

Effect of Essential Oils on Postharvest Decay and Some Quality Factors of Peach (*Prunus persica* var. Redhaven)

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ABSTRACT

The aim of the study was to determine the antifungal effects of the essential oils against fungal pathogen *Botrytis cinerea* the causal agent of grey mould disease of peach (*Prunus persica* L.) under in vitro and in vivo conditions. Treatments consisted of four essential oils (anise, ammi, ziziphora and cinnamon) and five concentrations (0, 200, 400, 600 and 800 $\mu\text{L.L}^{-1}$). Results of in vitro experiment showed that all of used essential oils at all applied concentrations inhibited grey mould growth. All of these essential oils in concentration 800 $\mu\text{L.L}^{-1}$ were without germination spores of grey mould. The essential oils application significantly decreased weight loss percentage and increased life storage fruits. Also, essential oils positively affected on postharvest quality factors including total soluble solids, titrable acidity, anthocyanin, carbohydrate content and pH value. It was observed that treated fruits with ammi essential oil at concentration 800 $\mu\text{L.L}^{-1}$ had the highest total soluble solids; titrable acidity, anthocyanin, and carbohydrate content and it had the lowest decay and acidity. Thus, these results showed that essential oils have strong impact on postharvest decay and fruit quality of peach.

Key Words: antifungal activity, essential oils, grey mould

INTRODUCTION

Botrytis cinerea Pers: Fr. (grey mould rot) is a ubiquitous pathogen, which causes severe damage in many fruits, vegetables and ornamental crops in pre- and postharvest (Elad 1997). Antimicrobial chemicals such as benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors are often used in control of plant disease in agriculture (Moorman and Lease 1992). Because of increasing concerns about chemical usage in food and the environment, there is also renewed interest in nonchemical approaches to postharvest disease control (Wilson *et al.*, 1987). There is therefore still a need for new and effective natural antimicrobials of reducing or eliminating fungus. In nature, essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reducing their appetite for such plants (Bakkali *et al.*, 2008). Essential oil bearing plants may be alternative to currently used disease control agents, since they constitute a rich source of bioactive chemicals (Isman 2000, Burt 2004). Takayuki *et al.* (2007) applied to measure the antifungal effects of 52 dried samples of spice and herbs against a soil-borne phytopathogenic fungus, *Fusarium oxysporum*. Essential oils of seven Moroccan Labiatae were chemically analysed and evaluated for their in vitro antifungal activity against *Botrytis cinerea* (Chebli *et al.*, 2003). Among them, *Origanum compactum* and *Thymus glandulosus* greatly inhibited the growth of the mycelium and the inhibition of *Botrytis cinerea* was 100% for both oils at 100 ppm. Soyulu *et al.* (2010) investigated antifungal activities of essential oils obtained from aerial parts of aromatic plants, such as origanum (*Origanum syriacum* L. var. bevanii), lavender (*Lavandula stoechas* L. var. stoechas) and rosemary (*Rosmarinus officinalis* L.), against *Botrytis cinerea*. They showed that complete growth of pathogen was inhibited by essential oil of lavender and rosemary. Peaches (*Prunus persica* L.) are susceptible to postharvest decay caused by several pathogenic fungi such as by *Botrytis cinerea*, is a major disease of peach. Thus, the objective of this study was to test and compare the inhibitory effects of the essential oils of ammi (*Carum copticum*), anise (*Pimpinella anisum*), ziziphora (*Ziziphora clinopodioides*) and cinnamon (*Cinnamomum zeylanicum*) against grey mould (*Botrytis cinerea*) and evaluate the potential application of essential oils to control postharvest spoilage on stored peach.

MATERIALS AND METHODS

Plant materials and Extraction of essential oils

The air-dried seeds of anise and ammi, headspace of ziziphora and bark tree of cinnamon were supplied from agricultural research fields of Ferdowsi University of Mashhad of Iran. After the plant materials were authenticated the air-dried materials (100 g) of medicinal plants were subjected to hydro distillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4 °C until further analysis.

