CHAPTER IV

EVALUATION OF POSTHARVEST TREATMENTS AND SAFE COMPOUNDS

4.1 INTRODUCTION

Postharvest washing of melons is essential for the control of storage decay and should be carried out immediately after harvest to ensure a high quality product (Bonnardeaux & Robinson 1994). In most countries this is achieved by dipping fruit in fungicides; however this has led to large amounts of fungicides being used for postharvest disease control throughout the world, with perceived or possibly real threats to human health, and unpredictable economic or environmental consequences (Schirra et al., 2000). Postharvest treatment of melons with fungicides is not recommended by the majority of countries importing melons, and hence there is a need for alternative methods of disease control in Australia. Consequently, there is a renewed interest in alternative postharvest disease management practices that can reduce consumer and environmental risks (Droby et al., 1998).

Biological control using antagonist micro-organisms has been extensively studied as a method of postharvest disease control (Filnonow et al., 1996; Janisiewicz and Marchi, 1992; Madrigal et al., 1994; Nunes et al., 2001; Vinas et al., 1998; Wei et al., 1999). The use of biological agents for the control of postharvest disease is limited by the relatively narrow spectrum of activity (Janisiewicz et al., 1992), poor economic feasibility (Nunes et al., 2002) and constraints regarding public acceptance (Wilson and Pusey, 1985).
Heat treatment applied by different means has also been examined as an alternative for the reduction of postharvest fungicide usage. The efficacy of fungicides has been found to increase many fold when used in hot water, allowing effective control of decay at lower dosages (Ben-Yehoshua et al., 2003). However, a negative side effect in one study shows that a postharvest treatment in hot fungicide left higher residues, than treatment in a cool temperature (Schirra et al., 1998).

Hot water treatment alone has been shown to be an effective physical method for the control of a wide range of pathogens for storage rots (Palou et al., 2001; Schirra et al., 2000; Teitel et al., 1989). Besides reduction of storage decay, dipping fresh produce in hot water is believed to improve the quality of fruit for prolonged storage (Ben-Yehoshua, 2003; Fallik, 2004). A heat shock host response from hot water treatment has been found to weaken fungal growth by inducing host antifungal compounds involved in resistance (Fallik et al., 1996). Water recycling of hot water is possible in this type of system as most of the pathogens cannot survive at temperatures above 50°C (Barkai-Golan and Phillips, 1991; Lurie, 1998), making better economic use of the water. More importantly the practice of hot water washing of fresh produce is environmentally friendly and involves no risk to health. The practice of hot water treatment would reduce production costs and would cost less for the consumers (Lurie, 1998).

In recent years, many chemicals that are used as food additives or for food processing, have been evaluated for their efficacy in controlling postharvest storage rots of fruit and vegetables. These are generally regarded as safe (GRAS) compounds which do not require expensive testing and validation by regulatory agencies (Aharoni et al., 1997). Many of them have been shown to have broad-spectrum antimicrobial properties (Palou et al.,
Sodium bicarbonate (Smilanick et al., 1999), molybdate and acetate salts (Palou et al., 2001) are such compounds. Likewise, elemental iodine is a biocidally active form of iodine (Chang, 1958) and its efficiency has been found to increase at high temperature (Ellis et al., 1993). Iodine has already been recommended for the postharvest treatment of fruit and vegetables as a general sanitizer and is currently being used commercially in Australia (Morris and Bokshi, 2002 unpublished report). These chemicals warrant further investigation for the postharvest treatment of melons as a safe alternative to fungicide.

Unfortunately, none of the physical or non-pesticide chemical treatments has been found to work as a stand-alone alternative for equivalent control to that of synthetic fungicides (Palou et al., 2002). The possibility of using hot water in combination with GRAS compounds for the control of storage diseases has been suggested for postharvest treatment on different commodities (Marquenie et al., 2002). The need to find a suitable replacement for fungicides has prompted research aimed at combining various alternatives into a control strategy that equates with the effectiveness of synthetic chemicals (Conway et al., 2004). Therefore, this research program was undertaken to evaluate new alternatives to fungicide that would lead to a treatment strategy for controlling postharvest diseases as well as maintaining quality of melons in long time storage.

4.2 MATERIALS AND METHODS

A number of experiments were conducted using chemical and non-chemical treatments to develop an effective and a safe postharvest-treatment-technology of melons. The chemicals that are currently being used for postharvest treatment were evaluated for their efficacy and were compare to safe alternatives. The safe chemicals and the non-chemical treatments
were evaluated separately and subsequently combined to develop an effective postharvest treatment.

Inoculation, dip treatment, incubation and disease assessment of melons

Freshly harvested unwashed honeydew and rockmelons were used in different postharvest dipping experiments to control storage rots. Storage diseases occurred either by inoculation with spores or from natural infections of pathogenic fungi. Fruit were challenge inoculated with 50 μl of spores of the fungi through scratch wounds (3 mm deep, 3 mm width and 10 mm long) at the concentrations mentioned in the Table 3.2.3.1 (Chapter III). There were eight wounds per fruit. Four hours after inoculation fruit were dipped for one min; one at a time in 25 litre solutions in a 60 litre plastic bucket. Fruit were allowed to air-dry for 30 min, before being put in to cartons. The cartons were covered with perforated plastic and incubated at 15° C. Fruit were examined for disease development after 10 days of incubation by measuring the area rotted at the point of challenge inoculation or using a 1-5 severity scale in case of natural infections. Rot severity scores were: 1 = very little or no infection or mould growth; 2 = approx. 2 - 5 % of the fruit skin covered with moulds and/or with a few small lesions (<1 cm diameter), 3 = approx. 6 - 15 % of the skin covered with moulds and/or with larger lesions (>1 to <3 cm diameter); 4 = approx. 16 to 50 % of the skin covered with moulds and/or with lesions (>3 to <5 cm diameter) and 5 = more than 50 % of the skin covered with moulds and/or with very severe lesions. The lesions consisted of one or several different types of storage rots.

4.2.1 Efficacy of fungicides for the control of storage rots of melons

Postharvest fungicides which are currently used on rockmelon in Australia to control virulent strains of *Fusarium acuminatum, Alternaria alternata* and *Rhizopus* spp. were
evaluated. The fungicides and commercially recommended concentrations used are listed below in Table 4.2.1. Five medium sized fruit were randomly selected for each fungicide treatment and for each of the pathogens. Eight scratch wounds were made on each fruit to challenge with the pathogen. Lesion development was assessed by measuring the area of rotted surface at the inoculation site.

