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Antibacterial activity of the essential oil and extracts of *Satureja hortensis* against plant pathogenic bacteria and their potential use as seed disinfectants

Recep Kotan[a,*], Fatih Dadasoğlu[b], Kenan Karagoz[b], Ahmet Cakir[c], Hakan Ozer[d], Saban Kordali[a], Ramazan Cakmakci[d], Neslihan Dikbas[e]

[a] *Atatürk University, Faculty of Agriculture, Department of Plant Protection, 25240 Erzurum, Turkey*
[b] *Ağrı İbrahim Çeçen University, Faculty of Arts and Sciences, Department of Biology, 04100 Ağrı, Turkey*
[c] *Kilis 7 Aralık University, Faculty of Arts and Sciences, Department of Chemistry, 79000 Kilis, Turkey*
[d] *Atatürk University, Faculty of Agriculture, Department of Field Crops, 25240 Erzurum, Turkey*
[e] *Atatürk University, Faculty of Agriculture, Department of Agricultural Biotechnology, 25240 Erzurum, Turkey*

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ABSTRACT

The aim of this study was to determine the antibacterial activity and potential use as seed disinfectant of *Satureja hortensis* L. essential oil, extracts (*n*-hexane, chloroform, acetone and methanol) and two pure compounds (carvacrol and thymol) against plant pathogenic bacteria. Antibacterial activity assays were carried out by disc diffusion method. Minimum inhibition concentrations (MICs) were determined by a twofold serial dilution method. The seeds were exposured to the essential oil vapors or directly soaked in the essential oil and extracts (hexane, methanol and hexane–methanol extracts mixture) suspensions at different concentrations. They were tested for their effectiveness on the percentage of the germinated seeds (tomato and/or lettuce) coated with some bacteria (*Clavibacter michiganensis* ssp. *michiganensis*, *Xanthomonas axanopodis* pv. *vesicatoria* and/or *Xanthomonas axanopodis* pv. *vitians*), the number of the infected seedling, disease severity and/or plant shoots and roots fresh weights in Petri plate or pot assays. Our results showed that the essential oil, carvacrol and thymol have a strong antibacterial activity against bacteria on Petri plates. However, the extracts showed weak antibacterial activity. The direct application of the some essential oil concentrations showed toxic effect on seed germination. On the other hand, the indirect application of the essential oil, direct or indirect applications of the hexane and methanol extracts were not toxic. The best successful result was obtained from 2.5 mg/ml concentration of hexane–methanol extract mixtures applications for lettuce seeds, infected with *X. axanopodis* pv. *vitians.* In this application, the disease severity rate was minimum level, and germination rate, shoots and roots fresh weights were maximum level. Our results show that hexane–methanol extract mixture of *S. hortensis* can be used as a seed disinfectant and as a botanical pesticide for management of plant bacterial disease.

1. Introduction

Plant diseases caused by plant pathogenic fungi, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants are one of the major problems of crop loss (Kotan et al., 2010). For this reason, avoiding or mitigating crop losses due to the plant diseases is an important consideration in plant production. Rapid and effective control of the plant disease in crop cultivation is generally achieved by use of synthetic pesticides and antibiotics. However, these chemicals are associated with undesirable effects on the environment due to their slow biodegradation in the environment and some toxic residues in the products for mammalian health (Barnard et al., 1997; Isman, 2000). The risk of developing resistance by microorganisms and the high cost–benefit ratio are also other disadvantages of synthetic pesticide uses (Brent and Hollomon, 1998; Roy and Dureja, 1998).

Management strategies of the phytopathogenic bacterial pathogens include the use of disease-free seed and seedlings, resistant cultivars, antibiotics and copper sprays. However, these strategies are not always effective, especially when environmental conditions are optimal for disease or inoculums levels are high. Spraying with the antibiotics and copper compounds have never been satisfactory. Their selective pressure may lead to select bacteria resistant to the antibiotic and the consequent possible

* Corresponding author at: Atatürk University, Faculty of Agriculture, Department of Plant Protection, TR-25240 Erzurum, Turkey. Tel.: +90 442 2311167; fax: +90 442 2311469.
E-mail address: rkotan@atauni.edu.tr (R. Kotan).

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horizontal transfer to human pathogens. Furthermore, their use may lead to select bacteria resistant to the antibiotic and the consequent possible horizontal transfer to human pathogens. As consequence antibiotics are forbidden in many countries and only in a few countries the use of oxytetracycline and streptomycin for the control of the bacterial diseases on important crops is allowed (McManus et al., 2002).

New awareness to reduce the usage of the chemical pesticides by developing alternative strategies or technologies in order to improve plant disease resistance and control of pathogens are being promoted. Therefore, there has been a growing interest in research concerning the alternative pesticides and antimicrobial active compounds, including the plant extracts and essential oils of aromatic plants (Pradhanang et al., 2003; Kotan et al., 2010). Among the aromatic plant species, genus *Satureja* L. occupies a special position because of the fact that essential oils and extracts of these species showed very significant antimicrobial activity against various species of bacteria and fungi.

During the last years, numerous studies have documented the antimicrobial effects of *Satureja hortensis* L. (summer savory) essential oil or extracts against plant pathogenic fungi (Güllüce et al., 2003; Sahin et al., 2003; Adiguzel et al., 2007; Dikbas et al., 2008), bacteria (Güllüce et al., 2003; Kotan et al., 2007; Özkalp and Özcan, 2009; Karami-Osboo et al., 2010; Mihajilov-Krstev et al., 2009, 2010) and virus (Dunkić et al., 2010) under in vitro conditions. To our knowledge, there is a lack of information on the antibacterial property of the extracts and/or essential oil obtained from *S. hortensis* against plant pathogenic bacteria under in vivo assays.

