Adv. Hort. Sci., 2024 38(2): 169-176 DOI: 10.36253/ahsc-15659

AHS
Advances in Horticultural Science

The effect of thymol and carvacrol rich-plant essential oils on controlling postharvest decay molds in orange fruit

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Key words: Antifungal effect, *Satureja* spp., spoilage, *Thymus* spp.

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Citation:
MOKHTARNEJAD L., FARZANEH M., 2024 - *The effect of thymol and carvacrol rich-plant essential oils on controlling postharvest decay molds in orange fruit.* - Adv. Hort. Sci., 38(2): 169-176.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 1 December 2022
Accepted for publication 2 February 2024

Abstract: The antifungal activity of essential oils of *Thymus daenensis*, *Thymus vulgaris*, *Satureja hortensis* and *Satureja khuzistanica* as well as their major compounds were studied against mold decays of orange fruit. According to GC-MS analysis, the major compounds of *T. danensis* essential oil were thymol (65.5%) and alpha-terpinene (11.9%) whereas *T. vulgaris* was rich in thymol (59%) and p-cymene (15.6%). Carvacrol (88.4%) in *S. khuzistanica* oil and carvacrol (51%), gamma-terpinene (20.8%) and p-cymene (13.7%) in *S. hortensis* oil were charecterized as major compounds. The oil of *S. khuzistanica* and its major compound carvacrol exhibited the strongest fungicide activity against *Penicillium digitatum, Rhizopus stolonifer* and *Colletotrichum gloeosporioides* at 300 µL/L. The results on orange fruits exhibited that the use of *S. khuzistanica* and *S. hortensis* EOs as spraying and dipping treatments could considerably reduce spoilages decays in the fruit.

1. Introduction

Post-harvest diseases of fruits are mainly caused by fungal species such as *Botrytis* spp., *Colletotrichum* spp., *Aspergillus* spp., *Alternaria* spp., *Rhizopus* spp. and *Penicillium* spp. (Agrios, 2005). The fruit decay caused by post-harvest diseases is usually more than what is thought, because with the decrease in yield the price of damaged fruits (Wills and Golding, 2016). Citrus fruits, especially oranges, are among the fruits that are highly sensitive to fungal infections. The use of fungicides, such as benomyl, thiabendazole and imazalil, is the most common method of controlling post-harvest decays of citrus fruits. These fungicides have health and environmental problems such as cumulative and carcinogenic properties in living organisms and acute or chronic poisoning effects. In addition, resistance to these fungicides is increasing in the population of pathogens (Sharifi-Tehrani and Farzaneh, 2018). Anyway, the increase in global demand for providing sufficient and healthy food, based on health standards, along with the policies of the World Food and Agriculture

Organization (FAO) and the Environmental Protection Organization (EPO) has caused extensive research to be carried out. According to the Food and Drug Administration (FDA), the essential oils (EOs) of some medicinal plants are known as natural and healthy alternatives to chemical fungicides and are more acceptable to the public (Brun *et al*., 2003; Carvalho de Sousa *et al*., 2004; Nazzaro *et al*., 2017).

EOs are volatile and natural complex compounds that are characterized by their sharp and strong smell and are formed as secondary metabolites in aromatic plants. Some EOs that have antiseptic properties (antibacterial, antiviral and antifungal properties) are used in food and pharmaceutical industries (Burt, 2004; Bolouri *et al*., 2022). In nature, EOs play an important role in protecting plants against bacteria, viruses, fungi and insects (Regnault-Roger *et al*., 2012; Zitzelsberger and Buchbauer, 2015). They may also attract a number of insects to disperse pollen and seeds (Bakkali *et al*., 2008). Medicinal plant EOs not only have no side effects (at the right concentration), but due to their antioxidant properties, may increase the quality and storage time of fruits (Arras and Usai, 2001; Anthony *et al*., 2003; Plotto *et al*., 2003; Plaza *et al*., 2004). Research has shown that aromatic plants belonging to the Lamiaceae and Asteraceae families are rich in antimicrobial and antioxidant compounds (Barroso and Ruberto, 1998; Farzaneh *et al*., 2006 a, b; Farzaneh *et al*., 2015). The antifungal property of EOs is also related to some of their compounds such as carvacrol, menthol, cymene, thymol, cinnamaldehyde, eugenol, pinene, and linalool, which are known as compounds with high antifungal effect (Cimanga *et al*., 2002).

