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Management of Postharvest Diseases of Washington Navel Orange Using Combined Treatments between Essential oils and Chitosan

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ABSTRACT

Citrus infected by *Penicillium* ssp. and *Geotrichum ssp.* caused different symptoms on fruits in the field, packinghouse, or during handling causing postharvest diseases. *Penicillium digitatum* Sacc. and *Penicillium italicum* Wehmer caused green and blue moulds, respectively, however, *Geotrichum citri-aurantii* Link ex Persn, caused sour rot. Controlling of postharvest diseases using essential oils and/or chitosan on Washington naval orange fruits during storage period were investigated. Effect of Thyme, Nerol and chitosan against the mycelial growth and spore germination of *Penicillium digitatum*; *P. italicum* and *Geotrichum citri-aurantii in vitro* were tried. Results showed that complete suppression of mycelial growth and spore germination of all tried fungi was achieved with Thyme and Nerol at 1.5 %. In addition to chitosan at 8.0 g/L. Thyme or Nerol at concentration of 1.5 % and chitosan at 8.0 g/ L were applied alone or in combination to study their protective effect against green, blue molds and sour rot diseases of Washington navel orange fruits. Results showed that the highest inhibition was obtained with Thyme or Nerol at 1.5 % followed by chitosan at 8.0g / L which inhibited disease incidence and severity more than 88.0 and 92.0 % respectively for green, blue molds and sour rot diseases. Single treatments showed satisfactory impact. All tested treatments had no negative effect on fruit quality. The most effective treatments are combined between Thyme or Nerol at 1.5 % followed by chitosan at 8.0g / L which reduced the fruit weight loss and fruit disorders percentage as compared with untreated fruits. Meanwhile, no significant effect on total soluble solids percentage (TSS) was observed.

Keywords: Valencia orange fruits, Green mould, blue mould, sour rot, caused, postharvest diseases, Thyme, Nerol, chitosan

Introduction

Postharvest diseases affecting citrus fruits causing highly losses in developing countries and less in industrialized countries (Nunes 2011; Deepmala *et al.,* 2015). Citrus fruits infected with several diseases that causing great losses during the postharvest. The most sever diseases of citrus are the blue and green moulds caused by *Penicillium digitatum* Sacc. and *Penicillium italicum* Wehmer, respectively, while, *Geotrichum citri-aurantii* Link ex Persn caused sour rot (Zhang *et al.,* 2005). It is necessary to alternative fungicidal application with several eco-chemical for controlling postharvest disease (Villers 2014).

Essential oils showed antimicrobial activities, low toxic, low environmental effect and eco-friendly. There are several investigated showed that satisfactory impact to control postharvest disease of different fruit using essential oils (Burt, 2004; Feng and Zheng 2007; Amiri *et al.,* 2008, Liu *et al.,* 2009).

Chitosan has antimicrobial activity against several food-borne pathogens and used as a preservative in the food industries (Ganan *et al.,* 2009; Gao *et al.,* 2013). Coating fruits with chitosan

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reduced water loss and reduced respiration and suppressed the postharvest diseases (Gao *et al.,* 2013). Chitosan is polysaccharide with antifungal properties (Abd-El-Kareem *et al.,* 2016; Liang *et al.,* 2017). To improvement the antimicrobial activities of chitosan could be mixed with essential oils (Yuan *et al.,* 2016).

The goal of this study are, evaluation of essential oils and chitosan alone or in combinations to control postharvest diseases of Washington navel orange fruits during storage.

2. Materials ad Methods

2.1. Washington navel orange fruits

The mature Washington navel orange used in this study were obtained from National Research Centre station. They were selected being free of damage and fungal infection.

2.2. Fungal isolates

Three pathogenic isolates of *Penicillium digitatum* Sacc. ; *Penicillium italicum* Wehmer, and *Geotrichum citri-aurantii* Link ex Persn the causal agents of green mould ; blue mould and sour rot respectively of citrus fruits were obtained from Plant Pathol. Dept., (NRC).

2.3. Preparation of spore suspension

Spore suspension was prepared by flooding 10 days old cultures of *Penicillium digitatum*; *P. italicum* and *Geotrichum citri-aurantii* using sterilized water containing 0.1% (v/v) Tween 80. Spores were counted using a hemacytometer slid, and the spore concentrations from the pathogenic fungi were adjusted to 1×10^6 spores / ml.

