ASSESSMENT OF THE ANTIFUNGAL ACTIVITY OF NATURAL COMPOUNDS TO REDUCE POSTHARVEST GRAY MOULD (*BOTRYTIS CINEREA* PERS.: FR.) OF KIWIFRUITS (*ACTINIDIA DELICIOSA*) DURING STORAGE

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Abstract: Essential oils obtained by hydrodistillation from thyme (*Thymus vulgaris* L.), ajowan (*Carum copticum* L.), fennel (*Foeniculum vulgare* L.) and summer savory (*Satureja hortensis* L.) were assessed under *in vivo* condition for antifungal activity against *Botrytis cinerea* on kiwifruits. Inoculated and oil-treated fruits were kept in storage, 90 days. Evaluation of the antifungal activity of essential oils showed that with the increase of their concentrations the antifungal activity was increased, but no significant differences were observed. In addition, the quality parameters such as total soluble solids (TSS), titrable acidity (TA) and vitamin C reduced in fruits treated with essential oil. Weightloss and firmness values were not affected by essential oil treatment and essential oil treated kiwifruits showed off-flavor in compare to control. Results of this study suggest that application of essential oil to control postharvest pathogens is worthy of future works.

Key words: kiwifruit (*Actinidia deliciosa* cv. Hayward), *Botrytis cinerea*, fungal decay, essential oil, antifungal, post-harvest

INTRODUCTION

Postharvest losses of fruits and vegetables is a serious problem, because the values of fresh product significantly increase while passing from the farm to the consumers table and due to overpopulation the demand for fruits and vegetables increases in the world. Fungal pathogens are mainly responsible for postharvest losses of fruits and vegetables (Korsten 2006).

Kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa* Hayward] is a climacteric and susceptible fruit to fungal decays in postharvest stage. Stem end rot caused by *Botrytis cinerea* Pers.: Fr. is the most serious pathogen of kiwifruit during storage period which is controlled traditionally by using synthetic fungicides (Eckert and Ogawa 1988; Wurms *et al.* 1999).

In the recent years, pesticide residues on horticultural products, especially the fungicides used in postharvest stage, are of major concerns to the horticultural industry. Also, the increase in consumer's awareness about hazards of pesticide residues on fresh products and their demand to non-residue products, human health, and environmental pollution are considered. Additionally, the reduction in efficacy of fungicides and consequently development of resistant strains of fungi causes that agriculture researches assay new methods and substances to develop effective and safer alternatives to agrochemicals. The use of natural products such as plant extracts, essential oils, salicylic acid and chitosan or integrated use of these products with other methods such as controlled atmosphere and modified storage atmosphere was investigated (Poole and McLeod 1994; Du *et al.* 1997; Ejechi *et al.* 1999; Shahi *et al.* 2003; Valero *et al.* 2006).

Although a lot of studies were carried out to examine the antifungal activity of essential oils under *in vitro* conditions (Rasooli and Owlia 2005; Yahyazadeh *et al.* 2008; Abdolahi *et al.* 2010), a few work were conducted to evaluate a possible use of antifungal property of essential oils to preservation of fruits and vegetables in postharvest stage. Thanassoulopoulos and Yanna (1997) screened antifungal activity of essential oils from origanum, sweet basil and thyme against gray mold rot on inoculated kiwifruits with *B. cinerea*. Tripathi *et al.* (2008) investigated the antifungal property of twenty six essential oils against *B. cinerea* under *in vitro* conditions and found a total growth inhibition

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by using extracts from *Chenopodium ambrosioides*, *Eucalyptus citriodora*, *Eupatorium*, *Cannabinum*, *Lawsonia inermis*, *Ocimum canum*, *O. gratissimum*, *O. sanctum*, *Prunus persica*, *Zingiber cassumunad* and *Z. officinale*. On the other hand, essential oils from *P. persica*, *O. sanctum* and *Z. officinale* increased the storage life of oil treated grapes 4, 5 and 6 days, respectively. Additionally, vapor of essential oils from peppermint and sweet basil against *Monilina fruticola*, *Rhizopus stolonifer* and *Aspergillus niger* showed a good antifungal activity under *in vitro* and *in vivo* conditions, reducing decay and maintaining quality parameters of peach fruits after prolonged storage (Ziedan and Farag 2008).

Therefore, based on the above mentioned points, the aim of this study was to assay a potential use of essential oils from fennel, thyme, ajowan and summer savory in order to control of gray mold fungal decay on kiwifruit and enhancing fruit quality and marketability by reducing the application of synthetic fungicides.

