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Influence of Summer Savory Essential Oil (*Satureja hortensis*) on Decay of Strawberry and Grape

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Abstract: The essential oil isolated from the aerial parts of wild Turkish *Satureja hortensis* L. (summer savory) was analyzed by GC-MS, and carvacrol (54.7 %), γ-terpinene (20.9 %), p-cymene (12.3 %), α-terpinene (2.0 %), and thymol (2.0 %) were found to be major components of the oil. Post-harvest decay of fruits caused by some fungi is still a most important problem and causes major crop losses during storage and shipment. High relative humidity during storage and marketing conditions accelerate development of fungal disease and decay of the grapes. The six different concentrations of the oil (0.01, 0.02, 0.04, 0.11, 0.22 and 0.45 μL/cm^3) were tested for their effectiveness in reducing decay of strawberry and grape fruits at three storage temperature (5, 10 and 20°C). The oil tested reduced decay of strawberry and grape fruits compared to controls, in particularly at low temperatures (5 and 10°C). Decay reducing effect of the oil on strawberry and grape fruits increased with increase in doses of the oil. However, microbial development in the fruits increased with increase in temperature. Nevertheless, the present results showed that *S. hortensis* essential oil has a significant reducing effect on the decay of strawberry and grape fruits during storage at low temperatures. Therefore, the essential oil of summer savory may be a potential source of alternative fungicides to protect strawberry and grape fruits as well as other stored products from pathogens and saprophytes.

Key words: *Satureja hortensis;* essential oil; strawberry; grape; fruit decay; deterioration.

Introduction: Recently, the possible use of natural compounds may be resulted to minimize our dependency on present chemicals to reduce decay and post-harvest losses of fruits and vegetables. Post-harvest decay of strawberry fruits caused by some fungi is still a most important problem and causes major crop losses during storage and shipment. Control of fungal growth in storage products during storage can be achieved by physical and chemical methods. Often used methods to reduce the

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fungal development in strawberry fruits are to exposure high CO_2 and to store at low temperatures.

However, prolonged exposure to high CO_2 can cause off strawberry fruits. Nevertheless, low temperature storage alone could be not adequate for prolonged storage life required the service distant markets [1]. The use of fungicides is the most effective method in reducing post-harvest diseases in strawberries and grapes [2-4], however it is associated with the chemical residues in the products [1,5,6]. Furthermore, the development of resistance of post-harvest pathogens to chemical fungicides is another disadvantage of fungicide usage [5,7]. The grape is a non-climacteric fruit which shows severe problems during post-harvest handling, storage and marketing. As many fruits, grapes have a relatively low pH and thus very sensitive to microbial disease, especially fungal disease [8]. In particular, high relative humidity during storage and marketing conditions accelerate development of fungal disease and decay of the grapes. Over recent decades, producers have used synthetic fungicides to solve this problem. The most common synthetic fungicide is SO_2. However, the high concentrations of SO_2 can affects product quality inducing bleaching, accelerated water loss, browning and possible sulphite residue [9,10].

On the other hand, it has been estimated that over 23 million kg of synthetic fungicides have been used annually worldwide and also generally accepted that production and marketing of fruit and vegetables can not be possible without use of synthetic chemicals [10-12]. Furthermore, there are an increasing consumer concern against the usage of the synthetic chemicals due to their possible carcinogenic effects, residual toxicity for mammalians, environmental pollutions and occurrence of microbial resistance [5-7]. Thus, in recent years there is an increasing interest in the food industry to search for new strategies as alternative control agents to control fruit post-harvest decay [1,3,10,12, 13,14].