2.4. Laboratory trails

2.4.1. Testing of essential oils and chitosan on mycelial growth and spore germination of pathogenic fungi

The effect of certain essential oils, such as thyme and nerol, at concentrations of 0.0, 0.5, 1.0, and 1.5 % (v/v), as well as chitosan at 0.0, 4.0, 6.0, and 8.0 g / L, on the mycelial growth of *Penicillium digitatum, P. italicum*, and *Geotrichum citri-aurantii*, was investigated at 25 2°C on potato dextrose agar (PDA). The sterilised PDA medium was spread in 100 ml portions into 250 ml Erlenmeyer flasks and autoclaved for 15 minutes at 121°C.

Individual treatments were prepared and then added to PDA medium before solidification to obtain final concentrations, which were then gently mixed with 0.1 % Tween 80 (Sigma) to improve solubility. Before solidification, each flask was divided in sterilised Petri plates (9 cm diameter). Individually plates were inoculated with equal discs (6 mm diameter) obtained from 10-day old cultures of each *Penicillium digitatum, P. italicum,* and *Geotrichum citri-aurantii,* and incubated at 25 2°C. When the control plates completed full growth, the average growth diameter was calculated and linear mycelial growth of the fungus was measured. As a replica, each treatment was represented by 5 plates. Meanwhile, the effect of same treatments against the spores germination of *Penicillium digitatum* ; *P. italicum* and *Geotrichum citri-aurantii* were tested on potato dextrose broth (PDB) at 25 ± 2°C according to Chien *et al.,* (2007).

The prepared potato dextrose broth (PDB) was dispersed in 5 ml portions into a 10 ml test tube and autoclaved at 121°C for 15 minutes to sterilise it. To obtain the final concentrations of potassium or sodium silicates were prepared as described before then added to PDB and gently mixed with 0.1 % Tween 80 (Sigma) to improve solubility. After that, each tube was injected with 1.0 ml of spore suspension containing 10^6 spores per ml. On a rotating shaker, inoculated test tubes were incubated at 25 2°C for 24 hours (200 rpm). The germination rate of germinated spores was determined microscopically. One hundred spores per replicate were used in the experiment, with five replicates for each treatment.

2.4.2. Testing of essential oils on postharvest disease of Washington navel orange fruits *in vivo*

Thyme and Nerol at different at 0.0, 0.5, 1.0 and 1.5 % were tested against green, blue molds and sour rot of Valencia orange fruits *in vivo*. Washington navel orange fruits were sterilized with 2 % sodium hypochlorite for 2 min then washed several times with sterilized water. Wounding fruits was

carried out using sterilized scalpel then dipped in Thyme or Nerol at concentrations of 0.0,0.5, 1.0 and 1.5 % and air dried. Inoculation of treated fruits was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *Penicillium digitatum*; *P. italicum* and *Geotrichum citri-aurantii* individually then air dried. Inoculated fruits were. A set of inoculated fruits with *Penicillium digitatum*; *P. italicum* and *Geotrichum citri-aurantii*, individually only were left as control. All treated or un-treated (control) fruits were placed into carton boxes at the rate of 10 fruits/box and stored for 15 days at 20±2oC and 90-95% relative humidity. The fruits were examined and expressed as percentage of fruit infected.

2.4.3. Testing of chitosan on postharvest disease of Washington navel orange fruits *in vivo*

Chitosan solution at different concentrations were applied against green, blue molds and sour rot of Valencia orange fruits *in vivo*. Washington navel orange fruits were surface-sterilized and wounded using sterilized scalpel. Wounded fruits were dipped in chitosan solution at 0.0,2.0, 4.0,6.0 and 8.0 g / L individually then air dried. Inoculation of treated fruits was carried out as mentioned before. All treated or un-treated (control) fruits were stored as mentioned before.

2.4.4. Testing of essential oils and chitosan on postharvest disease of Washington navel orange fruits *in vivo*

Thyme or Nerol at concentration of 1.5 % and chitosan at 8.0 g/L were applied alone or in combination to study their effect on green, blue molds and sour rot diseases of navel orange fruits. Fruits were surface-sterilized and wounded as mentioned before. Wounded fruits were subjected to the flowing treatments: (1) dipping in Thyme at 1.5 % 1.0 minute, (2) Dipping in Nerol for 1.0 minute (3) dipping in chitosan for 1.0 minutes (4) control (untreated fruits).