MATERIALS AND METHODS

Plant material and essential oils

Kiwifruits [*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa* Hayward] were obtained from a wholesale market in Urmia, Iran. Fruits were selected for uniformity in size, appearance, ripeness and the absence of physical defects. The selected fruits were randomized before being used for treatments with essential oils. Four locally available aromatic plants were selected for extraction of essential oils. Fruits of ajowan (*Carum copticum* L.), summer savory (*Satureja hortensis* L.), fennel (*Foeniculum vulgare* Mill.) and thyme (*Thymus vulgaris* L.) were used for extraction of essential oils by hydrodistillation for 3 h using a Clevenger-type apparatus. The oils were separated, dried over anhydrous sodium sulphate and kept in air tight sealed dark glass at 4°C until used.

GC and GC/MS analysis

The GC analyses were carried out on a Shimutzu 17A gas chromatograph and a DB-5 (non-polar and 95% dimethyl polysiloxane) capillary column (30 m x 0.25 mm; 0.25 μm film thickness). The oven temperature was held at 30°C for 3 min then programmed at 2.1 ml/min to 280°C. Other operating conditions were as follows: carrier gas He, with a flow rate of 2.1 ml/min; injector temperature 230°C; detector temperature 250°C; split ratio, 50 GC/MS analyses were performed on a Shimutzu 17A GC coupled with Shimutzu QGD5050 Mass system. The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Mass range was from *m/z* 50–450 amu.

The constituents of the oils were identified by calculation of their retention indices under temperature-programmed conditions for identification of individual *n-alkanes* (C_6–C_{24}) and the oil on DB-5 capillary column. Compounds were made by comparison of their mass spectra with those of the internal reference mass spectra library (NIST 98 and Wiley 5.0) or with authentic compounds or with those of reported in the literature (Davies 1990; Adams 2001). Quantitative data was obtained from FID area percentages without the use of correction factors.

Test pathogens

The tested fungus, *B. cinerea* was isolated from infected table grape fruits and provided by the Plant Protection Department, Urmia University. Stock cultures were maintained on Potato Dextrose Agar (PDA) slants at 4°C for sporulation. The fungus was grown on PDA medium and incubated at 25°C in the dark for 7 days. Conidia were scrapped out from agar surface and suspended in sterile distilled water and their concentration was adjusted to 10^6 conidia/ml using a haemocytometer.

Assessment of antifungal activity of essential oils in in vivo conditions

Kiwifruits were dipped in the solution of 1% sodium hypochlorite for 2 min, rinsed with distilled water, and air-dried before treatment. Fruits were inoculated with spore suspensions ($1x10^6$ spore/ml) of test pathogens and allowed to become dry. Inoculated fruits were sprayed with concentrations 0, 250, 500 and 750 μl/l of essential oil. Eight treated fruits were placed in a 1.5 l plastic box and the boxes were kept for 90 days in cold storage (0–1°C). Four replicates were used per each treatment.

Evaluation of fruit decay

At the end of storage period, disease severity on whole fruits was evaluated. The surface of fruit devised to 10 parts and percentage of decayed fruit was estimated.

Evaluation of quality sensors

Flavor analyses to compare the quality of treated and control kiwifruits were carried out by 6 trained panelists. A questionnaire was used to record the data for each treatment for the following characteristics: visual aspect (general aspect), firmness, sweetness, juiciness, sourness and crunchiness, on a 5-point scale: 1 – very low; 2 – low; 3 – medium; 4 – high and 5 – very high. Flesh firmness of all fruits was destructively measured. Firmness reading was taken by using a penetrometer (FT 327, International Ripening Company, Alfonsine, Italy) fitted with a flat-8 mm diameter tip. The tip was inserted after skin removal, at the fruit equator, in opposite sides to a depth of 7 mm. Weight loss was calculated by weighting the fruit at harvest and reweighting at the end of the storage period (90th day). Weight loss percentage was calculated as percentage loss of initial weight.

A random sample of 8 fruits was sampled per replicate, juiced, and filtered to get a clear sample. Total soluble solids content (TSS) was determined by means of digital refractometer (Atago, Tokyo, Co. Ltd, Japan) and results were expressed in ºBrix. Titrable acidity (TA) was titrated with 0.1 N sodium hydroxide (NaOH) to an end point of 8.1 and expressed as percentage of citric acid. TSS/TA was expressed as the ratio between TSS and TA. Ascorbic acid concentration (Vitamin C) was determined using the 2, 6 dichlorophenol indophenol titration method (Tian *et al.* 2002).

