Available online at www.sciencedirect.com
ScienceDirect
Biochemical Systematics and Ecology 36 (2008) 360–368
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Antimicrobial and insecticidal activities of essential oil isolated from Turkish *Salvia hydrangea* DC. ex Benth.

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Received 29 August 2006; accepted 9 December 2007

Abstract

The hydrodistillated essential oil of *Salvia hydrangea* was analyzed by GC–MS. Fifty-four different components representing 95.9% of the compounds in the oil were identified. Camphor (54.2%), α-humulene (4.0%), *cis*-sesquisabinene hydrate (2.8%), myrtenol (2.6%), β-bisabolol (2.2%) and 1,8-cineole (2.1%) were found to be predominant components. The oil was also characterized by relatively high amount of oxygenated monoterpenes (69.6%). The oil was tested for fungitoxic effects against 33 agricultural pathogenic fungi using in vitro microbial growth inhibition assays. The oil exhibited considerable antifungal activity against a broad spectrum of tested fungi. Antibacterial activity of the oil was determined against 30 bacterial strains using the disc diffusion method. The oil had a very wide spectrum of antibacterial activity. However, it was not as active as penicillin. The oil showed 68.3–75.0% mortality against adults of *Sitophilus granarius* and *Tribolium confusum*, the major pests of wheat and wheat products, respectively. It can be concluded that the oil of *S. hydrangea* has a potential against agricultural pathogenic fungi and two stored pests, *S. granarius* and *T. confusum*.

Keywords: Lamiaceae; *Salvia hydrangea*; Essential oil; Camphor; Antifungal; Antibacterial; Insecticidal; *Sitophilus granarius*; *Tribolium confusum*

1. Introduction

In recent years, scientists have focused on the increase of food production needed for the fast expansion of world population. Unfortunately, crop losses due to insects and plant diseases caused by fungi, bacteria and viruses are still a major problem. Food-borne diseases caused by microorganisms are also still a problem and result in major losses of economically important crops. Synthetic chemicals are widely used in the control of plant diseases. However, these chemicals may cause toxic residues in the treated products (Barnard et al., 1997; Isman, 2000). Some synthetic

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0305-1978/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2007.12.003

pesticides can also cause environmental pollution owing to their slow biodegradation in the environment (Barnard et al., 1997). In addition, the risk of developing resistance against the synthetic pesticides by microorganisms and the high cost–benefit ratio are other disadvantage of pesticide usage (Brent and Hollomon, 1998; Roy and Dureja, 1998). Therefore, there has been a growing interest in research concerning with the alternative pesticides, including plant extracts, essential oils and secondary metabolites (Misra and Pavlostathis, 1997; Roy and Dureja, 1998; Isman, 2000; Cakir et al., 2004; Kordali et al., 2005a,b).

Insect pests often cause extensive loss of the stored food grains and their products in tropical and semitropical environments (Isman, 2000). Therefore, there is a need for diverse kinds of safe insecticides or repellents for use in food grain. Plant-derived secondary metabolites play an important role in plant resistance to insects. Therefore, screening plant essential oils and plant extracts for insecticidal properties could lead to discovery of new agents for pest control (Isman, 2000; Kordali et al., 2006).

The genus *Salvia* (sage) is an important genus of the Lamiaceae family and comprises about 900 species, widespread throughout the world. Some members of this genus are also cultivated to use as flavouring agents in perfumery, cosmetics as well as food. For instance, *Salvia triloba* and *Salvia officinalis* are used for the commercial production of sage in Turkey (Baytop, 1999). There are about 90 species of *Salvia* in the Turkish flora, of which 45 are endemic (Davis, 1982; Baytop, 1999; Demirci et al., 2003). The species of *Salvia*, known as "*adaçayi*" in Anatolia, are used as antiseptics, stimulants, diuretics and for wound healing in Turkish folk medicine and for herbal teas and food flavoring (Baytop, 1999; Demirci et al., 2003; Tepe et al., 2005). *Salvia hydrangea* grows wildly in North-Eastern Anatolia and is used as an herbal tea, especially in the Iğdir region of Turkey. Recently, the chemical composition of Iranian *S. hydrangea* has been reported (Rustaiyan et al., 1997; Barazandeh, 2004). However, there is so far no report on the analyses of chemical composition of the essential oil of Turkish *S. hydrangea* and its antifungal, antibacterial and insecticidal activities. Thus, the objective of this study is to determine the chemical composition of Turkish *S. hydrangea* essential oil and its toxicity against 33 phytopathogenic fungi, 30 bacterial strains from plant, clinic and food origins, and two important stored crop pests, *Sitophilus granarius* and *Tribolium confusum*.