Meanwhile combined treatment was carried out by dipping fruits in Thyme or Nerol at concentration of 1.5 % followed by dipping fruits in chitosan at 8.0 g/L then are dried at room temperature. Inoculation of fruits was carried out as mentioned before. All treated or un-treated (control) were stored as mentioned before.

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

2.4.5. Testing of essential oils and chitosan on Washington navel orange quality *in vivo*

Thyme or Nerol at concentration of 1.5 % and chitosan at 8.0 g/ L were applied alone or in combination to study their effect on fruit quality *in vivo i.e.* fruit weight loss, total soluble solids (TSS) and fruit disorders. Fruits were subjected to the flowing treatments: (1) dipping in Thyme at 1.5 % for 1.0 minute, (2) Dipping in Nerol for 1.0 minute (3) dipping in chitosan for 1.0 minutes (4) control (untreated fruits). Meanwhile combined treatment was carried out by dipping fruits in Thyme or Nerol at concentration of 1.5 % followed by dipping fruits in chitosan at 8.0 g/L then are dried at room temperature.

Each treatment was about 10 kg weight put as one layer in three carton boxes (60×40×15cm). Experimental boxes were stored at 5±2˚C and 90% relative humidity for 40 days.

2.5. Effect of the tested treatments on Washington navel orange fruits were evaluated through the following determinations

2.5.1. Fruit weight loss percentage

The initial weight of Washington navel orange fruits was recorded in each treatment, then fruit weight loss% was calculated by weighing the same fruits at the end of cold storage duration using the following formula:

$$\text{Weight Loss \%} = \frac{\text{Initial weight} - \text{Weight at end experiment}}{\text{Initial weight}} \times 100$$

2.5.2. Total soluble solids percentage (TSS)

TSS% was determined in orange fruit juice using a hand refractometer according to Chen and Mellenthin (1981).

2.5.3. Fruit disorders percentage

Fruits affected with either pathological or physiological disorders were counted by visual and calculated as a percentage to the initial number of fruits per each treatment.

2.6. Statistical analysis

Tukey test for multiple comparison among means was utilized Neler *et al.,* (1984)

3. Results

***3.1. In vitro* trails**

3.1.1. Effect of essential oils on linear growth and spore germination of citrus postharvest fungi

Thyme and Nerol were examined *in vitro* against the linear mycelial growth and spore germination of *Penicillium digitatum, P. italicum,* and *Geotrichum citri-aurantii* at concentrations of 0.0, 0.5, 1.0, and 1.5 % (v/v). The results in Fig (1, 2) show that all concentrations of both essential oils significantly suppressed pathogenic fungus linear growth and spore germination. Thyme and Nerol, at a concentration of 1.5 %, completely inhibited linear growth and spore germination. With a concentration of 1.0 %, linear growth and spore germination were lowered by more than 86.7 and 91.3 %, respectively. Other concentrations, were less effective

3.1.2. Effect of chitosan on linear growth and spore germination of pathogenic fungi

In vitro, the effects of chitosan at concentrations of 0.0, 4.0, 6.0 and 8.0 g/L on the linear mycelial growth and spore germination of *Penicillium digitatum, P. italicum*, and *Geotrichum citri-aurantii* were tested. The results in Fig (1, 2) show that all concentrations tested significantly inhibited fungal linear growth and spore germination. With a dose of 8 g/L, linear growth was completely inhibited. Germination of spores was completely inhibited at 6.0 g/ L.

3.1.3. Effect of essential oils on postharvest disease of Washington navel orange fruits *in vivo*

Thyme and Nerol were evaluated *in vivo* against green, blue moulds, and sour rot of Washington navel orange fruits at varying concentrations of 0.0, 0.5, 1.0, and 1.5 %. Data in Tables (1 and 2) show that all tried concentrations of both essential oils considerably lowered the incidence and severity of all diseases studied. Thyme and Nerol, both at 1.5 %, were the most effective treatments, which reducing disease incidence ranged (77 to 82.0 %) and (74.0 to 83.0 %), respectively. Thyme and Nerol, both at 1.0 %, had a moderate effect, reducing disease incidence and severity more than 57.0 and 67.0 %, respectively.