Statistical analysis

Statistical analyses of the data were performed with MSTATC (Michigan State University, version 4.00/EM) program (Nissen 1989) and the means were separated by Duncan's multiple range test. Statistical differences at $p < 0.05$ were considered significant.

RESULTS

Essential oil analysis

The chemical composition of essential oils used in this study is listed in table 1. The major compounds found in *T. vulgaris* oil were β-ocimene (12.62%), thymol (10.56%) and carvacrol (6.85%). The oil of *C. copticum* was particularly rich in thymol (63.18%), ϱ-cymene (21.4%) and γ-terpinene (13.8%). Trans-anethole (64.72%), fenchone (14.59%) and methyl chavicol (6.67%) were the main components of *F. vulgare*. At the same time, *S. hortensis* oil contained carvacrol (54.14%), terpinolene (20.59%) and α-phellandrene (5.31%).

Assessment of antifungal activity of essential oils on inoculated kiwifruits

Evaluation of antifungal activity of essential oils against *B. cinerea* growth in inoculated kiwifruits showed that with the increase of oil concentration disease severity in oil-treated fruits decreased (Fig. 1), but without any significant differences within different essential oils (Table 2).

Assessment potential in preservation of quality sensors

Evaluation the effectiveness of various essential oils on sensory parameters showed that TSS and TA values decreased with the increase of essential oil concentration (Figs. 2, 3), and *C. copticum* oil treated fruits showed the

Table 1. The quality and quantity (%) of tested essential oils

No.	Compound[a]	RI[b]	*T. vulgaris*	*C. copticum*	*F. vulgare*	*S. savory*
1	α-pinene	936	5.24	–	–	–
2	limonene	964	–	–	3.37	–
3	β-pinene	971	3.84	–	1.46	–
4	champhene	988	2.56	–	–	–
5	γ-terpinene	996	–	13.71	1.43	–
6	α-phellandrene	997	8.5	–	–	5.31
7	β-ocimene	1030	12.62	–	–	–
8	ϱ-cymene	1040	–	21.4	–	3.56
9	fenchone	1051	–	–	14.59	–
10	terpinolene	1075	–	–	–	20.59
11	α-terpinolene	1080	1.24	–	–	–
12	camphor	1083	–	–	1.48	–
13	linalool	1090	4.18	–	–	–
14	nerol oxide	1103	3.24	–	–	–
15	borneol	1107	2.32	–	–	–
16	trans-Anethole	1240	–	–	64.42	–
17	linalyl acetate	1253	1.32	–	–	–
18	carvacrol	1285	6.85	–	–	54.14
19	thymol	1289	10.56	63.17	–	–
20	geranilacetate	1352	2.04	–	–	–
21	α-copaene	1358	1.38	–	–	–
22	β-caryophyllene	1420	6.13	–	–	–
23	germacrene D	1462	1.94	–	–	–
24	δ-cadinene	1517	2.08	–	–	–
25	spathulenol	1550	1.71	–	–	–
26	widrol	1569	2.07	–	–	–
27	caryophyllene oxide	1586	5.7	–	–	–
28	docosane	2300	2.77	–	–	–

[a] the compounds that present lower than 1% were not showed
[b] retention indices

Table 2. Means squares for the variance of the effects of essential oils on quality parameters of treated fruits

Significance	Disease severity	Flavor	Firmness	Weight loss [%]	TSS	TA	TSS/TA	Vitamin C
EO[a]	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**
Eon[b]	*	**	n.s.	n.s.	**	*	n.s.	*
EO* Eon	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	**

[a] EO – Essential oil, [b] Eon – Essential oil concentration, TSS – Total soluble solids, TA – Titrable acidity
Treatments with an associated asterisk were statistically significantly different
(*, **, and n.s. = $p < 0.05$, 0.001, and not significant, respectively)

Table 3. Effect of different concentration of various essential oils on flavor in treated fruits

Essential oil	Flavor				Vitamin C			
	0	250	500	750	0	250	500	750 [µl/l]
T. vulgaris	3.03 ab*	3.71 a	2.52 b	1.15 cd	25.59 ab	29.09 a	17.34 a–e	4.57 efg
C. copticum	1.9 bcd	0.93 d	1.15 cd	1.27 cd	9.33 d–g	5.41 d–g	9.15 d–g	6.5 d–g
F. vulgare	2.96 ab	2.4 b	0.99 d	2.65 ab	22.52 abc	17.94 a–d	3.67 fg	16.56 a–f
S. hortensis	2.56 b	0.84 d	2.21 bc	2.15 bc	14.03 b–f	0.6 g	15.77 b–f	11.38 c–g

