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Antibacterial effects of *Origanum onites* against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria

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ABSTRACT

The aim of this study was to determine the antibacterial activity of the essential oil, the extracts and pure metabolites of *Origanum onites* L. against plant pathogenic bacteria and potential use of the extracts against the diseases caused by some phytopathogenic bacteria in vitro and in vivo conditions. The essential oil and the extracts of *O. onites*, and its pure compounds isolated from the acetone extract were individually tested against a total of 14 phytopathogenic bacterial strains. The essential oil and hexane extract contained mainly carvacrol (70.50% and 80.50%), *p*-cymene (13.97% and 0.96%), thymol (2.19% and 7.53%, respectively). The oil showed potent antibacterial effect against all tested phytopathogenic bacteria, with inhibition zones of 22–40 mm. The hexane, chloroform and acetone extracts also inhibited the growth of the tested plant pathogenic bacteria exhibiting 12–24 mm inhibition zones, except *Xanthomonas campestris* pv. *zinniae*. MIC values for the oil and effective extracts were determined between 7.81 and 31.25 μl/ml and 40 and 100 mg/ml, respectively. However, methanol extract did not show any antibacterial effects against the pathogenic bacteria. Seven compounds were isolated from acetone extract by column and thin layer chromatography and their chemical structures were characterized by UV, IR, ^{1}H-NMR, ^{13}C-NMR and 1D and 2D NMR spectroscopic methods as caryophyllene oxide, carvacrol, *n*-tetracosanol, β-sitosterol, betulinic acid, ursolic acid, naringenin and aromadendrin. Among these compounds, carvacrol inhibited the growth of all the bacteria tested, whereas aromadendrin and naringenin inhibited only a few bacterial species. In the Petri plate assays, disease severity on the tomato and lettuce was reduced by the extracts as compared to control, whereas the high concentrations of the extracts showed a negative effect on seed germinations of the tomato and lettuce. The effects of the extracts on the germination, disease severity and seedling growth of tomato coated with *Clavibacter michiganensis* ssp. *michiganensis*, *Xanthomonos axonopodies* pv. *vesicatoria* and lettuce coated with *Xanthomonas campestris* pv. *vitians* were also studied on pots. The results showed that the extract applications significantly reduced the disease symptoms and they did not affect the germination and seedling growth of tomato and lettuce as compared to control. Furthermore, some applications increased the germination of tomato and lettuce. The current results showed that the extracts can be used in inhibiting some bacterial plant disease.

1. Introduction

Plant diseases caused by plant pathogenic bacteria are one of the major problems of crop loss (Kotan et al., 2005, 2010). Avoiding or mitigating crop losses due to the plant diseases is an important

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consideration in plant production. Rapid and effective control of the plant disease 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).

Bacterial diseases caused *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al., *Xanthomonas axonopodis* pv. (syn. *campestris*) *vesicatoria* (Doidge) Dye and *Xanthomonas campestris* pv. *vitians* (Brown) Dyes have devastated various host plants, leading to considerable losses in productivity and quality of harvests. They are seed-borne diseases and characterized by necrotic lesions on leaves, steams, and/or fruits. In warm and rainy weather, bacterial spot may cause severe defoliation of the plants that results in reduced yield, and diseased fruits may not be suitable for fresh-market sale.

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. Furthermore, antibiotics are forbidden in many countries because of their general toxicity and exert a negative impact on both yield and the environment. Only the United States and a few other countries allow the use of oxytetracycline and streptomycin for the control of the bacterial diseases on important crops (McManus et al., 2002).

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. 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 (Bajpai et al., 2010, 2011; Pradhanang et al., 2003; Kotan et al., 2010). Among the aromatic plant species, the genus *Origanum* L. has received great attention because of the fact that essential oil and extracts of members of this genus showed a very strong antimicrobial activity against various species of bacteria and fungi (Mosch et al., 1990; Lambert et al., 2001; Baydar et al., 2004; Arfa et al., 2006; Cavalcanti et al., 2006; Vasinauskiene et al., 2006; Sarac and Ugur, 2008; Wogiatzi et al., 2009; Copur et al., 2010; Bajpai et al., 2011; Arslan et al., 2012).

The genus *Origanum* (oregano) is an important genus of the Lamiaceae family and is represented in Turkey by about 23 species and 32 taxa (Baser, 2002; Skoula and Harborne, 2002; Gurbuz et al., 2011). *Origanum* species known "kekik" in Anatolia are aromatic and are used as condiment or herbal tea (Baser et al., 1993; Baser, 2002; Chan et al., 2010; Bagci et al., 2013). *Origanum onites* L. is the dominant species in the northwest of Turkey (Coskun et al., 2008). Recently, this spice plant has drawn more attention of consumers due to its antibacterial (Mosch et al., 1990, 1996; Baydar et al., 2004), antifungal (Reddy et al., 1998; Daferera et al., 2003; Dervis and Arslan, 2010), insecticidal (Isman, 2000; Aslan et al., 2005; Kordali et al., 2008; Ayvaz et al., 2010), acaricidal (Coskun et al., 2008) and antioxidative effects on human healthy (Kulisic et al., 2004; Bakkali et al., 2008). These activities have been mainly attributed to carvacrol, the major constituent of these oils. To our knowledge, there is a lack of information on the antibacterial property of the extracts and/or essential oil obtained from *O. onites* against plant pathogenic bacteria under in vivo assays. Therefore, this study was undertaken to assess the in vitro and in vivo antibacterial efficacy of the essential oil, *n*-hexane, chloroform, acetone and methanol extracts of the aerial parts of *O. onites* against plant pathogenic bacteria on the petri plate and pot assays.

2. Materials and methods

2.1. General experimental procedures

NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer, operating at 400 MHz and 100 MHz for ^{1}H and ^{13}C, respectively, using chloroform-d ($CDCl_3$) and dimethyl sulfoxide-d_6 (DMSO-d_6). Chemical shifts were expressed in δ (ppm) downfield from TMS as an internal standard and coupling constants reported in Hz. The IR spectra were determined on a FT-IR PerkinElmer Model 1600 spectrophotometer. Column chromatography (CC) was carried out using silica gel 60 (70–230 and 200–400 mesh), thin layer chromatography (TLC) and preparative TLC on silica gel 60 precoated plates, F-254 (Merck) (Cakir et al., 2003; Kotan et al., 2010). The spots on TLC were visualized by UV_{254}, UV_{366} and spraying with 1% vanillin-H_2SO_4 followed by heating (105 °C). Melting points were determined with a Thermo Scientific 9200 apparatus.

2.2. Plant material

Plant material was provided from the aerial parts of *O. onites* grown under field conditions at Hatay, Turkey in July 2009. The plant was collected at the flowering stage and dried in shade.

2.3. Extraction procedures

To obtain the essential oil, the dried plant samples (500 g) were powdered and 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. The yield of the oil was 3.67%.

Furthermore, to obtain plant extracts, the dried plant samples were powdered in a blender and then samples of 50 g 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 methanol extract was individually dissolved in distilled water (60 °C) and then filtered. Thus, chlorophyll was removed from the solution. Then, this solution was lyophilized in a Labconco 117 freeze-dryer at 5 m-Hg and −50 °C. The extract yields (w/w) of hexane, chloroform, acetone and methanol were 3.92, 7.04, 5.62 and 8.04, respectively.

2.4. Isolation procedures of pure compounds

To isolate compounds in the acetone extract responsible for antibacterial activity, the powdered plant sample (1125 g) was extracted with acetone (3L × 5). After filtration, acetone was evaporated under reduced pressure and temperature and a brown crude extract (72.64 g) was obtained.

The concentrated extract (35 g) was fractioned on silica gel CC (350 g, 200–400 mesh) using $CHCl_3$-hexane (8:2, 9:1 and 1:0) and $CHCl_3$-ethyl acetate (8:2, 7:3, 6:4, 1:1, 4:6, 2:8 and 0:1) as eluents. The fractions (50 ml each) were compared by TLC (silica gel) using $CHCl_3$-hexane (8:2) and $CHCl_3$-ethyl acetate (8:2, 6:4, 4:6, 2:8 and

0:1) solvent systems and the eluents giving similar spots on TLC were combined. Thus, eight fractions (A–H) were finally obtained.

Fraction A (2.51 g) was subjected to silica gel CC (70 g, 230–400 mesh) using hexane- $CHCl_3$ (1:1) and thus compound **1** (820 mg) was isolated. Fraction C (29.90 g) was rechromatographed over silica gel (230–400 mesh, 160 g) eluting with $CHCl_3$-hexane (1:1 and 0:1) and compound **2** (25.05 g) was isolated from the fractions 20–40. Fraction D (14.57 g) was solved using hot $CHCl_3$-hexane (9:1) (60 °C). After one day, white crystals occurred. The crystals were removed and controlled on TLC using $CHCl_3$-hexane (9:1). TLC showed that the crystals were pure compounds and thus compound **3** was isolated (1.25 g). Fraction E (5.92 g) was further chromatographed on silica gel CC (230–400 mesh) and $CHCl_3$-hexane (9:1) and $CHCl_3$. Compounds **4** (1.85 g) and **5** (1.50 g) isolated from the fractions 32–43 and 52–60, respectively. Fraction F (3.94 g) was further fractioned on silica gel CC (Silica gel 60, 230–400 mesh, 170 g) eluting with $CHCl_3$: methanol (98:2 and 96:4, v/v). Compound **6** precipitated during the elution of fractions 18–40. This precipitate was repeatedly washed over $CHCl_3$ and compound **6** (1.50 g) was isolated. Fraction G (4.07 g) submitted to a silica gel CC (Silica gel 60, 180 g, 230–400 mesh) using $CHCl_3$: methanol: acetone (95:5, v/v) as eluent and compounds **7** (1.25 g) and **8** (1.50 g) from fractions 24–31 and 56–75, respectively.

