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Evaluation of hot water and potassium sorbate for controlling sour rot disease of lemon fruits

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ABSTRACT

Evaluate of combined treatments between hot water and potassium sorbate for controlling sour rot disease of lemon fruits was studied. Results under in vitro trails revealed that the lethal temperatures to G. citri-aurantii were 56.0 and 58.0 ^{o}C when were exposed to temperatures for 30 and 10 seconds respectively. Meanwhile, complete reduction in linear growth of G. citri-aurantii was achieved with potassium sorbate at 15.0 g / L. The highest reduction was obtained with PS at 12.5 g / L which reduced the linear growth by 75.4%. Moreover, under in vivo trails results indicated that all tested hot water temperatures for all tested exposure times significantly reduced the sour rot incidence and severity of Lemon fruits. The most effective treatment was hot water at 60 ^{o}C for 30 seconds which reduced sour rot incidence and severity by 81.0 and 84.0 % respectively. On the other hand the highest reduction in sour rot incidence or severity was obtained with potassium sorbate at 12.5 and 15.0 g / L which reduced the disease incidence by 82.0 &82.5 and severity by 85.0 & 86.0 % respectively . Furthermore, combined treatments between hot water at temperatures of 60^{o}C for 30 seconds and potassium sorbate at 12.5 or 15.0 g /L. reduced the disease incidence by 91.0 & 92.0 and severity by 93.0 & 94.0 % respectively . Meanwhile, single treatment of hot water at temperatures of 60^{o}C for 30 seconds and potassium sorbate at 12.5 or 15.0 g /L. reduced the disease incidence and severity more than 76.0 and 78.0 % respectively. It could be suggested that hot water combined with potassium sorbate are excellent treatments for controlling sour rot disease of Lemon fruits .

Key words: Lemon fruits, sour rot disease, Hot water, Potassium sorbate, Postharvest disease

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INTRODUCTION

Significant losses can occur after the harvest during the storage and marketing of Lemon (*Citrus lemon*) fruit due to sour rot, caused by *Geotrichum citri-aurantii* Butler (syn = *Geotrichum candidum* Link [1], [2]. Applications of fungicides are used to manage postharvest diseases of citrus fruits [2] [3], [4]. It was found that chemical fungicides currently provide the primary mean for controlling postharvest decay in citrus fruit [5], [6]. However, the use of chemical fungicides for postharvest disease control in under scrutiny due to growing resistant isolates to pesticides [7], [8]. There is a growing need to develop alternative approaches for controlling postharvest diseases of citrus fruits [9].

Short-dipping period of hot water treatment is one physical method that can effectively reduce postharvest diseases on fresh fruits and vegetables [10], [11] [12], [13] [14] .. Lanza *et al*., [11] reported that hot water dip at 52°C for 180s was as effective as non heated imazalil fungicide in controlling postharvest decay of lemon fruits.

Sorbic acid and its water-soluble salts, especially potassium sorbate (PS), are the most widely used antimicrobial agents for food preservation worldwide .They are also used as preservation in animal feeds , pharmaceuticals and cosmetics [15], [16].

Using potassium sorbate (PS) for controlling postharvest diseases of tomato, apple, carrots, potato and citrus

fruits was reported by several investigators [2], [9] [17], [18], [19] [20]

.The objective of this study is to evaluate the effect of combination between hot water and potassium sorbate for controlling sour rot disease of lemon fruits .

EXPERIMETNAL SECTION

Lemon fruits

The mature Lemon fruits used in the experiment were grown in National Research center NRC orchards, and brought to the laboratory immediately after harvest. They were selected for their uniformity, size, color and shape, and for being free of damage and fungal infection.

Fungal isolate

Postharvest pathogen used in this study (*Geotrichum citri-aurantii* Butler (syn = *Geotrichum candidum* Link), was obtained from Plant Pathol. Dept., NRC , Egypt. The pathogenic isolate was maintained on potato dextrose agar medium for further study .

