control fruits were treated with 4 treatments, each treatment consisted of 60 fruits. Treatments as follows:

1- Acetic acid vapor 0.1 ml / L air (the middle concentration that prevent the germination penicillium spores)
2- Carnauba –wax as spray on surface of fruits as wax solution (Carnauba wax was obtained from the packing and exporting fruit station at the Kalyoubia governorate, Egypt.
3- Carnauba waxed fruits were evaporated with acetic acid 0.1 ml / L air vapor
4- Control fruits were fumigated with air vapor.
All treatments (Fig 2) were applied in three replicates, each of 20 fruits. They were placed in 10 L glass jars utilizing breathing pump (as mentioned in vitro), with acetic acid vapor or air control for time.

Storage conditions:

Each treatment of fruits were placed in three performed cartoon boxes (30×40×20 cm), to determine disease incidence, the second to determine weight loss and the third for fruits analysis, each box contained of 20 fruits was replicated three times, and the experiment was repeated twice (2010 and 2011 seasons). Mandarin fruits were subjected randomly to one of the above treatments and stored at 5°C and 90% RH for 45 days in laboratory of refrigeration, Agriculture Development Systems (ADS) project in the Faculty of Agriculture, Cairo University, then at room temperature (shelf life) for 5 days.

Data recorded in vitro:

The linear growth (mm) of each fungus was recorded after incubation period, whenever plates reach its maximum. Reduction percentages in colonies diameters caused by the tested treatments were calculated using the formula suggested by Fokemma (1973) as follows:

\[
\text{Reduction percentage} = \frac{(de - di)}{de} \times 100
\]

Where; \( de \) = linear growth in control set.
\( di \) = linear growth in treatment set.

Disease assessment:

Disease severity of the fruit and lesion diameter on each fruit was recorded. When the visible rot zone out-sides the wounded area on fruit was more than 0.5 mm wide, it was counted as decayed fruit. Disease incidence was measured according to the method of Zeng et al. (2006). Efficiency of the tested treatments in controlling the disease was calculated according to the following formula:

\[
\% \text{ Efficiency} = \frac{\text{Control disease severity} - \text{Treatment disease severity}}{\text{Control disease severity}} \times 100
\]

Physiochemical properties of stored mandarin:

Data recorded: was determined after 45 days of cold storage

Weight Loss%:

Fruits were periodically weighed and the loss in mass weight was recorded for each replicate. Data were calculated as percentage.

Fruit Firmness (lb/in\(^2\)):

It was measured on the two opposite sides of mandarin fruit samples by using a hand magness taylor pressure tester (lb/in\(^2\)).

Soluble Solids Content (SSC) %:

Individual mandarin fruits were ground in an electric juice extractor for freshly prepared juice. Soluble solids content was measured using Digital refract meter PR32 (0.32%)Atago Palete ATago .CO .LTD. Japan.

Titratable Acidity (TA) %:

Total acidity (expressed as citric acid %) was determined by titrating 5-ml juice with 0.1N sodium hydroxide using phenolphthalein as indicator (A.O.A.C., 1990).
Juice Ratio %:

Fruit weighed with an electronic balance. Juice was extracted by machine and weight was taken. The juice percentage was expressed as percentage of total weight at the time of measurement. Weight juice / weight fruit x 100(A.O.A.C., 1990).

Ascorbic Acid (Vitamin C):

Vitamin C content was measured using 2, 6 dichlorophenol indophenols' method described by (A.O.A.C., 1990).

Respiration Rate:

Individual fruits for each treatment were weighed and placed in 2-liter jars at 20°C. The jars were sealed for 3 h with a cap and a rubber septum. The resulting O2 and CO2 samples of the headspace were removed from the septum with a syringe and injected into Servomex Inst. Model 1450C (Food Pack Gas Analyzer) to measure oxygen and carbon dioxide production. Respiration rate was calculated as ml CO2 kg⁻¹ h⁻¹. (Lurie and Pesis, 1992).

Marketing Life:

At the end of storage 45 days at 5°C and 90% RH, about 20 fruits from every treatment of naturally fruits were held in ambient room at (20°C ± 1) and 75% RH for 5 days to simulate marketing life (percentage of decayed fruit: The number of decayed fruits due to fungus infection calculated as a percentage of the total number of fruits using the following equation: and quality measurements as physicochemical properties.