2. Material and methods

2.1. Plant material and isolation of essential oil

The aerial parts of *S. hydrangea* DC. ex Benth. were collected in Igdir region of Turkey (North-Eastern Anatolia) in July 2004 at the flowering stages and were dried in the shade. The plant sample was identified by Dr. Yusuf Kaya and voucher specimen (ATA-9792) has been deposited in the herbarium of Atatürk University, Erzurum (Turkey).

The dried plant samples (500 g) were subjected to hydrodistillation (plant material in boiling water) using a Clevenger-type apparatus for 4 h. Hydrodistillation of *S. hydrangea* yielded 0.53% (w/w) of essential oil. The yields were based on dry materials of plant samples.

2.2. GC–MS analysis

The analysis of the essential oil was performed with a Thermofinnigan Trace GC/Trace DSQ/A1300, (E.I Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m × 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 °C at 10 °C/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 individual 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 (Adams, 2007).

2.3. Fungal species and antifungal activity assays

The agricultural pathogenic fungi were obtained from the culture collection of Atatürk University (Faculty of Agriculture, Department of Plant Protection). Cultures of each of the fungi were maintained on potato dextrose agar (PDA) and were stored at +4 °C. The fungal species used in the experiments were given in Table 2.

Antifungal activity was determined as described previously (Kordali et al., 2005a,b, 2007). Briefly, potato dextrose agar (PDA) plates were prepared using 9 cm diameter glass Petri dishes. The oil was dissolved in ethanol (1/1; v/v) and 20 μl amount of the oil was added to each of PDA plates containing 20 ml of agar at 50 °C. A diameter disc 5 mm of the fungal species were cut from 1 week-old cultures on PDA plates and then mycelial surface of the disc was placed upside down on the centre of dish. Therefore, fungal species was contacting to growth medium on dish. Then, the plates were incubated in the dark at 22 ± 2 °C. The extension diameter (mm) of hyphae from centre to the sides of dishes was measured at 24 h intervals for 6 days. Mean growth measurements were calculated from three replicates of each of the fungal species. PDA plates containing ethanol (20 μl/Petri dish), without essential oil solutions, were used as negative control. In addition, PDA plates treated with benomyl (12.0 mg/Petri dish) were used as positive control.

2.4. Antibacterial activity assays

The oil was individually tested against 30 strains of bacteria (Table 3). Clinical bacterial strains were provided by the Department of Clinical Microbiology of Medicine Faculty, Atatürk University, Erzurum (Turkey). Phytopathogenic bacterial strains were isolated from some fruits and vegetables exhibiting typical bacterial disease symptoms. It was observed that hypersensitivity test (HR) results of some phytopathogenic bacterial strains were positive on tobacco plants (*Nicotiana tabacum* L. cv. Samsun) (Table 3). Bacterial cultures were preserved in Luria Broth and 15% glycerol solution at −80 °C prior to use.

Antibacterial activity assays were carried out by disc diffusion method on NA (Difco) medium. Bacterial suspension (50 μl) was adjusted to 10^8 cfu/ml final cell concentration, was poured into Petri dishes flasks (9 cm) containing 25 ml sterile NA and then was spread by a sterile swab. Amounts of 600, 900, 1200 and 1800 μl of the oil were individually dissolved in 1 ml of ethanol and these solutions were sterilized in 0.45 μm millipore filters. Sterilized discs (5 mm) were impregnated with 10 μl of these essential oil solutions, corresponding to 3.75, 4.75, 5.50 and 6.50 μl/disk, respectively, placed on the inoculated agar. These discs were put in the middle of plates. Penicillin was used as a positive control: 1 mg of penicillin was dissolved in 1 ml sterilized and distilled water and then sterilized disc was soaked with 10 μl of this solution (corresponding to10 μg/disc). Bacterial cultures of plant origins were incubated 6 days at 27 ± 2 °C, whereas the bacterial cultures of clinic and food origins were incubated at 35 ± 2 °C for the same period. At the end of 6 days, inhibition zones were measured in diameter (mm) around of the discs. All the tests were made in triplicate.