Seed-borne diseases can be spread with seed trade and control of them using commercial disease management methods is extremely difficult (Bradbury, 1986). Therefore, the use of healthy seeds is the most important manner for controlling the above diseases. Of course this matter is an important problem in organic agriculture. This study was undertaken to assess the in vitro and in vivo antibacterial efficacy of the essential oils and *n*-hexane, chloroform, acetone and methanol extracts of the aerial parts of *S. hortensis* and their two main phenolic components (carvacrol and thymol) of *S. hortensis* (Kotan et al., 2007; Tozlu et al., 2011) as a seed disinfectant against plant pathogenic bacteria on the Petri plate and pot assays.

2. Materials and methods

2.1. Phytopathogenic bacterial strains

The bacterial strains used in this study were obtained from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University, Erzurum, Turkey (Table 1). They had been isolated from some fruits and vegetables exhibiting typical bacterial disease symptoms on their respective host plants. The bacterial strains were identified by using the MIDI system (Microbial Identification System Inc., Newark, DE, version 5.0) and BIOLOG (Biolog Inc., Hayward, CA, USA) system (Sahin and Miller, 1997). The bacterial culture preserved in Luria Broth and 15% glycerol solution at −80 °C for using further studies.

2.2. Plant materials and pure compounds

The aerial parts of *S. hortensis* were collected from Erzurum province in eastern Anatolia region of Turkey in July 2009 at the flowering stages, and were dried in shade. The plant sample was identified by Dr. Kaya (Sciences Faculty, Department of Biology, Atatürk University, Erzurum) and Cakmakcı (Faculty of Agriculture, Department of Field Crops, Atatürk University, Erzurum). It has

Table 1
Antibacterial activities of the essential oil and extracts from *S. hortensis* and pure compounds against plant pathogenic bacteria.

Bacterium	Strains	Essential oil		Extracts								Pure compounds				Antibiotics	
				Hexane		Chloroform		Acetone		Methanol		Carvacrol		Thymol		OFX	
		IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	
Clavibacter michiganensis ssp. *michiganensis*	RK-Cmm	26[a]	15.63	11	80	–	ND	10	80	–	ND	23	31.25	41[a]	20.0	18	
Erwinia amylovora	RK-228	40	125	13	80	8	100	9	100	–	ND	28	31.25	26[a]	20.0	24	
Erwinia carotovora subsp. *atroceptica*	RK-462	41	125	7	100	–	ND	–	ND	–	ND	15[a]	15.63	21[a]	20.0	21	
Erwinia chrysanthemi	RK-421	24	31.25	8	100	–	ND	–	ND	–	ND	16[a]	15.63	20[a]	20.0	25	
Pseudomonas cichorii	RK-166	18	15.63	–	ND	–	ND	–	ND	–	ND	12[a]	31.25	21[a]	20.0	24	
Pseudomonas syringae pv. *syringae*	RK-204	36[a]	15.63	–	ND	–	ND	–	ND	–	ND	26[a]	15.63	29[a]	20.0	12	
Pseudomonas syringae pv. *tomato*	RK-Ps-tom	29[a]	31.25	10	80	–	ND	10	90	–	ND	25	15.63	26	20.0	13	
Xanthomonas axanopodis pv. *malvacearum*	RK-Xa-mal	42	31.25	10	80	–	ND	12	90	7	100	38	15.63	34	20.0	17	
Xanthomonas axanopodis pv. *vesicatoria*	Xcv-761	41[a]	31.25	14	80	–	ND	9	90	7	100	32	15.63	26	20.0	23	
Xanthomonas axanopodis pv. *campestris*	RK-Xa-cam	45	15.63	10	80	–	ND	8	90	–	ND	25	15.63	29	20.0	21	
Xanthomonas campestris pv. *raphani*	RK-Xc-rap	36[a]	31.25	9	90	7	100	10	90	–	ND	30	15.63	26	20.0	16	
Xanthomonas axanopodis pv. *vitians*	Xa-vit	50	15.63	10	80	–	ND	7	100	–	ND	28	15.63	38[a]	10.0	16	
Xanthomonas campestris pv. *zinniae*	Xc-zin	31	15.63	8	100	–	ND	7	100	–	ND	20[a]	31.25	33	20.0	24	
Xanthomonas axanopodis pv. *pelargonii*	RK-Xa-pel	42	15.63	–	ND	–	ND	–	ND	–	ND	29	15.63	30	20.0	22	

IZD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 12.5 μL of the essential oils and liquid monoterpene carvacrol solutions (1/1 ml DMSO), and the extracts and solid monoterpene thymol solutions (10 mg/ml DMSO); MIC, minimal inhibitory concentration as μL/ml for the essential oil and carvacrol, and in mg/ml for the extracts and thymol; ND, MIC value was not determined.
–, not active. OFX, ofloxacin (5 μg/disk).
[a] Bactericidal effect was observed.

been deposited in the herbarium of Atatürk University, Erzurum (Turkey). Pure carvacrol and thymol were purchased from Sigma.

2.3. Lettuce and tomato seeds

Lettuce (*Lactuca sativa* L. cv. Yedikule) and tomato (*Licopersicum esculentum* L. cv. H2274) seeds were purchased from a local market, and then stored in refrigerator (5 °C) and dry conditions. The minimum germination and purity rates of the lettuce and tomato seeds were 85–99% and 80–99%, respectively.

2.4. Extraction of the essential oil

The dried and powdered plant sample (500 g) was subjected to hydro-distillation using a Clevenger-type apparatus for 4 h. The oil was extracted with $CHCl_3$ and then were dried over anhydrous sodium sulfate (Na_2SO_4) and stored under N_2 atmosphere at 20 °C in a sealed vial until use.

2.5. Extraction procedures

The dried plant samples were powdered in a blender and then 50 g of each plant sample extracted individually with *n*-hexane, chloroform, acetone and methanol at room temperature. After filtration, the organic solvents were evaporated under reduced pressure and temperature. For the methanol extract of the plant sample, the concentrated MeOH extract was individually dissolved in distilled water (60 °C) and then filtered. The solution was extracted with *n*-hexane for three times to remove lipophilic compounds. Then, water solution was lyophilized in a Labconco 117 freeze-dryer at 5 μm-Hg and −50 °C.