Hot water treatment

The digital water bath apparatus (Neslab GP-300 Series Constant Temperature Bath, Union City, CA) were used *in vitro* and *in vivo* experiments.

Preparation of Fungal inoculum

The pathogen was grown on potato dextrose agar (PDA) at 20 ^{o}C for 10 days. To make *G. citri-aurantii* inoculum, sterilized water with 0.01% Tween 80 was added to inoculated plates and rubbed gently with a glass rod. The resulting suspension was filtered through one layer of cheesecloth and the concentration was adjusted with a hemacytometer. Arthrospore suspensions of *G. citri-aurantii* were prepared and adjusted to 1x10^{8} arthrospores/ml.

In vitro experiments

Effect of different hot water temperatures and exposure times on the viability of *G. citri-aurantii*

Viability of agar disks with mycelia of *G. citri-aurantii* was carried out according to the method described by **Whiting, *et al.*** [21] . Growth agar disks of pathogenic fungus was evaluated at different temperatures and exposure times using digital hot water bath (Neslab GP-300 Series Constant Temperature Bath, Union City, CA). Screw-cap glass vials, 20 cm long and 20 mm in diameter, containing 20.0 ml sterilized water were placed in water path at different temperatures.

Disks of agar with mycelia (6- mm diameter) , were cut from the grown edge of 10 days -old cultures *G. citri-aurantii* growing on PDA medium . Agar disks were transferred to Screw-cap glass vials, 20 cm long and 20 mm in diameter, containing 20.0 ml sterilized water placed in water path at 25 , 50, 52 , 54, 56 and 58 ^{o}C , for different exposures time *i. e.* 10, 20 , 30 and 40 seconds . Treated growth agar disks were dried using sterilized filter paper and transferred into Petri-plates containing PDA medium. Five Screw-cap glass vials , and 3 disks per each were used for each treatment . Viability of mycelia from agar disk that had been subjected to previous temperatures with different exposure times was assessed by planting treated disks on PDA medium and incubated at 20 ^{o}C for 5 days . Disks that showing growth or non- growth were recorded .

Inhibitory effect of potassium sorbate on linear growth of *G. citri-aurantii*

Potassium sorbate, at different concentrations *i.e* 0.0 , 2.5, 5.0 , 7.5, 10.0, 12.5 and 15.0 g / L were tested to study their inhibitory effect on linear growth of *G. citri-aurantii* .The prepared PDA medium was dispersed into 250 ml Erlenmyer flasks and sterilized by autoclaving at 121°C for 15 min. Potassium sorbate, at different concentrations was added in to PDA medium before its solidification to obtain the final concentrations of 0.0 , 2.5, 5.0 , 7.5, 10.0, 12.5 and 15.0 g / L then mixed gently with 0.1% Tween 80 (Sigma) to enhance solubility. Each flask was disbanded in sterilized Petri- plates (9- cm diameter) before its solidification. Plates were individually inoculated with equal disks (6- mm diameter) taken from 10-days old cultures of *G. citri-aurantii* then incubated at 20 ± 2°C. Linear mycelial growth of fungus was measured, when the control plates reached full growth and the average growth diameter was calculated. Each treatment was represented by 5 plates as replicates.

In vivo experiments

Effect of hot water and potassium sorbate on sour rot disease of Lemon fruits *in vivo* Inoculation of Lemon fruits

Lemon fruits were surface-sterilized with 2% sodium hypochlorite for 2 min at room temperature, and washed with sterilized water several times . Fruits were artificially wounded using sterilized scalpel. Inoculation of wounded

fruits was carried out by spraying fruits with Arthrospore suspensions of *G. citri-aurantii* ($1x10^8$ arthrospores/ml) then air dried.

a-Hot water treatment : Inoculated fruits were dipped in hot water at temperatures, *i.e.* 25, 50, 52,54,56,58 and 60^{o}C in addition to various exposure times, *i.e.* 10, 20 and 30 seconds.