2.5. Insects and bioassays

S. granarius adults were collected from the Eastern Anatolia storage house (Pasinler town), whereas *T. confusum* adults were collected from Ankara storage house and also from Plant Protection Central Research Institute, Ankara. The beetles were maintained in the Plant Protection Department, Faculty of Agriculture, Atatürk University and feed on wheat (*Triticum vulgare*) at 25 ± 1 °C, 64 ± 5% relative humidity and 12 h:12 h (L:D). Whatman no. 1 paper was stuck onto the top of Petri dishes (9 cm × 1.5 cm deep) from inside and then impregnated with 10, 20, 30 and 40 μl of the oil using an automatic pipette. A filter paper was placed on bottom of each of Petri dishes and 20 adults (4–6 day-old) of the insects were placed on this filter paper, containing 20 grains of wheat. Thus, there was no direct contact between the oils and the insects. Petri dishes were covered with a lid and transferred into incubator, and then kept under standard conditions at 16:8 (light:dark) photoperiod (25 ± 1 °C, 64 ± 5% relative humidity) for 4 days. After exposure, mortality of the adults was counted at 12, 24, 48 and 96 h. Control treatments without the oil was treated in the same way. Each experiment was replicated for three times at each dose.

2.6. Statistical analysis

Variance analyses were used to test the significant difference among the results from the antifungal and insecticidal assays (SPSS 10.0 software package, 1999). Differences between means were tested through LSD and values of $p < 0.05$ were considered significantly different.

3. Results and discussion

3.1. Essential oil composition

The essential oil of *S. hydrangea* was analyzed by GC–MS and 54 different components were identified (Table 1). Camphor (54.2%), α-humulene (4.0%), *cis*-sesquisabinene hydrate (2.8%), myrtenol (2.6%), β-bisabolol (2.2%), 1,8-cineole (2.1%) and α-humulene epoxide (2.1%) were found to be major components. The oil was characterized by relatively high amount of oxygenated monoterpenes (69.6%) as well as sesquiterpenes (23.4%). Essential oil composition of *S. hydrangea* collected from different localities of Iran has been previously reported (Rustaiyan et al., 1997; Barazandeh, 2004). The oil of *S. hydrangea* collected from Isfahan region of Iran contained β-caryophyllene (33.4%), caryophyllene oxide (25.4%), β-pinene (5.9%), limonene (3.3%) and (*E*)-β-ocimene (3.2%) as major components, and it was characterized by relatively high amount of sesquiterpenes and low content of oxygenated monoterpenes (Barazandeh, 2004). However, in the present study, these compounds were found to be relatively traces amounts in the Turkish *S. hydrangea* oil (Table 1) and it was also characterized by relatively high amount of oxygenated monoterpenes. Furthermore, the *S. hydrangea* oil collected from Guillan region of Iran consists of mainly spathulenol (23.1%), 1,8-cineole (12.3%), α-pinene (10.0%) and β-caryophyllene (9.9%) (Rustaiyan et al., 1997). As shown in Table 1, Turkish *S. hydrangea* oil has different chemical composition and major components in comparison to the oils isolated from Iranian *S. hydrangea*. These differences can be attributed to genotypic variation and climatic conditions.

3.2. Antifungal activity of the essential oil

The oil showed inhibitory activity against 32 of the 33 fungi tested (Table 2). It completely inhibited growth of *Fusarium incarnatum*, *Fusarium nivale*, *Alternaria solani*, *Pythium ultimum* and *Rhizoctonia solani*. In many cases, antifungal activity of the oil was also found similar to positive control, benomyl (Table 2). The oil of *S. hydrangea* contains camphor (54.2%) as major component (Table 1) and often major components are responsible for activity of essential oils. Recent reports showed that commercially obtained camphor had weak antifungal activity against limited number of plant pathogenic fungal species including the fungi tested in the present study (Kordali et al., 2005a, 2007). The weak antifungal activity of commercial camphor against five soilborne fungal pathogens has also been reported (Pitarokili et al., 2003). These results suggest that activity of the oil in the present study can not all be attributed to camphor. The oil of *S. hydrangea* contains the relatively high proportions of oxygenated monoterpenes (Table 1) and in general, oils containing relatively high proportion of oxygenated monoterpenes have stronger antifungal activities when compared to essential oils rich in relatively monoterpene hydrocarbons as well as sesquiterpenes (Pattnaik et al., 1997; Pitarokili et al., 2003; Kordali et al., 2005a,b, 2007). Thus, the potent antifungal activity of *S. hydrangea* oil could be attributed to its relatively high proportions of oxygenated monoterpenes.