2.6. Antibacterial activity assays

Antibacterial activity assays were carried out by disc diffusion method (Murray et al., 1995) with a minor modification using Triptic Soy Agar (TSA, Merck, Germany) medium. The essential oil, the extracts and pure phenolics solutions were prepared by dissolving using 10% dimethylsulfoxide (DMSO), and then were sterilized by filtration by 0.45 μm Millipore filters. Bacterial cultures were grown in Triptic Soy Broth (TSB, Merck, Germany) and their suspension (100 μL) containing 1×10^8 CFU/ml of bacteria spread by a sterile swab on TSA medium. The disks (6 mm in diameter) were impregnated with 12.5 μl of the emulsions of the essential oils and liquid monoterpene, carvacrol prepared in 10% DMSO-distiled water, and with 10.0 mg/ml suspensions of the extracts and solid monoterpene, thymol preperad in 10% DMSO-distiled water. Then, they were put in the middle of the inoculated plates. The bacterial cultures were incubated at 27 ± 2 °C for 48 h, and then inhibition zones were measured in diameter (mm) around of the discs. Furthermore, bactericidal and bacteriostatic activities were also determined. The Triptic Soy Agar samples taken from inhibited areas around of the disks were put into nutrient broth without essential oil, extracts and pure compounds, and incubated at 27 ± 2 °C for two days. After 48 h, whether there was no bacterial growth was observed in the broth culture, it was considered as bactericidal effect or not bactericidal. Ofloxacin antibiotic disks (5 μg/disk) were used as control. All the tests were made in triplicate.

2.7. Determination of minimum inhibition concentration (MIC)

Minimum inhibition concentrations (MICs) of the essential oils, the extracts and pure compounds (carvacrol and thymol) were tested by using a twofold serial dilution method (Bajpai et al., 2010; Kotan et al., 2010). Twofold serial dilutions of the liquid substances, essential oil and carvacrol (500 μl/ml) were prepared by diluting 10% DMSO to achieve a decreasing concentration ranging from 500 μl/ml to 3.125 μl/ml. However, solutions of the solid substances, the extracts and thymol were prepared by diluting 10% DMSO at concentrations ranging from 10 to 100 mg/ml. Using 100 μl of suspension containing 1×10^8 CFU/ml was measured spectrophotometrically at 600 nm of bacteria spread on TSA plates. The blank disks (Oxoid) were impregnated with 12.5 μl of the solutions tested. Then, they were put in the middle of inoculated TSA plates. The bacterial cultures were incubated at 27 ± 2 °C for 48 h. The lowest concentration of the essential oils, extracts and pure compounds showing a clear zone of inhibition was considered as the MIC. 10% DMSO was used as negative control. Each test was repeated at least twice.

2.8. Seed surface disinfection

The seeds were surface disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the seed surface. Seed disinfection was performed by dipping the seeds for 3 min in 3% sodium hypochlorite and washing four times in sterilized and distilled water (sd. H_2O). Seeds were left to dry on sterile What-man filter paper sheets overnight in the laminar flow hood for using further studies.

2.9. Coating procedure of pathogenic bacteria on the seeds

Pathogen bacteria were grown in 50 ml flasks containing 20 ml of TSB medium on a rotary shaker at 27 °C for 24 h. Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and appropriately diluted to 1×10^8 CFU/ml in sd. H_2O. Approximately, 0.2 g of sucrose (10 mg/ml) was added to each Erlenmeyer flasks, and 90 g of the surface-sterilized seeds were soaked separately in this suspension. The seeds were incubated in the flasks by shaking at 80 rpm for 2 h at 28 °C to coat the seeds with the pathogens. After shaking, the seeds were taken out and air-dried on sterile What-man filter paper sheets overnight in the laminar flow hood.

2.10. Determination of the germination percentage of tomato seeds treated with essential oil or extracts on pot assays

As stated above, the seeds were surface disinfected and coated with the pathogen (*C. m.* ssp. *michiganensis*). Then, the seeds were treated with the essential oil and extract two different ways. The concentrations of the essential oil (8 and 16 mg/ml), and hexane and methanol extracts (40 mg/ml) were prepared by dissolving in 10% DMSO: distilled-water in 10 ml flasks. In one of the applications, the seeds were directly soaked in the essential oil and extracts suspensions for 1, 2 and 4 h at 28 °C until the seeds coated with the oil and extract suspensions. In the other application, Whatman no. 1 papers (3 cm × 3 cm) were stuck onto the top of Petri dishes (9 cm × 1.5 cm deep) from inside and then impregnated with the oil suspensions using an automatic pipette. The seeds surface disinfected and coated with the pathogen put on in the Petri plate. Petri dishes were sealed with parafilm and incubated by shaking at 80 rpm for 1, 2 or 4 h at 28 °C. The seeds were exposured to the essential oil vapors. Thus, there was no direct contact between the oil and the seeds. As control applications, surface disinfected seeds coated with the pathogen handled similarly with the exception of the oil were exposed to ambient air. In the both applications, the seeds were left to dry on sterile Whatman filter paper sheets overnight in the laminar flow hood. We planted 10 seeds per plastic pots (6.5 cm × 6.5 cm) containing garden soil and sand (1:1). The experiment was conducted in a growth chamber with an average temperature of 23 ± 2 °C, relatively humidity of about 60% and

photoperiod of approximately 12–14 h day light. Pots were arranged in a randomized block design with three replications. Three pots of each treatment comprised a replicate. The seedlings were watered once every two days. The percentage of germinated seeds per treatment was determined by counting the number of germinated seeds twenty days after planting.