**Table 4.2.1** Fungicides, their sources and commercially recommended concentrations (a.i.) tested as postharvest dips for melons

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Product name</th>
<th>Source</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imazalil</td>
<td>Magnate</td>
<td>Colin Campbell, NSW, Australia</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Benomyl</td>
<td>Benlate</td>
<td>Colin Campbell, NSW, Australia</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>Spin-flo</td>
<td>RhonePoulenc Rural Australia PLtd</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Guazatine</td>
<td>Panoctine</td>
<td>RhonePoulenc Rural Australia PLtd</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Iprodione</td>
<td>Rovral</td>
<td>Colin Campbell, NSW, Australia</td>
<td>500 ppm</td>
</tr>
</tbody>
</table>

4.2.2 *Safe chemicals for the control of storage rots of melons*

The GRAS chemicals were compared for their effectiveness in controlling melon storage diseases with postharvest fungicide chemicals at 22° C. The chemicals and their concentrations were used as recommended by Palou *et al.*, (2002) on citrus fruit and Morris & Bokshi (2002, Sydney Postharvest Laboratory internal report) on different fruit and vegetables as shown in Table 4.2.2.
Table 4.2.2 Chemicals, their source and concentrations (a.i.) tested as postharvest dips for melons

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Source</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
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</tr>
<tr>
<td>Sodium acetate</td>
<td>AJAX chemicals, Sydney, Australia</td>
<td>0.5%</td>
</tr>
<tr>
<td>Ammonium molybdate</td>
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</tr>
<tr>
<td>Sodium molybdate</td>
<td>AJAX chemicals, Sydney, Australia</td>
<td>24 mM</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>Sara Lee, VIC, Australia</td>
<td>500 ppm</td>
</tr>
<tr>
<td>Iodine</td>
<td>Ioteq, Sydney, Australia</td>
<td>30 ppm</td>
</tr>
<tr>
<td>Guazatine (panoctine)</td>
<td>Rhone Poulenc Rural Australia Ptd</td>
<td>500 ppm</td>
</tr>
</tbody>
</table>

Five medium sized fruit were used per treatment and eight wounds were made on each to challenge with *F. acuminatum*. After 10 days of incubation rot development was assessed by measuring area of rotted surface at the inoculation site. Secondary effects of the chemicals, including ripening and phytotoxicity, appeared on the rind tissue of melons after 10 days of incubation and were assayed by scoring with a severity scale of 1-5 mentioned in Table 4.3.2.

4.2.3 GRAS chemicals in hot water for the control of storage rots of melons

The GRAS chemical treatments that were applied at 22° C and showed a reduction of storage rots in Experiment 4.2.2 were further evaluated for their efficacy as a hot solution. Ammonium molybdate, sodium molybdate and iodine were prepared at the recommended concentration (Table 4.2.2), in hot water (48° C) or water at ambient temperature (22° C, control) for a two min postharvest dip of melons. Five medium sized fruit were randomly selected per treatment and eight scratch wounds (3 mm deep, 3 mm width and 10 mm long) were made on each to challenge with *F. acuminatum*. After 10 days of incubation, rot...
development was assessed by measuring area of the lesions at inoculation site and also observed for effects on rind tissues of the melons.

4.2.4 Phytotoxicity due to pH adjustment of GRAS compounds

The effect of pH adjustment on phytotoxicity to the rind tissues of the GRAS compounds was tested on honeydew melons. The solutions of sodium molybdate (5830 ppm), ammonium molybdate (1234 ppm) and sodium acetate (5000 ppm) were prepared in duplicate. Each solution of the compound was either adjusted to pH 7 or left non-adjusted for pH. Five fruit for each dip treatment were scratch wounded on six points, left to air dry and four hours later were dipped in the solutions for two minutes. The treated fruit were incubated at room temperature for 48 hours and photographic record of rind staining (phytotoxicity) was taken.

4.2.5 Hot water dips of melons at different temperatures and times

Three separate experiments were conducted on rockmelon and honeydew melons to test for the effect of hot water temperature and duration of dipping, on rot development in storage. The experiments are as follows:

1. rockmelons dipped in water at 20°, 50°, 55°, 60° or 65° C for 1 min
2. rockmelons dipped in water at 20°, 60° and 65° C for 15, 30 or 60 seconds
3. honeydew melons dipped in water at 20°, 55° or 60° C for 1 or 3 min.

In the first experiment, three replications of five fruit were dipped without inoculation and incubated for 10 days at 15°C to develop diseases from natural inoculum. Disease control was assessed following a 1-5 severity scale described above (section 4.2). For the second and third experiments five fruit for each of the treatments were inoculated through eight
scratch wounds with *F. acuminatum*. Disease control was assessed by measuring the lesion area (cm$^2$) at the inoculation site after 10 days of incubation at 15°C.

### 4.2.6 Assessment of disease resistance and peroxidase activity on melons dipped in hot water

A post-dipping inoculation experiment with *Fusarium acuminatum* was conducted on both rockmelon and honeydew melon to examine the mode of action of hot water treatment by the assessment of rot development. Four replicates of four fruit per treatment were dipped at 20°C or 55°C for 1 min and allowed to stand at room temperature for 24 h. Each fruit was then inoculated with conidia through six scratch wounds. The severity of disease from inoculation was assessed after 10 days incubation at 15°C by measuring the rotted area (cm$^2$) on inoculation sites.

Peroxidase activity was examined on honeydew melons by taking three replicates of rind tissue from three fruit samples at 0, 6, 12, 24, and 48 h after they had been dipped in hot water. Samples were taken from 0.5-cm-thick rind tissues from 1.0 cm$^2$ at four points at the equator of each fruit and pooled as a replicate sample.

The activity of peroxidase was assayed following the method of Biles *et al.* (2000) with some modification. Samples (1 g for leaf FW or 2 g fruit FW) were ground in a mortar with liquid nitrogen and 1 ml of 0.1M sodium acetate buffer pH 5.0, 1M sodium chloride and 1mM EDTA was added. Extracts were centrifuged at 12,000 g at 4°C for 15 min and the supernatants transferred to microcentrifuge tubes and stored at -20°C until colorimetric assay.
Spectrophotometer readings at 470 nm were recorded 0, 1 and 2 min after 5 µl of crude extract was added to 1 ml of 10 mM guaiacol and 10 mM H₂O₂ in 50 mM sodium acetate buffer pH 5.0. Peroxidase activity was calculated from the polynomial equation of the absorbance curve for standard pure peroxidase from Sigma Chemicals Co. St Louis, USA (product number 77332). The specific activity of peroxidase was expressed as Units/mg total soluble protein.

Protein content of the crude extracts of peroxidase assay was determined by the Bradford method (Bradford, 1976) using Bio-Rad Protein assay reagent and bovine serum albumin was used as the standard.

### 4.2.7 Selected safe chemicals in hot water for the control of storage rots of melons

Hot chlorine and iodine solutions at 55° C were compared with a conventional fungicide, carbendazim (500 ppm) at room temperature for control of Fusarium rot of honeydew melons. Chlorine from sodium hypochlorite was used at 100 ppm and the iodine solution was prepared at 30 ppm. Inoculation of *F. acuminatum* was applied through six scratch wounds on each of the 15 fruit divided into three replicates. Five fruit of each replication were dipped in 15 L of solution for 1 min. Incubation and disease assessment was performed as described above (section 4.2).

