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Efficacy of Some Essential Oils on Controlling Green Mold of Orange and their Effects on Postharvest Quality Parameters

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Abstract: The effect of lime, thyme, comphore oils against *Penicillium digitatum*, the causes of green mould disease of orange fruits, was evaluated for their inhibitory effect *in vitro* and *in vivo* during storage conditions. *In vitro* experiments, different concentration of each essential oil at 1, 5 and 10% (v/v) was tested on the growth of *P. digitatum*. The best concentration at 10% showed the highest inhibition growth of *P. digitatum* for all tested oils. *In vivo* experiments, in 2009 and 2010 seasons, treating fruits 15 days before harvest and after harvest or only after harvest (natural and artificial infection of fruits) by essential oils at conc. of 10% (v/v) significantly reduced the disease severity of fruits compared with untreated fruits (control) at 5°C. There was no significant difference found between the two tested methods of applying oils on fruits (natural and artificial infection of fruits). All treatments significantly reduced the undesirable fruits percentage, fruit weight loss percentage during cooling storage for 14 weeks compared with control during the two investigated seasons. Also preceding harvest and post harvest spraying with lime essential oil at conc. of 10% caused significant increase in TSS% during cold storage for 14 weeks compared with control. Prolonged cooling storage at 5°C for nine weeks slightly increased total soluble solids percentage of both controls and decline towards the end of storage period (14 weeks) of both treatments and control. At the end of storage period, there was no significant difference on Total Acidity (TA) between all tested treatments and controls. Vitamin C was gradually decreased as storage prolonged, for both control and all treatments.

Key words: *Penicillium digitatum,* lime, thyme, camphor oils, green mould

INTRODUCTION

Orange is one of the most important citrus fruit produced in Egypt, *Penicillium digitatum* is the most devastating pathogen of orange fruits, being responsible for about 90% of production losses during post-harvest handling (Macarisin *et al*., 2007). Postharvest diseases cause significant economic losses for the citrus industry during storage, transport and marketing (Solaimani *et al*., 2009). Traditionally, plant diseases control is achieved mainly through the use of fungicides, postharvest due to decay may increase up to 50% without fungicide treatment, although decay can be reduced to 5-10% with postharvest fungicides. However, alternative methods are needed because of concerns about environmental contamination and human health. Elucidating non-chemical control methods to reduce postharvest decay is becoming increasingly important. Consumers are demanding less chemical residue on produce and many fungi are developing resistance to commonly used fungicides (Ragsdale and Sisler, 1994; Bull *et al*., 1997). Recently, researchers have shown an interest in the application of non-toxic alternatives or supplements to synthetic fungicides. Plant extracts, including essential oils, have been investigated as alternative measures against pathological breakdown (Klieber *et al*., 2002; Ahmed *et al*., 2007).

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The objective of our study was to determine the postharvest control of green mould decay of orange by using the essential oils lime, thyme, comphore oils and *in vitro* and *in vivo*. Also, physical (weight loss % and Fruit decay %) and chemical characteristics (Total Soluble Solids (TSS %), Titratable acidity and Vitamin C contents of treated fruits were also determined biweekly.

MATERIALS AND METHODS

Pathogen inoculum: *Penicillium digitatum* was isolated from infected orange fruits and cultured on Potato Dextrose agar medium (PDA). Spore suspensions were prepared by removing the spores from the sporulating edges of a 7 days old culture with a bacteriological loop and suspending them in sterile distilled water. Spore concentrations were determined with a hemacytometer. The spore concentrations of *Penicillium digitatum* were adjusted to 5×10^4 spores mL^{-1}.

***In vitro* studies:** The inhibitory effect of lime, thyme and camphor oils on the mycelial growth of *P. digitatum* were studied under *in vitro* conditions. The essential oils were added to conical flasks containing 100 mL of sterilized PDA medium before its solidification to obtain the proposed concentrations of 1, 5 and 10% using the prepared stock solutions. The supplemented media was poured into Petri dishes (9 cm), nearly 20 mL per each. Discs (5 mM diameter) were placed on the centre of Petri dishes, incubated for seven days at 25+28°C, then examined. Reduction in mycelial growth was calculated relative to the growth in check treatment.