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Design of experiments and treatments

All the experiments (in vitro and in vivo) were carried-out in randomized factorial design with two factors; including four essential oils (anise, ammi, ziziphora and cinnamon) and five concentrations (0, 200, 400, 600 and 800 $\mu\text{L.L}^{-1}$) with four replications.

Antifungal effects of the essential oils on mycelia growth in vitro

Antifungal activity was studied by using a contact assay (in vitro), which produces hyphal growth inhibition. The method was used for essential oils treatment on potato dextrose agar (PDA) medium "Solution Method" (SM) (Özden *et al.* 2002). In this method the essential oils were dissolved in tween 80-water solution (5% v/v) and required amounts of the solutions were added to each of the PDA plates containing 20 ml of agar at 45 °C in petri dish. A 0.5 mm disk of mycelium of 7-8 days old culture *B. cinerea* was located on PDA medium. The treated medium was incubated in 24°C and mycelium growth was determined daily. Inhibitory percentage was determined according to the following formula. $IP = \frac{dc-dt}{dc} \times 100$. IP= Inhibitory percent, dc= mycelium growth diameter in control and dt= mycelium growth diameter in essential oil treated petri dish. Four replication used for each treatment.

Spore germination assay

The effect of anise, ammi, cinnamon and ziziphora oils on spore germination were tested in potato dextrose Agar (PDA). The used oils were added to a 10 ml glass tube containing 5 ml PDA to obtain final concentrations 0, 200, 400, 600 and 800 $\mu\text{L.L}^{-1}$. Spore suspension (10^5 spores ml^{-1}) of grey mould was prepared from actively growing culture (7–8 days old) in distilled sterile water. At the same time, aliquots (1 ml) of spore suspensions of grey mould were added to each tube. After 20 h of incubation at 28 °C on a rotary shaker (200 rpm), at least 100 spores per replicate were observed microscopically to determine germination rate (Xu *et al.*, 2007). Four replication used for each treatment.

Effect of essential oils on postharvest decay and some qualities factors of grey mould inoculation on peach fruits

Grey mould was isolated from infected peach fruit. Spore suspensions were prepared by removing the spores from the sporulation edges of a 7-8days old culture with a bacteriological loop, and suspending them in sterile distilled water. Spore concentration was determined with a hemocytometer (1×10^5), and adjusted as required with sterile distilled water. At first, fruit were treated by sodium hypochlorite (100 $\mu\text{L.L}^{-1}$). Then fruits were dipped in prepared suspension and located in room temperature for 2 h in order to fixed fungal inoculation (Asghari *et al.*, 2009). According to the in vitro experiment, SM method (solution method) was used. Fruits were treated by required essential oils, treated the fruit, by dipping at concentrations of 0, 200, 400, 600 and 800 $\mu\text{L.L}^{-1}$ for 30 second and located in the packages separately (disinfection of plastic containers that full were closed). Essential oil-treated and untreated fruits together with control were transferred into packages and were steeked in order preventing loss of oils and then put into the cold storage (4°C).

Decay incidence in the cold storage conditions

The degree of infection on fruit was rated using a scale of 0 to 9, where 1= no infection; 2= trace infection low than 10%, 3= infection between 10- 20%, 4= infection between 20- 30%, 5= infection between 30- 40%, 6= infection between 40- 50%, 7= infection between 50- 65%, 8= infection between 65- 80% and 9 = infection more than 80% (Asghari Marjanlo *et al.*, 2009).

Quality Parameters

Titriable acidity (TA), Total soluble solids (TSS) and pH

The pH value of fruit was measured with a pH meter (model Jenway 3320) at 20°C. Titriable acidity (TA) was determined by titration with 0.1 N NaOH until pH 8.1 was reached and reported g 100 g⁻¹ of malic acid fresh weight using malic acid as a control (Horwitz 1975). Total soluble solids (TSS) was determined at 20°C with a digital Refractometer (model RFM340, UK) and reported as °Brix.