The efficacy of iodine solution in controlling disease was further evaluated by dipping fruit in water or 30 ppm iodine solution at 20° C or 55° C or 60° C for 1 min. Fifteen fruit of three replicates were used per treatment, challenge inoculated with *F. acuminatum* on eight scratch wounds per fruit. Fruit for each treatment were dipped in 15 L of solution for one min. Incubation and disease assessment was performed as described earlier (section 4.2).
4.2.8 *Storability of melons dipped in hot iodine or guazatine*

The cool storage life of rockmelons was determined for fruit treated with a postharvest dip in hot iodine solution or guazatine (500 ppm) at room temperature at 22°C (± 1°C). Undipped, freshly harvested ‘Hiline’ rockmelons from the grower’s field at Griffith, New South Wales, were dipped in iodine (30 ppm) at 55°C using a commercial unit (Bioteq, Sydney, NSW), providing a continuous delivery of iodine into the dip tank (200 litres) with an automatic controller (Plate 4.2.8). For dipping in guazatine or water, a dip tank of 200 litres was used. Twenty-seven melons per treatment were dipped in three replicates of nine fruits. After dipping, treated fruit were placed in cartons and stored at 5°C with high humidity (>90%) for four weeks followed by two days at 15°C to allow rot development from natural infections. Fruit were scored for the total rots caused by the common pathogens like *Fusarium*, *Alternaria*, and *Rhizopus* spp. using a 1-5 severity scale described earlier (Section 4.2). Fruit with a rot severity above 2 in the scale is considered not marketable.

Fruit firmness was also examined with a penetrometer at the end of four weeks plus two days of storage. Fruit were cut in half longitudinally through the points between upper surface and the soil mark to test firmness. Penetrometer readings were taken on the middle of the flesh (mesocarp) at three points on each side of one of the half cut; one at the equator and two at the polar ends 3 cm from external point.
Plate 4.2.8 Iodine delivering unit used for dipping melons in iodine at room temperature or high temperature at up to 60° C (Bioteq, Sydney Australia)

4.2.9 Viability of pathogen spores in iodine solutions

An experiment was conducted to test the survival of conidia of *Fusarium*, *Alternaria*, or *Rhizopus* spp. in water or iodine at room temperature or at 55° C. Glass test tubes (150 mm by 16 mm) containing 4.5 ml of 30 ppm iodine or water were immersed for 10 min in a water bath of the respective temperature to equilibrate. Conidial suspension (0.5 ml) containing $10^7$ cfu.ml$^{-1}$ was passed through a filter membrane to collect the conidia. After equilibration of solutions, the filter with conidia was placed into the test tube and gently agitated under water for 1 min. The test tube with filter and conidia was then quickly transferred to cold water (22° C) to bring it down to room temperature, and at the same time 0.5 ml of sodium thiosulphate solution was added to both treatment solutions to neutralize the iodine solution. The test tube contents were then vortexed for 30 s to disperse the conidia from the filter membrane. The conidium suspensions (100 µl) were then put on to potato dextrose agar (PDA) in Petri plates in a dilution series, and after incubation at 25° C, the number of colonies was counted.
4.2.10 Statistical analyses

Data were analysed with Simstat software version 2.04 (Provalis Research, Montreal, Canada) for a generalized linear model (GLM) of analysis of variance using a factorial treatment design or a completely randomised design. Replicates of four or five fruit were used in all the postharvest dipping tests for the control of storage diseases. Depending on the size used in the experiments, each fruit was inoculated at six or eight sites. For the assessment of peroxidase on heat treated fruit, there were three samples (replications) from three different fruit for each of the sampling times. There were three replications of 10 fruit per treatment for the testing of safe chemical experiments for the storage of rockmelon. The protected least significant product at 5% (P<0.05) was used to test the significant differences between the treatment means. Data showing significant differences are presented and discussed, unless otherwise stated.

4.3 RESULTS

4.3.1 Efficacy of conventional fungicides for the control of storage rots of melons

The fungicides used for the dipping of rockmelon against the three major pathogens gave a significant reduction of rots compared to the control (Figure 4.3.1). Imazalil was significantly effective to completely control all three pathogens – *F. acuminatum*, *A. alternata* and *Rhizopus* spp. The benomyl treatment significantly reduced disease compared to the water treatment, but controlled 60-70% of the pathogens. Complete control of *F. acuminatum* and *A. alternata* was observed with carbendazim treatment but *Rhizopus* sp. was only controlled about 70%. Substantial (90-95%) control of *F. acuminatum* and *A. alternata* occurred from dipping in guazatine, however, complete control of *Rhizopus* spp. was observed in this treatment. Iprodione controlled *Rhizopus*
spp. and *A. alternata* completely, but only gave 70% control of *F. acuminatum*. None of the chemicals was found to have any phytotoxic effect or any adverse effect on the skin of rockmelons.

![Graph showing rot development from Rhizopus spp., F. acuminatum and A. alternata on rockmelon fruit after postharvest dipping in fungicides.](image)

**Figure 4.3.1** Rot development from *Rhizopus* spp., *F. acuminatum* and *A. alternata* on rockmelon fruit after postharvest dipping in fungicides. Fruit were inoculated with fungal spores through wounds and four hours later dipped in fungicides or water for one min. Disease severity was assessed by measuring the areas of lesions after 10 days of incubation at 15°C. All the chemicals were used at 500 ppm a.i. Different letters indicate significant difference (*P*≤0.01; *n*=5).

### 4.3.2 Safe chemicals for the control of storage rots of melons

A significant reduction of storage rots in rockmelon and honeydew caused by *F. acuminatum* occurred due to dipping treatments in GRAS compounds (Figure 4.3.2). However, the conventional fungicide, guazatine and NaOCl (chlorine 500 ppm) were the most effective treatments for control of storage rot of rockmelons. Of the remaining treatments, iodine at 30 ppm was the most effective, followed by sodium bicarbonate (20,000 ppm). Sodium molybdate (5830 ppm) or ammonium molybdate (1234 ppm)
showed moderate reduction of storage rots of rockmelons. No reduction of Fusarium rot was observed from the treatment of sodium acetate (5000 ppm).

Reductions of Fusarium rot on honeydew melons due to postharvest dipping treatments in GRAS compounds or in fungicide were more or less similar to rockmelons. However, across the treatments, reduction of rots on honeydew melons was relatively less compared to rockmelons including water dip (Figure 4.3.2). Nevertheless, guazatine (500 ppm) or iodine (30 ppm) dips reduced the storage rot of honeydew melons most, followed by chlorine (500 ppm) and ammonium molybdate (1234 ppm). Least reduction of rot occurred in treatment with sodium bicarbonate (20000 ppm), sodium acetate (5000 ppm) and sodium molybdate (5830 ppm).