2.5. Phytopathogenic bacterial strains

The essential oil and the extracts of *O. onites* plant, and pure compounds isolated from the acetone extract were individually tested against a total of 14 phytopathogenic bacterial strains. All bacterial strains tested in the present study were obtained from Dr. Recep Kotan (Atatürk University, Faculty of Agriculture, Department of Plant Protection, Erzurum, Turkey). The used bacterial strains in this study were *Clavibacter michiganensis* ssp. *michiganensis* RK-Cmm, *Erwinia amylovora* RK-228, *Erwinia carotovora* ssp. *atroseptica* RK-462, *Erwinia chrysanthemi* RK-421, *Pseudomonas cichorii* RK-166, *Pseudomonas syringae* pv. *syringae* RK-204, *Pseudomonas syringae* pv. *tomato* RK-Ps-tom, *Xanthomonas axonopodis* pv. *malvacearum* RK-Xa-mal, *Xanthomonas axonopodis* pv. *vesicatoria* Xcv-761, *Xanthomonas axonopodies* pv. *campestris* RK-Xa-cam, *Xanthomonas axonopodis* pv. *vitians* Xa-vit, *Xanthomonas hortorum* pv. *pelargonii* RK-Xa-pel, *Xanthomonas campestris* pv. *raphani* RK-Xc-rap and *Xanthomonas campestris* pv. *zinniae* Xc-zin. The bacterial strains were stored at −80 °C in 15% glycerol and Luria Broth (LB) until use.

2.6. Lettuce and tomato seeds

Lettuce (*Lactuca sativa* L. cv. Yedikule) and tomato (*Lycopersicon 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.

2.7. Antibacterial activity tests

Antibacterial activity assays were carried out by disk diffusion method on tryptic soy agar (TSA, Merck, Germany) medium (Kotan et al., 2005, 2010). The oil, extracts and pure compounds were prepared by dissolving using 10% DMSO–distilled water, and then were sterilized by filtration by 0.45 μm Millipore filters. Bacterial cultures were grown in tryptic soy broth (TSB, Merck, Germany) and their suspension (100 μl) containing 1 × 108 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 oil and 1.25 mg of the extracts solutions. 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 the discs. Streptomycin sulfate (5 μg/disk) and 10% DMSO–distilled water were used as positive and negative controls, respectively. All the tests were made in triplicate.

2.8. Determination of minimum inhibition concentration (MIC)

Minimum inhibition concentrations (MICs) of the oil, extracts and pure compounds solutions were tested by a two-fold serial dilution method (Bajpai et al., 2010; Kotan et al., 2005, 2010). Two-fold serial dilutions of the oil and carvacrol (500 μl/ml) were prepared by diluting 10% DMSO to achieve a decreasing concentration ranging from 500 l/ml to 3.125 l/ml. However, solutions of the extracts, and pure compounds isolated were prepared at concentrations ranging from 10 mg/ml to 100 mg/ml. Using 100 l of suspension containing 1×10^8 cfu/ml was measured spectrophotometrically at 600 nm of bacteria spread on TSA plates. The disks were impregnated with 12.5 l of the solutions tested. Then, they were put in the middle of inoculated TSA agar plates. The bacterial cultures were incubated at 27 ± 2 °C for 48 h. The least concentration of each the solution showing a clear zone of inhibition was taken as the MIC value. All the tests were made in triplicate.

2.9. Seed surface disinfection with sodium hypochlorite

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% (v/v) sodium hypochlorite and by washing four times in sterilized and distilled water (sd. H_2O). Seeds were left to dry on sterile Whatman filter paper sheets.

2.10. 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 two days at 28 °C to coat the seeds with the pathogens. After shaking, the seeds were taken out and air-dried on sterile Whatman filter paper sheets.

2.11. Determination of the germination percentage of tomato seeds treated with the extracts on pot assays

As stated above, the seeds were surface disinfected and coated with the pathogens (*Clavibacter michiganensis* ssp. *michiganensis* and *Xanthomonas campestris* pv. *vesicatoria*). Then, the seeds were treated with the extracts at two different ways. The concentrations of hexane, chloroform and acetone extracts (5, 10, 15 and 20 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 extracts suspensions for 1, 2 and 4 h at 28 °C until the seeds uniformly coated with extract suspensions. Sterile Whatman no. 1 filter papers were placed on bottom of each of petri dishes (9 × 1.5 cm deep). The seeds surface disinfected and coated with the pathogen put on this filter paper sheets in the Petri plate. Whatman no. 1 paper was then stuck onto the top of petri dishes from inside and then impregnated with the extracts suspensions using an automatic pipette. Thus, there was no direct contact between thee extracts and the seeds.

In both applications, the seeds were left to dry on sterile Whatman filter paper sheets overnight in the laminar flow hood. The seeds were sown in plastic pots (ten seeds per pots) (6.5 × 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. The seedlings were watered once every two days. The percentage of germinated seeds per treatment was determined by counting the number of germinated seeds after 20 days. Streptomycin sulfate (0.5 mg/ml) by adding 0.5 mg of streptomycin to 1 ml sd. H_2O, disinfected seed infected with pathogen and sterilized seed not disinfected with pathogen were used as controls.

2.12. Determination of the germination percentage and number of infected seedlings of tomato seeds treated with the extracts on petri plate assays

The extracts suspensions (5, 10, 15 and 20 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. michiganensis* ssp. *michiganensis*, *X. axonopodies* pv. *vesicatoria* and *X. axonopodies* pv. *vitians*) soaked in the suspensions, and then incubated by shaking at 80 rpm for 3 h at 28 °C until the seeds were uniformly 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 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. If no, a few water-soaked lesions on the cotyledons in the seedling stage were observed after 10 days, these seedlings were considered as infected with pathogenic bacteria and vice versa. As stated above, streptomycin sulfate, the disinfected seed infected with pathogen and sterilized seed not disinfected with pathogen were used as controls.

2.13. Determination of the effect of the extracts on seed germination, disease severity and growth promotion on pot assays

The extracts (5, 10 and 20 mg/ml) were prepared by dissolving in 10% DMSO: distilled-water in 10 ml flasks. Lettuce and tomato seeds were coated with pathogens (*C. michiganensis* ssp. *michiganensis*, *X. axonopodies* pv. *vesicatoria* and *X. axonopodies* pv. *vitians*), and treated with different concentrations of the extracts (5, 10, 15 and 20 mg/ml) and streptomycin (0.5 mg/ml) as described above. Ten seeds were planted on each plastic pot (6.5 × 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 twenty days after planting. Disease severity was evaluated 40 days after planting using a 1 to 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 mean ± standard error (SE) of three replicates. The assays were repeated three times with 10 lettuce and tomato seeds per pots for each treatment. Streptomycin sulfate, disinfected seed infected with pathogen and sterilized seed not disinfected with pathogen were used as controls.

2.14. 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 mean ± SE.

3. Results

3.1. Chemical compositions and antibacterial effects of the essential oil and n-hexane extract

According to GC and GC-MS analyses, the chemical compositions of the essential oil and *n*-hexane extract of *O. onites* are shown in Table 1. As seen in this table, the essential oil and hexane extract contained carvacrol (70.50% and 80.50%, respectively), *p*-cymene (13.97% and 0.96%, respectively), thymol (2.19% and 7.53%, respectively). In general, the oil and hexane extract had qualitatively and quantitatively similar chemical composition. The oil and hexane extract were rich in aromatic monoterpenes. However, the oil was found to be qualitatively rich in oxygenated monoterpenes. As shown in Table 1, many oxygenated monoterpenes were not detected in the oil as compared with hexane extract. On the other hand, hexane extract also contains non-volatile compounds such as *n*-hexadecanoic acid, ethyl linoleate, *n*-heneicosane and *n*-tetracosanal.

As can be seen from Table 2, the oil and hexane extract of *O. onites* showed potent antibacterial effect against all tested phytopathogenic bacteria and exhibited inhibition zones of 36–8 mm. However, the hexane extract did not show any inhibition effect on the growth of *X. campestris* pv. *zinniae*, its growth inhibited by the oil with 33 mm inhibition. In particular, the oil was very effective against all tested bacteria. As shown in Table 2, the inhibition zones and MIC values of the oils against the pathogenic bacterial species were higher than those of the hexane extract.

3.2. Antibacterial activity of the extracts

Antibacterial effects of the chloroform, acetone and methanol extracts of *O. onites* against 14 bacterial plant pathogens were tested and the results are shown in Table 2. This table shows that chloroform and acetone extract inhibited the growth of all tested plant pathogens with inhibition zones of 12–24 mm. MIC values for chloroform and acetone extracts were determined between 40 and 100 mg/ml. However, methanol extract did not show any antibacterial effect against the plant pathogens.

3.3. Chemical composition of the acetone extract and antibacterial activities of its constituents

As can be seen from Table 2, acetone extract of *O. onites* showed potent antibacterial effect against all tested plant pathogens. Hence, the acetone extract was subjected to silica gel column chromatography to isolate the constituent(s) responsible for antibacterial activity. Thus, seven compounds isolated and their chemical structures were characterized by UV, IR, ^{1}H-NMR, ^{13}C-NMR and 1D and 2D NMR spectroscopic methods as caryophyllene oxide (**1**), carvacrol (**2**), tetracosanol (**3**), β-sitosterol (**5**), betulinic

Table 1
Chemical composition of the essential oil and *n*-hexane extract of aerial parts of *O. onites*.