2.11. Effect of S. hortensis hexane–methanol extracts mixture on seed germination, disease severity and growth promotion in Petri plate assays

The hexane–methanol extract mixture (1:1, w/w) suspensions (2.5, 5, 10, 20 and 40 mg/ml) were prepared by dissolving in 10% DMSO: distilled-water in 10 ml flasks. The seeds surface disinfected and coated with the pathogens separately (*C. m.* ssp. *michiganensis*, *X. a.* pv. *vesicatoria* and *X. a.* pv. *vitians*) soaked in the extract suspensions and streptomycin (0.5 mg/ml), and then incubated by shaking at 80 rpm for 3 h at 28 °C until the seeds were coated with the suspensions. Seeds were left to dry on sterile Whatman filter paper sheets overnight in the laminar flow hood. Two sterile Whatman filter papers were placed on the bottom of each Petri dish (9–1.5 cm deep) and 10 seeds were transferred on the filter papers. Then, 10 ml of sd. H_2O was added to each Petri plate. The Petri dishes containing 10 seeds were sealed with parafilm to prevent evaporation of water were incubated in growth chamber on supplied with 12 h of fluorescent light and humidity of 80% at 23 ± 2 °C. The treated seeds were allowed to germinate in Petri dishes. The assays were arranged in a completely randomized design with three replications including controls. The percentage of germinated seeds per treatment was determined by counting the number of germinated seeds after 7 days. If at least 2 mm of radicle had emerged, the seeds were considered germinated. When water-soaked lesions were observed on the cotyledons the seedlings were considered diseased. Streptomycin sulfate (500 μg/ml), disinfected seed infected with pathogen and sterilized seed not disinfected with pathogen were used as controls.

2.12. Effect of S. hortensis hexane–methanol extracts mixture on seed germination, disease severity and growth promotion in pot assays

The hexane–methanol extract mixture (1:1, w/w) suspensions (2.5, 5, 10, 20 and 40 mg/ml) were prepared by dissolving in 10% DMSO: distilled-water in 10 ml flasks. Lettuce and tomato seeds were coated with pathogens (*C. m.* ssp. *michiganensis*, *X. a.* pv. *vesicatoria* and *X. a.* pv. *vitians*), and soaked in different concentrations of the hexane–methanol extracts mixture (2.5, 5, 10, 20 and 40 mg/ml) and streptomycin (0.5 mg/ml) as described above. Ten seeds were planted on each plastic pot (6.5 cm × 6.5 cm) containing garden soil and sand (1:1), and the experiments were conducted in a growth chamber as described above. The seedlings were watered once every two days. Pots were arranged in a randomized block design with three replications. Three pots of each treatment comprised a replicate. The percentage of germinated seeds per treatment was determined by counting the number of germinated seeds 20 days after planting. Disease severity was evaluated 40 days after planting using a 1–5 scale, in which 1 = no disease, 2 = a few water-soaked lesions, 3 = many spots with coalescence and slight plant wilting, 4 = severe wilting and defoliation, and 5 = plants dead (Sahin and Miller, 1997). Forty-five days after planting, the seedlings were evaluated and data shoot fresh weight and root fresh weight recorded as average milligrams of biomass per plant. Values were reported as averages for the three replicates ± standard errors. The assays were repeated three times with 10 seeds of tomato and lettuce per pots for each treatment.

Table 2
Percentage of germinated tomato seeds coated with *C. m.* ssp. *michiganensis*, treated directly or indirectly with *S. hortensis* essential oil, hexane and methanol extract suspensions and then transferred in pots.

Treatments	Dose	Germinated seeds (%)		
		1 h	2 h	4 h
Direct seed treatments				
Essential oil	8 mg/ml	10.0 a	06.7 a	00.0 a
	16 mg/ml	06.0 a	03.3 a	00.0 a
Hexane extract	40 mg/ml	73.3 c	73.3 d	73.3 d
Methanol extract	40 mg/ml	63.3 b	63.3 c	63.3 c
Indirect seed treatments				
Essential oil	8 mg/ml	76.7 c	50.0 b	70.0 cd
Essential oil	16 mg/ml	60.0 b	60.0 bc	60.0 bc
Control				
Surface disinfected seeds	–	63.3 b	63.3 c	63.3 c

*Values are given as the mean of three experiments. Different letters in a column indicate statistically significant differences according to the Duncan test $p < 0.05$.

Inhibition of disease severity as percentage [IDS (%)] was calculated as follows: IDS (%) = $100 \times (A_{control} - A_{sample})/A_{control}$. Where $A_{control}$ is the disease severity of the control (only pathogen application) and A_{sample} is the disease severity of the essential oils, extracts and streptomycin sulfate applications.

2.13. Statistical analysis

SPSS for Windows, version 10.0, was used for statistical analysis. Analysis of variance (ANOVA) was used to determine the effects of treatment on disease incidence and growth measurements. Means were compared using Duncan's multiple range tests. Results were expressed as average ± standard errors (SE).

3. Results

3.1. Antibacterial activity assays

The essential oil and extracts of *S. hortensis* and two pure compounds, carvacrol and thymol were tested for antagonistic activity against plant pathogenic bacteria (Table 1). According to the in vitro test results, the essential oil showed a broad spectrum of strong antibacterial activity against all pathogens tested. The inhibition zones and MIC values of the oil changed from 18 to 50 mm and 15.63–125 μl/ml, respectively. However, the extracts showed weak antibacterial activity against limited number of tested pathogens. Their inhibition zones and MICs values changed from 7–14 mm and 80–100 μl/ml, respectively. Among the extracts, the most antibacterial effect was obtained from the hexane extract. Carvacrol and thymol, the major phenolics of the *S. hortensis* oil showed strong antagonistic activity changed from 12–41 mm of mean inhibition zone and 15.63–31.25 μl/ml of a MIC.