Satureja hortensis L. (summer savory) is a well known aromatic and medicinal plant, which is widely distributed in the Anatolia. Its leaves, flowers and stems are frequently used as tea or additives in commercial spice mixtures for many foods to offer aroma and flavor. It is known as "sater", "anik", "koç otu" and "anug" names by Anatolian people [15]. *S. hortensis* has also been traditionally used in the treatment of various ailments including craps, muscle pains, nausea, diarrhea and digestive and infectious diseases [15]. It has also shown antispasmodic, antidiarrheal, antioxidant, sedative and antimicrobial properties [16-18]. Recently, it has been shown that essential oils extracted from the species belonging to *Satureja*, *Thymus* and *Origanum* genus and their aromatic monoterpene constituents, thymol and carvacrol possess at a broad spectrum of potent antibacterial and antifungal activities [1,10,14,19-26]. Therefore, we investigated the chemical composition of the essential oil of Turkish *Satureja hortensis* and its potential use for its effect on the post-harvest life of strawberry and grape fruits.

Experimental

Plant material and isolation of essential oil: The aerial parts of wild *Satureja hortensis* L. were collected from Gaziler valley of Senkaya in the Eastern Anatolia region of Turkey in July 2008 at full flowering stage. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist, S. Kordali, in the Department of Plant Protection, Atatürk University, Erzurum (Turkey). The voucher specimen (ATA-9833) has been deposited in the Biotechnology Research and Application Centre at Atatürk University, Erzurum (Turkey). Collected plant materials were dried in shade and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation (plant material in boiling water) using a Clevenger-type apparatus for 4 hours. Hydrodistillation of *S. hortensis* yielded 2.3 % (v/w) of essential oil. The yield were based on dry material of plant sample.

GC analysis conditions: The analysis of the essential oils was performed using a Thermofinnigan Trace GC/A1300, (E.I) equipped with a SGE/BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 μm). Helium was the carrier gas, at a flow rate of 1 ml/min. Injector temperature was set at 220°C. The programme used was 50-150°C at a rate of 3°C /min, held isothermal for 10 minutes and finally raised to 250°C at 10°C /min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μl were

injected manually and in the splitless mode. Quantitative data of the oils was obtained from FID area percentage data (Table 1).

GC-MS analysis conditions: The analysis of the essential oil was performed with a Thermofinnigan Trace GC/Trace DSQ /A1300 at Atatürk University in Erzurum city of Turkey, (E.I Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 °C and 290°C, respectively. The oven temperature was programmed from 50°C to 150°C at 3°C/min, then held isothermal for 10 min and finally raised to 250 ºC at 10 ºC/ min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μL were injected manually in the splitless mode. The relative percentage of the oil constituents was expressed as percentages by FID peak area normalization.

The identification of the major compounds was based on comparison of their relative retention times with those of authentic samples on SGE-BPX5 capillary column, and by matching of their mass spectra of peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data [27].

Treatments of strawberry and grape fruits with essential oil under storage conditions: The experiments were carried out using the method of Tzortzakis [13]. Strawberry and grape fruits used in the experiments were purchased from local markets in Erzurum (Turkey). The ripe strawberry fruits of uniform size, free of physical damage and fungal decay were selected [12]. The ripe grape clusters (150-170 g) were selected to obtain homogeneous batches based on color, size, absence of injuries and healthy, greenish rachises. Ten strawberry berries and one grape cluster were separately placed into one polystyrene container (9x10x12 cm) with snap-on lids.

Six different concentrations of the essential oil (5, 10, 20, 50, 100 and 200 μL) dripped on to a filter paper (3 x 3 cm^2) were placed into individual small beakers, which were subsequently placed inside the plastic containers just before the lids were covered. The essential oil concentrations in the plastic containers (450 cm^3) for each experiment were 0.01, 0.02, 0.04, 0.11, 0.22 and 0.45 μL/cm^3, respectively. The containers were then transferred to storage at 5, 10 and 20°C in 70 % relative humidity under a photoperiod of 12-h light and 12-h dark under storage conditions. Control samples were handled similarly with the exception of the volatile treatments. Strawberry fruits and grape clusters exposed to "ambient air" were used control. All experiments were arranged in a completely randomized design with three replications including controls. The severity of decay was visually evaluated for visible fungal pathogens for each treatment after 26 th days for strawberry, and after 30 th days for grape exposure to the essential oil vapors at 5, 10 and 20 °C.