The purpose of this research is to investigate the potential of EOs of plants rich in thymol and carvacrol, such as *Thymus danensis*, *Thymus vulgaris*, *Satureja hortensis*, and *Satureja khuzistanica* in preventing rot and decay of orange fruit caused by *Colletotrichum gloeosporioides*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium digitatum*.

2. Materials and Methods

Inoculum preparation of pathogens

Four fungi that cause post-harvest decay of orange fruit, including *C. gloeosporioides*, *A. niger*, *R. stolonifer,* and *P. digitatum*, were obtained from the mycology collection of the Department of Plant protection, Agriculture and Natural Resources Campus, University of Tehran. In order to prepare the pathogen inoculum, 5 mL of distilled sterile water containing 0.05% Tween 80 was added to the seven-day old culture of each fungus on PDA medium and the surface of the colony was scraped to provide spores and mycelia suspension. The resulting suspensions were passed through four-layer cheesecloth, and then the spore population was adjusted to a concentration of $1*10^5$ spores per milliliter using a hemacytometer.

Plant EOs and their major compounds

The aerial parts of tow thyme species, *T. danensis* and, *Thymus vulgaris,* at the flowering stage were collected from Semirom region of Isfahan province, while the aerial parts of two savory species, *S. hortensis* and *S. khuzistanica* were collected from Pol-Dokhtar and Majin regions of Lorestan province, respectively. The collected plant parts were delivered to the Medicinal Plants and Drugs Research Institute (MPDRI), Shahid Beheshti University (SBU) in Tehran. After confirming the identity, the plants were dried at room temperature and shade. Each sample was powdered using a mill, and then their EOs was extracted by distillation with water in a Clevenger according to the method recommended in the British Pharmacopoeia (1988). The standard compounds of thymol, carvacrol, para-cymene and gamma-terpinene were purchased from Sigma-Aldrich Co.

Analysis and identification of EOs compounds

The EO obtained from each plant was identified with gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS) methods. First, one microliter of EO extracted from each plant was injected into the TRACE™ GC 2000 gas chromatograph (ThermoQuest Italia S.p.A., Rodano, Milan, Italy) with a flame ionization detector (FID) and fused silica capillary DB-1 column (60 m × 0.25 mmi.d.; film thickness= 0.25 µm). Injector and detector temperatures were 250˚C and 300˚C, respectively. Helium was used as the carrier gas at a flow rate of 1.1 ml/min; oven temperature was programmed from 60˚C to 250˚C at the rate of 4˚C/min, and finally held isothermally for 10 min. GC-MS analysis was also performed by using a ThermoQuest Finnigan Trace GC/MS (ThermoQuest Italia S.p.A., Rodano, Milan, Italy), equipped with a DB-1 column (60 m × 0.25 mmi.d.; film thickness= 0.25 µm). Gas chromatographic conditions and the thermal programming were as given for GC. Helium was used as carrier gas

with ionization voltage of 70 ev. Ion source and interface temperatures were 200˚C and 250˚C, respectively. Mass range was from *m/z* 43-456. Identification of individual compounds was done by comparison of their mass spectra with those of similar compounds from a database (Wiley/NBS library) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature. The percentage of each compound was determined according to its relative area percentages obtained by FID, without using correction factors (Adams, 2007).