Experimental design and statistical analysis:

The results of the parameters (The linear growth, Efficiency and Disease Severity were analyzed using the software Co Stat version 6.400- Co Hort Software. The mean of all treatments were compared by the least significant difference (L.S.D.) at 5% level of probability according to Gary (2010).

All results of physiochemical parameters were performed in triplicate using completely randomized factorial design and Marketing life using completely randomized design. Data were analyzed with the Analysis of variance (ANOVA) procedure of MSTAT-C program. When significant differences were detected, treatment means were compared by LSD range test at the 5% level of probability in the two investigated seasons (Snedecor and Cochran, 1980).

Results:

Effect of acetic acid fumigation on linear growth:

Vapors of acetic acids passing overhead the PDA medium inoculation with the disease of P. italicum (P.I) and P. digitatum (P.D) caused inhibited of the fungal linear growth as demonstrated in Table 1. Vaporization of the acetic acid solutions having concentrations of 0.05, 0.1 and 0.2 ml/L completely prohibited the fungal
growth of both tested Penicillium isolates. The concentration of 0.025 showed a very significant inhibitory effect compared with the control.

### Table 1: Effect of acetic acid fumigation on linear growth (mm) of P. italicum (P.I) and P. digitatum (P.D).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Con mL/L</th>
<th>P.I Linear growth (mm)</th>
<th>Inhibition (%)</th>
<th>P.D Linear growth (mm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>0.025</td>
<td>8.33</td>
<td>90.4</td>
<td>9.87</td>
<td>89.03</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.00</td>
<td>100</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.00</td>
<td>100</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.00</td>
<td>100</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td>90.00</td>
<td>0.0</td>
<td>90.00</td>
<td>0.0</td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td></td>
<td>0.10</td>
<td></td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

**Effect of fumigation with acetic acid and waxing on fruit rots:**

Exposing mandarin fruits to vapors of acetic acid obtained by air flow out of the solutions at the rate of 0.1ml/L air, highly reduced green mould incited by P. digitatum or blue mould incited by P. italicum on naturally infected or artificially inoculated ones as shown in Tables 2 and 4. The air flow through acetic acid caused great reduction in the severity of green mould and blue mould during the two successive seasons (2010-2011). Both treatments (acetic acid alone or with wax) were very effective against Penicillium infection and its development. However, wax as well gave significant results.

Data in table (2 and 4) showed that all treatments significantly suppressed the decay development on naturally infected mandarin fruits at the end of cold storage (seasons 2010-2011). It was observed that the efficacy of most treatments was almost constant along the two season results. Tested treatments proved their higher efficiency to control postharvest fruit rots on Mandarin fruits more than untreated fruits and fruits treated with wax only.

Results indicate that all treated fruits showed complete reduction in mold incidence. It is clear from (Table 2,4) that efficacy of fruit mandarin treated with acetic acid and acetic acid with wax were able to protect both mandarin fruits against mold incidence under stress of artificial inoculation with mould pathogens for 45 days in refrigerator, the period of experimental test. By the untreated control fruits there were percentage 100% green molds and blue molds infections, but diseases severity were 57.13 and 54.79 % at the end of storage, after shelf life for 5 days at room temperature disease severity were 82.43-85.13 % in the untreated fruits (control).

**Influence of fumigation with acetic acid and waxing treatments on physiochemical parameters:**

**Weight loss percent:**

Results in table (2 and 4) in both seasons show that with ending storage period 45days at 5°C, minimum decrease percentage of fruit weight was observed in treatment where acetic acid vapor and wax was used compared to control that show maximum weight loss percent. Decrease percentage of fruits weight were due to the tendency of the fruit samples to lose water and equilibrate with the surrounding environment and also as a result of respiration during storage time. No significant differences were observed between the acetic acid alone and control treatments; meanwhile, weight percent of naturally mandarin fruits significantly decrease than inoculated fruits with P. digitatum and P. italicum. The interaction between treatments and inoculation significantly caused decrease in weight loss% especially in treated fruits with acetic acid vapor and carnauba wax for naturally fruits, and for inoculated fruits with P. digitatum and P. italicum compared to control treatments.