RI[a]	Components	The oil (%)	Extract (%)	Identification methods
930	α-Thujene	0.26	–	GC, MS, RI
938	α-Pinene	0.61	–	GC, MS, RI
957	Camphene	0.23	–	GC, MS, RI
994	Myrcene	0.74	tr	GC, MS, RI
1023	α-Terpinene	0.15	tr	GC, MS, RI
1034	*p*-Cymene	13.97	0.96	GC, MS, RI
1067	γ-Terpinene	tr	0.81	GC, MS, RI
1079	*cis*-Sabinene hydrate	0.48	tr	GC, MS, RI
1099	2,5-Dimethyl styrene	0.06	–	GC, MS, RI
1117	*trans*-Sabinene hydrate	0.37	tr	GC, MS, RI
1130	*cis*-*p*-Menth-2-en-1-ol	0.07	–	GC, MS, RI
1147	*cis*-Verbenol	0.11	–	GC, MS, RI
1172	Borneol	0.89	2.42	GC, MS, RI
1178	Terpinen-4-ol	0.96	tr	GC, MS, RI
1185	*p*-Cymen-8-ol	0.25	–	GC, MS, RI
1190	α-Terpineol	0.30	–	GC, MS, RI
1197	*trans*-Dihydrocarvone	0.08	–	GC, MS, RI
1219	Thymol methyl ether	0.14	–	GC, MS, RI
1243	Carvone	0.18	–	GC, MS, RI
1251	Thymoquinone	0.23	tr	GC, MS, RI
1278	Bornyl acetate	tr	–	GC, MS, RI
1285	Thymol	2.19	7.53	GC, MS, RI
1296	Carvacrol	70.50	80.50	GC, MS, RI
1346	Thymol acetate	0.56	0.53	GC, MS, RI
1380	Carvacrol acetate	tr	tr	GC, MS, RI
1409	4aα,7α,7aβ-Nepetalactone	–	tr	MS, RI
1419	β-Caryophyllene	1.06	1.09	GC, MS, RI
1460	α-Humulene	0.16	–	GC, MS, RI
1461	Methyl vanillin	–	1.34	MS, RI
1478	γ-Muurolene	tr	–	GC, MS, RI
1494	Viridiflorene	–	tr	GC, MS, RI
1508	β-Bisabolene	0.20	0.45	GC, MS, RI
1513	γ-Cadinene	0.10	tr	GC, MS, RI
1562	Thymohydroquinone	–	tr	MS, RI
1574	Spathulenol	0.13	tr	GC, MS, RI
1579	Caryophyllene epoxide	2.10	0.58	GC, MS, RI
1609	Humulene epoxide	0.19	–	MS, RI
1636	δ-Cadinol	0.19	0.37	GC, MS, RI
1651	β-Eudesmol	0.30	tr	MS, RI
1671	Bulnesol	0.22	–	MS, RI
1844	(*Z*,*Z*)-Farnesyl acetone	–	tr	MS, RI
1923	*n*-Hexadecanoic acid	–	tr	GC, MS, RI
1969	Ethyl linoleate	–	1.33	GC, MS, RI
2100	*n*-Heneicosane	–	tr	GC, MS, RI
2624	*n*-Tetracosanol	–	0.87	GC, MS, RI
Grouped components (%)				
Aromatic monoterpenes		87.90	90.86	
Monoterpene hydrocarbons		1.99	0.81	
Oxygenated monoterpenes		3.44	2.42	
Sesquiterpene hydrocarbons		1.52	1.54	
Oxygenated sesquiterpenes		3.13	0.95	
Others		–	2.20	
Total identified		97.98	98.78	

tr, traces (less than 0.07%).

[a] Retention index relative to *n*-alkanes on SGE-BPX5 capillary column; 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 (Adams, 2007). RI, tentatively identified based on comparison of retention index of the compounds compared with published data (Adams, 2007).

acid (**4**), ursolic acid (**6**), naringenin (**7**) and aromadendrin (**8**) (Fig. 1).

These compounds were tested for antibacterial activity against the plant pathogens and carvacrol showed potent antibacterial activity ranging from 15.63 μl/ml to 31.25 μl/ml MIC values against all tested bacteria (Table 3). On the other hand, as can be seen from Table 3, naringenin and aromadendrin showed antibacterial activity against only a few pathogens. Other compounds did not show any antibacterial effect against the tested pathogens. Among the isolated compounds, only carvacrol showed potent antibacterial activity (Table 3) at a broad spectrum. Therefore, the antibacterial activity at a broad spectrum of the acetone extract can be attributed to high content of carvacrol in the acetone extract.

3.4. Effects of the essential oils and extracts on germination and disease severity of the tomato and lettuce seeds on petri plates

In the petri plate assays, especially the higher concentrations of the extracts showed a negative effect on seed germinations of the tomato. Generally, the number of the infected seedlings was reduced as compared to control (Tables 4 and 5

). As can be seen from Table 4, the germination of tomato seeds was reduced by about 50%. On the other hand, some applications of the extracts significantly reduced disease severity as compared to pathogen application. The higher concentrations of the extracts strongly reduced the disease severity (Table 4). Nevertheless, in the petri plate assays, the extracts did not exhibit any inhibition effects on seed germination of lettuce (Table 5). In particular, 20 mg/ml concentration of acetone extract completely inhibited bacterial disease symptoms on the lettuce seedling.

3.5. Effects of the essential oils and extracts on germination and disease severity of the tomato and lettuce seeds on pots

The results showed the effects of the extracts isolated from *O. onites* on the germination, disease severity, plant height, root fresh weight and shoot fresh weight of tomato seeds coated with *C. michiganensis* ssp. *michiganensis*, *X. axonopodies* pv. *vesicatoria* and lettuce seeds coated with *X. campestris* pv. *vitians* are presented in Tables 5–7. The present results show that the extract applications reduced the disease severity significantly and also they did not affect the germination and seedling growth of tomato and lettuce as compared to control (Tables 5–7). Furthermore, some applications increased the germination of tomato and lettuce (Table 6). The current results also indicated that some concentrations of the extracts caused a positive effect on the plant growth parameters (Tables 5–7). For instance, 15 mg/ml concentrations of the hexane extract of *O. onites* increased the plant height, root fresh weight and shoot fresh weight of tomato coated with *X. axonopodies* pv. *vesicatoria* and reduced the bacterial disease symptoms (57.74%) on pots (Table 7) as compared with control groups (streptomycin sulfate, only pathogen and no pathogen groups). However, appropriate dose of the extracts is dissimilar for different seed or pathogen on account of disease severity and plant growth parameters.

4. Discussion

The use of synthetic pesticides in plant disease protection programs around the world have resulted in disturbances of the environment, pest resurgences, and pest resistance to pesticides and lethal effect to non-target organisms in the agro-ecosystems in addition to direct toxicity to users (Prakash et al., 2008; Asogwa et al., 2010; Bajpai et al., 2010). It is also known that many plant pathogenic bacteria have acquired resistance to synthetic pesticides (White et al., 2002). 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. One of the alternative control methods of the disease is use of effective plant oils and extracts in conventional and organic agriculture.

There are numerous reports on the chemical composition of the essential oil of *O. onites* growing in Turkish flora and different flora of the world (Baydar et al., 2004; Ozel and Kaymaz, 2004; Figueredo et al., 2006; Kizil and Uyar, 2006; Sarac and Ugur, 2008; Kizil et al.,

Table 2
Antibacterial activities of the essential oil and extracts from *O. onites* against plant pathogenic bacteria.

Bacteria	Strains	Essential oil		Extracts								Antibiotic
				Hexane		Chloroform		Acetone		Methanol		STS
		IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD	MIC	IZD
Clavibacter michiganensis ssp. *michiganensis*	RK-Cmm	33	15.62	19	40	19	60	24	40	–	–	18
Erwinia amylovora	RK-228	28	31.25	15	40	16	60	21	40	–	–	24
Erwinia carotovora ssp. *atroceptica*	RK-462	33	15.62	15	40	14	50	11	60	–	–	21
Erwinia chrysanthemi	RK-421	22	15.62	12	60	11	60	13	60	–	–	25
Pseudomonas cichorii	RK-166	33	15.62	8	100	8	100	8	100	–	–	24
Pseudomonas syringae pv. *syringae*	RK-204	32	31.25	18	40	15	60	19	50	–	–	12
Pseudomonas syringae pv. *tomato*	RK-Ps-tom	25	31.25	15	50	16	60	21	50	–	–	13
Xanthomonas axonopodis pv. *malvacearum*	RK-Xa-mal	37	15.62	18	40	13	60	18	50	–	–	17
Xanthomonas axonopodis pv. *vesicatoria*	Xcv-761	34	15.62	15	40	14	60	20	40	–	–	23
Xanthomonas axonopodis pv. *campestris*	RK-Xa-cam	36*	15.62	15	40	14	60	15	50	–	–	21
Xanthomonas axonopodis pv. *vitians*	Xa-vit	34	15.62	12	70	12	60	13	60	–	–	16
Xanthomonas axonopodis pv. *pelargonii*	RK-Xa-pel	34	31.25	12	60	12	60	13	50	–	–	22
Xanthomonas campestris pv. *raphani*	RK-Xc-rap	40	7.81	16	50	14	60	20	50	–	–	16
Xanthomonas campestris pv. *zinniae*	Xc-zin	33	31.25	–	–	–	–	–	–	–	–	24

IZD: inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 1.25 mg of the extracts and 12.5 μl of the essential oil; MIC, minimal inhibitory concentration as μl/ml for the essential oil and in mg/ml for the extracts; *bactericidal effect was observed; –: not active. STS: streptomycin sulfate (5 μg/disk).