3.2. Effect of essential oil and methanol extract of S. hortensis on the tomato seed germination on Petri plate assays

The results of the effect of direct or indirect treatments with *S. hortensis* essential oil, hexane and methanol extracts suspensions on germination of tomato seeds coated with *C. m.* ssp. *michiganensis* are given in Table 2. Direct applications of the oil suspensions containing 8 and 16 mg/ml, on the contrary of the other applications, were toxic to tomato seed germination. Maximum percentage of germinated seedlings (76.7%) was obtained with indirect application of 8 mg/ml essential oil after 1 h treatment. The direct application of the hexane extract (40 mg/ml) was not toxic to the tomato seed germination, and the percentage of

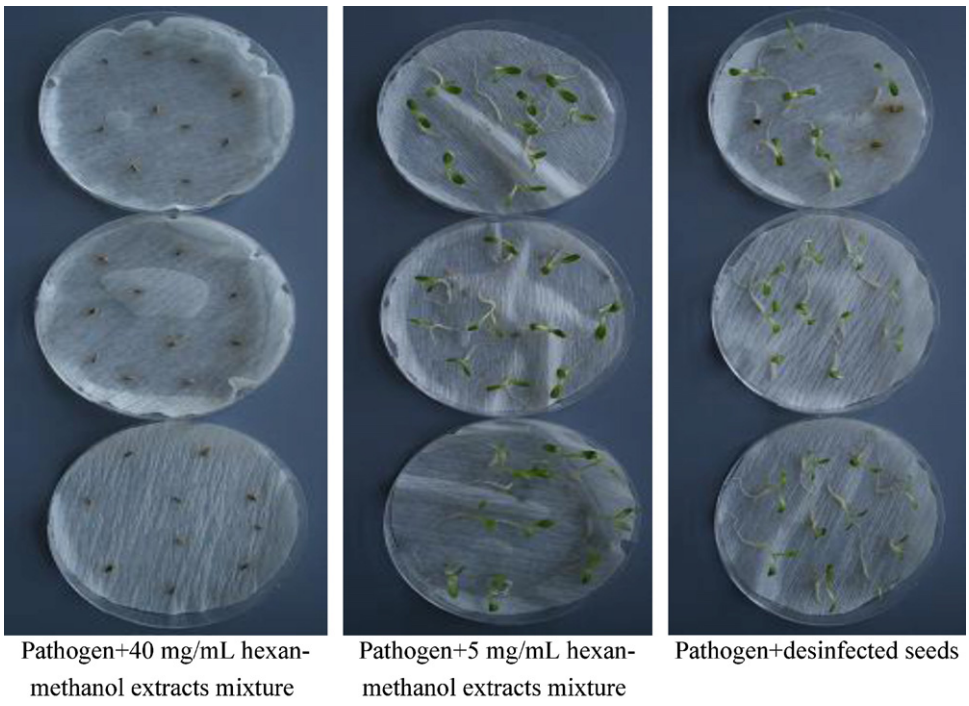

Fig. 1. The number of geminated lettuce seeds and infected lettuce seeds with pathogenic bacteria treated with *S. hortensis* hexan and methanol extracts mixture on the Petri plate.

germinated seedlings was 73.3% for 1, 2 and 4 h treatments resulting statistically significant compared to the control.

3.3. Effect of hexane–methanol extracts mixture of S. hortensis on tomato and lettuce seed germination and seedling infection in Petri plates assays

The effects of hexane–methanol extracts mixture (2.5, 5, 10, 20 and 40 mg/ml) of *S. hortensis* on Petri plate assays on germinations and infected seedling of the tomato and lettuce seeds coated with *C. m.* ssp. *michiganensis*, *X. a.* pv. *vesicatoria* and *X. a.* pv. *Vitians* are shown in Table 3. As can be seen from this table, in general, the hexane–methanol extracts mixture have weak/or no toxic effect on the germination of tomato seeds as compared with control group. However, high applications concentrations (10, 20 and 40 mg/ml) of the hexane–methanol extracts mixture were found to be toxic on the lettuce seed germination. On the other hand, as compared with control groups, the number of infected seedling of tomato and lettuce coated with Xcv-761 and Xcvit, respectively decreased by some applications of the extracts mixture. For instance, 5 and 10 mg/ml concentrations both reduced the infected seedlings growths of lettuce and tomato seeds infected with Xcv-761 and Xcvit, respectively and had weak or no toxic effect on the germination of the seeds (Fig. 1).

3.4. Effect of the hexane–methanol extracts mixture of S. hortensis on tomato seed germination, disease severity, shoot fresh weight and root fresh weight in pot assays

In pot assays, effect of *S. hortensis* hexane–methanol extracts mixture (2.5, 5, 10, 20 and 40 mg/ml) on the germination, disease severity, shoot and root fresh weights of tomato seeds infected with *C. m.* ssp. *michiganensis*, *X. a.* pv. *vesicatoria* and lettuce seeds infected with *X. a.* pv. *vitians* are shown in Tables 4 and 5, respectively. As shown in Table 4, the highest germination of tomato seeds infected with *C. m.* ssp. *michiganensis* was obtained in the treatment with 5 mg/ml concentration of the *S. hortensis* hexane–methanol extracts mixture and only pathogen application. However, there are no significant differences between all of the applications. The present results showed that on pot assays, all treatments of hexane–methanol extracts mixture were not toxic on the germination of tomato seeds infected with *C. m.* ssp. *michiganensis* as compared with control group. On the other hand, the lowest disease severity (75.6%) was observed with the treatment with 40 mg/ml hexane–methanol extracts mixture. A significant decrease of the disease severity was observed for the other hexane–methanol extracts mixture treatment except for 2.5 mg/ml (Table 4). As can be seen from Table 4, all applications did not significantly affect the plant height, whereas the highest plant height was determined by 10 mg/ml concentration of hexane–methanol extracts mixture. However, some applications of the hexane–methanol extract mixtures significantly decreased roots and shoots fresh weights. The highest root fresh weight was determined by 2.5 mg/ml concentration of hexane and methanol extract mixtures and only pathogen applications. In addition, the highest shoot fresh weight was obtained from 2.5 mg/ml concentration of hexane and methanol extract mixtures applications (Fig. 2). However, none of these positive effects on vegetative growth of plants were statistically significant different from control (only pathogen) application.