In vitro antifungal assay

The main ingredients of EOs, including thymol, carvacrol, paracymene and gamma terpinene, were obtained from the Phytochemistry Department of MPDRI. Antifungal effect of EOs and main compounds was investigated against four post-harvest decay fungi of fruit by mixing EO with PDA solid culture medium (Farzaneh *et al*., 2006 b). In short, Petri dishes containing concentrations of 75, 150, 300, 600 and 1200 microliters of EO/standard major -compound per liter of culture medium were prepared and after placing a fungal disk (with a diameter of 5 mm) in center of Petri dishes, they were kept at a temperature of 25 °C in darkness. The growth of each fungus colony was measured daily until the surface of control Petri dishes was completely occupied by the fungus. The percentage of growth inhibition was calculated. The minimum inhibitory concentration (MIC) of the EOs was calculated to prevent the growth of fungi. To investigate whether the EO shows fungicidal or fungistatic activity, the fungal disk of the treatments without fungal growth, was re-cultured on the PDA culture medium, and the growth or not growth of the fungus on the PDA was investigated after one week to calculate the minimum fungicidal concentration (MFC). In addition, the EC_{50} value (effective concentration causing 50% inhibition of mycelial growth) was calculated from the data by probit analysis.

Antifungal assay on orange fruit

The healthy orange fruits (Thomson cultivar) free of any chemical and physiological treatment and same in size and ripeness index were provided from Citrus and Subtropical Fruits Research Center, Ramsar, Mazandaran province, Iran. After disinfecting the fruits surface by 70% ethanol for one minute, a wound of 1 mm in diameter and 2 mm in depth (limited to the albedo part in the equatorial region of the fruit) was created on each fruit in sterile condition. Then the fruits were treated with the concentration of 1/1000 (1000 ppm) EO by two methods; dipping and spraying. Then treated fruits were inoculated by spraying of a suspension of 1×10^5 spores per milliliter. Control treatments included dipping and spraying of fruits with Tween 80 solution (0.05%) and thiabendazole fungicide. In this experiment, each treatment contained of 4 replicates and each replicate consisted of 8 experimental units (fruits). The surface of treated fruits was dried under air flow for 2 h and then they arranged on special fiber plates before transferring to a storage room of 25°C and darkness. After the storage period (10 days), the diameter of the decay area on orange fruit was measured using a caliper. The efficacy of the EOs was determined by the formula: IP =(C–T/C)*100), where IP is the inhibitory percentage of the of spoilage decay, and C and T are the spoilage decay area in control and treatment, respectively.

Statistical analysis

To analyze the data, three software were used. At first, the normality of the data was normalized by Mini-tab software Version 17.1 (Minitab Inc. state college, PA). Then SAS software Version 9.1.3 (SAS Institute Inc., Cary, NC) with GLM method was used for variance analysis. After analysis the variance, the mean of the data was compared using Duncan's multi-range test at the 5% level. The EC_{50} values were calculated from the data subjected to probit analysis using IBM SPSS statistics Version 26 (IBM Corp. Chicago, IL).

3. Results

The main compounds of EOs

The main compounds in *T. danensis* EO included thymol (65.5%), alpha-terpinene (11.9%) and paracymene (7.5%) (Table 1). Thymol (59%), paracymene (15.6%) and gamma-terpinene (4.2%) were the main compounds identified in the EO of *T. vulgaris* (Table 1). The main compounds in the EO of the *S. khuzistanica* included carvacrol (88.4%), para-cymene (3%) and gamma-terpinene (4.5%). Carvacrol (51%), gamma-terpinene (20.8%) and para-cymene (13.7%) were the main compounds identified in the EO of the *S. hortensis* species (Table 2).

Table 1 - The major constituents (%) of chemical composition of *Thymus daenensis* and *T. vulgaris* essential oils

No.	Compound	Retention indices	*T. daenenis* (%)	*T. vulgaris* (%)
1	Alpha-thujene	925	0.8	1.8
2	Alpha-pinene	933	1.6	1.6
3	Beta-pinene	974	0.7	2.6
4	Myrcene	981	1.1	1.9
5	Alpha-phelandrene	999	0.2	1.7
6	Para-cymene	1014	7.5	15.6
7	Gamma-terpinene	1053	-	4.2
8	Alpha-terpinene	1080	11.9	-
9	Thymol	1266	65.5	59.0
10	Carvacrol	1282	0.1	3.1
11	Carvacryl acetate	1345	2.5	2.0
12	Beta-caryophyllene	1424	3.8	1.5
13	Beta-bisabolene	1501	1.3	0.9
Total		-	97.0	95.9

* Retention indices relative to C6-C24 n-alkanes on the DB-1 column.