3.3. Antibacterial activity of the essential oil

The oil showed varied degrees of the antibacterial activity at a wide spectrum depending on tested antibacterial strains (Table 3). It was not active against only 5 of the 30 bacterial strains (Table 3). The oil showed antibacterial activity by producing a weak zone diameter of inhibition from 7 to 15 mm, depending on susceptibility of the tested bacteria. No correlation between oil concentration and antibacterial activity was also found. However, inhibition zones of the oil were lower than those of penicillin (8–60 mm). Furthermore, penicillin showed wide inhibition zones at very low concentrations compared with the oil.

The essential oils of various *Salvia* species, contained relatively high proportion of oxygenated monoterpenes showed varied antibacterial activities at a wide spectrum (Tepe et al., 2004, 2005; Norouzi-Arasi et al., 2005).

Table 1
The chemical composition of *Salvia hydrangea* essential oil

Peak no.	RI[a]	Constituents	%	Identification method
1	915	α-Pinene	0.3	GC, MS
2	938	Camphene	0.5	GC, MS
3	975	β-Pinene	0.4	GC, MS
4	1032	Limonene	0.9	GC, MS
5	1061	1,8-Cineole	2.1	GC, MS
6	1062	γ-Terpinene	0.1	GC, MS
7	1094	α-Pinene oxide	0.1	MS
8	1102	Linalol	0.2	GC, MS
9	1127	α-Capholenal	0.3	MS
10	1139	*cis*-Pinene hydrate	1.4	MS
11	1146	Camphor	54.2	GC, MS
12	1155	Pinocarvone	0.6	MS
13	1161	Borneol	1.9	GC, MS
14	1166	Terpinen-4-ol	1.4	GC, MS
15	1173	*p*-Cymen-8-ol	0.4	MS
16	1177	Myrtenol	2.6	GC, MS
17	1187	Verbenone	0.6	GC, MS
18	1192	*trans*-Carveol	1.2	GC, MS
19	1201	*cis*-Carveol	0.1	GC, MS
20	1209	Carvone	1.2	GC, MS
21	1228	Bornyl acetate	0.8	GC, MS
22	1232	(*E*)-Anethole	0.1	GC, MS
23	1238	Carvacrol	0.1	GC, MS
24	1248	*cis*-Pinocarvyl acetate	0.1	GC, MS
25	1251	*cis*-Piperitol acetate	0.5	MS
26	1259	α-Terpineol acetate	0.3	MS
27	1264	(*Z*)-β-Damascenone	0.1	MS
28	1271	α-Copaene	0.3	GC, MS
29	1274	β-Bourbonene	0.5	MS
30	1277	β-Elemene	0.2	MS
31	1284	Isoitalicene	0.2	GC, MS
32	1290	β-Caryophyllene	0.4	GC, MS
33	1303	(*Z*)-β-Farnesene	0.4	MS
34	1306	α-Humulene	4.0	GC, MS
35	1313	*ar*-Curcumene	0.6	MS
36	1315	γ-Curcumene	1.3	MS
37	1321	Bicyclogermacrene	0.5	MS
38	1325	(*E*,*E*)-α-Farnesene	1.2	MS
39	1329	δ-Cadinene	0.7	MS
40	1339	*cis*-Sesquisabinene hydrate	2.8	MS
41	1354	Spathulenol	1.8	MS
42	1356	Caryophyllene oxide	0.2	GC, MS
43	1370	α-Humulene epoxide	2.1	MS
44	1378	1-*epi*-Cubenol	1.1	MS
45	1383	β-Acorenol	0.5	MS
46	1386	Cubenol	0.4	MS
47	1392	α-Cadinol	1.3	MS
48	1400	β-Bisabolol	2.2	MS
49	1408	α-Bisabolol	0.5	MS
50	1411	(*Z*)-γ-Atlantone	0.3	MS
51	1439	(*Z*,*Z*)-Farnesyl acetone	0.3	MS
52	1452	Myristic acid	0.1	MS
53	1462	Phytol[b]	0.2	GC, MS
Grouped components				
Monoterpene hydrocarbons (%)			2.2	
Oxygenated monoterpenes (%)			70.2	