As shown in Table 4, the all concentrations of *S. hortensis* hexane–methanol extracts mixture caused low germinations with 50.0–73.3% germination rations as compared with the only pathogen and streptomycin sulfate applications (80.0%). However, all concentrations of the hexane–methanol extracts mixture and streptomycin sulfate significantly reduced disease severity caused by *X. a.* pv. *vesicatoria* in comparison to control (only pathogen). Furthermore, the all concentrations of the hexane–methanol extracts mixture, except 5 mg/ml dose, increased the plant height of tomato in comparison to the only pathogen application (Table 4). Table 4 also indicated that 20 and 40 mg/ml concentrations of the hexane–methanol extracts mixture showed an increasing effect on roots and shoots weights of tomato seeds infected by *X. a.* pv. *vesicatoria*. The highest plant height, root fresh and shoot fresh weights

Table 3
Number of the infected seedling and percentage of the germinated seeds (tomato and lettuce) coated with *Clavibacter michiganensis* ssp. *michiganensis* strain Cmm, *Xanthomonas axanopodis* pv. *vesicatoria* strain Xcv 761 and *Xanthomonas axanopodis* pv. *vitians* strain Xcvit, and than treated with *S. hortensis* hexane–methanol extracts mixture (2.5, 5.0, 10, 20 and 40 mg/ml) on Petri plate assays.

Treatments	Dose	Germinated seeds (%)			Number of the infected seedling		
		Cmm (tomato)	Xcv-761 (tomato)	Xcvit (lettuce)	Cmm (tomato)	Xcv-761 (tomato)	Xcvit (lettuce)
Hexane–methanol extracts mixture	40 mg/ml	66.7 ± 0.6 a	60.0 ± 0.4 a	0.0 ± 0.0 a	10 ± 0.00 b	4.67 ± 0.55 b	ND
	20 mg/ml	70.0 ± 0.7 ab	76.7 ± 0.4 bc	67.0 ± 0.4 a	10 ± 0.00 b	7.67 ± 0.42 d	0.67 ± 0.42 a
	10 mg/ml	73.3 ± 0.8 ab	76.7 ± 0.4 bc	40.0 ± 1.0 b	10 ± 0.00 b	7.67 ± 0.42 d	0.67 ± 0.21 a
	5 mg/ml	73.3 ± 0.4 ab	66.7 ± 0.2 ab	100.0 ± 0.0 c	10 ± 0.00 b	6.67 ± 0.21 cd	4.00 ± 0.96 b
	2.5 mg/ml	73.3 ± 0.4 ab	76.7 ± 0.2 bc	100.0 ± 0.0 c	10 ± 0.00 b	7.33 ± 0.42 d	10.00 ± 0.00 c
Control 1 (streptomisin)	500 μg/ml	76.7 ± 0.4 ab	56.7 ± 0.6 a	100.0 ± 0.0 c	10 ± 0.00 b	5.67 ± 0.55 bc	4.33 ± 0.55 b
Control 2 (sterilized seed + pathogen)	–	60.0 ± 0.4 ab	66.7 ± 0.6 ab	100.0 ± 0.0 c	10 ± 0.00 b	6.67 ± 0.55 cd	10.00 ± 0.00 c
Control 3 (sterilized seed + no pathogen)	–	83.3 ± 0.6 b	83.3 ± 0.6 c	100.0 ± 0.0 c	0.0 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a

Values followed by different letters in the same column are significantly different ($p < 0.05$) based on Duncan's multiple range test.
ND, not determined.

Table 4
The effects of *S. hortensis* hexane–methanol extracts mixture on germination of tomato seeds and disease severity caused of *C. michiganensis* ssp. *michiganensis* and *Xanthomonas axanopodis* pv. *vesicatoria* on pots.

Treatments	Dose (mg/ml)	GS (%)	DS	IDS (%)	PH (mm)	RFW (g/plant)	SFW (g/plant)
C. michiganensis ssp. *michiganensis*							
Hexane–methanol extracts mixture	40	43.3 a	1.22 ± 0.14 a	75.6 a	19.76 ± 1.37 a	0.77 ± 0.03 a	2.09 ± 0.20 a
	20	63.3 a	2.00 ± 0.28 b	60.0 b	18.47 ± 0.93 a	1.07 ± 0.24 ab	2.51 ± 0.61 a
	10	43.3 a	2.66 ± 0.16 c	46.8 c	22.33 ± 1.66 a	1.07 ± 0.02 ab	2.73 ± 0.20 a
	5	66.7 a	3.77 ± 0.14 d	24.6 d	18.49 ± 1.48 a	1.06 ± 0.15 ab	2.58 ± 0.40 a
	2.5	60.0 a	4.88 ± 0.11 e	2.4 e	18.72 ± 0.87 a	1.66 ± 0.37 b	4.90 ± 0.89 b
Streptomycin sulfate	0.5	63.3 a	4.77 ± 0.14 e	4.6 e	20.74 ± 1.54 a	1.14 ± 0.22 ab	3.02 ± 0.66 ab
Control (only pathogen)	–	66.7 a	5.00 ± 0.00 e	–	19.00 ± 1.00 a	1.66 ± 0.40 b	3.95 ± 0.92 ab
Xanthomonas axanopodis pv.*vesicatoria*							
Hexane–methanol extracts mixture	40	63.3 ab	1.33 ± 0.16 a	73.4 a	20.79 ± 2.24 b	0.61 ± 0.06 a	2.35 ± 0.27 a
	20	66.7 ab	1.66 ± 0.16 ab	66.8 ab	19.41 ± 1.30 ab	1.05 ± 0.02 b	3.19 ± 0.18 a
	10	50.0 a	2.88 ± 0.20 c	42.4 c	18.87 ± 0.64 ab	1.00 ± 0.05 ab	4.35 ± 0.17 b
	5	73.3 bc	2.00 ± 0.28 b	60.0 b	16.52 ± 1.01 a	0.94 ± 0.25 ab	2.42 ± 0.60 a
	2.5	60.0 ab	2.88 ± 0.26 c	42.4 c	18.40 ± 1.35 ab	0.76 ± 0.10 ab	2.83 ± 0.29 a
Streptomycin sulfate	0.5	80.0 c	2.11 ± 0.20 b	57.8 b	17.58 ± 0.41 ab	0.69 ± 0.01 ab	2.28 ± 0.06 a
Control (only pathogen)	–	80.0 c	5.00 ± 0.00 d	–	16.80 ± 0.47 ab	0.63 ± 0.12 a	2.45 ± 0.51 a