Table 2 - The major constituents (%) of the chemical composition of *Satureja khuzistanica* and *Satureja hortensis* essential oils

No.	Compound	Retention indices	S. Khuzistanica	S. Hortensis (%)
1	Alpha-thujene	925	-	2.5
2	Alpha-pinene	933	-	2.9
3	Beta-pinene	974	0.2	1.1
4	Myrcene	981	0.2	1.5
5	Para-cymene	1014	3.0	13.7
6	1.8-cineole	1023	0.7	1.0
7	Gamma-terpinene	1053	4.5	20.8
8	Carvacrol	1282	88.4	51.0
9	Carvacryl acetate	1345	0.1	1.3
Total		-	97.1	96.7

* Retention indices relative to C6-C24 n-alkanes on the DB-1 column.

Antifungal effect of EOs

The results of the antifungal effect of the EOs on the growth of fungi are shown in Table 3. In general, the more the concentration of EO increased, the more antifungal activity was seen. In addition, the intensity of the EOs inhibitory effects against *C. gloeosporioides* and *R. stolonifer* was more evident (Table 3).

According to the results (Table 3), for controlling the *A. niger* growth, only the EO of *S. khuzistanica* showed the highest antifungal activity with the MIC 300 µL/L. To inhibit the growth of *P. digitatum,* all EOs showed significant antifungal activity with the

Table 3 - The inhibitory activity (%) of four plant essential oils at different concentrations against spoilage fungi of citrus fruit by poisonous PDA medium method

Essential oil	concentration (µl/l)	Inhibitory activity (%)			
		A. niger	*P. digitatum*	*C. gloeosporioides*	*R. stolonifer*
T. daenensis	75	22.16	34.20	11.13	24.96
	150	57.35	56.33	34.20	96.05
	300	90.11	85.71	100	100
	600	100	100	100	100
	1200	100	100	100	100
T. vulgaris	75	20.94	27.11	3.38	0.60
	150	65.45	55.55	31.77	90.22
	300	75.01	73.34	82.29	100
	600	100	100	100	100
	1200	100	100	100	100
S. hortensis	75	16.22	28.92	2.49	14.25
	150	27.94	39.11	58.33	51.55
	300	88.32	78.92	100	100
	600	100	100	100	100
	1200	100	100	100	100
S. khuzistanica	75	24.10	11.50	26.32	25.81
	150	67.91	60.00	80.77	96.26
	300	100	90.66	100	100
	600	100	100	100	100
	1200	100	100	100	100

MIC 600 µL/L., whereas the EOs of three species, including *S. hortensis*, *S. khuzistanica* and *T. danensis* showed the great antifungal activity against *C. gloeosporioides* with the MIC 300 µL/L. To inhibit *R. stolonifer*, all four EOs with the MIC 300 µL/L showed the noticeable antifungal activity.

The results (Table 4) obtained from the re-culture of fungal disks, in the treatments which no fungal growth was observed, showed that none of the EOs had fungicide properties on the *A. niger*. Two EOs of *S. khuzistanica* and *S. hortensis* at a concentration of 1200 µL/L showed fungicidal properties against *P. digitatum,* whereas *T. daenensis* and *T. vulgaris* EOs showed the MFC values more than 1200 µL/L.

Essential oil of *S. khuzistanica* showed MFC against *C. gloeosporioides* at MFC 600 µL/L, while *S. hortensis* and *T. danensis* EOs exhibited MFC of 1200 µL/L of culture medium. Howevere, *T. daenensis* oil didn't show MFC value at the maximum concentration. To control of *R. stolonifer*, the EOs of *S. khuzistanica* and *S. hortensis* showed MFC at the concentrations 300 µL/L and 600 µL/L, respectively, while both *T. vulgaris* and *T. danensis* EOs exhibited MFC at the concentration of 1200 µL/L (Table 4).