b- Potassium sorbate treatment : Meanwhile, other inoculated fruits were dipped in potassium sorbate at concentrations of 0.0, 2.5, 5.0, 7.5, 10.0,and 15.0 g/L then air dried. A set of inoculated fruits with pathogenic fungus without any treatments were left as control.

c- Testing of combined treatments between hot water and potassium sorbate on sour rot disease of lemon fruits

Hot water at temperatures of 60^{o}C for 30 seconds and potassium sorbate at 12.5 and 15.0 g /L alone or in combination were applied to study their effect on sour rot of Lemon fruits.

All treated or un-treated (control) fruits were placed into carton boxes at the rate of 20 fruits/box and stored for 15 days at 20±2^{o}C for assessment. The fruits were examined regularly to detect mould and regarded as infected if a visible lesion was observed. Results were expressed as percentage of fruit infected.

Assessment of sour rot disease :

Disease incidence (%) were expressed as percentage of fruit infected, while disease severity (%) were expressed as percentage of rotted part of fruit which was calculated from the following formula:

$$\text{Percentage of rotted part (\%)} = \frac{\text{Rotted part weight of fruit}}{\text{Fruit weight}} \text{ X } 100$$

Statistical analysis

Tukey test for multiple comparison among means was utilized [22] **.**

RESULTS

In vitro experiments

Effect of different hot water temperatures and exposure times on the viability of *G. citri-aurantii*

Viability of agar disks with mycelia of *G. citri-aurantii* was tested against different temperatures *i.e.* 25 , 50, 52 , 54, 56 and 58 ^{o}C and exposure times *i. e*, 10, 20 , 30 and 40 seconds in digital water path . Results in Table (1) reveal that when exposures times increased the lethal temperatures of hot water decreased of tested fungus. The lethal temperatures to *G. citri-aurantii* were 56.0 and 58.0 ^{o}C when were exposed to temperatures for 30 and 10 seconds respectively.

Table 1. Viability of mycelia agar disks of *Geotrichum citri-aurantii*as affected with hot water temperatures and exposure times

Hot water (C^o)	Viability of pathogenic fungus			
	Exposure time (second)			
25	10	20	30	40
50	+	+	+	+
52	+	+	+	+
54	+	+	—	—
56	+	+	—	—
58	—	—	—	—

(+) = Indicate growth and (—) = Indicate no growth

Inhibitory effect of potassium sorbate on linear growth of *G. citri-aurantii*

Potassium sorbate (PS) at different concentrations *i.e* 0.0 , 2.5, 5.0 , 7.5, 10.0, 12.5 and 15.0 g / L were tested to study their inhibitory effect on linear growth of *G. citri-aurantii* .Results in Table (2) indicate that all tested concentrations of potassium sorbate significantly reduced the linear growth of *G. citri-aurantii*. Complete reduction in linear growth was achieved with potassium sorbate at 15.0 g / L. The highest reduction was obtained with PS at 12.5 g / L which reduced the linear growth by 75.4 %. Followed by PS at 10.0 g/ L which reduced the linear growth by 63.3 %. Meanwhile, other concentrations showed moderate effect .

Table 2). Inhibitory effect of potassium sorbate (PS) on linear growth of *Geotrichum citri-aurantii in vitro*

Potassium sorbate g/ L	*Geotrichum citri-aurantii*	
	Linear growth (mm)	Reduction %
0.0	90.0 a[(1)]	0.0
2.5	65.0 b	27.8
5.0	54.4 c	39.6
7.5	41.2 d	54.2
10.0	33.0 e	63.3
12.5	22.1 f	75.4
15.0	00.0 g	100.0

Figures with the same litter are not significantly different (P =0.05)