GS, germinated tomato seeds; DS, disease severity; IDS (%), inhibition of disease severity according to control; PH, plant height; RFW, root fresh weight; SFW, shoot fresh weight on pots. Values followed by different letters in the same column for each pathogen are significantly different ($p < 0.05$) based on Duncan's multiple range test.

were obtained from 40, 20 and 10 mg/ml concentrations, respectively.

As shown in Table 5, 40, 20 and 10 mg/ml concentrations of *S. hortensis* hexane–methanol extracts mixture showed potent toxic effect on the germination of lettuce seeds. On the other hand, as compared with control group (no pathogen), 5 and 2.5 mg/ml concentrations were no toxic effect on the germination of the lettuce seeds. Moreover, these concentrations also significantly reduced disease severity with 77.5% inhibition caused by *X. a.* pv. *vitians* in lettuce in comparison to only pathogen applications. There was also no significant difference in disease severity in comparison to the control group (no pathogen application). As can be seen from Table 5, 2.5 and 5 mg/ml dose applications of the hexane–methanol extracts mixture also increased the roots and shoots growth of lettuce as compared with controls. The maximum plant root fresh weight and shoot fresh weight were obtained from 2.5 mg/ml concentration of hexane–methanol extracts mixtures. However, 10, 20 and 40 mg/ml doses, which were inhibited the seed germinations of lettuce, potently reduced roots and shoots fresh weight of lettuce.

Table 5
The effects of *S. hortensis* hexane–methanol extracts mixture on the germination of lettuce seeds and disease severity caused by *Xanthomonas axanopodis* pv. *vitiens* on pots.

Treatments	Dose (mg/ml)	GS%	DS	IDS (%)	RFW	SFW
Hexane–methanol extracts mixture	40	0.0 ± 0.0 a	ND	–	ND	ND
	20	3.3 ± 0.0 a	1.00 ± 0.00 a	77.5 a	0.04 ± 0.04 a	0.07 ± 0.07 a
	10	13.3 ± 2.1 a	1.00 ± 0.00 a	77.5 a	0.19 ± 0.19 ab	0.07 ± 0.07 a
	5	93.3 ± 7.4 c	1.00 ± 0.00 a	77.5 a	0.70 ± 0.03 d	1.20 ± 0.06 d
	2.5	100 ± 0.0 c	1.00 ± 0.00 a	77.5 a	0.81 ± 0.02 d	1.23 ± 0.04 d
Streptomycin sulfate	0.5	86.7 ± 6.3 c	2.66 ± 0.33 b	40.1 b	0.57 ± 0.06 cd	0.77 ± 0.08 c
Control (no pathogen)	–	87.7 ± 6.3 c	1.00 ± 0.00 a	77.5 a	0.34 ± 0.06 bc	0.53 ± 0.05 b
Control (only pathogen)	–	63.3 ± 5.8 b	4.44 ± 0.17 c	–	0.63 ± 0.04 d	0.61 ± 0.03 bc

GS, germinated lettuce seeds; DS, disease severity; IDS, inhibition of disease severity according to control (only pathogen application); PH, plant height (PH); RFW, root fresh weight; SFW, shoot fresh weight. Values followed by different letters in the same column are significantly different ($p < 0.05$) based on Duncan's multiple range test. ND, not determined.

Fig. 2. Effect of the hexane and methanol extract mixtures of *S. hortensis* on lettuce seed germination, plant growth and disease severity caused by *X. a.* pv. *vitians* on pots (A: 2.5 mg/ml extract, B: 40 mg/ml extract, C: desinfected seeds).

4. Discussion

In recent years, the use of synthetic pesticides in plant disease protection programs around the world has resulted in disturbances of the environment, pest resurgences, and pest resistance to pesticides and lethal effect to nontarget organisms in the agro-ecosystems in addition to direct toxicity to users (Prakash et al., 2008; Asogwa et al., 2010; Bajpai et al., 2010). Therefore, considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms. The use of plant essential oil and extracts as antimicrobial agents is one of the first choices after plant bacterial disease outbreaks. Besides, interest has been generated in the development of safer antibacterial agents to control plant pathogenic bacteria in agriculture which also include essential oils and extracts (Bajpai et al., 2010; Lo Cantore et al., 2009).