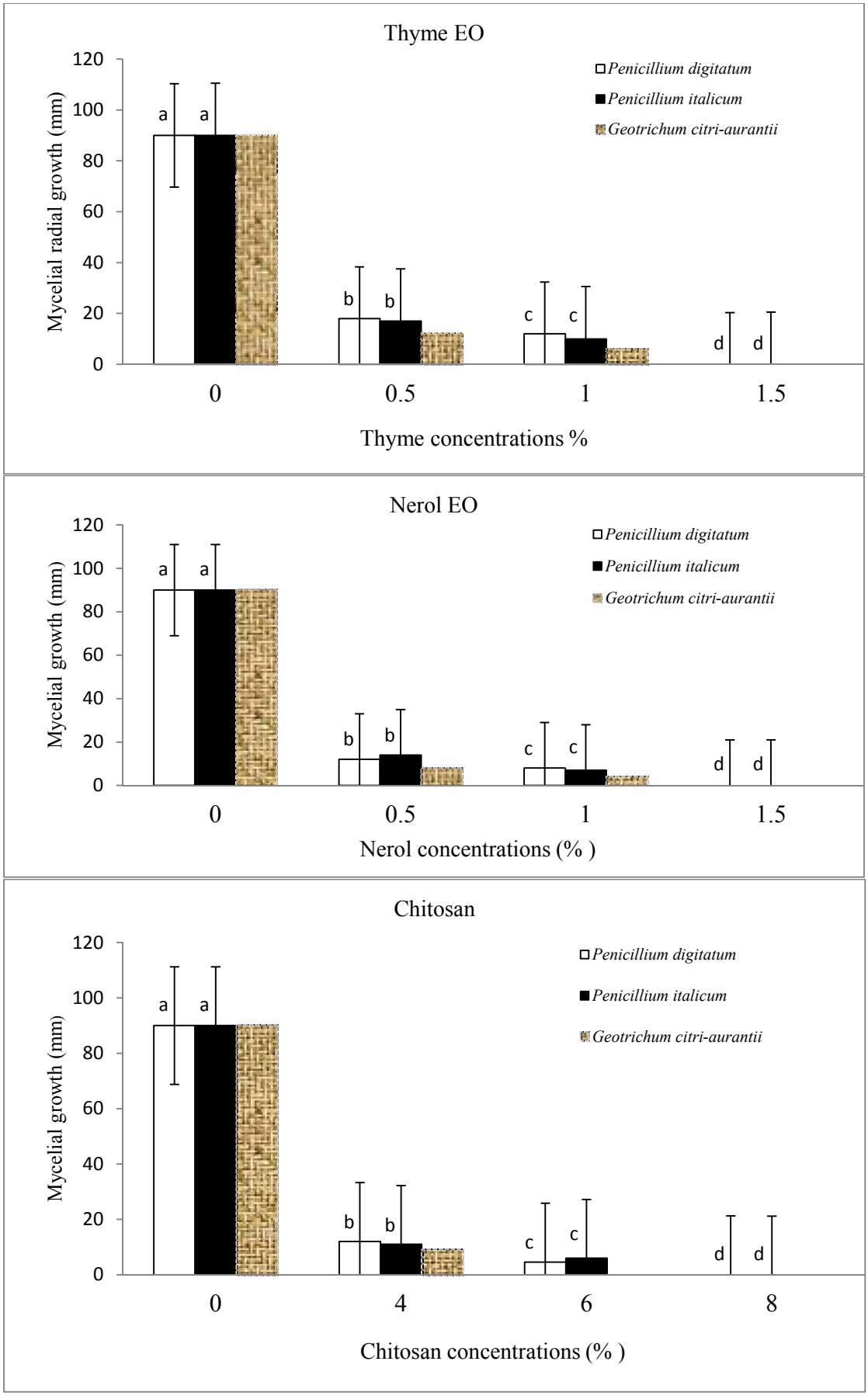

Fig. 1: Effect of different concentrations of some essential oils and chitosan on linear growth of pathogenic fungi.