In vivo: Orange trees (*Citrus sinensisl*) Baladi cultivar forty years old, in good physical condition, free of insects, damage and diseases are selected and used as plant material of this investigation. Three trees for each treatment were used as a replicates (three branches were used of each tree). Trees were spraying 15 days before harvested (preharvest treatments) time by five liter of each oils per tree afternoon at the concentration of 10% v/v.

Orange fruits (Baladi cultivar) were harvested at commercial maturity. Fruits were used immediately after harvested, surface washed with tap water and then air dried. Fruits randomly divided into 2 equal groups; first group was wounded in three points around the entire equatorial region of each orange in depth of 5.0 mM with a 1.25 mM diameter needle at the equator of each fruit to prepare for inoculation. The second group, fruits were used without wound. Seven treatments were carried out as following:

- Preceding harvest spraying with lime oil+post harvest spraying with lime oil
- Post-harvest spraying with lime oil isolate
- Preceding harvest spraying with thyme+postharvest spraying with thyme oil
- Post harvest spraying with thyme oil
- Preceding harvest spraying with comphoreoil+post harvest spraying with comphore oil
- Post-harvest spraying with comphore oil
- Healthy Control (spraying with tap water)

This protocol was repeated in a separate trial and conducted for two seasons (2009 and 2010). All treatments were inoculated by dipping in suspension of *P. digitatum* (5×10^4 spores mL^{-1}). Fruits were air dried, then put into 440x300x100 mM plastic trays, retained high humidity (about 85-90%) and stored at 5°C. Infection incidence was observed periodically up to 30 days in the first group and 60 days in second group percentage of disease severity was calculated by dividing the weight of infected area by weight of orange. There were three replicate trials of 5 fruits per replicate.

Representative samples of five fruits per replicate were taken biweekly storage period until the percentage of decay reached 50%. Physical and chemical fruit properties were estimated biweekly.

Physical characteristics

Weight loss%: This character was determined by weighing 5 fruits in each replicate fortnightly. Percentage of weight loss was calculated by determination of progressive reduction in fruit weight during storage period relative to the original fresh weight at the beginning of storage.

Undesirable fruits percentage: Calculated by dividing the number of decayed fruits by the total number of fruits as following Equation:

$$\text{Undesirable fruits\%} = (\text{The number of undesirable fruits}/\text{Total number of fruit}) \times 100$$

Chemical characteristics

Total Soluble Solids (TSS%): Total Soluble Solids (TSS%) in fruit juice was determined by using a hand refractometer.

Total acids: Total acidity in fruit juice was determined by titrating fruit juice against 0.1 N NaOH with phenol phethalin as an indicator and calculated as gram citric acid per 100 mL fruit juice.

Ascorbic acids: Ascorbic acid content was determined by using 2.6 dichlorophenol. Indophenol as discribed in the AOAC (1985).

Statistical analysis: All data obtained throughout this study were tabulated and statistically analyzed, according to methods described by Snedecor and Cochran (1990) and using LSD test to recognize the significance of the differences among various treatments means.

RESULTS AND DISCUSSION

Biological control studies

***In vitro*:** Results presented in Table 1 show that all tested essential oils (lime, thyme and camphor oils) decreased growth of *P. digitatum* compared with control *in vitro* condition at concentration 1, 5 and 10% (v/v). The mycelial growth of *P. digitatum* decreased with increasing the concentration of each tested oils. The concentration at 10% for all tested oils was the best concentration used in this study. Lime oil was significant decrease growth of *P. digitatum* than other oils.

In vivo:

Effect of oils on artificially inoculated infection: In 2009 and 2010 seasons (Table 2), treated fruits with lime, thyme and camphor oils exhibited significantly decrease in disease severity of green mold caused by *P. digitatum* at 5°C compared with control. There was no significant difference found between the two methods of applying oils on fruits. However, spraying fruits with oils, 15 days before and after harvest exhibited higher reduction in disease severity than spraying one time after harvest. Results also indicated that, treated fruits with lemon oil before and after harvest or after harvest significantly decreased disease severity of green mold of orange fruits than other tested oils.