Table 1 (*continued*)

Peak no.	RI[a]	Constituents	%	Identification method
Sesquiterpene hydrocarbons (%)			10.4	
Oxygenated sesquiterpenes (%)			13.2	
Diterpenes (%)			0.2	
Others (%)			0.4	
Total identified (%)			96.6	

GC, identification based on retention times of authentic compounds on SGE-BPX5 capillary column; MS, tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data (Adams, 2007); tr, traces (less than 0.1%).

[a] Retention index relative to *n*-alkanes on SGE-BPX5 capillary column.

[b] The exact isomer was not identified.

In the present study, *S. hydrangea* oil was characterized by high content of oxygenated monoterpenes (Table 1). Therefore, antibacterial activity of the oil can be attributed to its oxygenated monoterpenes. It has been also demonstrated that oxygenated monoterpenes such as camphor, 1,8-cineole, terpinen-4-ol, and borneol, which were detected in *S. hydrangea* oil as major components, have antibacterial activity (Pattnaik et al., 1997; Kordali et al.,

Table 2
Inhibition of mycelial growth of fungal species by the essential oil of *Salvia hydrangea*

Fungal species	The oil (20 μl/Petri dish)		Benomyl (12 mg/Petri dish)		Control
	Growth[a] (mm)	Inh. (%)	Growth[a] (mm)	Inh. (%)	Growth[a] (mm)
Alternaria alternata	9.8 ± 0.9	68.4***	14.7 ± 1.2	52.6***	31.0 ± 3.5
Alternaria solani	5.1 ± 0.1	75.8***	12.9 ± 1.2	38.9*	21.1 ± 2.7
Aspergillus sp.	12.4 ± 1.8	47.7*	5.2 ± 0.1	78.1***	23.7 ± 4.4
Botrytis sp.	11.4 ± 1.5	83.4***	5.0 ± 0.0	92.7***	68.7 ± 7.6
Colletotrichum sp.	12.2 ± 1.4	49.0**	13.7 ± 1.4	42.7*	23.9 ± 3.8
Drechslera sp.	5.8 ± 0.2	81.5***	9.7 ± 0.8	69.0***	31.3 ± 2.8
Fusarium acuminatum	6.7 ± 0.5	77.1***	5.0 ± 0.0	82.9***	29.2 ± 3.2
Fusarium chlamydosporum	7.3 ± 0.6	71.7***	5.0 ± 0.0	80.6***	25.8 ± 3.5
Fusarium culmorum	6.7 ± 0.5	74.7***	5.0 ± 0.0	81.1***	30.9 ± 2.7
Fusarium equiseti	5.3 ± 0.2	68.5***	5.0 ± 0.0	70.2***	16.8 ± 2.1
Fusarium graminearum	7.2 ± 0.5	81.1***	5.0 ± 0.0	86.9***	38.1 ± 5.1
Fusarium incarnatum	5.0 ± 0.0	68.2***	5.0 ± 0.0	68.2***	15.7 ± 1.7
Fusarium nivale	5.0 ± 0.0	83.9***	5.0 ± 0.0	83.9***	31.1 ± 4.1
Fusarium oxysporum	8.9 ± 0.8	67.9***	5.0 ± 0.0	81.9***	27.7 ± 3.7
Fusarium proliferatum	7.4 ± 0.5	72.7***	5.0 ± 0.0	81.5***	27.1 ± 3.0
Fusarium sambucinum	6.8 ± 0.4	85.3***	5.0 ± 0.0	89.2***	46.2 ± 6.1
Fusarium scirpi	7.8 ± 0.6	52.7***	5.0 ± 0.0	69.7***	16.5 ± 2.8
Fusarium semitectum	6.6 ± 0.4	83.1***	5.0 ± 0.0	87.2***	39.1 ± 5.3
Fusarium solani	17.1 ± 1.7	26.6	5.0 ± 0.0	78.5***	23.3 ± 2.7
Fusarium tabacinum	6.7 ± 0.5	59.1***	5.0 ± 0.0	69.5***	16.4 ± 2.0
Fusarium verticillioides	6.1 ± 0.3	74.6***	5.0 ± 0.0	79.2***	24.0 ± 2.9
Nigrospora sp.	5.3 ± 0.1	51.8**	5.0 ± 0.0	54.5**	11.0 ± 0.9
Penicillium jensenii	14.7 ± 2.0	61.2***	5.2 ± 0.1	86.3***	37.9 ± 7.1
Phoma sp.	6.2 ± 0.4	67.5***	5.0 ± 0.0	73.8***	19.1 ± 2.4
Pythium ultimum	5.0 ± 0.0	93.5***	19.7 ± 3.1	74.4***	77.1 ± 5.5
Phytophthora capsici	6.8 ± 0.4	78.3***	5.2 ± 0.1	83.4***	31.4 ± 3.3
Rhizoctonia solani	5.0 ± 0.0	91.2***	5.0 ± 0.0	91.2***	56.9 ± 7.4
Sclerotinia sclerotiorum	8.1 ± 0.9	87.0***	5.0 ± 0.0	92.0***	62.2 ± 8.3
Sclerotinia sp.	5.5 ± 0.2	85.7***	29.8 ± 3.9	22.4	38.4 ± 6.1
Trichothecium sp.	6.7 ± 0.6	81.1***	5.0 ± 0.0	85.9***	35.4 ± 4.7
Verticillium albo-atrum	7.1 ± 0.6	69.7***	5.0 ± 0.0	77.4***	22.1 ± 2.7
Verticillium dahliae	5.9 ± 0.3	55.3***	5.0 ± 0.0	62.1***	13.2 ± 1.4
Verticillium tenerum	5.5 ± 0.8	74.3***	5.0 ± 0.0	76.6***	21.4 ± 2.9