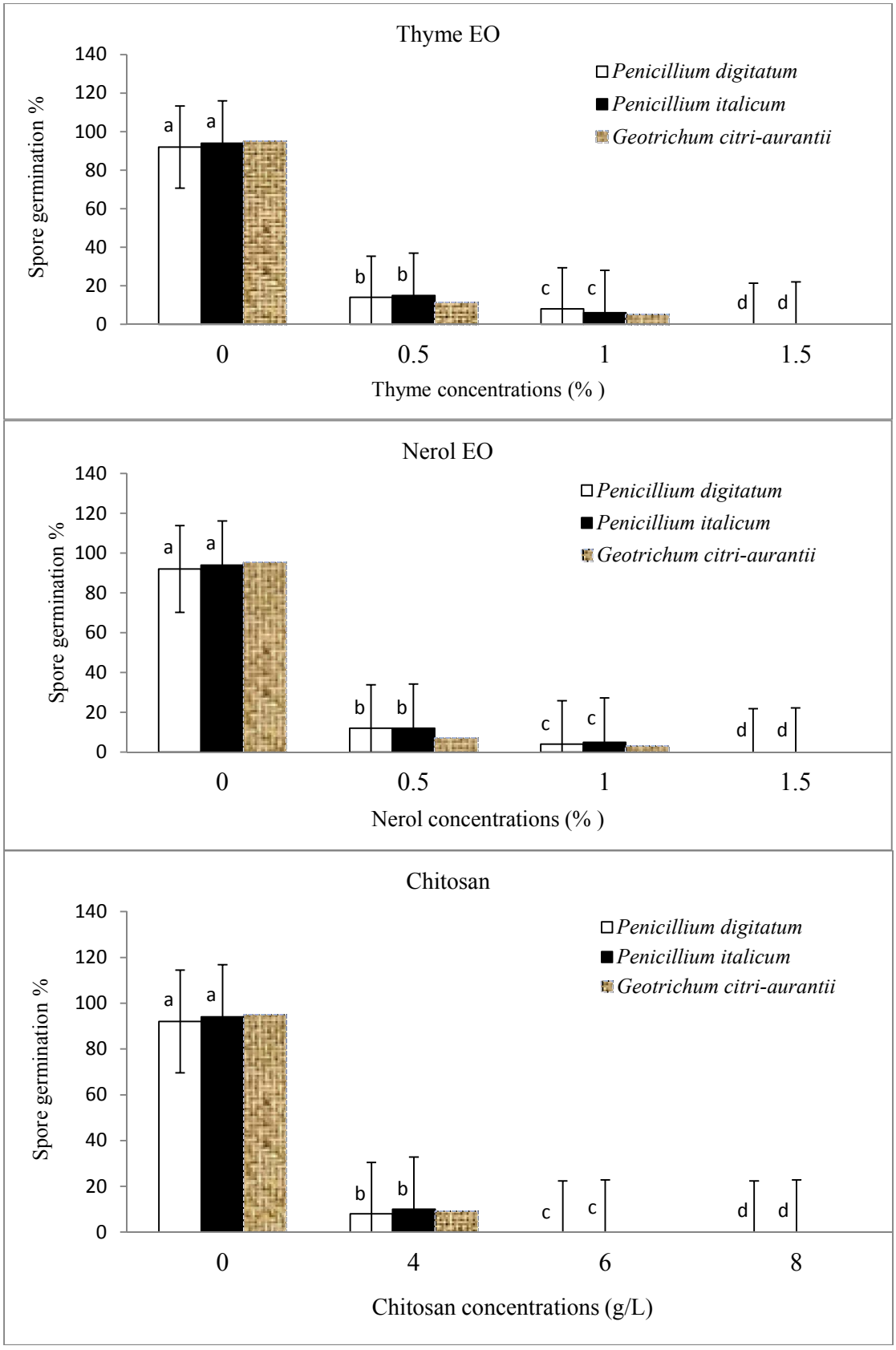

Fig. 2: Effect of different concentrations of some essential oils and chitosan on spore germination of pathogenic fungi.

Table 1: Effect of different concentrations of some essential oils on disease incidence of citrus postharvest diseases

Essential oils	Conc.	Postharvest diseases incidence					
		Green mould		Blue mould		Sour rot	
		Disease incidence	Reduction %	Disease incidence	Reduction %	Disease incidence	Reduction %
Thyme	0.5	54.0 b	46.0	62.0b	38.0	50.0 b	50.0
	1.0	32.0 c	68.0	43.0c	57.0	28.0 c	72.0
	1.5	23.0 d	77.0	28.0 d	72.0	18.0 d	82.0
Nerol	0.5	56.0 b	44.0	60.0b	40.0	48.0 b	52.0
	1.0	35.0 c	65.0	39.0 c	61.0	25.0 c	75.0
	1.5	24.0 d	76.0	26.0 d	74.0	17.0 d	83.0
Control	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a	0.0

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

Table 2: Effect of different concentrations of some essential oils on disease severity of citrus postharvest diseases