Effect of oils on natural infection: Data presented in Table 3 shows that treatments with lemon, thyme and camphor oils to control green mold of fruits stored at 5°C showed significant lower disease severity than non treated fruits (control). Results also demonstrated that spraying lime oil before and after harvest or after harvest significantly suppressed green mold disease. Also, there is no significant differences observed between the two tested methods of applying oils on fruits. Results reported herein are in accordance and confirm those reported by several researches. Some success has been achieved using essential oils as volatiles to combat decay of postharvest fruits and vegetables (Tabanca *et al*., 2007; Tunc *et al*., 2007; Serrano *et al*., 2008). The incorporation of essential oils into fruit coatings primarily applied to retain moisture, is gaining popularity. The advantage of using coatings amended with essential oils, rather than vapour, is that there is closer contact between the essential oils and fruit surfaces (Du Pooly *et al*., 2009). Furthermore, fruit displaying wounds with clear signs of pathogen stasis confirmed the fungicidal activity of the oils and their components. The essential oils and individual terpenoids appeared to enhance the ability of the coatings to control gas exchange, thereby reducing moisture loss and increasing juiciness. Essential oils

Table 1: Percentage of *P. digitatium* growth inhibition caused by Lemon thyme and camphor oils *in vitro*

	Inhibition (%)			
Oils	1	5	10	Mean
Lemon	7.3c	5.6f	3.2h	5.4c
Thyme	7.3c	6.2e	4.8g	6.1b
Camphor	7.8b	6.9d	4.7g	15.2c
control	8.5a	8.5a	8.5a	8.5a
Mean	7.7a	6.8b	5.3c	

Values in the column followed by different letters indicate significant differences among treatments according to least significant differences test (p = 0.05)

Table 2: Effect of spraying Lemon thyme and camphor oils at different times of harvest on developing citrus mold disease of citrus wounded fruits inoculated by *P. digitatum*

	2009 season		2010 season	
Oils	Before and after spraying (%)	After spraying (%)	Before and after spraying (%)	After spraying (%)
Lemon	1.27*d	1.87c	1.58b	1.83c
Thyme	2.03c	2.97b	2.13c	2.90b
Camphor	2.13c	3.37b	1.77c	3.23b
Control	48a	50a	51.67a	51.67a
Mean	13.59a	14.55a	14.48a	14.72a

*Disease index %, Values in the column followed by different letters indicate significant differences among treatments according to lease significant differences test (p = 0.05)

Table 3: Effect of spraying lemon, thyme and camphor oils 15 days before harvest and after harvest or after harvest to natural infection of unwounded citrus fruits *P. digitatum*

	2009 season		2010 season	
Oils	Before and after spraying (%)	After spraying (%)	Before and after spraying (%)	After sparing (%)
Lemon	0.10*f	1.00c	0.27e	1.13c
Thyme	1.07e	1.60d	1.33cd	1.67b
Camphor	1.33de	1.90df	1.0d	1.73
Control	42.0a	42.0a	42.67a	58.0a
Mean	11.21a	11.63a	11.40a	11.8a

*Disease index %, Values in the column followed by different letters indicate significant differences among treatments according to least significant differences test (p = 0.05)

