

ORIGINAL ARTICLE

Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*

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Abstract**Aims:** The antifungal effects of essential oils of oregano (*Origanum syriacum* var. *bevanii*) and fennel (*Foeniculum vulgare*) were evaluated against *Sclerotinia sclerotiorum*. Effects of the essential oils on morphological structures of hyphae and sclerotia were studied under light and scanning electron microscopes (SEM).**Methods and Results:** Inhibitory effects of volatile and contact phases of the essential oils used were determined on hyphae and sclerotia. Both essential oils have a marked antifungal effect against *S. sclerotiorum*. Soil amendment with essential oils has significant effect on reducing sclerotial viability. Both essential oils significantly inhibited the fungal growth in soil, thereby increasing the number of surviving tomato seedling by 69.8% and 53.3%, respectively. Light and SEM observations on pathogen hyphae and sclerotia revealed considerable morphological alterations in hyphae and sclerotia.**Conclusions:** The significant reduction in the mycelial growth and germination of sclerotia would greatly reduce the pathogen inoculum source. This may influence the rate of disease development in soil.**Significance and Impact of the Study:** Considering the reduction in the number of diseased plants in infested soil amended with essential oils, we concluded that oregano and fennel essential oils could be used as possible bio fungicides alternative to synthetic fungicides against phytopathogenic fungi.**Introduction**

Sclerotinia sclerotiorum (Lib.) de Bary causing *Sclerotinia* stem and root rot (syn. white rot) of tomato has been considered as an important soil-borne disease of over 400 species of plants including a wide range of economically important crops world-wide and several glasshouse crops (Boland and Hall 1994). This fungus is among the world's most dangerous plant pathogen due to their effects on flowers, leaves, fruits or stems under high humidity or when free moisture is present on the plant surface (Zhou and Boland 1998). The pathogen produces over-wintering structures known as sclerotia. Sclerotia are vegetative structures composed of a mass of mycelium protected by a well-developed differentiated rind. Sclerotia of *S. sclerotiorum* reside in the soil for several years

and, when appropriate environmental conditions exist, can germinate either in a myceliogenic manner, giving rise to infective hyphae, or by carpogenic germination to produce apothecia which release millions of sexually produced, air-borne ascospores (Coley-Smith and Cooke 1971).

For disease management, several strategies have been applied against the soil-borne pathogens to reduce the survival of the resting fungal structures such as sclerotia. Fungicide sprays can prevent infection by ascospores; however, due to difficulty in achieving spray penetration of the crop canopy, disease can still occur. Once the pathogen has become established in the soil, steam sterilization or fumigation with methyl bromide can be used to kill the sclerotia. The high cost of steam sterilization and pesticides, development of fungicides resistance

pathogen isolates, governmental restriction on the use of fumigants with environmental concerns over regular use of fungicides and the difficulty in finding suitable rotation crops to reduce pathogen inoculum have led to increase in the search for efficient alternative to chemical fungicide management of *S. sclerotiorum* (Staub 1991; Köhl and Fokkema 1998; Zhou and Boland 1998). Therefore, interest in secondary metabolites from plant extracts and mainly essential oils as potential antimicrobial agents for use in food preservation, crop protection and pharmacological applications has increased during the past decade (Isman 2000; Burt 2004). Furthermore, the rapid rise in demand for organically produced fruits and vegetables will increase the demand for natural pesticides such as essential oils. Recently, many studies on the antifungal activities of essential oils against fungal pathogens have been reported (Kalemba and Kunicka 2003). Very few studies, however, have focused on the antifungal activities of essential oils against this soil-borne pathogen (Edris and Farrag 2003; Pitarokili *et al.* 2003; Soyly *et al.* 2005a). Although there have been numerous reports on the antifungal activities of essential oil *in vitro* conditions, there is no research on the antifungal activity of the essential oil towards soil-borne fungal pathogen *in vivo* conditions.

In the study described herein, we assessed *in vitro* and *in vivo* antifungal effects of the essential oils obtained from medicinal plant oregano (*Origanum syriacum* L. var. *bevanii*), and fennel (*Foeniculum vulgare* Mill.) against *S. sclerotiorum*. The broad aims of this study were (i) to investigate antifungal effects of the essential oils on hyphal growth on Petri plates and on the viability of sclerotia of *S. sclerotiorum* in soils, (ii) to assess potential biocontrol capacities of the essential oils against disease suppression *in vivo* conditions and (iii) to reveal effects of the essential oils on morphological structures of fungal hyphae and sclerotia under light and scanning electron microscopes (SEM).