![Figure 4.3.2](image)

**Figure 4.3.2** Rot development from *F. acuminatum* on rockmelon and honeydew fruit after postharvest dipping in GRAS chemicals. Melons were inoculated with *F. acuminatum* through wounds and four hours later dipped in sodium bicarbonate (Na-bicarbonate) 20000 ppm or sodium acetate (Na-acetate) 5000 ppm or ammonium molybdate (NH₄-molybdate) 1234 ppm or sodium molybdate (Na-molybdate) 5830 ppm or sodium hypochlorite (chemicals) 500 ppm or iodine 30 ppm or guazatine 500 ppm or water for 2 min. Disease severity was assessed by measuring lesions area (cm²) after 10 days of incubation at 15° C. Different letters show significant difference among the treatments (*P≤0.01; n=5*).
Melons dipped in GRAS chemicals or guazatine were also examined at the time of the scoring for general mould growth or rots growing on fruit other than at inoculation sites. Effects from dipping such as induction of ripening in storage and staining or phytotoxicity to skin also scored (Table 4.3.2). Mould growth on rockmelons was lowest in the guazatine (500 ppm) dip followed by chlorine (500 ppm) and iodine (30 ppm). The highest growth of general mould was observed on rockmelons dipped in water. There was no reduction in mould growth from dipping in sodium bicarbonate, but a moderate reduction of mould growth was observed in the dipping treatments of sodium molybdate, ammonium molybdate and sodium acetate. On honeydew melons the growth of general moulds was less than on rockmelons across all treatments including water. However, the water treatment of honeydew melons had increased growth of general mould compared to the chemical dip treatments.

Dipping melons in GRAS compounds or guazatine or water induced ripening by yellowing the rind of the fruit during incubation in storage compared to water treatment. The maximum ripening of rockmelon during storage occurred in sodium acetate, molybdate salts, chlorine and guazatine treatments (Table 4.3.2). The lowest ripening of rockmelons was observed in water or iodine or sodium bicarbonate treatments (Table 4.3.2 and Plate 4.3.2A). Similarly, ripening of honeydew melons was affected by various dip treatments (Table 4.3.2 and Plate 4.3.2B). Treatment with chlorine accelerated the ripening of honeydew melons most, followed by sodium bicarbonate and ammonium molybdate treatments respectively. Sodium acetate induced ripening of honeydew melons moderately, whereas iodine or guazatine did not induce ripening of honeydew melons compared to the water dip.
Some GRAS treatments caused staining with brown colouration on the rind resembling a phytotoxic effect (Table 4.3.2 and Plate 4.3.2B). On rockmelons there was no phytotoxic effect on the rind of fruit from dipping in GRAS chemicals (Plate 4.3.2A). On honeydew melons, sodium molybdate caused the most rind discolouration, followed by ammonium molybdate and sodium bicarbonate treatments respectively. Dipping in iodine, chlorine or guazatine did not result in any staining or phytotoxic effect on the rind tissues of honeydew melon.
Table 4.3.2 Effect of dipping rockmelons and honeydew melons in GRAS compounds and fungicide on mould growth, ripening in storage and staining on rind tissues. Mould growth, ripening and phytotoxicity were scored following a severity scale of 1-5 where, 1 = symptoms not visible; 2 = symptoms covered up to 5% of the surface; 3 = symptoms covered 11 – 25% surface; 4 = symptoms covered 26 – 50% surface and 5 = symptoms covered more than 50% of the surface area.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Rockmelon Mould growth (1-5 scales)</th>
<th>Rockmelon Ripening (1-5 scales)</th>
<th>Rockmelon Phytotoxicity (1-5 scales)</th>
<th>Honeydew Mould growth (1-5 scales)</th>
<th>Honeydew Ripening (1-5 scales)</th>
<th>Honeydew Phytotoxicity (1-5 scales)</th>
</tr>
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<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td>3.8 de</td>
<td>2.8 a</td>
<td>1.2</td>
<td>1.4 a</td>
<td>3.8 bc</td>
<td>2.2 b</td>
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<tr>
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<td>3.8 c</td>
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<td>1.2 a</td>
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<tr>
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<td>1.2</td>
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<td>1.0</td>
<td>1.0 a</td>
<td>4.4 c</td>
<td>1.2 a</td>
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<tr>
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<td>2.6 ab</td>
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<td>1.0 a</td>
</tr>
<tr>
<td>Guazatine</td>
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<td>3.4 bc</td>
<td>1.0</td>
<td>1.0 a</td>
<td>2.6 a</td>
<td>1.0 a</td>
</tr>
<tr>
<td>Water</td>
<td>4.2 e</td>
<td>2.2 a</td>
<td>1.0</td>
<td>2.2 b</td>
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**Level of significance**

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<th>NS</th>
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<td>0.908</td>
<td>0.798</td>
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</table>

NS = not significant

Means followed by same letter in a column are not significantly different by LSD (P < 0.05, n=5).
Plate 4.3.2A Disease developed on rockmelons treated with NaHCO$_3$ 2% (A) or CH$_3$COONa 0.5% (B) or (NH$_4$)$_2$MoO$_4$ 1 mM (C) or Na$_2$MoO$_4$ 24 mM (D) or NaOCl 500 ppm (E) or iodine 30 ppm (F) or guazatine 500 ppm (G) or water (H). Fruit were inoculated with *F. acuminatum* spores and dipped into solutions four hours later. Fruit were incubated at 15° C for 10 days after dipping and then measured for rot size at the inoculation site after removing the upper skin for clear visualization of rotten parts.
Plate 4.3.2B  Disease developed on honeydew melons treated with NaHCO$_3$ 2% (A) or CH$_3$COONa 0.5% (B) or (NH$_4$)$_2$MoO$_4$ 1 mM (C) or Na$_2$MoO$_4$ 24 mM (D) or NaOCl 500 ppm (E) or iodine 30 ppm (F) or guazatine 500 ppm (G) or water (H). Fruit were inoculated with *F. acuminatum* spores and dipped into solutions four hours later. Fruit were incubated at 15° C for 10 days after dipping and then measured for rot size at the inoculation sites.
4.3.3  *Comparison of hot GRAS treatments for the control of storage rots of melons*

GRAS compounds that showed significant reduction of Fusarium rot of melons at room temperature were further examined in combination with hot water. For all GRAS treatments, dipping in 48°C solutions significantly reduced the storage rot of both rockmelon and honeydew melon caused by *F. acuminatum* compared with 20°C (Figure 4.3.3). On rockmelons, the greatest reduction of rot from application of GRAS compound was observed in the sodium molybdate dip at 48°C, followed by iodine and ammonium molybdate respectively at 48°C. In contrast, on honeydew melons, the greatest rot reduction from GRAS compounds occurred in the iodine and sodium molybdate followed by ammonium molybdate at 48°C. However, sodium and ammonium molybdate again caused rind discoloration as observed in the previous experiment (Plate 4.3.3A and 4.3.3B).