Table 3
Antibacterial effects of the pure compounds isolated from acetone extracts of *O. onites*.

Bacteria	Caryophyllene oxide (**1**)		Carvacrol (**2**)		Tetracosanol (**3**)		β-Sitosterol (**5**)		Ursolic acid (**6**)		Naringenin (**7**)		Aromadendrin (**8**)	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
C. michiganensis subsp. *michiganensis* Cmm	–	–	23*	31.25	–	–	–	–	–	–	–	–	–	–
E. amylovora RK-228	–	–	28*	31.25	–	–	–	–	–	–	–	–	–	–
E. carotovora subsp. *atroceptica* RK-462	–	–	15	15.63	–	–	–	–	–	–	–	–	–	–
E. chrysanthemi RK-421	–	–	16	15.63	–	–	–	–	–	–	–	–	–	–
P. cichorii RK-166	–	–	12	31.25	–	–	–	–	–	–	–	–	–	–
P.syringae pv. *syringae* RK-204	–	–	26	15.63	–	–	–	–	–	–	–	–	–	–
P. syringae pv. *tomato* RK-Ps-tom	–	–	25*	15.63	–	–	–	–	–	–	–	–	8*	100
X. axonopodies pv. *malvacearum* RK-Xa-mal	–	–	38*	15.63	–	–	–	–	–	–	–	–	8*	100
X. axonopodies pv. *vesicatoria* Xcv110c	–	–	28*	15.63	–	–	–	–	–	–	–	–	8*	100
X. axonopodies pv. *campestris* RK-Xa-cam	–	–	25*	15.63	–	–	–	–	–	–	–	–	–	–
X. axonopodies pv. *vitians* Xa-vit	–	–	32*	15.63	–	–	–	–	–	–	9	100	12*	90
X. axonopodies pv. *pelargonii* RK-Xa-pel	–	–	20	31.25	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *raphani* RK-Xc-rap	–	–	30*	15.63	–	–	–	–	–	–	–	–	–	–
X. campestris pv. *zinniae* Xc-zin	–	–	28*	15.63	–	–	–	–	–	–	–	–	–	–

IZD, inhibition zone in diameter (mm) around the disks (6 mm); MIC, minimal inhibitory concentration as μl/ml; –, not active; *Bactericidal effect was observed.

Table 4
Percentage of the germinated seed and the number of infected seedling of the tomato seeds coated with *C. m.* ssp. *michiganensis*, *X. axonopodies* pv. *vesicatoria* and *X. campestris* pv. *vitians*, and than treated with *O. onites* extracts (hexane, acetone and chloroform) on petri plate assays.

		C. michiganensis ssp. *michiganensis*		*X. axonopodies* pv. *vesicatoria*		*X. campestris* pv. *vitians*	
Treatments	Dose (mg/ml)	Germination (%)	Infected seedling (%)	Germination (%)	Infected seedling (%)	Germination (%)	Infected seedling (%)
Hexane Extract	20	53.3 a	40.0 b	46.6 a	100.0 d	100.0 a	90.0 c
	15	70.0 b	63.3 c	63.3 bc	78.9 c	100.0 a	100.0 d
	10	53.3 a	40.0 b	76.6 c	86.9 d	100.0 a	100.0 d
	5	53.3 a	36.6 ab	63.3 bc	63.1 b	100.0 a	100.0 d
Streptomycin sulfate	0.5	83.3 c	80.0 d	56.6 ab	64.6 b	100.0 a	83.3 b
Surfaces disinfected	–	56.6 ab	100.0 d	63.3 bc	100.0 d	100.0 a	100.0 d
No pathogen (Control)	–	90.0 d	0.0 a	90.0 d	0.0 a	100.0 a	0.0 a
Acetone extract	20	70.0 b	42.8 b	83.3 ab	79.9 de	100.0 a	0.0 a
	15	70.0 b	47.6 b	86.6 abc	65.3 cd	100.0 a	9.0 b
	10	73.3 bc	63.8 c	76.6 a	65.2 c	100.0 a	40.0 c
	5	76.6 bc	95.6 d	80.0 a	25.0 b	100.0 a	100.0 d
Streptomycin sulfate	0.5	83.3 cd	96.0 d	93.3 bc	100 f	100.0 a	83.3 cd
Surfaces disinfected	–	56.6 a	100.0 d	80.0 a	95.7 e	100.0 a	100.0 d
No pathogen (Control)	–	90.0 d	0.0 a	96.6 c	0.0 a	100.0 a	0.0 a
Chloroform extract	20	56.6 a	23.4 a	56.6 a	94.1 c	10.0 a	100.0 c
	15	76.6 b	47.7 b	80.0 b	95.7 d	100.0 b	86.6 b
	10	60.0 a	77.6 b	86.6 bc	46.1 b	100.0 b	96.6 c
	5	73.3 b	63.5 b	90.0 cd	51.7 bc	100.0 b	96.6 c
Streptomycin sulfate	0.5	83.3 bc	96.0.0 c	93.3 cd	100 e	10.00 b	83.3 b
Surfaces disinfected	–	56.6 ab	100.0 c	80.0 b	95.7 d	10.00 b	100.0 c
No pathogen (Control)	–	90.0 c	0.0 a	96.6 d	0.0 a	100.0 b	0.0 a

Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

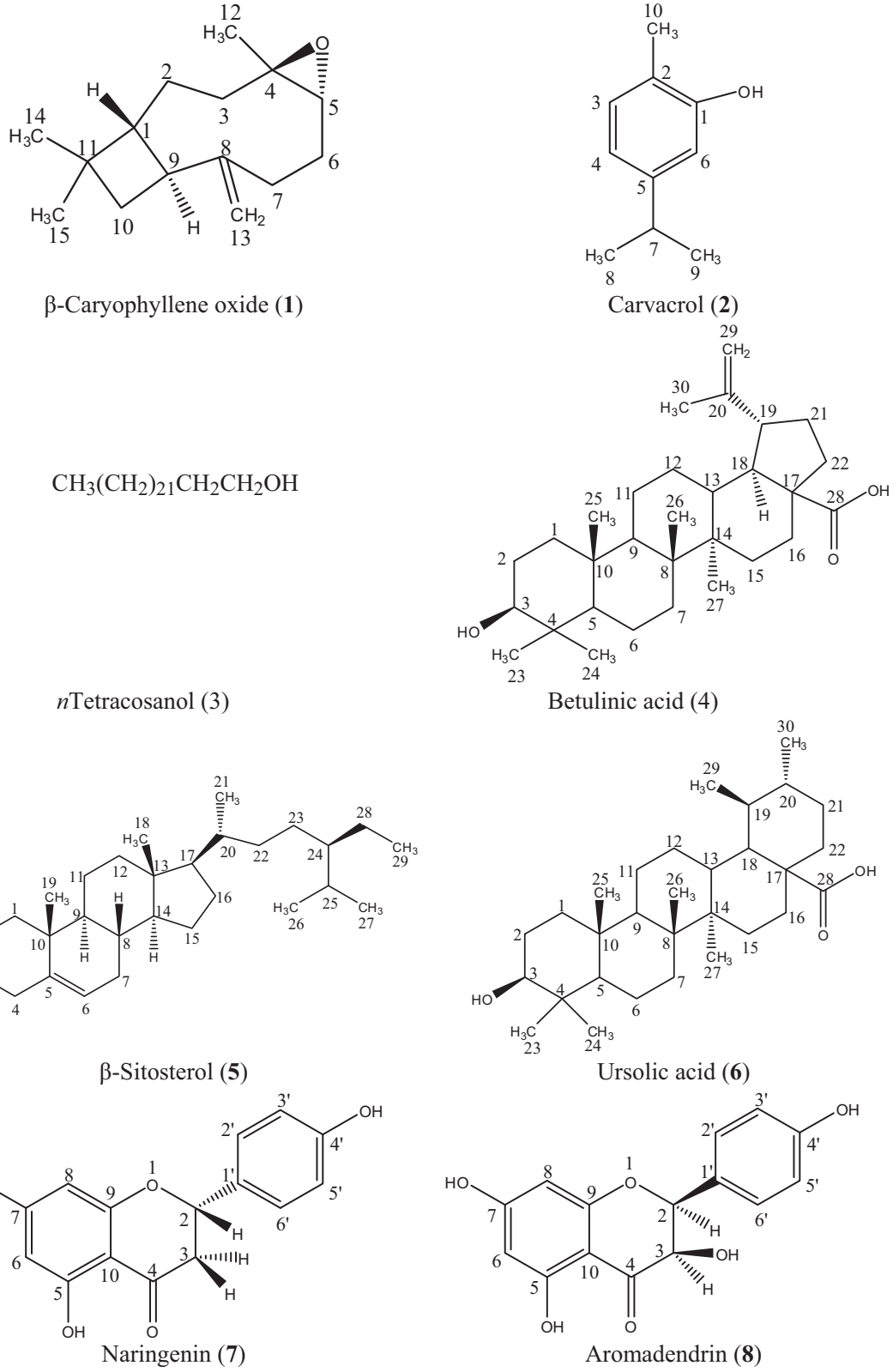

Fig. 1. Chemical structures of the compounds isolated from the aerial parts of *O. onites*.