Essential oils	Conc.	Postharvest diseases severity					
		Green mould		Blue mould		Sour rot	
		Disease severity	Reduction %	Disease severity	Reduction %	Disease severity	Reduction %
Thyme	0.5	48.0 b	52.0	51.0 b	49.0	45.0 b	55.0
	1.0	30.0 c	70.0	33.0 c	67.0	22.0 c	78.0
	1.5	20.0 d	80.0	24.0 d	76.0	16.0 d	84.0
Nerol	0.5	50.0 b	50.0	53.0 b	47.0	44.0 b	56.0
	1.0	31.0 c	69.0	32.0 c	68.0	23.0 c	87.0
	1.5	22.0d	78.0	24.0 d	76.0	15.0 d	85.0
Control	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a	0.0

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

3.1.4. Effect of chitosan solution on postharvest disease of Washington navel orange fruits *in vivo*

Green, blue moulds, and sour rot of Washington navel orange fruits were examined *in vivo* with chitosan solution at varied doses of 0.0, 2.0, 4.0, 6.0, and 8.0 g/L. The results in Table (3) show that all tested chitosan concentrations considerably reduced the incidence and severity of all tested diseases. Chitosan at 8.0 g/ L was the most effective treatment, reducing disease incidence by 80.0, 83.0, 80.0 and disease severity by 83.8, 82.0, and 82.0 % for green mould, blue moulds, and sour rot, respectively. Chitosan at 6.0 g/L had a moderate effect, reducing disease incidence and severity by more than 76.0 and 78.0 %, respectively. Other concentrations were less effective.

Table 3: Effect of different concentrations of chitosan solution on citrus postharvest diseases

Treatment	Conc.	Disease incidence					
		Green mould		Blue mould		Sour rot	
		Disease incidence	Reduction %	Disease incidence	Reduction %	Disease incidence	Reduction %
Chitosan	2.0	44.0 b	56.0	48.0b	52.0	42.0 b	58.0
	4.0	31.0 c	69.0	36.0 c	64.0	29.0 c	71.0
	6.0	24.0 d	76.0	29.5 d	70.5	24.0d	76.0
	8.0	18.0 e	82.0	20.0 e	80.0	20.0 e	80.0
	0.0	100.0a	0.0	100.0a	0.0	100.0a	0.0
				Disease severity			
Chitosan	2.0	40.0 b	60.0	44.0 b	56.0	42.0 b	58.0
	4.0	28.0 c	72.0	32.5 c	67.5	28.0 c	72.0
	6.0	22.4 d	78.0	24.5 d	75.5	22.0 d	78.0
	8.0	16.2 e	83.8	18.0 e	82.0	18.0 e	82.0
	0.0	100.0a	0.0	100.0a	0.0	100.0a	0.0

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

3.1.5. Effect of combined treatments between essential oils and chitosan on postharvest disease of Washington navel orange fruits *in vivo*

Thyme or Nerol at a concentration of 1.5 % and chitosan at 8.0 g/L were applied alone or in combination to Washington navel orange fruits to see if they might protect them from green, blue moulds, and sour rot diseases. The results in Tables (4 and 5) show that all of the treatments examined, used alone or in combination, significantly reduced the incidence and severity of all of the tested diseases.

Thyme or Nerol at 1.5 % followed by chitosan at 8.0g / L reduced disease incidence and severity more than 88.0 and 92.0 % for green, blue moulds, and sour rot diseases, respectively. Single treatments showed moderate effect

Table 4: Effect of integrated treatments between thyme or nerol and chitosan on disease incidence of Washington navel orange fruits

Essential oils	Conc.	Disease incidence					
		Green mould		Blue mould		Sour rot	
		Disease incidence	Reduction %	Disease incidence	Reduction %	Disease incidence	Reduction %
Sigel treatments							
Thyme	1.5	26.0 b	73.0	30.0b	70.0	20.0b	80.0
Nerol	1.5	24.0 b	76.0	28.0b	72.0	18.0 b	82.0
Chitosan	8.0	22.0 b	78.0	20.0 c	80.0	18.0 b	82.0
Combined treatments							
Thyme + chitosan		10.0 c	90.0	10.0 d	90.0	8.0 c	92.0
Nerol + chitosan		11.0 c	89.0	11.0 d	89.0	10.0 c	90.0
Control		100.0 a	0.0	100.0a	0.0	100.0a	0.0

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

Table 5: Effect of integrated treatments between thyme or nerol and chitosan on disease severity of Washington navel orange fruits