Decay evaluation: The decay severity were rated using a scale of 1 to 5, where 1-clean, 2-trace decay (about 25 %), 3-moderate decay (about 50 %), 4-severe decay (about 75 %), and 5-extensive decay (about 100 %).

Statistical analysis: Data obtained from the experiments were subjected to analysis of variance (ANOVA) using SPSS 10.0 software package. Significant differences between mean values were tested through Duncan Multiple Range Test (p=0.05).

Results and discussion: The essential oil isolated from the aerial parts of Turkish *S. hortensis* was analyzed by GC-MS, carvacrol (54.7 %), γ-terpinene (20.9 %), p-cymene (12.3 %), α-terpinene (2.0 %) and thymol (2.0 %) were found to be major components of the oil (Table 1). As can be seen

from Table 1, it was characterized by high content of aromatic monoterpenes such as carvacrol, thymol, p-cymene and thymol acetate, comprises 69.1 % of the total oil. It has been shown that the essential oils of *S. hortensis* growing in Turkey and other regions of the world are rich in carvacrol, thymol, γ-terpinene and p-cymene [18,28-34]. However, *S. hortensis* from different regions of the world are divided into "carvacrol type" and "thymol type". In general, relatively higher amount of carvacrol than thymol have been found in some essential oil of *S. hortensis* from different localities in Turkey and world [29-32,34]. In accordance with these findings, as shown in Table 1, the essential oil of *S. hortensis* contain relatively higher amount of carvacrol (54.7 %) than thymol (2.0 %). However, it has been documented that some chemotypes of this species from different localities in Turkey contained mainly thymol [18,28,33]. As addressed before, essential oil content may be affected by local, climatic and seasonal factors. For example, severe water stress was reported to alter carvacrol/γ-terpinene contents [35].

The decay rating of strawberry and grape fruits treated with the essential oil from *S. hortensis* were given in Table 2. As shown in Table 2, applied high doses of the oil completely inhibited the microbial growth on the strawberry fruits at 5 and 10°C after 26 days of treatment as compared with control (Fig. 1). Furthermore, the similar results were obtained for grape fruits. The decay of the grape fruits was significantly reduced by the 0.11, 0.22 and 0.45 μL/cm^3 of the oil at low temperatures (5°C and 10°C) as compared with the control. The highest doses of the oil (0.45 μL/cm^3) reduced decay of grape fruits after 30 th days of exposure at all storage temperature (Fig. 2). However, at 20°C, the other concentrations of the oil were not effective on the decay of grape fruits in comparison to low storage temperature.

The oil reduced fruit decay development of both strawberry and grape fruits compared to controls, in particular high concentrations (0.22 and 0.45 μL/cm^3 oil) and low temperatures (5 and 10°C). In general, effect of the oil on decay development of strawberry and grape fruits increased with increase in doses of the oil. However, fungal development in the fruits increased with increase in temperature and treatment days.

Fig. 1. Decayed strawberry fruits (left) that was not exposured to the essential oil vapor, and not decayed strawberry fruits (right) that was exposured the vapor of the oil (0.45 μl/ml) and stored at +5°C throughout 26 days

Fig. 2. decayed grape fruits (left) that was not exposured to the essential oil vapor, and not decayed grape fruits (right) that was exposured the vapor of the oil (0.45 µl/ml oil) at +5°C and stored at +5°C throughout 30 days

Consumers demand safe products, containing less or no synthetic chemicals as a mean of food preservation and food-borne disease. However, there is increasing incidence of food-borne diseases from pathogenic microorganisms and have been resulted in a major health impact on the world [3]. In addition, considerable post-harvest losses of fruit and vegetables are still an important problem in processing, preservation, distribution and marketing [8,10,12,14].