Inh., inhibition.

*Significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$ according to control.

[a] The growth of fungal species is given as mean ± standard error of three replicates.

Table 3
Antibacterial activities of the essential oil of *Salvia hydrangea*

Bacterial strains	Gram	Inhibition zones[a]				
		The oil				Penicillin
		3.75 μl/disk	4.75 μl/disk	5.50 μl/disk	6.50 μl/disk	10 μg/disk
Plant origins						
Clavibacter michiganense	+	12	14	15	15	40
Curtobacterium flaccumfaciens	+	8	10	13	14	60
Agrobacterium tumefaciens	–	8	8	8	8	12
Erwinia amylovora	–	7	8	9	9	31
Erwinia ananas	–	7	8	10	10	30
Erwinia caratovora	–	12	14	15	19	54
Erwinia chrysanthemi	–	7	7	7	8	22
Pseudomonas syringae pv. *glycinea*	–	7	7	7	8	24
Pseudomonas syringae pv. *maculicola*	–	7	9	9	10	32
Pseudomonas syringae pv. *phaseolicola*	–	7	11	10	10	27
Pseudomonas syringae pv. *populans*	–	–	–	–	–	29
Pseudomonas syringae pv. *syringae*	–	8	9	10	11	29
Pseudomonas syringae pv. *tabaci*	–	10	12	12	10	40
Pseudomonas syringae pv. *tomato*	–	–	–	–	–	–
Xanthomonas axanopodis pv. *vesicatoria*	–	9	9	10	9	–
Xanthomonas hortorum	–	7	8	9	9	23
Clinic and food origins						
Enterococcus fecalis ATCC 29122	+	7	7	8	8	24
Micrococcus luteus	+	–	–	–	–	59
Staphylococcus aerous ATCC 29213	+	8	8	7	8	21
Streptococcus pyogenes ATCC 176	+	7	8	8	8	42
Acinetobacter calcoaceticus	–	7	8	9	10	25
Chromobacterium violaceum	–	8	9	9	9	21
Enterobacter cloaceae	–	–	–	–	–	–
Esherichia coli	–	–	–	–	–	–
Klebsiella pneumonia	–	8	8	7	7	16
Proteus vulgaris	–	8	10	11	11	21
Pseudomonas aeruginosa ATCC 27859	–	8	10	11	11	29
Ralstonia pickettii	–	8	8	10	12	35
Salmonella enteritidus ATCC 13076	–	7	7	7	7	8
Salmonella typhimurium	–	8	7	8	9	36

–: Not active.
[a] Zones are means of three replicates.