In vivo experiments

Effect of hot water and potassium sorbate on sour rot disease of Lemon fruits

a-Effect of hot water treatment : Inoculated Lemon fruits were dipped in hot water at temperatures, *i.e.* 25, 50, 52,54,56,58 and 60^{o}C in addition to various exposure times, *i.e.* 10, 20 and 30 seconds to study their effect on sour rot disease . Results in Table (3) indicate that all tested hot water temperatures for all tested exposure times significantly reduced the sour rot incidence and severity of Lemon fruits. The most effective treatment was hot water at 60 ^{o}C for 30 seconds which reduced sour rot incidence and severity by 81.0 and 84.0 % respectively. Followed by hot water at 58 ^{o}C for 20 second which reduced sour rot incidence and severity by 69.3 and 77.6 % respectively. While other temperatures was less effective.

Table 3. Sour rot disease of lemon fruits in response to hot water temperatures and exposure times

Hot water (C^{o})	Sour rot disease					
	Exposure time (second)					
	10	20	30	10	20	30
	Disease incidence %			Disease severity		
25	100 a[(1)]	100 a	100 a	100.0 a	100.0 a	100.0 a
56	55.0 c	47.5 b	42.1 c	51.0 b	42.0 b	39.5 b
58	46.0 d	35.0 d	30.7 c	40.0 c	30.0 c	22.4 c
60	33.0 e	22.4 e	19.0 d	29.0 d	19.0 d	16.0 c

Figures with the same litter are not significantly different (P =0.05)

b- Effect of potassium sorbate treatment : inoculated fruits were dipped in potassium sorbate at concentrations of 0.0, 2.5, 5.0, 7.5, 10.0,and 15.0 g/L to study their effect on sour rot disease . Results in Table (4) indicate that all tested concentrations of potassium sorbate significantly reduced the disease incidence and severity of sour rot disease . The highest reduction either disease incidence or disease severity was obtained with potassium sorbate at 12.5 and 15.0 g / L which reduced the disease incidence by 82.0 &82.5 and severity by 85.0 & 86.0 % respectively . Followed by PS at 10.0 g/ L which reduced the disease incidence and severity by 75.5 and 79.5 % respectively . Meanwhile, other concentrations showed moderate effect .

Table 4. Sour rot disease of lemon fruits in response to different concentrations of potassium sorbate

Potassium sorbate (g/L)	Sour rot disease			
	Disease incidence		Disease severity	
	Incidence	Reduction (%)	Severity	Reduction (%)
0.0	100.0 a[(1)]	0.0	100.0 a	0.0
7.5	44.0 b	56.0	41.0 b	59.0
10.0	24.5 c	75.5	20.5 c	79.5
12.5	18.0 d	82.0	15.0 d	85.0
15.0	17.5 d	82.5	14.0 d	86.0

1- Figures with the same litter are not significantly different (P =0.05)

b- Effect of combined treatments between hot water and potassium sorbate

Hot water at temperatures of 60^{o}C for 30 seconds and potassium sorbate at 12.5 and 15.0 g /L alone or in combination were applied to study their effect on sour rot of Lemon fruits. Results in table (5) reveal that all tested treatments significantly reduced the disease incidence and severity of sour rot disease . The highest reduction either disease incidence or disease severity was obtained with combined treatments between hot water at temperatures of 60^{o}C for 30 seconds and potassium sorbate at 12.5 or 15.0 g /L. which reduced the disease incidence by 91.0 &92.0 and severity by 93.0 & 94.0 % respectively . Meanwhile, single treatment of hot water at temperatures of 60^{o}C for 30 seconds and potassium sorbate at 12.5 or 15.0 g /L. reduced the disease incidence and severity more than 76.0 and 78.0 % respectively.