Weight loss percentage

In order to determine any weight loss during the storage, both treated and untreated fruit were weighted at the previous and end of the storage day.

Anthocyanin

Total anthocyanin content was determined by the pH differential method (Rapisarda 2000). An aliquot of peach extract (1 ml) was diluted up to 10 ml with pH 1.0 solution (125 ml of 0.2 M KCl and 375 ml of 0.2 M HCl). A second aliquot (1 ml) was diluted up to 10 ml with a pH 4.5 buffered solutions (400 ml of 1 M CH₃CO₂Na, 240 ml of 1 M HCl, and 360 ml of H₂O). Absorbance of the solution was measured at 510 nm and the concentration of anthocyanins was calculated by the equation: $C_{mg/100\ g} = [(Abs_{pH1.0} - Abs_{pH4.5}) \times 484.82 \times 1000 / 24825] \times DF$. Where the term in parentheses is the difference of absorbance at 510 nm between pH 1.0 and pH 4.5 solution, 484.82 is the molecular mass of cyanidin-3-glucoside chloride, 24825 is its molar absorptivity (ϵ) nm in the pH 1.0 solution, and DF is the dilution factor.

Carbohydrate

Carbohydrate content was measured according to the method of Yemm and Willis (1954) using anthrone reagent. Sugars were extracted with 80% ethanol at 45 °C, followed by centrifugation at 5000 × g for 10 min. The reaction mixture consisted of 0.5 ml of extract and 5 ml of anthrone reagent which was boiled at 100°C for 30 minutes. Absorbance was determined at 620nm. The carbohydrate content is expressed as mg per g dry weight.

Statistical analysis

Data were analyzed using SASS 9.1 statistical software and means were compared by Duncan's multiple range test (DMRT) at 5% level of confidence.

RESULTS

Effect of essential oils on radial growth of grey mould and spore germination

The effects of different concentrations of these essential oils on the radial growth of grey mould are shown in Fig1. All used essential oils were found to inhibit the growth of grey mould in a dose-dependent manner. The result indicated that the highest radial growth was observed in control (without essential oil application), while the lowest radial growth were in ammi, anise and cinnamon essential oils at 600 $\mu\text{L.L}^{-1}$ and ziziphora oil at 200 and 400 $\mu\text{L.L}^{-1}$ that these treatments had no infection (Fig. 1). Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control, that the ammi oil had the highest percentage of inhibition with 73.18% and control had the lowest inhibition percentage on radial growth of grey mould (Fig. 2). Germination spores of grey mould were inhibited by ammi, cinnamon, anise and ziziphora essential oils (Fig. 3). All of these essential oils except ziziphora at 800 $\mu\text{L.L}^{-1}$ were without germination spores of grey mould, while ziziphora oil had the most inhibition in concentration 200 $\mu\text{L.L}^{-1}$.

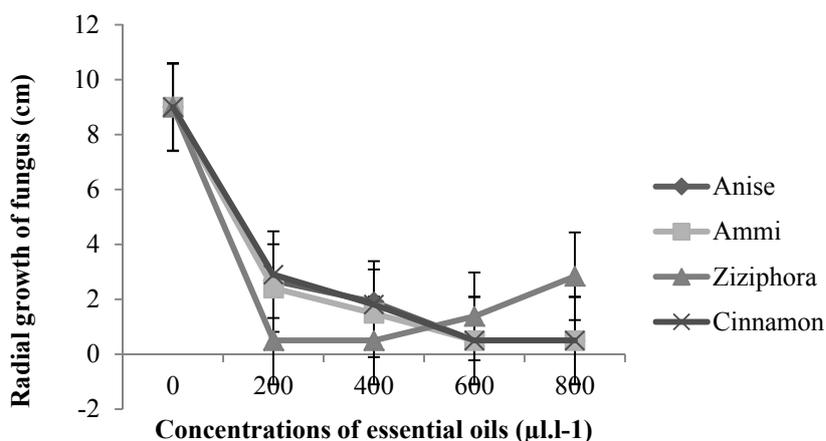


Figure 1. Effect of different concentrations of essential oils on radial growth (cm) of grey mould