2009; Korukluoglu et al., 2009; Kutlular and Ozel, 2009; Toncer et al., 2009; Wogiatzi et al., 2009; Sertkaya et al., 2010; Economou et al., 2011; Ozkan and Erdogan, 2011; Arslan et al., 2012; Orhan et al., 2012). In accordance with our results, many reports show that *O. onites* essential oil contain mainly aromatic monoterpenes, carvacrol and thymol (Skoula and Harborne, 2002; Baydar et al., 2004; Ozel and Kaymaz, 2004; Figueredo et al., 2006; Kizil and Uyar, 2006; Sarac and Ugur, 2008; Kizil et al., 2009; Korukluoglu et al., 2009; Kutlular and Ozel, 2009; Toncer et al., 2009; Wogiatzi et al., 2009; Sertkaya et al., 2010; Economou et al., 2011; Ozkan and

Table 5
Effects of the extracts from *O. onites* on the germination of tomato seeds and disease severity caused by *C. michiganensis* ssp. *michiganensis* on pots.

Treatments	Dose (mg/ml)	GS (%)	DS	IDS (%)	Plant height (mm)	Root fresh weight (g/plant)	Shoot fresh weight (g/plant)
Hexane Extract	20	50.0 a	1.33 ± 0.50 b	68.03	12.25 ± 1.33 c	0.23 ± 0.31 b	0.59 ± 0.20 ab
	15	46.6 a	1.00 ± 0.00 b	75.96	12.20 ± 1.30 c	0.05 ± 0.02 a	0.64 ± 0.23 c
	10	43.3 a	2.00 ± 0.63 c	51.92	11.35 ± 2.17 b	0.07 ± 0.02 a	0.76 ± 0.18 d
	5	50.0 a	3.33 ± 0.51 e	19.95	11.84 ± 0.50 b	0.06 ± 0.01 a	0.76 ± 0.20 d
Streptomycin sulfate	0.5	40.0 a	2.66 ± 0.81 d	–	12.45 ± 3.12 c	0.07 ± 0.04 a	0.91 ± 0.55 e
Control (only pathogen)	–	40.0 a	4.16 ± 0.75 f	–	10.08 ± 1.37 a	0.05 ± 0.01 a	0.49 ± 0.07 a
Control (no pathogen)	–	33.3 a	1.00 ± 0.00 a	–	11.80 ± 0.43 b	0.05 ± 0.01 a	0.56 ± 0.07 ab
Chloroform extract	20	30.0 a	2.33 ± 1.21 c	43.72	9.62 ± 0.40 abc	0.04 ± 0.00 ab	0.46 ± 0.04 a
	15	26.6 a	1.33 ± 0.51 b	67.87	10.73 ± 0.88 bc	0.05 ± 0.01 ab	0.60 ± 0.12 ab
	10	40.0 a	1.33 ± 0.51 b	67.87	8.59 ± 1.39 b	0.04 ± 0.01 ab	0.42 ± 0.12 a
	5	50.0 a	2.66 ± 0.51 c	35.75	7.66 ± 1.31 a	0.03 ± 0.00 a	0.30 ± 0.06 a
Streptomycin sulfate	0.5	40.0 a	2.66 ± 0.81 c	–	12.46 ± 3.11 c	0.07 ± 0.04 b	0.92 ± 0.55 b
Control (only pathogen)	–	40.0 a	4.14 ± 0.75 d	–	10.16 ± 1.46 abc	0.05 ± 0.01 ab	0.48 ± 0.06 a
Control (no pathogen)	–	33.3 a	0.00 ± 0.00 a	–	11.80 ± 0.43 c	0.05 ± 0.00 ab	0.55 ± 0.07 ab
Acetone extract	20	30.0 ab	2.83 ± 0.98 c	31.64	12.66 ± 0.35 c	0.22 ± 0.30 a	0.86 ± 0.04 d
	15	26.6 a	2.16 ± 0.98 bc	47.83	13.00 ± 1.00 c	0.16 ± 0.21 a	0.80 ± 0.14 d
	10	40.0 ab	2.33 ± 0.81 bc	43.72	10.33 ± 2.08 ab	0.03 ± 0.01 a	0.59 ± 0.20 abc
	5	53.3 b	1.50 ± 0.54 b	63.77	9.03 ± 1.81 a	0.02 ± 0.01 a	0.38 ± 0.13 a
Streptomycin sulfate	0.5	40.0 ab	2.66 ± 0.81 c	–	12.46 ± 3.11 c	0.07 ± 0.04 a	0.60 ± 0.14 abc
Control (only pathogen)	–	40.0 ab	4.14 ± 0.75 d	–	10.08 ± 1.37 ab	0.05 ± 0.01 a	0.49 ± 0.06 ab
Control (no pathogen)	–	33.3 ab	1.00 ± 0.00 a	–	11.80 ± 0.43 ab	0.05 ± 0.00 a	0.67 ± 0.14 bc

GS: Germinated tomato seeds; DS: disease severity, IDS: inhibition of disease severity according to only pathogen control (surfaces disinfected); –: Not evaluated.
Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

Erdogan, 2011; Arslan et al., 2012; Orhan et al., 2012). It has also been reported that the wild populations of the *O. onites* contain two different chemotypes, carvacrol and linalool. For instance, Ozkan and Erdogan (2011) documented the essential oil of *O. onites* growing in Turkish flora contained linalool (50.53%) as main components besides carvacrol (24.52%) and thymol (15.66%). As can be seen from Table 2, the oil and hexane extract of *O. onites* showed potent antibacterial effect against all tested phytopathogenic bacteria and exhibited inhibition zones of 36–8 mm. However, the inhibition zones and MIC values of the oil against the pathogenic bacterial species were higher than those of the hexane extract (Table 2). This potent antibacterial activity of the oil and hexane extract of *O. onites* can be attributed to their high carvacrol contents. On the other hand, synergistic and/or antagonistic interactions of the components in the oil and the extracts could be taken into account. In literature, it is well known that the essential oil of the plants rich in this compounds have strong antimicrobial effects (Baydar et al., 2004; Kizil and Uyar, 2006; Vasinauskiene et al., 2006; Kordali et al., 2008; Sarac and Ugur, 2008; Korukluoglu et al., 2009; Wogiatzi et al., 2009; Copur et al., 2010; Karami-Osboo et al., 2010; Kotan et al., 2010; Dadasoglu et al., 2011; Keskin and Toroglu, 2011; Orhan et al., 2011, 2012). Hence, our results are in agreement with the previous literature reports on *O. onites* essential oil.

Our results showed that the strong antibacterial effect of the essential oil and hexane extract of *O. onites* is probably related to its carvacrol and thymol constituents. 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). It has also been documented that these compounds disturb the structure of the cellular membrane

Table 6
Effects of *O onites* the extracts on the germination of tomato seeds and disease severity caused by *X. axonopodies* pv. *vesicatoria* on pots.

Treatments	Dose (mg/ml)	PGS (%)	DS	IDS (%)	Plant height (mm)	Root fresh weight (g/plant)	Shoot fresh weight (g/plant)
Hexane Extract	20	30.0 a	1.66 ± 0.51 a	61.66	12.25 ± 1.56 ab	0.09 ± 0.07 a	0.97 ± 0.18 b
	15	33.3 a	1.83 ± 0.40 a	57.74	13.58 ± 1.18 b	0.10 ± 0.05 a	0.68 ± 0.27 a
	10	36.6 a	2.50 ± 0.54 b	42.26	10.86 ± 1.25 a	0.05 ± 0.02 a	0.58 ± 0.07 a
	5	43.3 ab	3.16 ± 0.75 b	27.02	12.03 ± 1.25 ab	0.05 ± 0.01 a	0.59 ± 0.09 a
Streptomycin sulfate	0.5	50.0 a	2.83 ± 0.75 b	–	10.60 ± 0.52 a	0.04 ± 0.00 a	0.46 ± 0.06 a
Control (only pathogen)	–	63.3 c	4.33 ± 0.51 c	–	12.06 ± 0.55 ab	0.05 ± 0.00 a	0.63 ± 0.06 a
Control (no pathogen)	–	33.3 a	1.00 ± 0.00 a	–	11.80 ± 0.43 ab	0.05 ± 0.01 a	0.57 ± 0.05 a
Chloroform Extract	20	83.3 b	1.00 ± 0.00 a	76.91	9.08 ± 1.12 a	0.06 ± 0.02 bc	0.37 ± 0.21 a
	15	76.6 b	1.50 ± 0.54 a	65.36	10.16 ± 0.43 ab	0.04 ± 0.01 ab	0.74 ± 0.05 c
	10	73.3 b	1.50 ± 0.54 a	65.36	10.72 ± 0.38 ab	0.09 ± 0.03 c	0.76 ± 0.14 c
	5	76.6 b	3.00 ± 0.63 b	30.72	9.11 ± 0.78 a	0.02 ± 0.00 a	0.47 ± 0.13 ab
Streptomycin sulfate	0.5	56.6 a	2.83 ± 0.75 b	–	10.60 ± 0.52 bc	0.04 ± 0.00 ab	0.46 ± 0.06 ab
Control (only pathogen)	–	83.3 b	4.33 ± 0.51 c	–	12.06 ± 0.55 d	0.05 ± 0.00 ab	0.63 ± 0.06 bc
Control (no pathogen)	–	76.6 b	1.00 ± 0.00 a	–	11.80 ± 0.43 c	0.05 ± 0.00 ab	0.57 ± 0.05 abc
Acetone extract	20	70.0 abc	2.00 ± 0.63 bc	53.81	10.03 ± 2.83 ab	0.30 ± 0.21 b	0.63 ± 0.11 b
	15	76.6 bcd	2.33 ± 0.50 cd	46.19	9.29 ± 0.80 a	0.04 ± 0.01 a	0.51 ± 0.02 ab
	10	63.3 ab	1.50 ± 0.54 b	65.36	9.33 ± 0.94 a	0.03 ± 0.00 a	0.56 ± 0.03 ab
	5	90.0 d	1.66 ± 0.81 bc	61.66	9.73 ± 1.55 ab	0.04 ± 0.01 a	0.55 ± 0.10 ab
Streptomycin sulfate	0.5	56.6 a	2.83 ± 0.75 c	–	10.60 ± 0.52 ab	0.04 ± 0.00 a	0.46 ± 0.06 a
Control (only pathogen)	–	83.3 cd	4.33 ± 0.51 d	–	12.06 ± 0.55 b	0.05 ± 0.00 a	0.62 ± 0.06 b
Control (no pathogen)	–	76.6 bcd	1.00 ± 0.00 a	–	11.80 ± 0.43 ab	0.05 ± 0.00 a	0.57 ± 0.05 ab

PGS: Percentage of germinated tomato seeds; DS: disease severity; IDS: inhibition of disease severity according to only pathogen control (surfaces disinfected); –: not evaluated.
Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

Table 7
Effects of *O onites* the extracts on the germination of lettuce seeds and disease severity caused by *X. campestris* pv. *vitians* on pots.