2005b; Kotan et al., 2007). However, essential oils consisting of numerous components and other major and/or minor compound(s) in oils may affect antibacterial activity.

3.4. Insecticidal activity of the oil

The toxicity of 10, 20, 30 and 40 μl/Petri dish doses of the oil on two stored product pests, *S. granarius* and *T. confusum* were determined (Table 4). The mortality increased with increase in the exposure times. The oil caused 68.3–75.0% mortality of *S. granarius* and *T. confusum* adults, respectively, at a dose 40 μl/Petri dish after 96 h of exposure. Previous studies showed that some plant essential oils and their major components possessed various toxic effects against *S. granarius* and *T. confusum* (Obeng-Ofori et al., 1998; Tunc and Erler, 2003; Erler, 2005; Kordali et al., 2006). In general, toxicity of essential oils against pests is attributed to their main components. In the present study, *S. hydrangea* oil containing camphor (54.2%), α-humulene (4.0%), *cis*-sesquisabinene hydrate (2.8%), myrtenol (2.6%), β-bisabolol (2.2%) and 1,8-cineole (2.1%) as major components was found to be toxic against two stored pests (*S. granarius* and *T. confusum*). Toxic effect of camphor against *S. granarius* and *T. confusum* had been previously reported (Obeng-Ofori et al., 1998; Erler, 2005; Kordali et al., 2006). Therefore, in the present

Table 4
The toxicity of essential oil of *Salvia hydrangea* against the adults of *S. granaries* and *T. confusum*

Insects	% Mean mortality[a]				
	Exposure time (h)	Dose (μl/Petri dish)			
		10	20	30	40
S. granarius	12	6.7 ± 1.7	3.3 ± 1.7	15.0 ± 0.0**	11.7 ± 1.7*
	24	18.3 ± 1.7***	15.0 ± 2.9**	25.0 ± 2.9***	23.3 ± 3.3***
	48	21.7 ± 1.7***	28.3 ± 4.4***	33.3 ± 4.4***	35.0 ± 5.0***
	96	33.3 ± 4.4***	45.0 ± 5.8***	56.7 ± 6.0***	68.3 ± 10.9***
Control	–	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
T. confusum	12	5.0 ± 2.9	11.7 ± 4.4*	11.7 ± 1.7*	16.7 ± 4.4**
	24	18.3 ± 1.7**	20.0 ± 2.9***	20.0 ± 2.9***	25.0 ± 2.9***
	48	31.7 ± 4.4***	35.0 ± 5.8***	41.7 ± 4.4***	36.7 ± 5.4***
	96	51.7 ± 7.3***	71.7 ± 4.4***	75.0 ± 5.0***	75.0 ± 8.4***
Control	–	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

*Significant at $p < 0.05$; **significant at $p < 0.01$; ***significant at $p < 0.001$ according to control.
[a] Mean ± SE of three replicates, each set up with 20 adults.

study, the toxicity of the oil against the pests could be attributed to its major component, camphor. As it was previously reported, 1,8-cineole, terpinen-4-ol, borneol and bornyl acetate which were determined in the oil of *S. hydrangea* in the present study showed more toxic effects against *S. granarius* as well as *T. confusum* compared to the toxic effects of camphor (Obeng-Ofori et al., 1997; Tunc and Erler, 2003; Erler, 2005; Kordali et al., 2006). Therefore, these compounds and/or other components determined in *S. hydrangea* oil may possess a synergistic and/or antagonistic effect on toxicity of the oil.

In conclusion, *S. hydrangea* oil has a potential against agricultural pathogenic fungi and two stored pests adults, *S. granarius* and *T. confusum*. However, further studies will need to be addressed to evaluate cost, efficacy and safety of the oil.

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