![Graph showing the effect of GRAS compounds on melon rot](image)

**Figure 4.3.3** Effect of GRAS compounds at room temperature (20°C) or in hot water (48°C) for the control of Fusarium rot of melons. Melons were inoculated with *F. acuminatum* through wounds and four hours later dipped in ammonium molybdate 1234 ppm (NH4-mol.) at 20°C or 48°C or sodium molybdate 5830 ppm (Na-mol.) at 20°C or 48°C or iodine 30 ppm at 20°C or 48°C or water at 20°C or 48°C for 2 min. Disease severity was assessed by measuring the areas of lesions after 10 days of incubation at 15°C. Different letters show significant difference among the treatments (*P*≤0.01; *n*=5).
Plate 4.3.3A  Disease developed on rockmelons treated with (NH₄)₂MoO₄ 1 mM at 20° C (A) or (NH₄)₂MoO₄ 1 mM at 48° C (B) or Na₂MoO₄ 24 mM at 20° C (C) or Na₂MoO₄ 24 mM at 48° C (D) or iodine 30 ppm at 20° C (E) or iodine 30 ppm at 48° C (F) or water at 48° C (G) or water at 20° C (H). Fruit were inoculated with *F. acuminatum* spores and dipped four hours later. Fruit were incubated at 15° C for 10 days after dipping and then measured for rot size at the inoculation site after removing the upper skin for clear visualization of rotten parts.
Plate 4.3.3B  Disease developed on honeydew melons treated with (NH$_4$)$_2$MoO$_4$ 1 mM at 20° C (A) or (NH$_4$)$_2$MoO$_4$ 1 mM at 48° C (B) or Na$_2$MoO$_4$ 24 mM at 20° C (C) or Na$_2$MoO$_4$ 24 mM at 48° C (D) or iodine 30 ppm at 20° C (E) or iodine 30 ppm at 48° C (F) or water at 20° C (G) or water at 48° C (H). Fruit were inoculated with *F. acuminatum* spores and dipped four hours later. Fruit were incubated at 15°C for 10 days after dipping and then measured for rot size at the inoculation site.
4.3.4  *Phytotoxicity due to pH adjustment of GRAS compounds*

Adjustment of pH to neutral (pH 7) of the GRAS solutions for the treatment of honeydew melon, did not reduce the phytotoxicity of the compounds to the rind tissues (Plate 4.3.4). Both pH-adjusted and non-adjusted solutions of sodium molybdate resulted in severe phytotoxicity after 48 hours of treatment and incubation at room temperature. Moderate phytotoxicity was observed from ammonium molybdate dipping while low phytotoxicity developed in the sodium acetate dipped fruit.

4.3.5  *Hot water dips of melons at different temperatures*

*Experiment 1*: Rot development on rockmelons from natural inoculum was significantly reduced by dipping in hot water at 50°C and above, compared to dipping fruit at 20°C (Figure 4.3.5). As the temperature increased from 50°C to 60°C, control of rot increased. At 65°C dipping temperature, no further advantage in disease control was observed. However, despite good disease control at higher temperatures, dipping rockmelons in water at 60°C or 65°C caused pitting damage to the fruit (Plate 4.3.5). Ripening induced by hot water treatment was less on fruit dipped in hot water at or above 55°C water dip than at 22°C (Plate 4.3.5).
Plate 4.3.4 Phytotoxicity effects on honeydew melons dipped in GRAS compounds sodium molydate at a concentration (A), ammonium molybdate and (B) sodium acetate (C). Melons were dipped for 2 min and then incubated at room temperature for 48 hours before being photographed.

(A) Honeydew melons dipped in sodium molydate at a concentration of 5830 ppm with natural pH (8.4) (left) or adjusted pH (7.0) (right).

(B) Honeydew melons dipped in ammonium molydate at a concentration of 1234 ppm with natural pH (4.9) (left) or adjusted pH (7.0) (right).

(C) Honeydew melons dipped in sodium acetate at a concentration of 5000 ppm with natural pH (8.5) (left) or adjusted pH (7.0) (right).
Figure 4.3.5 Effect of hot water dipping temperature for rockmelons against storage rots. Freshly harvested melons were dipped in water at different temperature for one min. After dipping, fruit were incubated for 10 days at 15°C to develop rots from natural inoculum. Disease development was assessed using the severity scale of 1-5. Different letters show significant difference among the treatments (P≤0.01; n=3).
Plate 4.3.5 Freshly harvested melons were dipped in water at different temperatures for one min. Treated fruit were incubated at 15° C for 10 days for the development of rots from natural infections. Disease development was scored by using a severity scale of 1-5.
4.3.6 Effect of temperature and duration of water dip for the control of storage rots of melon

Experiment 2: Storage rot of rockmelons caused by *F. acuminatum* was reduced due to hot water treatments at 60°C or 65°C. As the dipping time increased from 15 sec to 60 sec, a greater degree of disease control was observed for each hot water treatment (Figure 4.3.6.1). The greatest reduction of rot in rockmelons occurred at 65°C or 60°C when dipped for 60 sec or a 30 sec dip at 65°C. Similarly a 15 sec dip at 65°C resulted in the same level of control as the 60°C treatment with 30 sec dip. In this experiment, there was a little rind damage to the fruit due to hot water dip at 65°C for 60 sec (no data recorded).

![Figure 4.3.6.1](image)

**Figure 4.3.6.1** Effect of hot water temperature and dipping duration on the Fusarium rot of rockmelons. Melons were inoculated with *F. acuminatum* through wounds and four hours later dipped in water at 20°C or 60°C or 65°C for 15 or 30 or 60 sec. Disease severity was assessed by measuring the areas of lesions after 10 days of incubation at 15°C. Different letters show significant difference among the treatments (*P*≤0.01; n=5).

Experiment 3: Storage rot of honeydew melons caused by *F. acuminatum* was reduced by hot water dips of 55°C and 60°C (Figure 4.3.6.2). Similar to disease control observed in rockmelon, a further reduction was observed in disease of honeydews, when the dipping
time was increased from one min to three minutes for these temperatures. The results show that dipping honeydew melons at 60°C for three min can completely control Fusarium rot. In this experiment, hot water dip of honeydew melons was not phytotoxic either at the higher temperature of 60°C or at longer dip time of three min. However, dipping at 60°C for three min slightly accelerated the ripening of melons during incubation (data not presented).

![Figure 4.3.6.2](image)

**Figure 4.3.6.2** Effect of hot water treatment at different temperatures and dipping time on the Fusarium rot of honeydew melons. Melons were inoculated with *F. acuminatum* through wounds and four hours later dipped at 55°C or at 60°C for either 1 min or 3 min. Disease severity was assessed by measuring the areas of lesions after 10 days of incubation at 15°C. Different letters show significant difference among the treatments (*P*≤0.01; *n*=9).
4.3.7  Effect of hot water on disease resistance mechanisms

Hot water dipping of *F. acuminatum* inoculated melons at 55° C for one min reduced storage rot. The reduction of rot on rockmelon from hot water treatment was 25%, whereas on honeydew melon the reduction was 21% compared with the 20° C dip (Figure 4.3.7.1).

![Figure 4.3.7.1](image)

**Figure 4.3.7.1** Rot development on rockmelon and honeydew melons dipped in water at 20° C or 55° C for 1 min. Melons were inoculated with *F. acuminatum* through the scratch wounds 24 hours after dipping. Inoculated fruit were stored at 15° C for 10 days before measuring the lesion areas for rot severity. Different letters show significant difference among the treatments (*P*≤0.01; *n*=12).