Treatments	Dose (mg/mL)	PGS (%)	DS	IDS (%)	Plant height (mm)*	Root fresh weight (g/plant)	Shoot fresh weight (g/plant)
Hexane Extract	20	60.0 a	1.33 ± 0.51 b	70.44	–	0.01 ± 0.00 a	0.20 ± 0.03 ab
	15	83.3 b	1.83 ± 0.75 bc	59.33	–	0.02 ± 0.01 a	0.27 ± 0.03 bc
	10	83.3 b	2.33 ± 0.51 cd	48.22	–	0.02 ± 0.01 a	0.27 ± 0.08 bc
	5	83.3 b	2.66 ± 0.51 d	40.89	–	0.03 ± 0.01 a	0.39 ± 0.05 c
Streptomycin sulfate	0.5	83.3 b	2.33 ± 0.81 cd	–	–	0.03 ± 0.03 a	0.21 ± 0.04 ab
Control (only pathogen)	–	96.6 b	4.50 ± 0.83 e	–	–	0.01 ± 0.00 a	0.12 ± 0.05 a
Control (no pathogen)	–	93.3 b	1.00 ± 0.00 a	–	–	0.02 ± 0.01 a	0.32 ± 0.11 bc
Chloroform Extract	20	3.3 a	5.00 ± 0.00 c	−11.1	–	0.01 ± 0.00 a	0.01 ± 0.02 a
	15	93.3 c	2.16 ± 1.47 b	52.00	–	0.02 ± 0.00 bc	0.28 ± 0.07 cd
	10	83.3 b	1.50 ± 0.54 b	66.67	–	0.03 ± 0.00 c	0.38 ± 0.10 d
	5	96.6 c	1.83 ± 0.75 b	59.33	–	0.03 ± 0.00 c	0.40 ± 0.02 d
Streptomycin sulfate	0.5	83.3 b	2.33 ± 0.81 b	–	–	0.02 ± 0.00 bc	0.22 ± 0.03 bc
Control (only pathogen)	–	96.6 c	4.50 ± 0.83 c	–	–	0.01 ± 0.00 ab	0.12 ± 0.05 ab
Control (no pathogen)	–	93.3 c	1.00 ± 0.00 a	–	–	0.03 ± 0.00 c	0.32 ± 0.12 c
Acetone extract	20	90.0 bc	1.33 ± 0.51 a	70.44	–	0.02 ± 0.00 ab	0.29 ± 0.06 bc
	15	76.6 a	1.16 ± 0.40 a	74.22	–	0.03 ± 0.00 b	0.45 ± 0.14 c
	10	96.6 d	1.50 ± 0.54 a	66.67	–	0.02 ± 0.00 ab	0.28 ± 0.09 ab
	5	93.3 bc	2.50 ± 0.54 b	44.44	–	0.02 ± 0.00 ab	0.23 ± 0.03 ab
Streptomycin sulfate	0.5	83.3 ab	2.33 ± 0.81 b	–	–	0.02 ± 0.00 ab	0.21 ± 0.03 ab
Control (only pathogen)	–	96.6 d	4.50 ± 0.83 c	–	–	0.01 ± 0.00 a	0.12 ± 0.05 a
Control (no pathogen)	–	93.3 bc	1.00 ± 0.00 a	–	–	0.03 ± 0.01 b	0.31 ± 0.12 bc

PGS: percentage of germinated lettuce seeds; DS: disease severity; IDS: inhibition of disease severity according to only pathogen control (surfaces disinfected); –: not evaluated.
Values followed by different letters in the same column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test.

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).

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; Pradhanang et al., 2003; Nguefack et al., 2005; Cavalcanti et al., 2006; Vasinauskiene et al., 2006; Lo Cantore et al., 2009; Karami-Osboo et al., 2010, yeni referanslar eklendi). 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 (Keyhani and Keyhani, 2006). In this study, some applications of the extracts caused a positive effect on the plant growth parameters. For instance, all concentrations of the hexane extract, 5 and 10 mg/ml concentrations of chloroform and acetone extracts of *O. onites* increased the germination and plant height of tomato (Table 5) as compared with control. Therefore, this increase can be related to host defense mechanisms in plants against plant pathogens.

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 the current study, the extract applications reduced the disease severity significantly and also they did not affect the germination and seedling growth of tomato and lettuce as compared to control (Tables 5–7). Some applications increased also the germination of tomato and lettuce and resulted in a positive effect on the plant growth parameters (Tables 5–7). In tomato seed assays infected with *C. michiganensis* spp. *michiganensis* and *X. axonopodies* pv. *vesicatoria*, 15 and 20 mg/ml concentrations of the hexane extract inhibited the disease severity with 75.96–57.74% and these applications also increased the germinations and seedling growths of tomato seeds (Tables 5 and 6). As can be seen from Table 6, 15 mg/ml concentrations of the hexane extract of *O. onites* increased the plant height (13.58 ± 1.18), root fresh weight (0.10 ± 0.05) and shoot fresh weight (0.68 ± 0.27) of tomato coated with *X. axonopodies* pv. *vesicatoria* and reduced the bacterial disease symptoms (57.74%) on pots as compared with control groups (streptomycin sulfate, only pathogen and no pathogen groups) in the current study. The similar results were also found for the acetone and chloroform extracts (Tables 5 and 6). Likewise, all application concentrations of the hexane, chloroform and acetone extracts significantly reduced the disease severity with 74.22–40.89% inhibition (Table 7). However, 20 mg/ml doses of hexane and chloroform extracts reduced the germination of the lettuce seeds.

As can be seen from Table 2, acetone extract of *O. onites* showed potent antibacterial effect against all tested plant pathogens. Hence, the acetone extract was subjected to silica gel column chromatography to isolate the constituent(s) responsible for antibacterial activity. Thus, seven compounds isolated and their chemical structures were characterized by UV, IR, ^{1}H-NMR, ^{13}C-NMR and 1D and 2D NMR spectroscopic methods as caryophyllene oxide (**1**), carvacrol (**2**), tetracosanol (**3**), β-sitosterol (**5**), betulinic acid (**4**), ursolic acid (**6**), naringenin (**7**) and aromadendrin (**8**) (Fig. 1). The chemical structures of the known compounds were also confirmed by comparison of their spectral data with that reported in the literature, i.e. as β-caryophylene oxide (**1**) (Yang and Deinzer, 1994; Adams, 2007; Noma et al., 2010), carvacrol (**2**) (Arfa et al., 2006; Adams, 2007; Mastelic et al., 2008), betulinic acid (**4**) (Ikuta and Itokawa, 1988; Chatterjee et al., 1999; Gutierrez, 2005; Bhattachartya et al., 2009), β-sitosterol (**5**) (Kovganko et al., 2000; Cakir et al., 2003; Huang et al., 2004; Nakamura et al., 2005), ursolic acid (**6**) (Moghaddam et al., 2006; Silva et al., 2008), naringenin (**7**) (Markham and Geiger, 1993; Hasan and Tahir, 2005; Ibrahim et al., 2007) and aromadendrin (**8**) (Markham and Geiger, 1993; Gautam et al., 2005; Iwashina et al., 2007; Freitas et al., 2008) and long range ^{1}H–^{13}C correlations from their HMBC spectra. In our study, these compounds were also tested for antibacterial activity against the plant pathogens and carvacrol showed potent antibacterial activity against all tested bacteria with MIC values, 15.63 μl/ml and 31.25 μl/ml (Table 3). On the other hand, as can be seen from Table 3, naringenin and aromadendrin showed antibacterial activity against only a few pathogens. Other compounds did not show any antibacterial effect against the tested pathogens. Among the compounds isolated from the acetone extract, only carvacrol showed the potent antibacterial activity at a broad spectrum

(Table 2). Therefore, the antibacterial activity at a broad spectrum of the acetone extract can be attributed to high content of carvacrol in the acetone extract. In the current study, 25.05 g of carvacrol was isolated from 35 g acetone extract by chromatographic methods. Previous study showed that carvacrol is an effective antibacterial component on some plant pathogenic bacteria (Dadasoglu et al., 2011; Kotan et al., 2007, 2010). Furthermore, this is the first report for the antibacterial activities of naringenin and aromadendrin against the plant pathogenic bacteria.