* values in columns followed by unlike letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$)

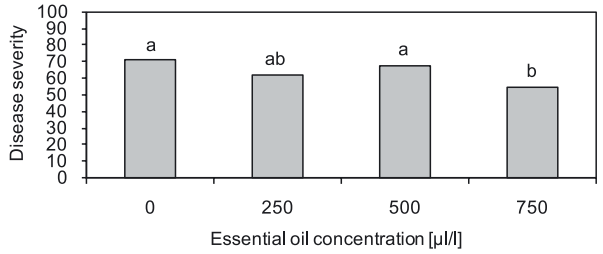

Fig. 1. Effect of different concentration of essential oils on disease severity in treated fruits.
Columns with unlike letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$)

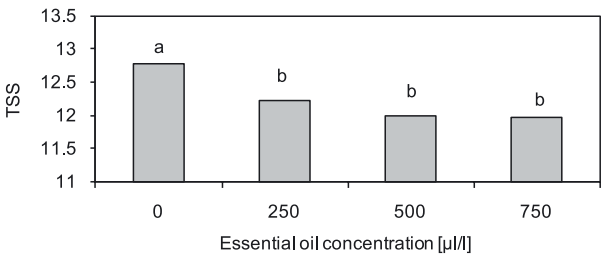

Fig. 2. Effect of different concentration of essential oils on total soluble solids (TSS) in treated fruits.
Columns with unlike letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$)

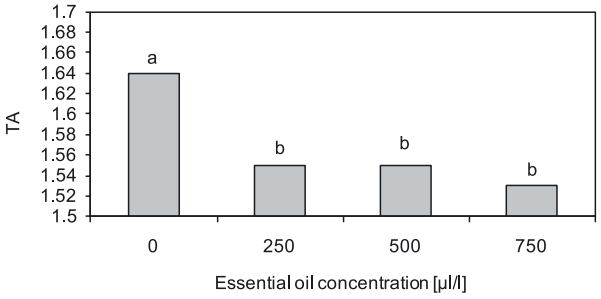

Fig. 3. Effect of different concentration of essential oils on titrable acidity (TA) in treated fruits.
Columns with unlike letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$)

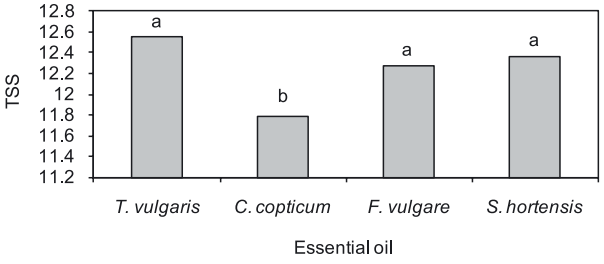

Fig. 4. Effect of different essential oils on total soluble solids (TSS) in treated fruits.
Columns with unlike letters differ significantly according to Duncan's Multiple Range Test ($p < 0.05$)

lowest TSS, but the other essential oils had no significant effect on TSS content (Fig. 4). In addition, essential oil treatment decreased flavor and vitamin C values in comparison with controls (Table 3).

DISCUSSION

Essential oils are natural mixtures of hydrocarbons and oxygen- (alcohols, aldehydes, ketones, carboxylic acids, esters, and lactones) containing organic substances of plants. Their constituents and derivatives have a long history of application as antimicrobial agents in the areas of food preservation and medicinal antimicrobial production (Voda *et al.* 2003). Biological activities of essential oils depends on the qualitative and quantitative characteristics of their components, which is affected by the plant genotype, plant chemotype, organ of plant, geographical origin, season, environmental, agronomic conditions, extraction method and storage condition of plant and essential oils (Marotti *et al.* 1992; Suhr and Nielsen 2003).

The effect of essential oils on reduction of disease severity and possibility of the use their preservative property was studied to maintain postharvest quality of kiwifruits inoculated with *B. cinerea*. The results confirmed that the tested essential oils showed antifungal activity against *B. cinerea* in the highest concentration (750 µl/l) compared to lower concentrations (250 and 500 µl/l). Generally, the results of our study were in accordance with previous study that showed origanum, sweet basil and thyme oils and had not significant effect in control of gray mold rot on inoculated kiwifruits with *B. cinerea* (Thanassoulopoulos and Yanna 1997).