**Firmness:**

Fumigation improved firmness of mandarin fruits compared with un fumigated fruit (control) at the end of 45 days storage at 5°C. Firmness of ‘Fortune’ mandarin was decreased in all treatments compared with harvest sample values 10 (lb/in²). Acetic acid and wax treatment maintained highest fruit firmness level followed by wax alone and there was no effect on firmness between acetic acid alone compared with control treatments in both seasons (Table 2 and 4). Highest firmness was achieved by naturally fruit compared with inoculated fruits with blue and green molds. As for the interaction, data referred that acetic acid vapor and wax significantly increased firmness parameter and it was the most effective on natural inoculated fruits with pencillium rots for keeping fruit firmness compared with control treatments.
Table 2: Effect of acetic acid fumigation and/or waxing on disease severity ratio and physiochemical properties of natural and inoculated mandarin fruits with *Pencillium* spp at end of the storage during 2010 season

<table>
<thead>
<tr>
<th>Inoculation Treatments</th>
<th>Disease severity %</th>
<th>Weight loss %</th>
<th>Firmness (lb/in²)</th>
<th>SSC %</th>
<th>Juice %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.I</td>
<td>P.D</td>
<td>N.I</td>
<td>Mean</td>
<td>P.I</td>
</tr>
<tr>
<td>Wax + Acetic Acid</td>
<td>1.52</td>
<td>2.31</td>
<td>0.00</td>
<td>5.2</td>
<td>5.4</td>
</tr>
<tr>
<td>Wax</td>
<td>2.29</td>
<td>4.26</td>
<td>0.89</td>
<td>5.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>2.68</td>
<td>4.01</td>
<td>0.90</td>
<td>12.7</td>
<td>12.9</td>
</tr>
<tr>
<td>Control</td>
<td>32.6</td>
<td>35.89</td>
<td>25.18</td>
<td>12.6</td>
<td>13.0</td>
</tr>
<tr>
<td>Mean</td>
<td>8.9</td>
<td>9.2</td>
<td>7.4</td>
<td>4.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>

L.S.D at 0.05%

- Treatments (A) 1.98
- Inoculation (B) 0.2

(P.I= *Penicillium italicum*

P.D= *Penicillium digitatum*

N.I=naturally infection)

Table 3: Effect of acetic acid fumigation and/or waxing on physiochemical properties of natural and inoculated mandarin fruits with *Pencillium* spp at end of the storage during 2010 season

<table>
<thead>
<tr>
<th>Characters</th>
<th>acidity%</th>
<th>Vitamin C (mg/100ml juice)</th>
<th>Respiration rate (CO₂ ml/kg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation Treatments</td>
<td>P.I</td>
<td>P.D</td>
<td>N.I</td>
</tr>
<tr>
<td>Wax + Acetic Acid</td>
<td>0.83</td>
<td>0.80</td>
<td>0.87</td>
</tr>
<tr>
<td>Wax</td>
<td>0.78</td>
<td>0.76</td>
<td>0.83</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.71</td>
<td>0.70</td>
<td>0.73</td>
</tr>
<tr>
<td>Control</td>
<td>0.75</td>
<td>0.74</td>
<td>0.77</td>
</tr>
<tr>
<td>Mean</td>
<td>0.75</td>
<td>0.74</td>
<td>0.77</td>
</tr>
</tbody>
</table>

L.S.D at 0.05%

- Treatments (A) 0.02
- Inoculation (B) 0.1

(P.I= *Penicillium italicum*

P.D= *Penicillium digitatum*

N.I=naturally infection)

Table 4: Effect of acetic acid fumigation and/or waxing on disease severity ratio and physiochemical properties of natural and inoculated mandarin fruits with *Penicillium* spp at end of the storage during 2011 season