Essential oils	Conc.	Disease severity					
		Green mould		Blue mould		Sour rot	
		Disease severity	Reduction %	Disease severity	Reduction %	Disease severity	Reduction %
Sigel treatments							
Thyme	1.5	22.0 b	78.0	24.0 b	76.0	18.0 b	82.0
Nerol	1.5	24.0 b	76.0	25.0 b	75.0	18.0 b	82.0
Chitosan	8.0	16.2 c	83.8	18.0 c	82.0	16.0 b	84.0
Combined treatments							
Thyme + chitosan		8.0 d	92.0	9.0 d	91.0	7.0	93.0
Nerol + chitosan		8.0 d	92.0	8.0 d	92.0	6.0	94.0
Control		100.0 a	0.0	100.0 a	0.0	100.0 a	0.0

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

3.1.6. Effect of combined treatments between essential oils and chitosan on Washington navel orange quality *in vivo*

Thyme or Nerol, at a concentration of 1.5 %, and chitosan, at 8.0 g/L, were used alone or in combination to investigate their effects on fruit quality, including weight loss, total soluble solids (TSS), and fruit diseases. Table (6) shows that none of the treatments examined had a no negative impact on fruit quality. When compared to untreated fruits, the most effective treatments were a combination of Thyme or Nerol at 1.5 % followed by chitosan at 8.0g/L, which greatly reduced fruit weight loss percentage and fruit disorders percentage. Meanwhile, there was no significant effect on total soluble solids percentage (TSS).

Table 6: Effect of integrated treatments between thyme or nerol and chitosan on fruit quality of Washington navel orange quality

Treatment	Washington navel orange quality %		
	Fruit weight loss	Total soluble solids (TSS)	Fruit disorders
	Sigel treatments		
Thyme	3.0 b	14.2 a	7.0 b
Nerol	3.2 b	14.1 a	8.0 b
Chitosan	1.4 c	14.2 a	6.0 c
	Combined treatments		
Thyme + chitosan	1.3 c	14.6 a	2.0 d
Nerol + chitosan	1.3 c	14.5 a	2.0 d
Control	5.5ba	14.0 a	21.0 a

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

4. Discussion

Citrus fruits are susceptible to a variety of mould diseases that cause significant quantity and quality losses as well as damage to the fruits throughout the postharvest period. The green and blue moulds caused by P. *digitatum and P. italicum*, respectively, are the most frequent and deadly diseases of citrus. Sour rot caused by *G. citri-aurantii* is next in importance.
(Palou *et al.,* 2002; Zheng *et al.,* 2005).

The extracted oils have a number of benefits, including: (1) It has an antibacterial effect. (2) A secure and healthy environment (3) Minimal toxicity (4) Biodegradable characteristics (5) Ecologically friendly (Burt, 2004, Tzortzakis and Economakis, 2007, Bosquez-Molina *et al.,* 2010).

In the present study results showed that *in vitro,* trails, complete suppression of linear growth and spore germination of all tested fungi was obtained with Thyme and Nerol at 1.5 %. While, *in vivo* Thyme and Nerol at 1.5 % significantly reduced the disease incidence and severity.

In this respect many successful research have shown that employing biological agents such as essential oils, postharvest disease control of many fruit can be achieved (Feng *et al.,* 2007; Amiri *et al.,* 2008; Liu, *et al.,* 2009).

Essential oils (EOs) are an effective control tool for reducing fruit production's environmental impact (Burt 2004 ; Bakkali *et al.,* 2008), but a most of these studies still under greenhouse condition (Lopez-Reyes *et al.,* 2010 and 2013).

The chemical structure of EOs, such as aldehydes, phenols, and ketones, determines their action on pathogens. These compounds effectively suppress pathogen growth. In addition, fungicidal compounds such as thymol, carvacrol, and b-anisaldehyde exist, and environmental materials rich in these compounds have the strongest inhibitory effect against *Penicillium digitatum*(Daferera *et al.,* 2000), *Colletotrichum gloeosporioides* (Barrera-Nacha *et al.,* 2008) and *R. stolonifera* (Spadaro *et al.,* 2014).

In general, the efficiency of EOs is confirmed through direct contact with the fruit, spraying, or dipping (Elshafie *et al.,* 2016). Furthermore, chitosan is the most flexible biopolymer, with antimicrobial action against a variety of foodborne pathogens, generating interest as a possible preservative (Ganan *et al.,* 2009).