Many publications have been previously documented on the antimicrobial activity of the essential oil or extracts of *S. hortensis* (Güllüce et al., 2003; Sahin et al., 2003; Kizil and Uyar, 2006; Adiguzel et al., 2007; Kotan et al., 2007; Dikbas et al., 2008; Mihajilov-Krstev et al., 2009; Özkalp and Özcan, 2009; Karami-Osboo et al., 2010). The present study showed that the essential oil of *S. hortensis* and its characteristic two phenolic compounds (carvacrol and thymol) posses the strong antibacterial activity based on the zone of inhibition against all the tested plant pathogenic bacteria on Petri plate assays. These results are in agreement with the previous literature reports on *S. hortensis* essential oil, carvacrol and thymol (Kizil and Uyar, 2006; Adiguzel et al., 2007; Kotan et al., 2007, 2010; Dikbas et al., 2008; Mihajilov-Krstev et al., 2009; Bajpai et al., 2010; Dadasoglu et al., 2011). In addition, the extracts of *S. hortensis* had also antagonistic activity against tested some of the plant pathogenic bacteria. The most successful result was obtained from the hexane extract. But, their antibacterial activities were weaker than that of the essential oil, carvacrol and thymol.

The composition of the essential oil and hexane extract isolated from the aerial parts of *S. hortensis* has previously been reported (Mihajilov-Krstev et al., 2009; Alizadeh et al., 2010; Najafia et al., 2010). Tozlu et al. (2011) stressed that the main components of the essential oil were carvacrol (54.74%), γ-terpinene (20.94%) and *p*-cymene (12.30%), whereas carvacrol (59.37%), γ-terpinene (7.82%), *p*-cymene (6.59%), *n*-eicosane (6.54%) and thymol (5.97%) were identified as the major components in the hexane extract of *S. hortensis*. Likewise, Güllüce et al. (2003) reported that the essential oil of *S. hortensis* contain mainly thymol (29.0%), carvacrol (26.5%), γ-terpinene (22.6%) and *p*-cymene (9.3%). In another study, Adiguzel et al. (2007) showed that the main constituents of the *S. hortensis* oil were thymol (40.54%), γ-terpinene (18.56%), carvacrol (13.98%), and *p*-cymene (8.97%). These results indicated that the oil and hexane extract contain the high content of carvacrol and thymol and therefore, the potent antibacterial activity of the oil and hexane extract can be attributed the high content of these compounds. On the other hand, it is known that antimicrobial effects or biological activities of essential oils and extracts of medicinal plants may be subjected to a change, based on the variations in the chemical composition of an essential oil that may be observed due to the origin, the locality, the environmental conditions, and the stage of development of the collected plant material (Sahin et al., 2003).

The mechanism of the action of the essential oil is probably related to the outer membrane disintegrating properties of thymol and carvacrol (Helander et al., 1998). Our results also confirmed that the potent antibacterial effect of the essential oil and hexane extract of *S. hortensis* is probably related to its carvacrol and thymol constituents. In the literature, some investigations suggest that these compounds penetrate inside the cell, where they interfere with cellular metabolism (Lambert et al., 2001; Marino et al., 2001; Karami-Osboo et al., 2010). Other studies also indicated that they disturb the structure of the cellular membrane and react with the active sites of enzymes or act as an H^+ carrier, depleting adenosine triphosphate pool (Farag et al., 1989; Ultee et al., 2002). In the current study, the direct application of the essential oil showed toxic effect on germination of the tomato seeds on pot assays. However, the indirect application of the essential oil, direct or indirect applications of the extracts mixture or their mixture did not toxic on germination of the tomato seeds. These results correlated with the allelopathic effects of the essential oil tested. Daizy et al. (2007) stressed that allelochemicals presented in the essential oil and extracts of different plant species have been reported to affect different physiological processes through their effects on enzymes responsible for phytohormone synthesis and were found to associate with inhibition of nutrients and ion absorption by affecting plasma membrane permeability. For this reason, *S. hortensis* hexane–methanol extracts mixture were tested for the effect on the lettuce and tomato seed germination, disease severity caused by *C. m.* ssp. *michiganensis*, *X. a.* pv. *vesicatoria* and *X. a.* pv. *vitians*, plant height, root fresh weight and shoot fresh weight on pots assays in the present study. Our results showed that some concentration of hexane–methanol extracts mixture from *S. hortensis* was not toxic on seed germination on pot assays. Furthermore, these concentrations reduced disease severity, increased germination percent of seeds, plant heights, shoots and roots fresh weights on pot assays. The best successful result was obtained from 2.5 mg/ml concentration of hexane–methanol extract mixtures applications for lettuce seeds, infected with *X. a.* pv. *vitians.* In this application, the disease severity rate was minimum level, and germination rate, shoots and roots fresh weights were maximum level.

Many plant essential oils, extracts and their pure compounds are considered to play a role in host defense mechanisms against plant pathogens. It is stated that biologically active compounds present in plant products act as elicitors and induce resistance in host plants resulting in reduction of disease development (Mihaliak et al., 1991; Vidhyasekaran, 1992). In addition, peroxidases have diverse functions in plant life such as defense against pathogen, cross-linking of cell wall components, formation of lignin and suberin, auxin catabolism, and antioxidant defense. Because of the antioxidant properties found in *S. hortensis*, it is important to characterize the peroxidases present in this labiatae (Keyhani and Keyhani, 2006). In this study, some of the concentration of hexane–methanol extracts mixture caused a positive effect on the plant growth parameters. Therefore, this increase can be related to host defense mechanisms in plants against plant pathogens.

The essential oil and extracts of *S. hortensis* have been recognized as having antibacterial effects, but their efficacy as seed disinfectants on plant pathogenic bacteria has not been studied. Therefore, this study was undertaken to assess the in vitro and in vivo antibacterial efficacy of the essential oil and extracts of *S. hortensis* against plant pathogenic bacteria. Application of the plant extracts as a seed disinfectant is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers. In this study, some concentrations of hexane–methanol extracts mixture of *S. hortensis* were not toxic to lettuce and tomato seeds, and could be used as potential disinfection agents against plant pathogenic bacteria. The current results show that hexane–methanol extract mixture of *S. hortensis* can be used as a seed disinfectant and as potential control agents for management of bacterial disease. Further studies on the combined effects of many local plant essential oils, extracts and their components as seed disinfectant are in progress in our model systems.

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