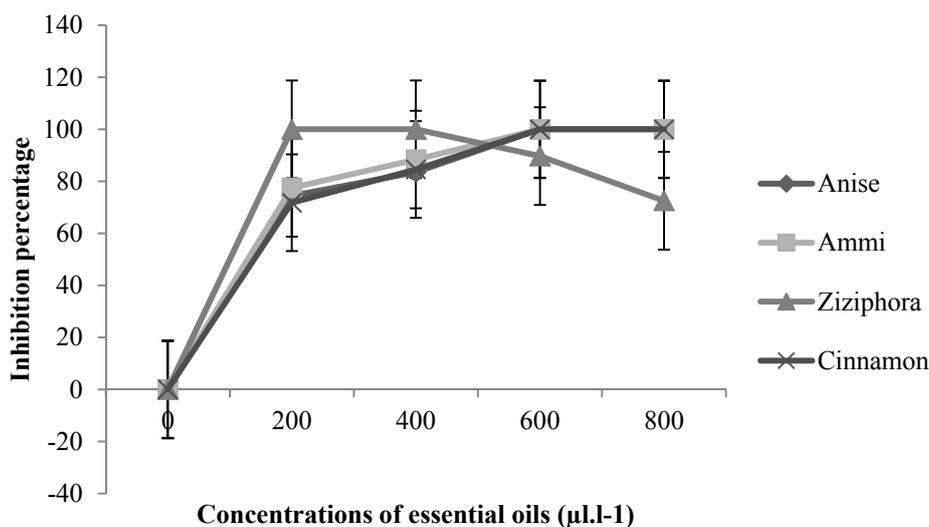


Figure 2. Effect of different concentrations of essential oils on mycelial growth inhibition (%) of grey mould

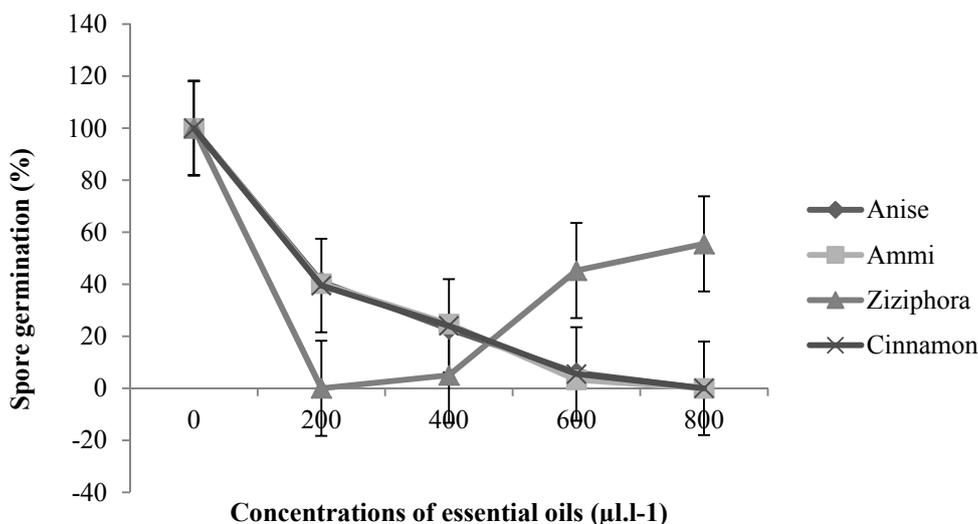


Figure 3. Effect of different concentrations of essential oils on spore germination (%) of grey mould