Table 5. Sour rot disease of lemon fruits in response to hot water and potassium sorbate alone or in combination

Treatment	Sour rot disease			
	Disease incidence (%)		Disease severity (%)	
	Incidence	Reduction (%)	Severity	Reduction (%)
	Single treatment			
Hot water at 60°C for 30 s	24.0b[(1)]	76.0	22.0 b	78.0
Potassium sorbate at 12.5 g/L	22.5 b	77.5	18.4 b	81.6
Potassium sorbate at 15.0 g/L	21.0 b	79.0	17.5 b	82.5
	Combined treatment			
Hot water at 60°C for 30 s + Potassium sorbate at 12.5 g/ L	9.0 c	91.0	7.0 c	93.0
Hot water at 60°C for 30 s + Potassium sorbate at 15.0 g/ L	8.0 c	92.0	6.0 c	94.0
Control	100.0 a	0.0	100.0 a	0.0

Figures with the same litter are not significantly different (P =0.05) -1

DISCUSSION

Sour rot disease caused by *Geotrichum citri-aurantii* is the most important disease attacks Lemon fruits during the storage and marketing of Lemon fruits [1], [2].

Short-dipping period of hot water treatment is one physical method that can effectively reduce postharvest decay on fresh fruits and vegetables 10], [11] [12], [13] [14] . In the present study, results revealed that when exposures times increased the lethal temperatures of hot water decreased of tested fungus. The lethal temperatures to *G. citri-aurantii* were 56.0 and 58.0 °C when were exposed to temperatures for 30 and 10 seconds respectively. While under in vivo conditions, results indicated that the most effective treatment was hot water at 60 °C for 30 seconds which reduced sour rot incidence and severity by 81.0 and 84.0 % respectively. Followed by hot water at 58 °C for 20 second which reduced sour rot incidence and severity by 69.3 and 77.6 % respectively. In this respect, Lanza *et al*. (2000) reported that hot water dip at 52°C for 180s was effective as non heated imazalil fungicide in controlling postharvest diseases of lemon fruits. In addition, brushing grapefruit for 20s with 56, 59 or 62°C water reduced decay by 20, 5 or 1 %, respectively, compared to the untreated fruits [5] .. Several fruit ripening processes are affected by heat, such as color, ethylene synthesis, respiration, fruit softening and cell wall metabolism, volatile production [11]. Postharvest heat treatment also can reduce chilling injury in many kinds of fruits during subsequent low temperature storage as well as reduce pathogen level and disease infection [14]. Heat treatments may affect postharvest quality in several ways. It has a direct effect on fungal growth, it may induce antifungal substances and the wax layer may melt into wounds and stomata of treated fruits [23].

Sorbic acid and its water-soluble salts, especially potassium sorbate (PS), are the most widely used antimicrobial agents for food preservation. They are also used as preservation in animal feeds and pharmaceuticals [15], [16]. Results in the present study indicated that the highest reduction in sour rot incidence or severity was obtained with potassium sorbate at 12.5 and 15.0 g / L which reduced the disease incidence by 82.0 &82.5 and severity by 85.0 & 86.0 % respectively . Furthermore, the highest reduction in disease incidence and severity was obtained with combined treatments between hot water at temperatures of 60°C for 30 seconds and potassium sorbate at 12.5 or 15.0 g /L. which reduced the disease incidence by 91.0 &92.0 and severity by 93.0 & 94.0 % respectively .

Inhibition of pathogens by sorbic acid and its salts may be caused by alternation of cell transport function, inhibition of enzymes involved in the glycolytic pathway or tricarboxylic acid cycle by inhibition of RNA, DNA , and protein synthesis , and by uncoupling of the oxidative phosphorylation in mitochondria [15], [16].

The depletion of AT P was reported in conidia of various fungi after exposure to sorbic acid[24]**.** The very low levels of mammalian toxicity of potassium sorbate (LD 50 in the rate: 4-7 g / kg body weight, equals 500 g for an adult human) and its wide application as a food preservative would make it an excellent chemical for postharvest treatment [16] **.**Using potassium sorbate (PS) for controlling postharvest diseases of several fruits was reported by [2], [9] [17], [18], [19] [20].

It could be suggested that hot water combined with potassium sorbate are make them an excellent treatment for controlling sour rot disease of Lemon fruits .

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