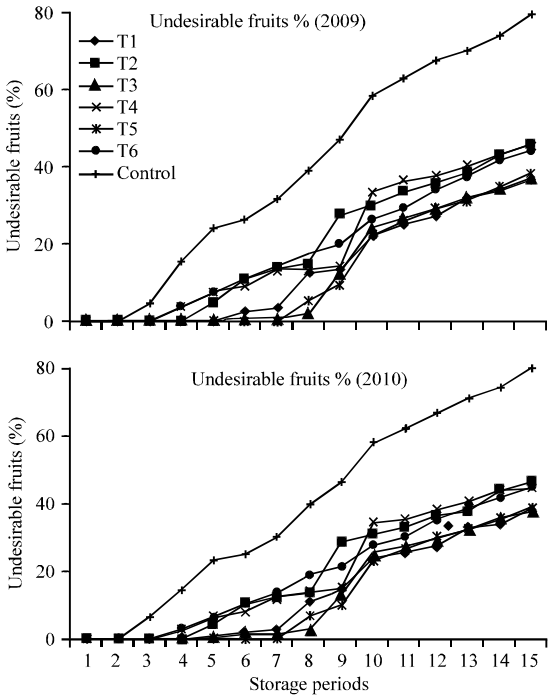

Fig. 1: Effect of lime thyme and camphor essential oils applications in controlling undesirable fruits percentage of "balady" oranges, during cold storage for 14 weeks, in 2009 and 2010 seasons T1 = Preceding harvest+post harvest spraying with lime (*Citrus lemon* L.) essential oil at 10%. T2 = Post-harvest spraying with lime essential oil at 10%. T3 = Preceding harvest+post harvest spraying with thyme (*Thymus vulgaris* L.) essential oil at 10% T4 = Post-harvest spraying with thyme essential oil at 10% T5 = Preceding harvest+post harvest spraying with Camphor essential oil at 10% T6 = Post harvest spraying with Camphor essential oil at 10% T7 = Control (spraying with Tap water)

could therefore, be extremely valuable to prevent shrivelling during prolonged export periods extend shelf-life and preserve the outer appearance of the fruit (Du Pooly *et al*., 2009).

Undesirable fruits percentage%: Data illustrated in Fig. 1 showed that undesirable fruit percentage increased by extending cooling storage period. The reason of fruit decay was the growth of mould on fruits. These finding are in agreement with Shehata (1998).

Generally, results indicated that all treatments significantly reduced the undesirable fruits percentage during cooling storage for 14 weeks, compared with controls. The best treatments are preceding harvest+post harvest spraying with lime essential oil at conc. of 10% significantly reduced undesirable fruits percentage by 53.58 and 52.50%, respectively compare with controls, in the two tested seasons. Preceding harvest+post harvest spraying with thyme essential oil at 10% wish significantly reduced undesirable Fruits by 53.16 and 52.91%, respectively compare with controls in 2009 and 2010. Then preceding harvest+post-harvest treatment camphor essential oil at 10% was significantly reduced undesirable fruits percentage by 51.48 and 51.66%, respectively compare with controls in two tested seasons. These finding are in agreement with Samra *et al*. (2006). They reported that spraying peach trees two weeks before harvest with Camphor oil at conc. of 0.1% reduced fruit decayed at cold storage.

The biological activity of the essential oils may be due to the action of their compounds against spore germination of pathogens such as *Penicillium italicum* and *P. digitatum*. These results are in agreement with Mari and Guizzardi (1998). They reported that the fungicidal of oil obtained from thymus against several post harvest pathogens may reveals the marked fungicidal activity of carvacrol in thyme. Also Samra *et al*. (2006) reported that the basic component of camphor essential oil such as carven found to completely inhibit 3-Hydroxy-3-methyl Glutrayl coenzyme reductase (HMGR), the key enzyme of mevalonate pathway. Mevalonate known to be the main pathway of gibberellins biosynthesis .

Yigit *et al*. (2000) found that the concentration of 900 ppm thyme essential oil prevented citrus fruit mould. Moreover, Aly *et al*. (2003) reported that lime fruit peel essential oil components inhibit linear growth on spore germination of *P. italicum*, *P. digitatum* and *Geotrichum canium*.

Fruit weight loss%: Data illustrated in Fig. 2 showed that fruit weigh loss percentage increased by extending cold storage period, during the two investigated seasons. These results could be due to the loss in moisture content. This agrees with the finding of Elshiekh and Abo-Goukh (2008).

Generally, results indicated that all treatments significantly decreased fruit weight loss percentage during cooling storage for 14 weeks, compare with controls during the two investigated seasons. In the two tested seasons the best treatments are preceding harvest+post harvest spraying with lime essential oil at 10% significantly reduced fruit weight loss percentage by 23.16 and 22.98%, respectively compare with controls. Also, preceding harvest+post harvest spraying with