<table>
<thead>
<tr>
<th>Inoculation Treatments</th>
<th>Disease severity %</th>
<th>Weight loss %</th>
<th>Firmness (lb/in²)</th>
<th>SSC %</th>
<th>Juice %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.I</td>
<td>P.D</td>
<td>N.I</td>
<td>Mean</td>
<td>P.I</td>
</tr>
<tr>
<td>Wax + Acetic Acid</td>
<td>1.93</td>
<td>2.35</td>
<td>0.00</td>
<td>6.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Wax</td>
<td>0.86</td>
<td>0.80</td>
<td>0.87</td>
<td>0.83</td>
<td>32.2</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.74</td>
<td>0.73</td>
<td>0.78</td>
<td>0.75</td>
<td>29.3</td>
</tr>
<tr>
<td>Control</td>
<td>0.73</td>
<td>0.72</td>
<td>0.78</td>
<td>0.74</td>
<td>29.2</td>
</tr>
<tr>
<td>Mean</td>
<td>0.73</td>
<td>0.72</td>
<td>0.78</td>
<td>0.73</td>
<td>31.9</td>
</tr>
</tbody>
</table>

L.S.D at 0.05%

- Treatments (A) 0.02
- Inoculation (B) 0.1

(P.I= *Penicillium italicum*

P.D= *Penicillium digitatum*

N.I=naturally infection)

Table 5: Effect of acetic acid fumigation and/or waxing on physiochemical properties of natural and inoculated mandarin fruits with *Penicillium* spp at end of the storage during 2011 season

<table>
<thead>
<tr>
<th>Characters</th>
<th>Acidity%</th>
<th>Vitamin C (mg/100ml juice)</th>
<th>Respiration rate (CO₂ ml/kg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation Treatments</td>
<td>P.I</td>
<td>P.D</td>
<td>N.I</td>
</tr>
<tr>
<td>Wax + Acetic Acid</td>
<td>0.85</td>
<td>0.83</td>
<td>0.90</td>
</tr>
<tr>
<td>Wax</td>
<td>0.82</td>
<td>0.80</td>
<td>0.85</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.74</td>
<td>0.73</td>
<td>0.78</td>
</tr>
<tr>
<td>Control</td>
<td>0.73</td>
<td>0.72</td>
<td>0.78</td>
</tr>
<tr>
<td>Mean</td>
<td>0.73</td>
<td>0.72</td>
<td>0.78</td>
</tr>
</tbody>
</table>

L.S.D at 0.05%

- Treatments (A) 0.02
- Inoculation (B) 0.1

(P.I= *Penicillium italicum*

P.D= *Penicillium digitatum*

N.I=naturally infection)
Soluble Solids Content (SSC):

As shown in Table (2 and 4) soluble solids content % of the control fruits rapidly increased at the end of storage period. The changes in SSC content were more slowly in acetic acid and wax or wax alone treatments than in control treatment. Naturally infected fruit attained significantly lower in changes of SSC % content than inoculated fruits. The highest averages of SSC % content were observed with acetic acid alone and control treatments. Interaction results show that, acetic acid and wax treatment tends to maintain significantly (P < 0.05) lower levels of SSC on naturally fruits and inoculated fruits with both *P. digitatum* and *P. italicum* compared with that in the control at the end of storage period in both seasons.

Juice content:

Statistical analyses (Table 2 and 4) indicated that juice recovery decreased much higher in control inoculated fruits with *pencillium spp* compared with naturally infected fruits after 45 days of storage at 5°C. The highest juice content obtained from the acetic acid and wax treatment followed by wax treatment alone. Also, Interaction data clarify that, acetic acid vapor and wax treatment maintained juice ratio % in maximum averages in non-inoculated fruits and inoculated fruits with *P. italicum* and *P. digitatum* in both seasons of study.

Juice Titratable Acidity:

According to (Table 3 and 5), the quantity of organic acids expressed as citric acid was decreased in mandarin fruits with ending of fruit maintenance period in cold storage for 45 days. There were significant differences in the levels of titratable acidity (TA) between fumigation fruits with acetic and wax or wax alone. Meanwhile, there were insignificant differences between acetic acid and control treatment. The control and acetic acid alone had a significant decrease in TA % but acetic acid vapor with wax treatment inhibited its decline juice of natural infected fruits which has more content of organic acids than fruits inoculated with blue and green molds. Interaction data were significant in both considered seasons at the end of the storage period.