Table 4 - Minimum fungicidal concentration (µl/l) of four essential oil against citrus fruit spoilage fungi. The experiments were carried out *in vitro* by Poisonous PDA Medium method

Fungi	*S. khusiztanica*	*S. hortensis*	*T. danensis*	*T. vulgaris*
A. niger	>1200	>1200	>1200	>1200
P. digitatum	1200	1200	>1200	>1200
C. gloeosporioides	600	1200	1200	>1200
R. stolonifer	300	600	1200	1200

Antifungal properties of the main components of EOs

In general, by increasing the concentration of the EO/standard main-component its antifungal activity increased (Table 5).

Among the main compounds, carvacrol exhibited the highest antifungal activity. The MIC of carvacrol against the growth of *R. stolonifer* was 150 µL/L. Carvacrol at a concentration of 300 µL/L prevented the growth of other fungi as well. Thymol was another main compound in the EOs, especially thyme, which showed considerable antifungal activity. Thymol at the MIC of 300 µL/L completely prevents

Table 5 - The inhibitory activity (%) of four major compounds of essential oils at different concentrations against spoilage fungi of citrus fruit by poisonous PDA medium method. The percentage of inhibition in each treatment corresponds to 4 repetitions (4 Petri dishes with a diameter of 8 cm)

Compound	Concentration (µl/l)	Inhibitory activity (%)			
		A. niger	*P. digitatum*	*C. gloeosporioides*	*R. stolonifer*
Thymol	75	10.20	6.70	14.65	14.50
	150	55.48	55.75	66.30	58.86
	300	95.34	100	100	100
	600	100	100	100	100
	1200	100	100	100	100
Carvacrol	75	22.71	29.63	31.88	36.40
	150	64.95	65.50	88.84	100
	300	100	100	100	100
	600	100	100	100	100
	1200	100	100	100	100
Para-cymene	75	0	0	0	0
	150	30.87	28.44	36.50	33.96
	300	61.54	55.89	69.74	62.94
	600	93.38	94.61	100	100
	1200	100	100	100	100
Gamma-terpinene	75	0	0	0	4.69
	150	19.85	15.32	26.58	27.63
	300	63.35	88.84	89.12	85.38
	600	96.48	100	100	100
	1200	100	100	100	100

the growth of three fungi; *C. gloeosporioides*, *R. stolonifera,* and *P. digitatum* whereas the growth rate of *A. niger* was inhibited by 95.3%. Para-cymene had also showed antifungal activity that was able to inhibit the growth of all the fungi at the concentration of 1200 µL/L. Among the fungi, *R. stolonifer* and *C. gloeosporioides* were more sensitive to para-cymene and their growth was completely inhibited at the concentration of 600 µL/L. It is also necessary to mention that this compound did not show any significant antifungal effect against any of the fungi at the low concentrations (<150 µL/L). Gamma-terpinen is one of the main components of EOs, especially in the savory plants that at the MIC concentration of 600 µL/L caused a complete inhibition of the growth of all fungi except *A. niger.* In the other hand, the fungus *A. niger* was the most resistant fungus to this compound, whose MIC was 1200 µL/L. Low concentrations of this compound did not show the inhibitory effect on the growth of the fungi (Table 5).

The results of the MFC indicated that gamma terpinene at any of the concentrations did not cause the death of the fungi. In addition, carvacrol and thymol showed strongest fungicidal activity with MFC 600 µl/l against *R. stolonifer*. Carvacrol also exhibited strong fungicidal activity (MFC 600 µl/l) against *C. gloeosporioides.* However, *A. niger* had the highest resistance to the compounds, and its MFC value was often more than 1200 µl/l (Table 6).

In addition, the antifungal potency of each EO and its main compound was determined according to EC_{50} value as well (Table 7). The lower the EC_{50} indicates the less the concentration of antifungal compound that is required to inhibit 50% of fungal growth. In general, the lowest EC_{50} values were achieved by *S. khuzistanica* EO that showed EC_{50} values of 95.14, 108.00, and 120.93 µL/L against *R. stolonifer, C. gloeosporioides*, and *A. niger*, respectively. In confirmation of it, carvacrol showed the lowest EC_{50} values of 80-124 µL/L against four citrus fruit spoilage fungi.