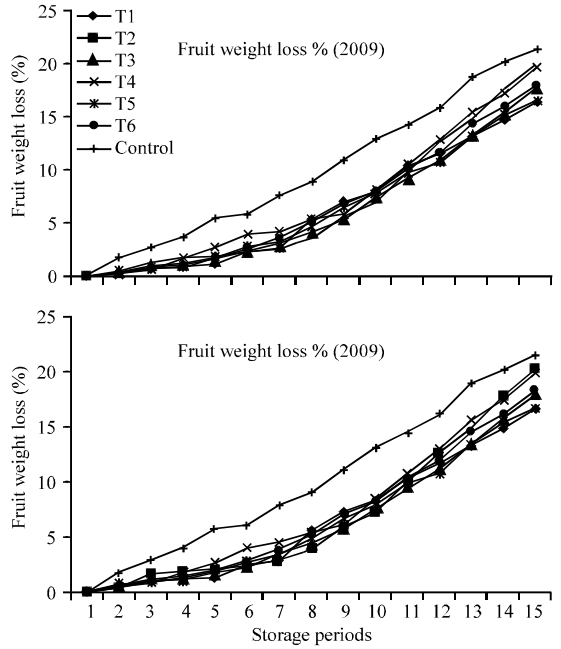

Fig. 2: Effect of lime thyme and camphor essential oils applications on fruit weight loss% of "Balady" Oranges, during cold storage for 14 weeks, in 2009 and 2010 seasons. Whereas T1-T6 as described in footnote of Fig. 1

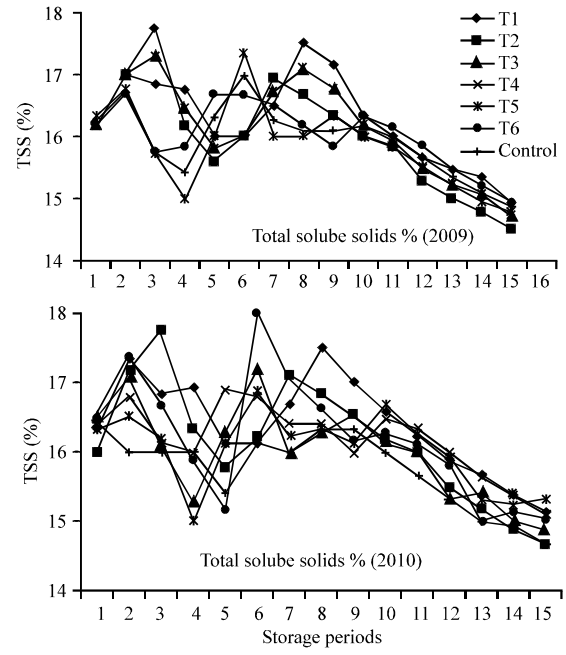

Fig. 3: Effect of lime thyme and camphor essential oils applications on Total Soluble Solids percentage (Tss%) of "Balady" Oranges, during cold storage for 14 weeks, in 2009 and 2010 seasons. Whereas, T1-T6 as described in footnote of Fig. 1

camphor essential oil at 20% with significantly reduced fruit weight loss percentage by 22.74 and 22.18%, respectively compare with controls then preceding harvest+post harvest treatment with thyme essential oil at 10% significantly reduced fruit weight loss percentage by 16.81 and 16.82%, respectively compare with controls.

The positive effects of oils on decreasing fruit weigh loss might be attributed to make a thin film of oil surrounding the fruit peel and induced a modification of microclimatic of fruits. These results are in agreement with Golam Rabbany and Mizutani (1996) and Samra *et al.* (2006).

Total Soluble Solids percentage (TSS%): From Fig. 3 it is clear to notice that preceding harvest+post-harvest spraying with lime essential oil at conc. of 10% caused significant increase in TSS % during cold storage for 14 weeks compared with controls, for the two experimental seasons, while the other treatments had no significant effects on TSS%.

From this figure we can notice that prolonging cold storage at 5°C for nine weeks slightly increased total soluble solids percentage and a decline towards the end of storage period (14 weeks) of both treatments and controls in 2009 and 2010 seasons. The decrement may be due to the transformation in part of TSS to acids.

Total acidity: From Fig. 4 it was clear to notice that at harvesting time, preharvest treated fruits content total acidity less than controls. This results are in agreement with Salih and Abdalla (1982), Martinez *et al.* (1991) and Mohamed and Abo-Goukh (2003). They reported that waxing decreases acidity during storage of orange and mango fruits, while at the end of storage period (14 weeks) there were no significant differences between all treatments and controls, on fruit Total Acidity (TA) content for the two experimental seasons 2009 and 2010 took similar trend.