The exact antimicrobial mechanism(s) action of essential oils, especially against phytopatogenic fungi is not clarified yet. Several reasons about this action of essential oils is presented: in some of reports on the antifungal activity of essential oils attributed to their most abundant components, especially phenolic compounds (Nychas 1995; Tripathi *et al.* 2009) and the major compounds detected in essential oils used in this study were thymol, carvacrol, trans-anethole and β-ocimene. The antifungal activity of

some phenolic compounds such as eugenol, thymol and carvacrol on apricot, plum, sweet cherry and table grape was investigated previously and their potential in the reduction of fungal decay was confirmed (Liu *et al.* 2002; Serrano *et al.* 2005; Valverde *et al.* 2005). Phenolic components available in essential oils that have lipophilic character act in cell wall and interfere in action of membrane catalyzed enzymes and enzymes responsible for energy and protein production, as a result cause cell death (Kalemba and Kunicka 2003). Also, the antimicrobial activity of phenolic compounds could be related to the presence of an aromatic nucleus and OH group in their structure that caused cell wall degeneration (Farag *et al.* 1989). Additionally, the antimicrobial activity of plant extracts and essential oils may be related to total constituents available in their composition and existence of synergistic correlation between total components (Daferera *et al.* 2003). It is generally recognized that the antimicrobial action of essential oils depends on their lipophilic character. Rasooli and Owlia (2005) showed that cell wall and cell membrane are the main target of *T. eriocalyx* and *T. x-porlock* essential oils against *Aspergillus parasiticus* and treatment with essential oils caused a severe damage to cell wall, cell membrane, and cellular organelles such as mitochondria.

Until now several studies were planned to evaluate the antifungal property of plant essential oils, but a few was carried out to evaluate the effect of essential oils to maintain quality parameters of fruits (Ranasinghe *et al.* 2005; Serrano *et al.* 2005; Tzortzakis 2007). Serrano *et al.* (2005) reported that sweet cherry fruits treated with essential oil constituents such as eugenol, thymol and menthol showed benefits in terms of reduced weight loss and maintenance of fruit firmness in comparison to controls. However, sweet cherries treated with eucalyptol garnered off-flavor. Some of workers stated that cinnamon and clove essential oils did not change organoleptic and physicochemical properties of banana fruits (Ranasinghe *et al.* 2005). But our work results are not accordance to previous reports and showed that the essential oils treatment had a negative effect on flavor of kiwifruits, reduced TSS, TA and vitamin C in fruits. However, firmness and weight loss values were not affected by essential oils treatment. The reason(s) of this effective/ineffective was not determined.

CONCLUSION

Results of this study showed that essential oils treatments had a low preservation and antifungal potential against *B. cinerea* on kiwifruits, thus further studies are needed to find out a better and effective method for the application of essential oils on fruits or combination of these compounds integrated with other tools and methods and screened in the future.

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POLISH SUMMARY

BADANIA NAD MOŻLIWOŚCIĄ UŻYCIA NATURALNYCH WYCIĄGÓW ROŚLINNYCH W CELU OGRANICZENIA POZBIOROWO WYSTĘPUJĄCEJ SZAREJ PLEŚNI (*BOTRYTIS CINEREA* PERS. FR.) NA OWOCACH KIWI (*ACTIVIDIA DELICIOSA*) PODCZAS MAGAZYNOWANIA

Olejki eteryczne otrzymywane poprzez hydrodestylację z tymianku (*Thymus vulgaris*), kminku koptyjskiego (*Carum copticum*), kopru (*Foeniculum vulgare*) i cząbru ogrodowego (*Satureja hortensis*), były badane *in vivo* pod względem antygrzybowej aktywności przeciwko *Botrytis cinerea* na owocach kiwi. Zaszczepione i potraktowane olejkiem owoce trzymano w przechowalni 90 dni. Ocena przeciwgrzybowej aktywności tych olejków wykazała, że wraz ze wzrostem ich stężenia wzrastała aktywność przeciwgrzybowa, lecz nie obserwowano istotnych różnic. Dodatkowo parametry jakościowe takie, jak: rozpuszczalne składniki stałe, miareczkowana kwasowość i witamina C, zmniejszały się w traktowanych olejkami owocach. Waga i jędrność nie zmniejszały się pod wpływem olejków, a traktowane nimi owoce mniej pachniały w porównaniu z kontrolą. Uzyskane wyniki sugerują, że warto podjąć dalsze badania nad aplikowaniem olejków eterycznych w celu ograniczenia pozbiorowych patogenów.