Effect of essential oils on postharvest quality factors of peach

Essential oils-treated fruit better maintained than untreated fruit (control) and had low severity of decay scores, whereas non-treated fruit showed increased fruit deterioration (Fig. 4). Among essential oils, treated fruits with ammi essential oil had the lowest decay scores, while control had the highest decay rate. Treated fruits with ammi, anise and cinnamon essential oils had no infection at 800 µL.L⁻¹. The effect of these essential oils on TSS content fruit showed in Table 1. Result indicated that significant differences were observed in TSS content among treated fruits and control. Data showed that treatment with ammi essential oil at 800 µL.L⁻¹ had the highest of TSS content (16.17°Brix). There was significant difference in TA content of treated fruits with control fruits. (Table 1). Among essential oils, ammi oil at 800 µL.L⁻¹ was the best treatment (0.81 g100 g⁻¹), but there was no significant difference in TSS/TA content among treatment (data not shown). There was significant difference in pH value, among treatments with control (Table 1). Treated fruits with the ammi essential oil in concentration 800 µL.L⁻¹ had the lowest pH value with 3.86, while control fruits had the highest pH value concentrations (4.43). The percentage of weight loss was very low for fruit treated by these essential oils and had significant difference comparison to control (p < 0.01). Among samples, treated fruits with ammi essential oil at

600 $\mu\text{L.L}^{-1}$ had the lowest the percentage of weight loss (5.74 %), while control, followed by 800 $\mu\text{L.L}^{-1}$ concentration all of essential oils had the highest the percentage of weight loss (Table 1). The anthocyanin content of the treated peach fruits had significantly different in among essential oils and control (Table 1). Treated fruits with the ammi and cinnamon essential oils had the highest anthocyanin content other than essential oils. The greatest anthocyanin content was observed in treated fruits with 600 $\mu\text{L.L}^{-1}$ ammi and cinnamon essential oils (23.31 $\text{mg}100\text{g}^{-1}$), while control had the least value. Treated fruits with ammi essential oil had the highest carbohydrate content, but not significantly different showed among effect other essential oils (Table 1). Results showed the best treatment was ammi oil at 600 $\mu\text{L.L}^{-1}$, while control fruits had the lowest carbohydrate content (131 $\text{mg}100\text{g}^{-1}$).

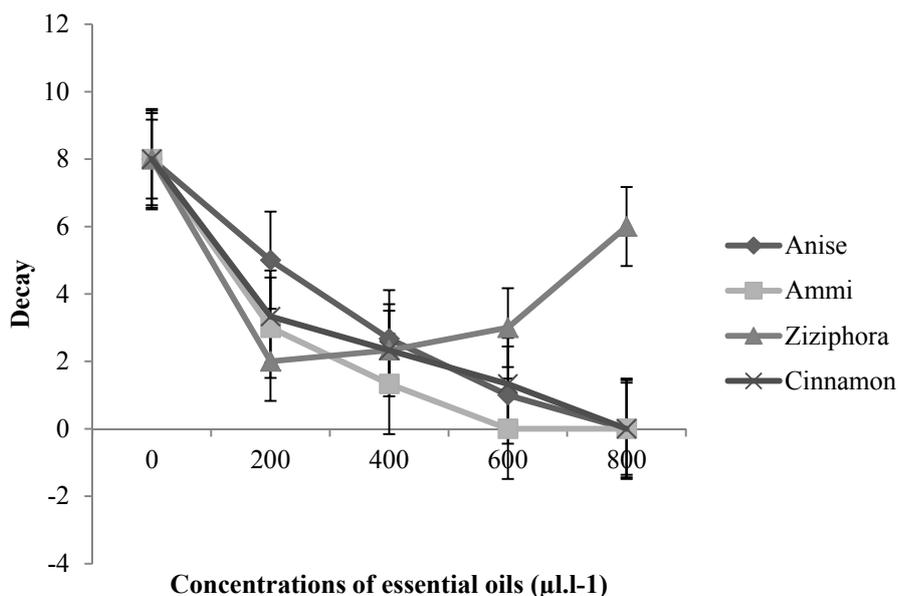


Figure 4. Effect of different concentrations of essential oils on decay rate of peach during storage.

Table 1. Effect of essential oils on postharvest quality factors of peach.