In conclusion, the hexane, chloroform and acetone extracts applications of *O. onites* significantly reduced the disease symptoms. They also did not affect the germinations and seedling growths of tomato and lettuce they can be used the inhibition of some plant disease when it was used appropriate doses, 10, 15 and 20 mg/ml doses of the hexane, chloroform and acetone extracts. The antibacterial effects of the extracts can be attributed to their phenolic content, carvacrol. Further studies are required to understand the positive effect of the essential oil and extracts on plant growth parameters.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2014.03.016.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition. Allured Publishing Corp, Carol Stream, IL, USA, pp. 803.
Arfa, A.B., Combes, S., Preziosi-Belloy, L., Gontard, N., Chalier, P., 2006. Antimicrobial activity of carvacrol related to its chemical structure. Lett. Appl. Microbiol. 43, 149–154.
Arslan, A., Uremis, M.I., Demirel, N., 2012. Effects of leafhopper feeding damage on herbage colour, essential oil content and compositions of Turkish and Greek oregano. Exp. Agric. 48, 428–437.
Aslan, I., Calmasur, O., Sahin, F., Caglar, O., 2005. Insecticidal effects of essential plant oils against *Ephestia kuehnielia* (Zell.), *Lasioderma serricome* (F.) and *Sitophilus granarius* (L.). J. Plant Dis. Prot. 112, 257–267.
Asogwa, E.U., Ndubuaku, T.C.N., Ugwu, J.A., Awe, O.O., 2010. Prospects of botanical pesticides from neem, *Azadirachta indica* for routine protection of cocoa farms against the brown cocoa mirid *Sahlbergella singularis* in Nigeria. J. Med. Plants Res. 4, 1–6.
Ayvaz, A., Sagdic, O., Karaborklu, S., Ozturk, I., 2010. Insecticidal activity of the essential oils from different plants against three stored-product insects. J. Insect Sci. 10, 1–13.
Bagci, Y., Saadia, Z., Ozcan, M.M., 2013. Aroma profile of *Origanum vulgare* L. subsp. viride (Boiss.) Hayek, *Satureja hortensis* L. and *Thymbra sintenisii* Bornm. & Aznav. subsp. *isaurica* P.H. Davis used as condiment and herbal tea in Turkey. J. Essent. Oil Bear. Plants 8, 304–311.
Bajpai, V.K., Kang, S., Xu, H., Lee, S.G., Baek, K.H., Kang, S.C., 2011. Potential roles of essential oils on controlling plant pathogenic bacteria *Xanthomonas* species: a review. Plant Pathol. J. 27, 207–224.
Bajpai, V.K., Cho, M.J., Kang, S.C., 2010. Control of plant pathogenic bacteria of *Xanthomonas* spp. by the essential oil and extracts of *Metasequoia glyptostroboides* Miki ex Hu in vitro and in vivo. J. Phytopathol. 158, 479–486.
Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils: a review. Food Chem. Toxicol. 46, 446–475.
Barnard, M., Padgitt, M., Uri, N.D., 1997. Pesticide use and its measurement. Int. Pest Control 39, 161–164.
Baser, K.H.C., 2002. Aromatic biodiversity among the flowering taxa of Turkey. Pure Appl. Chem. 74, 527–545.
Baser, K.H.C., Ozek, T., Tümen, G., Sezik, E., 1993. Composition of the essential oils of Turkish *Origanum* species with commercial importance. J. Essent. Oil Res. 5, 619–623.
Baydar, H., Sagdic, O., Ozkan, G., Karadogan, T., 2004. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. Food Control 15, 169–172.
Bhattachartya, S., Tarafdar, S., Saha, C.N., 2009. Triterpenoids and steroids from *Holarrhena pubescens* seeds. Pharmacogn. Mag. 5, 407–411.
Bradbury, J.F., 1986. Guide to Plant Pathogenic Bacteria. CAB International, Wallingford.
Brent, K.J., Hollomon, D.W., 1998. Fungicide Resistance: The Assessment of Risk. FRAC. Global Crop Protection Federation, Brussels, pp. 1–48, Monograph No. 2.
Cakir, A., Mavi, A., Yildirim, A., Duru, M.E., Harmandar, M., Kazaz, C., 2003. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. J. Ethnopharmacol. 87, 73–83.
Cavalcanti, F.R., Resende, M.L.V., Carvalho, C.P.S., Silveira, J.A.G., Oliveira, J.T.A., 2006. Induced defence responses and protective effects on tomato against *Xanthomonas vesicatoria* by an aqueous extract from *Solanum lycocarpum* infected with *Crinipellis perniciosa*. Biol. Control 39, 408–417 (yeni eklendi).
Chan, E.W.C., Lim, Y.Y., Chong, K.L., Tan, J.B.L., Wong, S.K., 2010. Antioxidant properties of tropical and temperate herbal teas. J. Food Comp. Anal. 23, 185–189.
Chatterjee, P., Pezzuto, J.M., Kouzi, S.A., 1999. Glucosidation of betulinic acid by *Cunninghamella* species. J. Nat. Prod. 62, 761–763.
Copur, G., Arslan, M., Duru, M., Baylan, M., Canogullari, S., Aksan, E., 2010. Use of oregano (*Origanum onites* L.) essential oil as hatching egg disinfectant. Afr. J. Biotechnol. 9, 2531–2538.
Coskun, S., Girisgin, O., Kurkcuoglu, M., Malyer, H., Girisgin, A.O., Kırımer, N., Baser, K.H., 2008. Acaricidal efficacy of *Origanum onites* L. essential oil against *Rhipicephalus turanicus* (Ixodidae). Parasitol. Res. 103, 259–261.
Daferera, D.J., Ziogas, B.N., Moschos, G.M., 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Prot. 22, 39–44.
Dervis, S., Arslan, M., 2010. Antifungal activity of essential oils against three vegetative compatibility groups of *Verticillium dahliae*. World J. Microbiol. Biotechnol. 26, 1813–1821.
Dadasoglu, F., Aydin, T., Kotan, R., Cakir, A., Ozer, H., Kordali, S., Cakmakci, R., Dikbas, N., Mete, E., 2011. Antibacterial activities of extracts and essential oils of three *Origanum* species against plant pathogenic bacteria and their potential use as seed disinfectants. J. Plant Pathol. 93, 271–282.
Economou, G., Panagopoulos, G., Tarantilis, P., Kalivas, D., Kotoulas, V., Travlos, I.S., Polysiou, M., Karamanos, A., 2011. Variability in essential oil content and composition of *Origanum hirtum* L., *Origanum onites* L., *Coridothymus capitatus* (L.) and *Satureja thymbra* L. populations from Greek Island Ikaria. Ind. Crops Prod. 33, 236–241.
Farag, R.S., Daw, Z.Y., Abo-Raya, S.H., 1989. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. J. Food. Sci. 54, 74–76.
Figueredo, G., Cabassu, P., Chalchat, J.C., Pasquier, B., 2006. Studies of Mediterranean oregano populations. VII: chemical composition of essential oils of carvacrol-rich oregano of various origins. J. Essent. Oil Res. 18, 244–249.
Freitas, M.O., Ponte, F.A.F., Lima, M.A.S., Silveira, E.R., 2008. Flavonoids and triterpenes from the nest of the stingless bee *Trigona spinipes*. J. Brazil. Chem. Soc. 13, 1–7.
Gautam, L.N., Awale, S., Kalauni, S.K., Shrestha, K., Gewali, M.B., 2005. Phytochemical and biological studies on *Podocarpus neriifolius* D. Don: a Himalayan conifer of Nepal. Sci. World 3, 22–25.
Gutierrez, R.M.P., 2005. Pentacyclic triterpenes from *Cirsium pascuarense*. J. Chil. Chem. Soc. 50, 587–589.
Gurbuz, B., Ipek, A., Ayvaz, N., 2011. Türkiye Florasındaki *Origanum* türlerinin yayılış alanları ve ticareti. Türk Bilimsel Derlemeler Dergisi 4, 55–58.
Hasan, A., Tahir, M.N., 2005. Flavonoids from the leaves of *Impatiens bicolor*. Turk. J. Chem. 29, 65–70.
Huang, L., Zhong, T., Chen, T., Zhao, Y., Chen, G., 2004. Identification of β-sitosterol, stigmasterol and ergosterin in *A. roxburghii* using supercritical fluid extraction followed by liquid chromatography/atmospheric pressure chemical ionization ion trap mass spectrometry. Rapid Commun. Mass Spectrom. 21, 3024–3032.
Ibrahim, L.F., El-Senousy, W.M., Hawas, U.W., 2007. NMR spectral analysis of flavonoids from *Chrysanthemum coronarium*. Chem. Nat. Compd. 43, 659–662.
Ikuta, A., Itokawa, H., 1988. Triterpenoids of *Paeonia japonica* callus tissue. Phytochemistry 27, 2813–2815.
Isman, B.M., 2000. Plant essential oils for pest and disease management. Crop Prot. 19, 603–608.
Iwashina, T., Githiri, S.M., Benitez, E.R., Takemura, T., Kitajima, J., Takahashi, R., 2007. Analysis of flavonoids in flower petals of soybean near-isogenic lines for flower and pubescence color genes. J. Hered. 98, 250–257.
Karami-Osboo, R., Khodaverdi, M., Ali-Akbari, F., 2010. Antibacterial effect of effective compounds of *Satureja hortensis* and *Thymus vulgaris* essential oils against *Erwinia amylovora*. J. Agric. Sci. Technol. 12, 35–45.
Keskin, D., Toroglu, S., 2011. Studies on antimicrobial activities of solvent extracts of different spices. J. Environ. Biol. 32, 251–256.
Keyhani, J., Keyhani, E., 2006. Peroxidase activity in *Satureja hortensis* L. roots. Acta Hortic. 723, 209–214.
Kizil, S., Uyar, F., 2006. Antimicrobial activities of some thyme (*Thymus*, *Satureja*, *Origanum* and *Thymbra*) species against important plant pathogens. Asian J. Chem. 18, 1455–1461.