Assessment of peroxidase activity indicated an increased activity of the enzyme within 6 h after hot water dipping (Figure 4.3.7.2). A greater increase in peroxidase activity was measured 12 h after heat treatment, reaching a maximum at 24 h. The activity of peroxidase remained stable up to 48 h after heat treatment. However, peroxidase activity was not analysed beyond 48 hours after hot water treatment.
Figure 4.3.7.2 Peroxidase activities in honeydew melons after dipping in water at room temperature (20° C) or at 55° C for 1 min. Samples for enzyme analysis were taken at 0 hour through to 48 hours after treatment. Rind and flesh samples were taken from 4 different points at the equator of each fruit and pooled as a replicate for assay. Data are the mean values of two separate assays of three replicates per treatment. Vertical bars represent SE (standard error) between treatments at each time point (n=6)

4.3.8 Combination of hot water and safe chemicals

Experiment 1: Dipping treatments of hot iodine and hot chlorine (30 ppm and 100 ppm respectively) and carbendazim 500 ppm at 20° C significantly reduced the storage rots of *F. acuminatum* inoculated honeydew melons compared to 20° C water dips. The effectiveness of disease control was similar in the treatments with 55° C iodine or fungicide carbendazim at room temperature (Figure 4.3.8.1). A significant reduction of Fusarium rot also occurred with 55° C chlorine dip but much less than hot iodine or carbendazim. Similarly, dipping honeydew melons in iodine 30 ppm at room temperature (20° C) gave better control of rot than the fruit dipped in chlorine 100 ppm at same temperature.
Figure 4.3.8.1 Effect of iodine 30 ppm or chlorine 100 ppm at 55\(^\circ\)C for the control of Fusarium rot of honeydew melons in comparison with fungicide carbendazim 500 ppm at 20\(^\circ\)C. Melons were inoculated with *F. acuminatum* through scratch wounds and 4 hours later were dipped for 1 min. Disease development was assessed by measuring the rotted areas at inoculation sites after 10 days of incubation at 15\(^\circ\)C. Different letters show significant difference among the treatments (*P*≤0.01; *n*=9).

*Experiment 2:* Dipping honeydew melons for 1 min in 30 ppm iodine heated to 55\(^\circ\)C or 60\(^\circ\)C reduced the development of rot (Figure 4.3.8.2). A 1 min dip in hot water at 60\(^\circ\)C controlled the rots to a substantial level, as did iodine at 55\(^\circ\)C. However, adequate disease control was obtained with iodine at the lower temperature, and was comparable to hot water at 60\(^\circ\)C.
Figure 4.3.8.2 Effect of water and iodine 30 ppm at different temperatures for the control of Fusarium rot on honeydew melons. Melons were inoculated with *F. acuminatum* through scratch wounds and 4 hours later were dipped for 1 min. Disease development was assessed by measuring the rotted areas at inoculation sites after 10 days of incubation at 15° C. Different letters show significant difference among the treatments (*P*<0.05; *n*=9).

4.3.9 *Storability of rockmelons after dipping in safe chemical in hot water*

Postharvest dipping of fruit with 30 ppm of hot iodine at 55° C or 500 ppm of guazatine at room temperature substantially reduced the development of storage rots from natural infection of major pathogens (Figure 4.3.9.1). Furthermore, fruit treated with the hot iodine and guazatine dips retained flesh firmness (penetrometer measurement) after 4 weeks of cold storage (Figure 4.3.9.2). Flesh firmness measurements were higher on fruit treated with iodine at 55° C (10.22%) or guazatine (9.67%) compared to the water dip at 22° C. Dipping in hot iodine kept fruit fresher with less rots (Plate 4.3.9) than guazatine dip, indicating a better marketability (data not presented).
Figure 4.3.9.1 Effect of postharvest dip (1 min) treatment on the storage rots developed on rockmelons. Fruit were dipped in water at room temperature (RT) or hot iodine 30 ppm (55° C) or guazatine 500 ppm at room temperature. After postharvest treatment melons were stored 4 weeks at 5° C followed by 2 days at 15° C. Disease development was examined by using a severity scale of 1-5. Different letters show significant difference among the treatments (P<0.05; n=3).

Figure 4.3.9.2 Fruit firmness on rockmelons dipped in water or hot iodine or guazatine, after four weeks of storage at 5° C followed by 2 days at 15° C. Mean firmness was measured by penetrometer at three points of the middle of the flesh, one at the equator and two in between equator and polar ends. Different letters show significant difference among the treatments (P<0.05; n=3).
Plate 4.3.9 Development of rots on rockmelons during 4 week of storage at 5° C plus two days at 15° C. Before storage fruit were dipped in (A) water at room temperature or (B) 500 ppm of guazatine at room temperature or (C) 30 ppm of hot iodine at 55° C. Rot developed in storage from natural infections.
4.3.10 Efficacy of hot iodine in killing pathogenic fungal spores

The viability of conidia of *F. acuminatum*, *A. alternata* and *Rhizopus* spp. was significantly affected by hot water at 55° C or iodine at room temperature or hot iodine at 55° C (Figure 4.3.10). The efficacy in killing spores was greater for hot iodine compared with hot water or iodine at room temperature. Hot iodine at 55° C was best for killing the spores of the pathogens followed by iodine at room temperature. The least number of spores were killed with the hot water treatment at 55° C.

![Figure 4.3.10](image)

**Figure 4.3.10** Mean reduction relative to treatment in water at room temperature at 22° C in number of viable conidia of *F. acuminatum*, *A. alternata* and *Rhizopus* spp. as measured by colonies counted after treatment in hot water (water 55° C), iodine 30 ppm at room temperature (Iodine RT), or hot iodine 30 ppm at 55° C (Iodine 55° C). Different letters show significant difference among the treatments (*P*<0.05; n=4).
4.4 DISCUSSION

Fungicide applications are the principal management practice to control fungal pathogens throughout the world (McGrath, 1996). The current practice of control of storage rots of melons is through dipping harvested fruit in fungicides. There are a number of chemical fungicides recommended and used by growers as postharvest treatment, however, no single chemical has a sufficiently wide spectrum of activity to control all postharvest diseases of rockmelons (Morris and Wade, 1983).

Results of our experiments using a range of recommended fungicides confirms that postharvest diseases of melons caused by the major pathogens are not controlled uniformly by all fungicides. Imazalil was found to be very effective against all the major pathogens of rockmelons *F. acuminatum, A. alternata* and *Rhizopus* spp. However, other fungicides were found to have selective control against the pathogens. For example, carbendazim, guazatine and iprodione were effective against some of the pathogens and had reduced control for others. The study suggests that other than imazalil, none of the fungicides can completely control the storage rots of melons caused by the major pathogens. A trial by Wade and Morris (1982), conducted some time ago, found that several of the postharvest pathogens of melons cannot be controlled by any one of the commercial fungicides. Morris and Wade (1983) found that a mixture of benomyl and guazatine gave the most effective control of all postharvest diseases of rockmelons compared with treatment with a single fungicide. Interestingly, in this study, benomyl gave poor control of storage diseases of rockmelons, but was found to be effective by Morris and Wade (1983). The reason for the ineffectiveness of benomyl in this trial may be because of fungal resistance against the fungicide.
Although fungicides are effective, there is still a concern about environmental hazards and residual effects on human health. Given the use of synthetic chemicals for the postharvest treatment of fruit and vegetables throughout the world, residues may well represent a major threat to human health, with unpredictable consequences for the economy and the environment (Schirra et al., 2000). The effective doses of fungicides could be minimised to a quarter or less with respect to conventional treatment by using them in combination with hot water on apples or grapefruits (Sharma and Kaul, 1990; McDonald et al., 1991). However, it has been reported that thiabendazole in hot water leaves similar or even higher levels of residues on blood oranges, than dips in normal temperatures of water (Schirra, et al., 1998). Consequently, our research has been focused on finding a safe alternative method for the postharvest treatment of melons other than the use of fungicides.