The possible use of natural substances may be resulted to minimize our dependency on present hazardous chemicals to reduce decay and post-harvest losses of fruits and vegetables. In the present study, the effectiveness of *S. hortensis* essential oil on the decay and quality of strawberry and grape fruits was investigated. The current results showed that the essential oil was significantly reduced the decay of strawberry and grape fruits.

The oil contains carvacrol (54.7 %), γ-terpinene (20.9 %), p-cymene (12.3 %), α-terpinene (2.0 %) and thymol (2.0 %) as major components (Table 1). The essential oil components of Turkish wild *S. hortensis* were previously reported [18,21,28,30,33,34,36] and it has been found that the oils contained various amount of thymol, carvacrol, p-cymene and γ-terpinene as major components. In general, major components are responsible for activity of essential oils. Thymol, carvacrol and p-cymene are the characteristic major components of many essential oils isolated from *Satureja*, *Thymus* and *Origanum* species, and their antimicrobial activity are often attributed to these components [18,20-22,25,26,33,36]. Potent fungi-toxic effects against various post-harvest and soil-borne fungal pathogens were also previously reported [20,22,25,37]. However, weak antifungal activity of p-cymene against limited number of fungal pathogens has been recently reported [25]. These results indicated that the inhibitory effect of *S. hortensis* essential oil on the decay of strawberry and grape fruits could be attributed to its relatively high content of carvacrol and thymol comprises 56.7 % of the total oil. As to mechanism of action of carvacrol, thymol and essential oils of *Satureja*, *Thymus* and *Origanum* species, which contain mainly thymol and carvacrol, it has been documented that these agents cause alterations in the hyphal

morphology and hyphal aggregates, resulting reduced hyphal diameters and lyses of hyphal wall interacting with the cell membrane of the fungal pathogen [23,37,38].

The potential use of essential oils and their components in the preservation of food products has been recently reviewed [39]. It has been documented that essential oils isolated from some *Satureja*, *Thymus* and *Origanum* species, which contain predominantly carvacrol and thymol, and its components (thymol and carvacrol) were effective in reducing of food spoiling microorganisms, food borne pathogens and decay of some fruits and vegetables caused by fungi [1,3,4,10,12,13,14,24,25]. For instance, Dikbas *et al*. have controlled the growth of *Aspergillus flavus* with *S. hortensis* essential oil on lemon fruit infected with this pathogen [24]. Likewise, in a different report, the role of carvacrol and thymol vapor atmosphere on *Botrytis cinerea* inoculated in PDA and in grape berries have been reported [10,12] . In these reports, these compounds potently reduced the fungal growth, decay of fruits and losses of quality in terms of sensory, nutritional and functional properties developing an active packaging by adding these compounds to table grapes stored 56 days under modified atmosphere. The similar results have also been reported in strawberry fruits using the essential oil of *Thymus vulgaris* against *B. cinerea* and *Rhizopus stolonifer* [1]. As can be seen from Tables 2, our present results are in accordance with the previous results.

This study showed that *S. hortensis* essential oil has a significant reducing effect on the decay of strawberry and grape fruits during storage especially at low temperatures. Therefore, it may be a potential source of alternative eco-friendly fungicides to protect strawberry and grape fruits as well as other stored products from pathogens and saprophytes.

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Table 1. Chemical composition of the essential oil of *Satureja hortensis*