Kizil, S., Ipek, A., Arslan, N., Khawar, K.M., 2009. Some agronomical characteristics and essential oil content of Oregano (*Origanum onites* L.) as influenced densities. J. Essent. Oil Bear. Plants 12, 172–180.
Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., Mete, E., 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. Bioresour. Technol. 99, 8788–8795.
Korukluoglu, M., Gurbuz, O., Sahan, Y., Yigit, A., Kacar, O., Rouseff, R., 2009. Chemical characterization and antifungal activity of *Origanum onites* L. essential oils and extracts. J. Food Saf. 29, 144–161.
Kotan, R., Sahin, F., Ala, A., 2005. Identification and pathogenicity of bacteria isolated from pome fruits trees in eastern Anatolia region of Turkey. J. Plant Dis. Prot. 113, 8–13.
Kotan, R., Cakir, A., Dadasoglu, F., Aydin, T., Cakmakci, R., Ozer, H., Kordali, S., Mete, E., Dikbas, N., 2010. Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria. J. Sci. Food Agric. 90, 145–160.
Kotan, R., Dadasoglu, F., Kordali, S., Cakir, A., Dikbas, N., Cakmakci, R., 2007. Antibacterial activity of essential oils extracted from some medicinal plants, carvacrol and thymol on *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) dye causes bacterial spot disease on pepper and tomato. J. Agric. Technol. 3, 299–306.
Kovganko, N.V., Kashkan, Z.N., Borisov, E.V., 2000. ^{13}C-NMR spectra of functionally substituted 3β-chloroderivatives of cholesterol and β-sitosterol. Chem. Nat. Compd. 36, 595–598.
Kulisic, T., Radoni, A., Katalinic, V., Milos, M., 2004. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. 85, 633–640.
Kutlular, O., Ozel, M.Z., 2009. Analysis of essential oils of *Origanum onites* by superheated water extraction using GC × GC-TOF/MS. J. Essent. Oil Bear. Plants 12, 462–470.
Lambert, R.J.W., Skandamis, P.N., Coote, P., Nychas, G.J.E., 2001. A study of the minimum inhibitory concentration and mode of action of Oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91, 453–462.
Lo Cantore, P., Shanmugaiah, V., Iacobellis, N.S., 2009. Antibacterial activity of essential oil components and their potential use in seed disinfection. J. Agric. Food Chem. 57, 9454–9461 (eklendi).
Marino, M., Bersani, C., Comi, G., 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. Int. J. Food Microbiol. 67, 187.
Markham, K.R., Geiger, H., 1993. ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In: Williams, C.A., Harborne, J.F. (Eds.), Flavone and Flavonol Glycosides. Chapman and Hall, London, UK, pp. 441–497.
Mastelic, J., Jerkovic, I., Blazevic, I., Poljak-Blazi, M., Borovic, M.S., Ivancic-Bace, I., Smrecki, V., Zarkovic, N., Brcic-Kostic, K., Vikic-Topic, D., Muller, N., 2008. Comparative study on the antioxidant and biological activities of carvacrol, thymol and eugenol derivatives. J. Agric. Food Chem. 56, 3989–3996.
McManus, P.S., Stockwell, V.O., Sundin, G.W., Jones, A.L., 2002. Antibiotic use in plant agriculture. Annu. Rev. Phytopathol. 40, 443–465.
Mihaliak, C.A., Gershenzo, J., Croteau, R., 1991. Lack of rapid monoterpene turnover in rooted plants, implication for theories of plant chemical defense. Oecologia 87, 373–376.
Moghaddam, F.M., Farimani, M.M., Salahvarzi, S., Amin, G., 2006. Chemical constituents of dichloromethane extract of cultivated *Satureja khuzistanica*. J. Evid. Based Complementary Altern. Med. 4, 95–98.
Mosch, J., Klingauf, F., Zeller, W., 1990. On the effect of plant extracts against fireblight (*Erwinia amylovora*). Acta Hortic. 273, 355–361.
Mosch, J., Zeller, W., Rieck, M., Ullrich, W., 1996. Further studies on plant extracts with a resistance induction effect against *Erwinia amylovora*. Acta Hortic. 411, 361–366.
Nakamura, Y., Yoshikawa, N., Hiroki, I., Sato, K., Ohtsuki, K., Chang, C.C., Upham, B.L., Trosko, J.E., 2005. Beta-sitosterol from psyllium seed husk (*Plantago ovata* Forsk) restores gap junctional intercellular communication in Ha-ras transfected rat liver cells. Nutr. Cancer 51, 218–225.
Nguefack, J., Somda, I., Mortensen, C.N., Zollo, P.H.A., 2005. Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (*Oryza sativa* L.). Seed Sci. Technol. 33, 397–407 (eklendi).
Noma, Y., Hashimoto, T., Uehara, S., Asakawa, Y., 2010. Microbial transformation of isopinocampheol and caryophyllene oxide. Flav. Frag. J. 25, 161–170.
Orhan, I.E., Ozcelik, B., Kan, Y., Kartal, M., 2011. Inhibitory Effects of various essential oils and individual components against extended-spectrum beta-lactamase (ESBL) produced by *Klebsiella pneumoniae* and their chemical compositions. J. Food Sci. 76, 538–546.
Orhan, I.E., Ozcelik, B., Kartal, M., Kan, Y., 2012. Antimicrobial and antiviral effects of essential oils from selected Umbelliferae and Labiatae plants and individual essential oil components. Turk. J. Biol. 36, 239–246.
Ozel, M.Z., Kaymaz, H., 2004. Superhetaed water extraction, steam distillation and soxhlet extraction of essential oils of *Origanum onites*. Anal. Bioanal. Chem. 379, 1127–1133.
Ozkan, A., Erdogan, A., 2011. A comparative evaluation of antioxidant and anticancer activity of essential oil from *Origanum onites* (Lamiaceae) and its two major phenolic components. Turk. J. Biol. 35, 735–742.
Pradhanang, P.M., Momol, M.T., Olson, S.M., Jones, J.B., 2003. Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. Plant Dis. 87, 423–427.
Prakash, A., Rao, J., Nandagopal, V., 2008. Future of botanical pesticides in rice, wheat, pulses and vegetables pest management. J. Biopest. 1, 154–169.
Reddy, M.V.Bh., Angers, P., Gosselin, A., Arul, J., 1998. Characterization and use of essential oil from *Thymus vulgaris* against *Botrytis cinerea* and *Rhizopus stolonifer* in strawberry fruits. Phytochemistry 47, 1515–1520.
Roy, N.K., Dureja, P., 1998. New ecofriendly pesticides for integrated pest management. Pest World 3, 16–21.
Sahin, F., Miller, S.A., 1997. Identification of the bacterial leaf spot pathogen of lettuce, *Xanthomonas campestris* pv. *vitians*, in Ohio, and assessment of cultivar resistance and seed treatment. Plant Dis. 81, 1443–1446.
Sarac, N., Ugur, A., 2008. Antimicrobial activities of the essential oils of *Origanum onites* L., *Origanum vulgare* L. subspecies hirtum (Link) letswaart, *Satureja thymbra* L., and *Thymus cilicicus*. J. Med. Food 11, 568–573.
Sertkaya, E., Kaya, K., Soylu, S., 2010. Acaricidal activities of the essential oils from several medicinal plants against the carmine spider mite (*Tetrancyhus cinnabarinus* Boisd.) (Acarina: Tetranychidae). Ind. Crops Prod. 31, 107–112.
Silva, M.G.V., Vieira, I.G.P., Mendes, F.N.P., Albuquerque, I.L., dos Santos, R.N., Silva, F.O., Morais, S.M., 2008. Variation of ursolic acid content in eight *Ocimum* species from Notheastern Brazil. Molecules 13, 2482–2487.
Skoula, M., Harborne, J.B., 2002. Taxonomy and chemistry. In: Kintzios, S.E. (Ed.), Oregano: The Genera Origanum and Lippia. Medicinal and Aromatic Plants Industrial Profiles, 25. Taylor & Francis/CRC Press, USA, pp. 67–108.
Toncer, O., Karaman, s., Kizil, S., Diraz, E., 2009. Changes in essential oil composition of oregano (*Origanum onites* L.) due to diurnal variations at different development stages. Not. Bot. Horti. Agrobo. 37, 177–181.
Ultee, A., Bennik, M.H.J., Moezelaar, R., 2002. The phenolic hydroxyl group of carvacrol is essential for action against the foodborne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68, 1561–1568.
Vasinauskiene, M., Radusiene, J., Zitikaite, I., Surviliene, E., 2006. Antibacterial activities of essential oils from aromatic and medicinal plants against growth of phytopathogenic bacteria. Agron. Res. 4 (special issue), 437–440 (eklendi).
Vidhyasekaran, P., 1992. Principles of Plant Pathology. CBS Printers and Publishers, New Delhi.
White, D.G., Zhao, S., Simjee, S., Wagner, D.D., McDermott, P.F., 2002. Antimicrobial resistance of food-borne pathogens. Microbes Infect. 4, 405–412.
Wogiatzi, E., Gougoulias, N., papachatzis, a., Vagelas, I., Chouliaras, N., 2009. Chemical composition and antimicrobial effects of Greek *Origanum* species essential oil. Biotechnol. Biotechnol. Equip. 23, 1322–1324.
Yang, X.G., Deinzer, M., 1994. Hydrolysis and rearrangement reactions of caryophyllene oxide. J. Nat. Prod. 57, 514–517.