During storage period there were a decrease in acidity during cold storage up to 3 weeks of storage, then an increase in acidity up to 8 weeks and a decline towards the end of the storage period, for both treated fruits and controls .These results are in agreement with El-Zeftawi (1976) and Elshiekh and Abo-Goukh (2008).

Vitamin C contents: Results in Fig. 5 indicate that compared with controls there were no significant effects between all treatments and controls during cold storage in vitamin C contents for 11 week in 2009 and 13 weeks in 2010. Prolonging storage period up to 14 week caused significant decrease for all treatments compared with controls in the two experimental seasons.

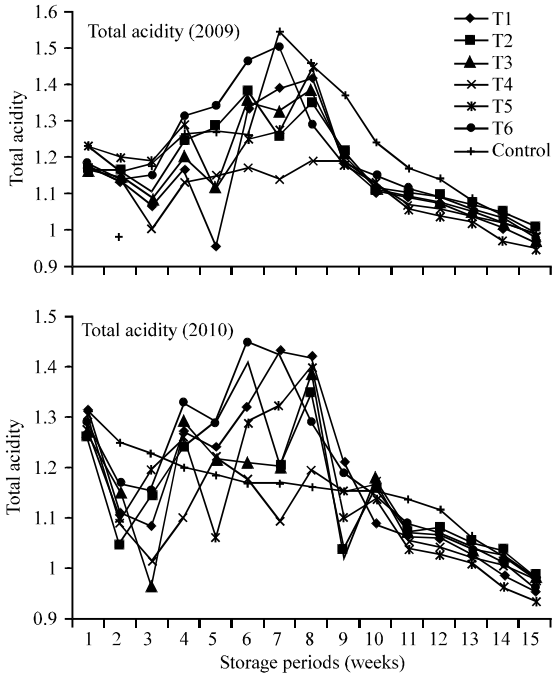

Fig. 4: Effect of lime thyme and camphor essential oil's applications on Total acidity, of "Balady" Oranges, during cold storage for 14 weeks, in 2009 and 2010 seasons. Whereas, T1-T6 as described in footnote of Fig. 1

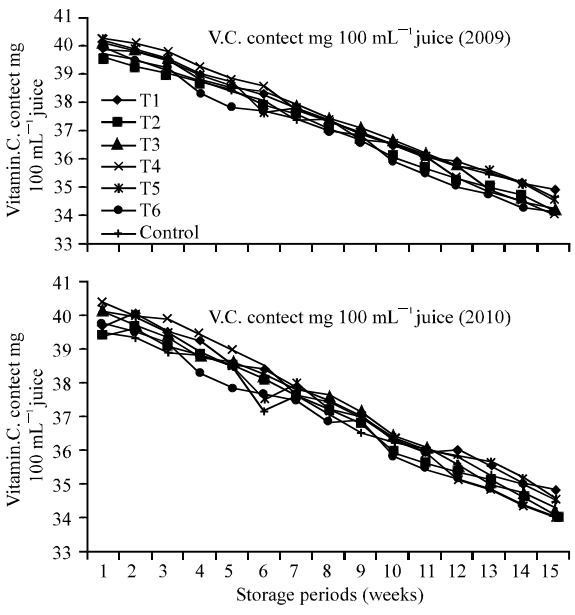

Fig. 5: Effect of lime thyme and camphor essential oils applications on Vitamin C. content, of "Balady" Oranges, during cold storage for 14 weeks, in 2009 and 2010 seasons. Whereas, T1-T6 as described in footnote of Fig. 1

During cold storage for 14 weeks it is clear from Fig. 5 that Vitamin C was gradually decreased as storage prolonged, for both control and all treatments. This agree with Trifiro *et al.* (1995), who describe maximum decrease of 8% in ascorbic acid in fresh juices of blood Oranges stored at 3°C for 30 days. Kabasakalis *et al.* (2000) and Del Caro *et al.* (2004). They reported that cold storage of fresh Orange juice for 31 days lost 7 to 13% of its ascorbic acid content. Also, they reported that segments of Minneola and salustiana Orange cultivars showed a significant decrease in ascorbic acid after 12 days of storage at 4°C. Ajibola *et al.* (2009), who found a significant decrease in ascorbic acid after 4 weeks of cold storage at 4±1°C.

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