In this research aimed at finding alternatives to fungicides, melons were treated with chemicals generally regarded as safe (GRAS) to control storage rots caused by common pathogens. The GRAS chemicals were compared for effectiveness against the commercially recommended guazatine. The results indicate that dipping melons in any of GRAS compounds did not control Fusarium rot as effectively as guazatine when used at room temperature. Previous reports using sodium bicarbonate, sodium and ammonium molybdate and sodium acetate for the control of postharvest diseases of several commodities are variable, with some diseases being controlled very well and others not so well (Aharoni et al., 1997; Palou et al., 2002). For example, a substantial reduction of blue mould incidence on oranges was reported after a postharvest dip of 2-4% of sodium bicarbonate solution for 150 sec at room temperature (Palou et al., 2001). However, sodium bicarbonate was observed to control naturally inoculated citrus fruit more effectively than artificially inoculated fruit (Smilanick et al., 1997), and the effect was found to be primarily fungistatic and not very persistent.
Of the GRAS compounds tested, a postharvest dip of iodine at room temperature significantly reduced rots, however not to the level achieved with the fungicide guazatine or chlorine treatments. Although our results suggest better disease control using chlorine, than iodine, chlorine is not desirable because high organic load results in production of different complex compounds which can become significant environmental hazards. Iodine, on the other hand, has no environmental or health risk and gave moderate control of storage rots. Even with a high organic load, efficacy of iodine has been reported to be greater than chlorine against a number of organisms in water (Koponen et al., 1993).

The ability of some of the GRAS compounds such as sodium molybdate and ammonium molybdate to reduce mould growth from natural infections indicates their wide spectrum efficacy against pathogens. However, the effectiveness of sodium molybdate, ammonium molybdate and sodium acetate to control storage diseases of melons has been overshadowed by staining of the rind and/or inducing ripening in storage. The phytotoxicity of molybdate salts causing staining on citrus fruit has also been reported earlier by Palou et al., (2002). Phytotoxicity evident at low concentrations of sodium bicarbonate indicates that a higher concentration of the compound would not be safe for the treatment of melons and indeed, phytotoxicity of sodium bicarbonate at three percent on melons has previously been reported by Aharoni et al., (1997). Postharvest dips of iodine did not cause staining of the rind in either honeydews or rockmelons. Coupled with its ability to reduce mould growth on both honeydew and rockmelons, such a performance by iodine further indicates its efficacy as a sanitizer for the control of superficial spores of fungal pathogens.

The induction of ripening on rockmelons and honeydew melons from the treatment of sodium molybdate, ammonium molybdate, sodium acetate and chlorine further questions
the use of these GRAS compounds for the postharvest treatment of melons. However, dissimilarities in ripening occurred between honeydew or rockmelons for the various dipping treatments, indicating the different composition in rind tissues may have had an effect (Simandjuntak et al., 1996). Further research on treatment of melons with sodium bicarbonate, sodium acetate, sodium molybdate, ammonium molybdate, chlorine is needed to prevent treatment related ripening of both types of melon. However, no effect on ripening was observed due to iodine treatment on either variety of melon.

Enhancement of the efficacy of GRAS compounds was investigated by combining them with hot water (Ellis et al., 1993; Marquenie et al., 2002; Palou et al., 2001; Palou et al., 2002; Smilanick and Sorenson, 2001). The results show that greater control of Fusarium rot on melons was achieved by dipping in heated solutions of the selected GRAS compounds at 48° C. This result supports the findings of Marquenie et al., (2002) who suggest that the use of GRAS compounds with hot water can increase their efficacy against the pathogens of storage rots. Others found that the molybdate salts of sodium and ammonium in hot water at 48° or 50° C gave satisfactory control of green and blue mould of lemon and oranges (Palou et al., 2002). Although hot water dip of both iodine and sodium molybdate gave significant reduction of Fusarium rot of melons the phytotoxicity of sodium molybdate on honeydew melons made it unsuitable for postharvest treatment. Hence, further trials were not performed with this compound. Dipping melons in iodine in combination with hot water increased its efficacy dramatically and had the added advantage of not having any adverse effect on the fruit. As a common sanitizer iodine was found to increase its effectiveness when used in hot water for postharvest dipping of fruit and vegetables (Morris and Bokshi, SPL internal reports, 2002).
Evaluation of hot water treatment alone without added chemicals was conducted to assess the control of storage rots and also to find the tolerable temperature level to maintain fruit quality. Postharvest dipping of melons in hot water 55° C - 60° C was found to be very effective, and increased with higher temperatures. The greater reduction of rots with the increased level of temperature, suggests that the hot water may have affected spore viability of the melon’s storage pathogens. Rot reduction following hot water treatment has previously been explained as a reduction of spore survival of various decay causing pathogens (Williams et al., 1994). In this study hot water below 55° C was not suitable in the control of storage rots caused by F. acuminatum. This is in line with earlier studies where hot water dips at 50°-53° C were shown to be ineffective in killing dormant spores of storage rots (Barkai-Golan and Phillips, 1991; Dettori et al., 1996).

Damage on the rind tissues due to dipping rockmelons at or above 60° C suggests the possibilities of injuries from hot water treatment. This is in contrast to Mayberry and Hartz (1991) who reported that dipping muskmelon at 60° C for three min did not damage the rind, but in agreement with Teitel et al., (1991) who showed that ‘Galia’ melons were damaged when dipped in hot water at 60° C even for a short period of time of 30 sec. The differences on the effect of hot water dipping of melons for the control of storage rots even within the same species may exist largely because of variations in growing areas (Mayberry and Hartz, 1991). They suggested that a crop grown in warmer climatic conditions develops greater resistance in response to hot water treatment than a crop grown under cooler climatic conditions.

Hot water treatment of melons was shown to delay ripening in storage. This may be due to the hot water treatment attenuating postharvest senescence and in some way causing inhibition or interruption of some of the activities of the ripening processes. Paull and
Chen (2000) suggested that heat treatment to climacteric fruit can cause an alteration of gene expression which inhibits or accelerates the postharvest ripening process. Paull (1990) earlier reported delay in ripening and increase of storage life of fruit and vegetables when treated in hot water. Delay in ripening due to heat treatment has been attributed to a delay in the degradation rate of mRNA which can maintain resistance against decay pathogens (Lurie et al., 1997).

The effectiveness of hot water was dependent on the temperature and dipping time of the fruit. Results of this study suggest that dipping melons in hotter water requires less duration of treatment for the control of storage rot pathogens. Hence, a one min dip in 60°C water was found to be as effective as a 30 sec dip in 65°C water. A similar report was made by Mayberry and Hartz (1991), who stated that a shorter exposure treatment was less effective and required higher water temperature (>60°C) for effective control of storage diseases of melons. But dipping melons at 60°C or over may cause damage to the skin even for a shorter dipping time of 30 sec (Teitel et al., 1991). Therefore, to maintain fruit quality, it is suggested to dip melons in the lower temperature of 55°C (Halloran et al., 1999).