RI[a]	Components	(%)[b]	Identification Methods
938	α-Pinene	1.8	GC, MS, RI
983	β-Pinene	1.0	GC, MS, RI
995	Myrcene	1.4	GC, MS, RI
1012	α-Phellandrene	0.2	GC, MS, RI
1023	α-Terpinene	2.0	GC, MS, RI
1034	p-Cymene	12.3	GC, MS, RI
1067	γ-Terpinene	20.9	GC, MS, RI
1172	Borneol	0.3	GC, MS, RI
1178	Terpinen-4-ol	0.4	GC, MS, RI
1190	α-Terpineol	0.1	GC, MS, RI
1219	Thymol methyl ether	tr	GC, MS, RI
1228	Carvacrol methyl ether	0.2	GC, MS, RI
1267	Thymoquinone	0.1	GC, MS, RI
1285	Thymol	2.0	GC, MS, RI
1296	Carvacrol	54.7	GC, MS, RI
1346	Thymol acetate	tr	GC, MS, RI
1373	4aα,7α,7aβ-Nepetalactone	tr	MS, RI
1419	β-Caryophyllene	1.1	GC, MS, RI
1442	Aromadendrene	0.2	GC, MS, RI
1478	γ-Muurolene	0.2	GC, MS, RI
1494	δ-Selinene	0.2	GC, MS, RI
1508	β-Bisabolene	0.3	GC, MS, RI
1512	γ-Cadinene	tr	GC, MS, RI
1517	δ-Cadinene	0.4	GC, MS, RI
1530	α-Cadinene	tr	GC, MS, RI
1574	Spathulenol	0.1	GC, MS, RI
1579	Caryophyllene epoxide	0.2	GC, MS, RI
	Grouped Components (%)		
	Aromatic monoterpenes	69.1	
	Monoterpene hydrocarbons	27.4	
	Oxygenated monoterpenes	0.4	
	Sesquiterpene hydrocarbons	2.2	
	Oxygenated sesquiterpenes	0.4	
	Total identified	98.1	

[a] Retention index relative to *n*-alkanes on SGE-BPX5 capillary column
[b] Relative area was given according to FID area percentage data
tr: traces (less than 0.1 %)
GC, identification was based on retention times of authentic compounds on SGE-BPX5 capillary column.
MS, identification was based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data [27].
RI, tentatively identified based on comparison of retention index of the compounds compared with published data [27].

Table 2. The decay severity of strawberry (measured after 26th days) and grape (measured after 30th days) fruits treated with six different concentrations of the essential oil from *S. hortensis* at three different temperatures

Treatments	The decay severity*					
	Strawberry fruits			Grape fruits		
	5°C	10°C	20°C	5°C	10°C	20°C
Control	5.0 ± 0.0 ^{c}	5.0 ± 0.0 ^{c}	5.0 ± 0.0 ^{a}	1.3 ± 0.0 ^{b}	2.3 ± 0.0 ^{b}	5.0 ± 0.0 ^{c}
0.01 µL/cm^3 oil	4.6 ± 0.3 ^{c}	5.0 ± 0.0 ^{c}	5.0 ± 0.0 ^{a}	1.3 ± 0.0 ^{b}	2.0 ± 0.3 ^{b}	5.0 ± 0.0 ^{c}
0.02 µL/cm^3 oil	4.6 ± 0.3 ^{c}	5.0 ± 0.0 ^{c}	5.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	4.3 ± 0.0 ^{c}
0.04 µL/cm^3 oil	3.6 ± 0.3 ^{b}	5.0 ± 0.0 ^{c}	5.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	4.3 ± 0.0 ^{c}
0.11 µL/cm^3 oil	3.0 ± 0.0 ^{b}	4.3 ± 0.3 ^{c}	5.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	4.3 ± 0.0 ^{c}
0.22 µL/cm^3 oil	1.3 ± 0.3 ^{a}	2.3 ± 0.3 ^{b}	5.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	2.0 ± 0.0 ^{a}
0.45 µL/cm^3 oil	1.0 ± 0.0 ^{a}	1.3 ± 0.3 ^{a}	5.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}	1.0 ± 0.0 ^{a}

* Mean values in the same column by the same letter are not significantly different to the test of Duncan Multiple Range Test (p=0.05)
Control: without any essential oil treatments; Scoring (mean value ± standard error means) represents a visual decay rating on fruits using 1-5 scale with 1: clean, 2: trace decay (about 25 %), 3: moderate decay (about 50 %), 4: severe decay (about 75 %), and 5: extensive decay (about 100 %).