Treatment	TSS ($^{\circ}\text{Brix}$)	TA ($\text{g}100\text{g}^{-1}$)	pH	Weight loss (%)	Anthocyanin ($\text{mg}100\text{g}^{-1}$)	Carbohydrate ($\text{mg}100\text{g}^{-1}$)
0(control)	11.5 ^h	0.60 ^h	4.43 ^a	12.3 ^a	12.67 ^g	131.33 ^b
An ₂₀₀	12.0 ^{gh}	0.66 ^{gh}	4.15 ^{bc}	9.22 ^{b-d}	15.38 ^{fg}	158.0 ^{fg}
An ₄₀₀	13.67 ^{de}	0.72 ^{e-h}	4.04 ^{c-e}	8.42 ^{b-e}	18.32 ^{c-f}	176.67 ^{c-f}
An ₆₀₀	15.0 ^{bc}	0.82 ^{c-e}	3.99 ^{d-f}	7.58 ^{b-f}	20.63 ^{a-d}	197.33 ^{a-c}
An ₈₀₀	15.5 ^{ab}	0.88 ^{b-d}	3.92 ^{ef}	9.55 ^{bc}	22.75 ^a	188.0 ^{a-e}
Am ₂₀₀	14.17 ^{cd}	0.77 ^{d-g}	4.02 ^{c-e}	7.07 ^{c-f}	18.02 ^{d-f}	168.33 ^{e-g}
Am ₄₀₀	14.33 ^{cd}	0.87 ^{b-d}	4.0 ^{c-e}	6.02 ^{ef}	20.47 ^{a-e}	192.33 ^{a-d}
Am ₆₀₀	12.83 ^{e-g}	0.79 ^{d-f}	4.12 ^{cd}	7.94 ^{b-f}	18.45 ^{c-f}	179.33 ^{c-f}
Am ₈₀₀	12.5 ^{f-h}	0.69 ^{f-h}	4.26 ^b	9.55 ^{bc}	16.86 ^{ef}	169.33 ^{d-g}
Z ₂₀₀	13.0 ^{e-g}	0.7 ^{e-h}	4.07 ^{c-e}	9.58 ^{bc}	17.96 ^{d-f}	182.33 ^{b-e}
Z ₄₀₀	13.5 ^{d-f}	0.81 ^{d-f}	4.02 ^{c-e}	7.37 ^{b-f}	18.87 ^{b-f}	198.0 ^{a-c}
Z ₆₀₀	15.5 ^{ab}	0.93 ^{a-c}	3.98 ^{d-f}	5.74 ^f	23.31 ^a	209.67 ^a
Z ₈₀₀	16.17 ^a	1.007 ^a	3.86 ^f	8.55 ^{b-d}	22.10 ^{ab}	206.33 ^a
C ₂₀₀	12.83 ^{e-g}	0.66 ^{gh}	4.28 ^b	9.69 ^b	16.90 ^{ef}	148.33 ^{gh}
C ₄₀₀	13.5 ^{d-f}	0.75 ^{e-g}	4.11 ^{cd}	8.99 ^{b-d}	20.30 ^{a-e}	191.33 ^{a-e}
C ₆₀₀	13.83 ^{de}	0.94 ^{ab}	4.08 ^{cd}	6.88 ^{d-f}	23.22 ^a	203.67 ^{ab}
C ₈₀₀	15.83 ^{ab}	1.0 ^a	3.97 ^{d-f}	7.86 ^{b-f}	21.66 ^{a-c}	195.33 ^{a-c}

An: Anis, Am: Ammi, Z: Ziziphora, C: Cinnamon essential oils, 200, 400, 600 and 800 $\mu\text{L.L}^{-1}$ concentrations of essential oils. Within each column, same letter indicates no significant difference between treatments at 5% levels.