In this study a three min dip at 55°C or a one min dip at 60°C almost completely controlled the storage pathogens of melons. The result suggests that hot water dip for a longer duration at a lower temperature of 55°C can compensate for dipping at a higher temperature that exposes fruit to possible heat injury at 60°C. Taitel et al., (1991) suggested a hot water dip at 55°C for 1-2 min as optimum for a postharvest anti-fungal treatment for ‘Galia’ melons. A similar report by Barkai-Golan et al. (1994) also found that hot water treatment of ‘Galia’ melons at 52°-55° C can effectively prevent storage rots caused by a number of postharvest pathogens. Furthermore, it revealed that longer
treatment time in hot water at a lower temperature does not affect the quality and ripening of melons (Teitel et al., 1989).

Reduction of storage rots in melons challenged with inoculum 24 hours after hot water treatment suggests an induction of resistance in fruit due to heat shock. This was shown by an increase in peroxidase activity in the rind. Our results confirm the work of Madi and Katan, (1998) and Reuveni et al., (1990) who showed that an increase in resistance was evidenced by increased activity of peroxidase, denoting a heightened state of resistance in the plant tissues. However, heat treatment has been reported to reduce the development of storage diseases both by inhibiting the fungal penetration to the host tissue as well as developing resistance by the formation of physical and chemical barriers against fungal growth (Ben-Yehoshua, 2003; Schirra et al., 2000).

Although hot water treatment appears to be a practical alternative to fungicidal treatments, it is reported to be of limited value, unless applied in combination with fungicides (Carter, 1981). Previously it has been found that hot water treatment alone would not provide acceptable decay control in a commercial packaging situation (Mayberry and Hartz, 1992). Furthermore, although there are a number of positive responses from heat treatment on commodities, there are also possible dangers from hot water treatment such as tissue damage (Shellie et al., 1993) and accelerated ripening (Lurie, 1998). Therefore, it is important to maintain a safe level of temperature for a useful postharvest hot water treatment of melons. This could be achieved by combining the GRAS compound(s) with hot water, and in the earlier experiments, iodine has been shown to be a real candidate for the purpose.
The efficacy of iodine was significantly increased when used in heated water up to 60°C for the control of storage disease of melons. Results from this study suggest that a one min dipping of melon in 30 ppm of iodine solution at 55°C can give equivalent control of storage rots of melons to that achieved by conventional fungicide. Similarly a hot water dip for 1 min at 60°C or a 3 min dip at 55°C heated water can give equivalent control to that achieved by using a fungicide. Further, the effect of heated iodine at 30 ppm is superior to heated chlorine at 100 ppm. A greater extent of iodine activity in an increased temperature has also been shown by Ellis et al., (1993) and Wyss and Strandskou (1945). The real benefit of combining iodine with hot water for the control of storage decay is that it reduces the risk of phytotoxicity by enabling a much lower temperature and shorter duration of dipping.

Finally, hot iodine at 55°C as a postharvest dip demonstrated equivalent control of storage rots of rockmelons during four weeks of storage at 5°C as did the conventional fungicide guazatine. Substantial control of different rots from the major pathogens as well as total rots by treatment with hot iodine reflects its wider effect against a group of melon pathogens. The wide spectrum efficacy of iodine as a general sanitizer has been described earlier (Koponen et al., 1993; Oliver et al., 1991; Morris and Bokshi, SPL internal reports, 2002).

Besides controlling the storage disease of melons, iodine in hot water was found to maintain fruit marketability with better firmness, similar to that of conventional fungicides after prolonged (4 weeks) storage. Hot water dipping of fresh produce has been reported to enhance fruit quality in storage without deterioration in firmness (Fallik, 2004). However, a longer dip in hot water may damage the firmness and reduce the quality of melons (Halloran et al., 1999). Hence, the treatment with hot iodine acts synergistically in the
maintenance of postharvest quality of melons. Furthermore, an effective and safe postharvest treatment with hot iodine dip has the benefit of a shorter dipping time with better efficacy than a fungicide and it also has no phytotoxic effects on the melons.

The efficacy of dip treatments can be attributed in part to reduced viability of the spores (Schirra et al., 2000). In this study a reduction in spore viability of the different storage pathogens was shown with iodine at room temperature as well as in hot water treatments. Although the sensitivity of pathogens like *Rhizopus* spp., *A. alternata* or *F. acuminatum* has been reported to vary with different treatments (Fallik et al., 2000), this does not always necessarily happen (Schirra et al., 2000). However, the increased efficacy of hot iodine was certainly the expression of the combined effects of both the treatments of hot water or iodine at room temperature when treated separately. Postharvest treatments of fresh produce with hot water only are reported to be about half as effective as a conventional fungicide (Olesen et al., 2004; Johnson et al., 2002) a result which could be significantly improved by using the benefit of iodine in combination.

The above results obtained by *in vitro* experiment could also help explain how the direct application of these treatments for postharvest dipping of melons works. The germicidal effects of hot water or iodine at room temperature or hot iodine reflects the fact that most of the reduction of rots occurred by the reduction of inoculum, when these treatments were applied as postharvest dips. Iodine at room temperature has not been demonstrated to be very effective against the aggressive pathogens, while hot water treatments have been reported to be ineffective in killing dormant spores (Barkai-Golan and Phillips, 1991). In a combined treatment for postharvest dip the weakness of one treatment can be compensated by the other, thereby causing substantial reduction of various inoculums of different levels of dormancy and helping to control rots during storage.
4.5 SUMMARY

Concerns about environmental hazards and human health call for urgent search for effective and suitable alternatives for postharvest treatment of melons. The GRAS compounds tested at room temperature as alternatives to fungicide demonstrated promising effects for the reduction of rots from challenge inoculation. However, difficulties with most of the GRAS compounds due to phytotoxicity on melons made them unsafe and unusable for the control of postharvest rots. The pH of most of the GRAS compounds was very high or low in the solutions and this might have affected the phytotoxicity of melons. As the adjustment of pH of the compounds did not change their effect on phytotoxicity, hence, these compounds were avoided for further trials. Of the GRAS chemicals tested iodine was found to be the most promising, due to the lack of adverse effect on melons and its ability to control diseases well.

The present results suggest that a postharvest dip of melons in hot water at 55°C causes substantial reduction of storage rots. Although the effectiveness of hot water increased with the increase in temperature there is always a risk of damage to the rind tissues of melons above 55°C. Similarly, although longer dip time results in greater disease control, there is also risk of tissue damage from hot water dip. Both the risk of tissue damage and phytotoxicity from chemicals could be minimized by the use of iodine in hot water at 55°C for the control of postharvest diseases of melons and for maintaining fruit quality during storage. Therefore, hot water treatment at or above 55°C with iodine at a concentration of 30 ppm can provide a safe alternative treatment for control of postharvest storage diseases of melons. When these new disease control treatments are combined with resistance inducing treatments applied in the field, the total benefit is likely to be at least equal and almost certainly better than any of the conventional postharvest fungicides used for melons.