In the present study results revealed that, complete suppression of linear growth and spore germination of all tested fungi was obtained with chitosan at 8.0 g / L while *in vivo*, trails, the highest reduction was obtained with chitosan at 8.0 g/ L which significantly reduced the disease incidence and severity. In other trails, the most effective treatments are thyme or nerol at 1.5 % followed by chitosan at 8.0g / L which reduced disease incidence and severity for all tested disease.

In this respect chitosan has been shown to have fungicidal effect against a variety of fungi and oomycetes in this regard (Vasyukova *et al.,* 2005; El-Mohamedy *et al.,* 2013). Some compounds also reduced spore growth at relatively high concentrations in other applications (Badawy *et al.,* 2005). Chitosan has recently been discovered to be able to penetrate the plasma membrane of Neurospora crassa and destroy cells. In the laboratory, chitosan has been shown to inhibit the growth of a variety of fungi and oomycetes (Palma-Guerrero *et al.,* 2008 and 2009).

Although the mechanism of chitosan's effects on the growth of many pathogenic fungi has not been fully elucidated, several hypotheses have been proposed, the first of which is that due to its polymorphic nature, chitosan interferes with the negative charge residues of large molecules exposed

on the fungal cell's surface. Electrolytes and protein components leak into the cells as a result of this process (El Hassni *et al.,* 2004).

Second, circulating hydrolysis products interact with microbial DNA, causing chitosan to bind with fungal DNA and RNA, inhibiting mRNA and protein production (Vasyukova *et al.,* 2005; Palma-Guerrero *et al.,* 2008).Third metal chelation, spore element chelation, and vital nutrient chelation (Rabea *et al.,* 2005).

Fourth, fungal mycelial malformation. Chitosan not only inhibits the growth of harmful fungus, but it also produces significant morphological, structural, and molecular disarray in fungal cells (El Ghaouth *et al.,* 2002 ; Ait Barka *et al.,* 2004).

Moreover, El Hassni *et al.,* (2004) found that chitosan caused morphological alterations in *B. cinerea* mycelium, such as big vesicles or empty cells devoid of cytoplasm. Furthermore, several applications found that by observing chitosan-treated fungi under a microscope, they can affect the morphology of hyphae (Banos *et al.,* 2006).

This decrease in fruit weight loss during storage is due to the exchange of water between the inner and outer atmosphere, in which the rate of transpiration is accelerated due to cellular breakdown (Woods 1990).Results in the present study indicated that all tested treatments had no negative effect on fruit quality. Combined between thyme or nerol at 1.5 % followed by chitosan at 8.0g / L which significantly reduced the fruit weight loss percentage and fruit disorders percentage as compared with untreated fruits. In this respect, chitosan has a broad range of applications in the food industry (Gao *et al.,* 2013). Chitosan coating creates a semi-permeable barrier that can limit water loss and disrupt the normal circulation of gases between the fruit and the outside atmosphere, reducing respiration, slowing ageing, and preventing microbial decomposition in fruits and vegetables (Gao *et al.,* 2013). Chitosan is the only naturally occurring alkaline polysaccharide with a diverse range of biological and biodegradable properties (Abd-El-Kareem *et al.,* 2016; Liang *et al.,* 2017). Some research have demonstrated that chitosan can be combined or encapsulated with EOs to improve its antibacterial action (Yuan *et al.,* 2016).

5. Conclusion

Penicillium digitatum Sacc. and *Penicillium italicum* Wehmer caused green and blue moulds, respectively, however*, Geotrichum citri-aurantii* Link ex Persn, caused sour rot. Disease of citrus fruits. Complete inhibition of linear growth and spore germination of all tested fungi was obtained with Thyme and Nerol at 1.5 %. In addition to chitosan at 8.0 g/L.

In vivo experiments, the most effective treatments are Thyme and Nerol at 1.5 % which reduced the disease incidence rating (77 % to 82.0 %) and (74.0 % to 83.0%) respectively.

Moreover, the highest reduction was obtained with chitosan at 8.0 g/ L which reduced the disease incidence by 80.0, 83.0, 80.0 and disease severity by 83.8, 82.0, 82.0 % for green mould, blue molds and sour rot respectively.

In other trails, the highest reduction was obtained with Thyme or Nerol at 1.5 % followed by chitosan at 8.0g / L which reduced disease incidence and severity more than 88.0 and 92.0 % respectively for green, blue molds and sour rot respectively. All tested treatments had no negative effect on fruit quality.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Statement of human and animal rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

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