Vitamin C:

As shown in Table 3 and 5 there were significant differences between control and tested treatments except acetic acid alone treatment (P < 0.05). The vitamin C content was high in acetic acid and wax or wax alone treatments. Maximum averages of vitamin C content were achieved by naturally fruits (non-inoculated fruits) with significant differences from inoculated mandarin fruits with both *P. italicum* and *P. digitatum*. Interaction data show that the highest vitamin C content was obtained from mandarin fruits fumigated with acetic acid and wax in both seasons.

Respiration Rate:

It can be seen from Table 3 and 5 that there was a noticeable decrease in values of respiration rate at the end of cold storage (45 days) compared with the initial respiration rate value at harvest[10.5 ml CO₂ kg⁻¹ h⁻¹] during the two seasons of investigation. Acetic acid vapor and carnauba wax treatment tended to have the effective role in reducing the rate of respiration of mandarin fruits than control ones. Also, naturally infected fruits had the lowest respiration rate compared with inoculated fruits with *P. italicum* and *P. digitatum*. Interaction data show a significant effect of various treatments on natural and inoculated fruits. The lowest rate of respiration was obtained from mandarin fruits treated with wax only.

Marketability of mandarin fruits:

Detection of experiments efficiency of the treatments after simulation of marketability life for 5 days at room temperature indicated on always reduction in disease severity as a result of high efficacy of such treatments particularly acetic acid + wax (Table 6) for season 2010 as it was 96.39% for P.I and 94.95% for P.D. Almost same results were obtained in season 2011 (96.14% for P.I.) and (96.1% for P.D.) of complete reduction in mandarin fruit rots and 100% efficiency for such treatment in naturally infected mandarin fruits. Concerning the effect of market period at (20°C ± 1) and 75% RH for 5 days on fruit parameters of naturally fruits (non - inoculated), data presented in (Table 6) showed significant increasing in parameters of weight loss % and SSC % in control fruits. Meanwhile, there was a significant decrease in titratable acidity, Vitamin C and juice ratio level in the two seasons. The best treatment kept quality of mandarin fruits after 5 days cold storage as simulation for marketing period, was acetic acid vapor and wax.
treatment followed by wax treatment alone, as fruits had low weight loss, high firmness, Vitamin C, juice ratio and keep on levels of SSC % and TA% without high changes.

Table 6: Effect of fumigation with acetic acid on disease severity ratio and physiochemical properties of natural mandarin fruits after 5 days from end of storage as marketing life during 2010 and 2011 seasons.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Disease severity %</th>
<th>Weight loss %</th>
<th>Firmness (lb/in²)</th>
<th>SSC %</th>
<th>Juice %</th>
<th>acidi%</th>
<th>Vitamin C (mg/100ml juice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculation</td>
<td>Penicillium digitatum</td>
<td>Penicillium italicum</td>
<td>N.I</td>
<td>2.68</td>
<td>3.01</td>
<td>3.72</td>
<td>3.85</td>
</tr>
<tr>
<td>Wax</td>
<td>14.66</td>
<td>14.84</td>
<td>14.75</td>
<td>14.75</td>
<td>27.8</td>
<td>28.2</td>
<td>28.5</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>6.27</td>
<td>6.27</td>
<td>6.27</td>
<td>6.27</td>
<td>6.27</td>
<td>6.27</td>
<td>6.27</td>
</tr>
<tr>
<td>Control</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
</tr>
<tr>
<td>Mean</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
<td>31.8</td>
</tr>
<tr>
<td>L.S.D 0.05%</td>
<td>2.58</td>
<td>2.58</td>
<td>2.58</td>
<td>2.58</td>
<td>2.58</td>
<td>2.58</td>
<td>2.58</td>
</tr>
</tbody>
</table>

Discussion:

Disease severity was mentioned as one of the limiting factors for postharvest life of citrus fruit because of removing the natural wax of citrus peel through the handling in packing houses (Dou, 2004). The inoculation experiments in vitro revealed that acetic acid vapor can reduce or inhibit the infection with P. digitatum and P. italicum. Acetic acid vapor on concentrations of 0.1 and 0.2ml/1L air caused complete inhibition of liner growth and spore germination of P. digitatum and P. italicum on potato dextrose agar medium. Efficiency of the control was 100% at 0.05, 0.1 and 0.2ml/L air acetic acid vapor and disease incidence was the least at 0.1/1L air acetic acid vapor. The inhibitory effect of acetic acid vapor kills fungal spores and it does not injure the fumigated fruits surface, and it is effective at low temperatures which mean that fruit in 5°C cold storage could be effectively treated with acetic acid vapors (Sholberg and Gaunce, 1995). Acetic acid vapors were more effective for controlling postharvest decay, the mechanisms of acetic acid inhibition for microorganisms apparently that it may affect the cell membrane interfering with the transport of metabolites and maintenance of membrane potential (Sholberg et al., 1998).

Also, in this study mandarin fruits fumigated at 0.1ml/L air acetic acid vapor and wax treatment can reduce or inhibit the infection with P. digitatum and P. italicum and more effective for controlling natural and artificial infection of mandarin fruits as fruit injured, inoculated and treated with AA vapor and wax, the fruit decays only slower than non-treated fruit. The absence of spores on the fruit surface would result in less decay because wounds would not become infected unless contaminated with a new source of inoculums. Acetic acid work, a protective layer of mycelium growth and therefore stopped up fruit damage and maintains quality. These results are in agreement with Sholberg et al., 2000 who reported that AA vapor is also very effective against Penicillium spp. (Waks et al., 1985) also reported that waxing of citrus fruits reduces mold caused by P. digitatum and P. italicum during cold storage and the lower incidence of mold rots in fruit waxed could be related to the action of wax as barrier, which prevents contact between the spores and wounds on the fruit peel caused during picking or handling.

The results showed that acetic acid (AA) at 0.1ml/L air vapor and wax delayed the changes in weight loss, firmness, soluble solids content, titratable acidity, vitamin C, and juice ratio and respiration rate compared with the control.

Also, acetic acid (AA) vapor treatment alone do not affect negatively the quality attributes as no significant differences were observed between acetic acid (AA) vapor alone and control treatment, in terms of weight loss, firmness, soluble solids content, titratable acidity, vitamin C. Weight loss is mainly associated with respiration and moisture evaporation through the skin. Wax action due to the blocking of transpiration from the skin of fruits by giving an inhibited an layer blocked the fruit water decrease from skin surface, also acetic acid works as a protective layer of growth mycelium and therefore stopped up fruit damage and maintains quality. These results are agreed with Bayindirli et al. (1995) who reported that wax may resulted in a high modification of the internal atmosphere of mandarin fruit.

In the present investigation, fumigating treatment with acetic acid+ wax gave effective results on fruit firmness as wax slowed down fruit softening process as well as pectin degradation. The findings are in agreement with Barman et al. (2011) who established that wax provided a high retention of fruit firmness, probably due to the less dehydration occurred and also to a slower degradation of cell wall components. Wax-treated fruits kept vitamin C in a higher level than in control, similar to is reported by Dang et al. (2010) who found that the content of vitamin C waxed fruits was higher than that of the control.
The results obtained from this study indicated that, mandarin fruits treated with acetic acid vapor and wax then cold stored for 45 days could kept for 5 days at 20°C at room temperature (marketable life). Treatments kept the fruit quality and effective for reducing the decay, weight loss and fruits had a good firmness, delay changes in SSC, acidity, juice ratio, vitamin C, so fruits were in healthy appearance and preferred by consumers. Using wax as surface coating material can more maintain the quality, taste and increase the storage capacity in mandarin (Porat et al., 2005).

Conclusion:

Waxed fumigated fruits with acetic acid at 0.1 ml / L air treatment can control decay of mandarin fruits with *P. digitatum* and *P. italicum*, and play a very effective and vital role to control compositional changes by delaying the ripening process and with a minimum quality loss and longer storage and market life as compared with the control sample. It is safe and can replace the chemical fungicides also can control the decay without producing any serious quality deficiencies. Mandarin tolerate AA vapor, it would be feasible to fumigate citrus in commercial quantities to prevent postharvest decay.

References