DISCUSSION

In vitro data presented in this study indicated that used essential oils had fungicidal effect in upper concentrations except ziziphora essential oil was the better in lower concentrations. Similarly, the growth of *Botrytis cinerea*, *Fusarium sp.* and *Clavibacter michiganensis subsp. michiganensis* bacteria were completely inhibited by oregano, thyme, dictamnus and marjoram essential oils at relatively low concentrations (Dimitra *et al.*, 2003). Chebli *et al.* (2003) indicated that essential oils of *Origanum compactum* and *Thymus glandulosus* inhibited the growth of the mycelium of *Botrytis cinerea*. Also, spore germination and germ tube elongation were also inhibited by the essential oils tested. The effect of essential oils on microbial growth has been reported by Fung *et al.* (1977). They thought it may be the result of phenolic compounds of essential oils that cause an altering of microbial cell permeability by interaction with membrane proteins. This would cause a deformation in cell structure and functionality, and permit the loss of macromolecules from their interior (Rattanapitigorn *et al.*, 2006). Moreover, each of the essential oil components has its own contribution on biological activity of the oil. For example, carvacrol was found as the main compound in ammi oil, while anethole was found in anise as the main compound, and these compounds have more fungicidal effect (Takayuki *et al.*, 2007). The results showed that used essential oils had the positive effect on storage life and reduce decay content. Previous reports indicated that reduced fruit decay during postharvest treatments with volatile compounds including raspberry and kiwifruit (Wang *et al.*, 2003, Williamson *et al.*, 2007). Essential oils mainly conjugated to phenolic compounds that accumulate in some plants cells and show useful effect for pathogen control (Plotto *et al.*, 2003). It is known those oxidations products of phlorisidzin (an ortho-dihydroxyphenolic compound) inhibit growth the apple scab fungus *Venturia inaequalis* (Asghari *et al.*, 2009). Fungal pectinases hydrolyze pectin, a cell wall compound that is abundant in the middle lamella and plays a role in cell adhesion. Thus, by inhibiting pectinases, the ability of the fungus to hydrolyze and invade the plant cell wall would be compromised (Vermerris *et al.*, 2006). It seems that similar role was done by phenolic compound of essential oils. Thus, these findings reveal that exogenous essential oils may have a positive influence on shelf life and reduce decay peach fruits. This study showed that essential oils were effective to maintain fruit quality. Treated fruits with essential oils had more total soluble solids, TA, anthocyanin and carbohydrate content comparison to control, which was in agreement with previous reports were shown that cinnamon and eucalyptus vapor had significant effect on TSS of strawberry infected to grey mould and increased TSS of fruits (Tian *et al.*, 2011). Also, Asghari *et al.* (2009) reported that titrable acidity of strawberry infected to grey mould, increased with cumin essential oil application. These results indicate that essential oils application significantly decreased weight loss percentage. Previous experiments using natural antifungal compounds (eugenol, thymol and menthol vapors) revealed benefits due to reduced weight loss percentage in cherries and grapes (Serrano *et al.*, 2005). Similar results were finding with eucalyptus and cinnamon oil in strawberry and tomato on reducing weight loss percentage (Tian *et al.*, 2011). In fact, there was a linear correlation between ethylene and damage, and thus the fungus was responsible for the majority of ethylene production, a part of the basal level typical of non-climacteric fruits (Cristescu *et al.*, 2002). Accordingly, it has been reported that grey mould produced greater amounts of ethylene as the concentration of conidia inoculated in vitro or in the climacteric tomato fruit increased. The respiration rate was clearly affected by these essential oils concentrations and dimension of infection (Cristescu *et al.*, 2002), Similar this experiment. It could be concluded that essential oils with reducing respiration rate of fruit had a positive influence on weight loss percentage of peach fruit.

CONCLUSIONS

Considering the reduction in the mycelial growth and germination of conidia in vitro and incidence of disease symptoms on essential oil treated fruits and increasing shelf life, we concluded that essential oils could be used as possible bio fungicides alternative to synthetic fungicides against phytopathogenic fungi. However, more studies are required to recommendation of these essential oils as a commercial and natural antifungal for increase postharvest on horticultural crops.

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