Table 6 - Minimum Fungicidal Concentration (µl/l) of major compounds of essential oils; thymol, carvacrol, para-cymene and gamma-terpinene; against spoilage fungi of citrus fruit. The experiments were carried out *in vitro* by poisonous PDA medium method

Fungi	Thymol	Carvacrol	Para-cymene	Gamma-terpinene
A. niger	>1200	>1200	>1200	>1200
P. digitatum	1200	1200	>1200	>1200
C. gloeosporioides	1200	600	>1200	1200
R. stolonifer	600	600	1200	>1200

Spoilage decay control on fruit

In general, the application of EOs by dipping method showed the greatest effect in reducing spoilage and fruit rot, whereas the spraying method also had significant effect. In addition, *S. khuzistanica* essential oil was the most effective oil to reduce *A. niger* (95.4%) and *P. digitatum* (86.8%) decays area on the fruit in dipping method. The EO of *S. khuzistanica* had the greatest effect against *R. stolonifer* and *C. gloeosporioides* decays on the fruits by both methods of dipping and spraying of the fruit which could completely (100%) inhibit the both decays

Table 7 - The EC_{50} value (effective concentration causing 50% inhibition of mycelial growth) of each essential oil and its major compounds against spoilage fungi of citrus fruit on PDA (µL/L) calculated by probit analysis

Essential oil/compound	*A. niger*	*P. digitatum*	*C. gloeosporioides*	*R. stolonifer*
T. danensis	154.40 (118.69-197.18) [z]	151.86 (97.52-219.75)	164.02 (151.83-178.53)	95.84 (88.81-103.094)
T. vulgaris	172.02 (80.34-324.16)	179.65 (108.88-283.32)	210.16 (194.97-226.88)	119.23 (111.86-125.91)
S. hortensis	191.92 (176.59-209.02)	186.28 (136.57-256.56)	141.95 (134.20-150.60)	143.68 (132.68-156.79)
S. khusiztanica	120.93 (111.04-131.95)	160.21 (118.11-213.54)	108.00 (99.28-117.03)	95.14 (88.11-102.40)
Thymol	156.31 (131.03-187.02)	143.14 (133.79-154.54)	128.59 (119.27-139.13)	135.97(125.77-147.96)
Carvacrol	124.70 (114.56-136.16)	119.49 (109.03-131.12)	97.53 (89.30-105.78)	80.92 (78.78-83.06)
Para-cymene	289.02 (191.82-459.22)	230.03 (171.91-329.54)	296.60 (213.73-432.76)	245.27 (183.04-365.97)
Gamma-terpinene	284.61 (213.66-403.15)	220.44 (206.91-234.68)	207.22 (193.60-221.86)	207.85 (193.04-224.10)

[z] Numbers in parentheses indicate 95% confidence limits determined by probit analysis.

(Table 8). In addition, *S. hortensis* could completely inhibit *R. stolonifera* decay. However, *T. danensis* and *T. vulgaris* couldn't completely inhibit of the any fruit fungal decay and exhibited weak fungicide activity on the fruit. In addition, fungicide tiabendazole could completely control *R. stolonifera* decay. It seems that *P. digitatum* and *A. niger* are the most resistance fungi to these EOs on the orange fruit.

4. Discussion and Conclusions

In our study, all four plants EOs (belong to Thymus and Satureja geniuses, Lamiaceae) exhibited considerable antifungal activity against postharvest spoilage fungi. It has been found that some medicinal plants of the Lamiaceae family have high antifungal properties (Bakkali *et al*., 2008; Adeyinka and Richard, 2015). Thymol was included the main part (more than 50%) of *T. danensis* and *T. vulgaris* EOs whereas S. *khuzistanica and S. hortensis* EOs were rich in carvacrol (more than 50%). In addition, their major compounds and specially thymol and carvacrol resulted in strong fungictatic and fungicide activities. However, the lowest MFC and EC_{50} values were obtained by *S. khuzistanica* oil and carvacrol. The antibacterial and antimicrobial properties of the main components of EOs such as cinnamaldehyde, eugenol, thymol and carvacrol have been identified in several studies (Bakkali *et al*., 2008; Adeyinka and Richard, 2015). The antimicrobial and antifungal activity of the EO may be due to the characteristics of terpenes/terpenoids compounds, which, due to their high lipophilic nature and low molecular weight, that enable them destroying cell membranes, and inhibiting spore germination (Bakkali *et al*., 2008; Nazzaro *et al*., 2017). However, the dominant composition of the EO may cause the antifungal activity of the EO alone or in synergic manner with other compounds (Plotto *et al*., 2003). Therefore, in our study, the antifungal property of these EOs can be contributed to their thymol or carvacrol content, although other EO constitutes may act synergistically and increase the antifungal activity of the main compound. Research has shown that aromatic plants belonging to the families *Lamiaceae* and *Asteraceae* are rich in antimicrobial and antioxidants compounds and increase the quality of the fruit and the length of its storage period as well (Tajkarimi *et al*., 2010; Hyldgaard *et al*., 2012; Gyawali and Ibrahim, 2014). In addition, EOs could control post-harvest diseases due to their antifungal effects on the both vapor and non-vapor phases (Tripathi *et al*., 2008).

In our study, the application of EOs by dipping method showed the more fungicide activity than the spraying method in terms of reducing spoilage and fruit rot. None of the four EOs and their dominant compounds at the maximum concentration studied in this research (1200 µl/l) could completely controlled *A. niger in vitro* and on fruit conditions, which indicates the high tolerance of this fungus to EO compounds. In addition, although both savory oils could completely kill *P. digitatum* by 1200 µl/l *in vitro*, they couldn't completely inhibit the *P. digitatum* decay on fruit. On the other hand, the sensitivity of *P. digitatum* to EO would be reduced on fruit. However, savory oils could completely inhibit *R. stolonifer* and *C. gloeosporioides* decays on fruit.

Although, the significant *in vitro* antifungal activity of the EOs studied in this research depended on the content of carvacrol and thymol, the EOs of both

Table 8 - The control of orange fruit fungal decays by four medicinal plants essential oils (1 per 1000) trough spraying and dipping methods, after 10 days' incubation in the dark condition at 25°C.

Treatment	Disease incidence (%)							
	Spraying method				Dipping method			
	A.n	P.d	C.c	R.s	A.n	P.d	C.c	R.s
T. daenensis	25.8 c*	38.8 b	9.4 ef	11.0 e	16.3 d	17.6 d	4.5 fg	7.2 f
T. vulgaris	25.4 c	33.5 b	13.7 de	12.4 de	16.1 d	25.4 c	5.0 fg	6.6 f
S. hortensis	9.6 ef	27.2 c	0.0 g	3.3 fg	7.2 f	16.3 d	0.0 g	0.0 g
S. khuzistanica	7.2 f	22.0 c	0.0 g	0.0 g	4.5 fg	12.5 de	0.0 g	0.0 g
Tiabendazole	7.6 f	21.5 c	3.8 fg	3.3 fg	3.5 fg	7.8 f	3.3 fg	0.0 g
Infected Control	97.5 a	97.5 a	95.0 a	95.0 a	97.5 a	95.0 a	95.0 a	95.0 a

An= *Aspergilus niger*; Pd= *Penicillium digitatum*; Cc= *Colletotrichum gloeosporioides*; Rs= *Rhizopus stolonifer*.
Means followed by the same letter within a column are not significantly different at *P≤0.05*.

savory species (rich in carvacrol) were more effective than thyme species oils (rich in thymol) in terms of controlling fungal decays on fruit. Finally, plant EOs rich in carvacrol are introduced as promising candidates for the commercial production of natural fungicides to disinfection and management of post-harvest decay molds of citrus fruits.

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