Materials and methods

Plant material and isolation of essential oils

For the extraction of essential oils, oregano plants were collected from the eastern Mediterranean Region of Turkey and fennel seeds were purchased locally. Leaves of oregano were used for extraction of the essential oils and in the case of fennel, seeds were used for essential oil extraction. Air-dried plant materials (200 g) were placed in a 5 l round-bottom distillation flask and 3 l double distilled water added. The essential oils were obtained by steam distillation for 3 h using Clevenger-type apparatus (İldam, Ankara), according to European Pharmacopoeia method (1997). The oils were separated, dried over anhy-

drous sodium sulfate and stored in an amber bottle at 4°C until used. The average yields of oregano and fennel oils were about 6.7% and 7.9% (v/w), respectively.

Test micro-organism

The *S. sclerotiorum* used in this study was isolated from sclerotia produced infected stems of tomato exhibiting symptoms of Sclerotinia stem rot. Surface disinfected sclerotia were plated on potato dextrose agar (PDA, Merck, Germany) amended with antibiotics (streptomycin sulfate 50 µg ml⁻¹, rifampicin 50 µg ml⁻¹). The plates were incubated at 20°C for 5–7 days to allow mycelium to grow into the medium. Small agar blocks containing hyphal tips were cut from the colony margins and transferred to fresh PDA. Replicate plates were incubated at 20°C. Fungal isolate was re-inoculated on to tomato seedling and found to be highly pathogenic. Stock cultures were maintained on PDA and kept at 4°C and subcultured once a month. The pure culture of pathogen has been deposited in the culture collection of the Plant Protection Department, MKU (No. Sst12).

Determination of antifungal effects of the essential oils on mycelial growth

The antifungal properties of essential oils were evaluated for assessing its volatile and contact phase effects towards mycelial growth of *S. sclerotiorum* as described previously (Soyly *et al.* 2006). Glass Petri plates (90 × 20 mm; Isolab, Istanbul, Turkey, which offer 80 ml air space after additions of 20 ml agar media) were used for the determination of volatile phase effect of the essential oil. Different concentrations of essential oils (8, 12, 16, 20, 24 µg ml⁻¹) were added to sterile filter papers (10 mm diameter, Whatman No. 1) and placed on the inner surface of the inverted lid of Petri dishes to obtain final concentrations of 0.1, 0.15, 0.2, 0.25 and 0.3 µg ml⁻¹ air. The petri plates were inoculated with *S. Sclerotiorum* as described above, and the plate sealed immediately with parafilm to prevent loss of essential oils from the plates and incubated at 20°C. For the determination of contact phase effect of essential oil, PDA medium was autoclaved and cooled in a water bath at 40°C. Different concentrations of essential oil were prepared by dissolving the requisite amounts in sterile Tween 20 (0.1%, v/v) solution, and mixed in the flasks with warm sterile molten medium (40°C) to obtain final concentrations of 0.4, 0.8, 1.6, 2.4 and 3.2 µg ml⁻¹. The PDA agar with essential oil was poured into sterile 90 mm glass Petri plates (≈20 ml/plate). Agar discs (7 mm diameter) from the edge of a 7-days-old *S. sclerotiorum* culture were placed at the centre of the each Petri plate and incubated at 20°C.

In the control, equal amounts of sterilized ethanol and Tween 20 were either mixed in the medium (for contact phase effect) or added to filter papers and placed onto the lid of Petri plates for volatile phase effect. The mean radial mycelial growth of the pathogen was determined by measuring the diameter of the colony in two directions at right angles. The growth was compared to the control plate in which the fungus covered the plate 7 days after inoculation. For each concentration, five replicate plates were used. The mean growth values were obtained and then converted in to the inhibition percentage of mycelial growth (MGI) in relation to the control treatment by using the formula, $MGI (\%) = [(d_c - d_t)/d_c] \times 100$, where d_c and d_t represent mycelial growth diameter in control and treated Petri plates, respectively. The experiments were conducted twice.

Determination of antifungal effects of the essential oils on sclerotial viability

Antifungal effects of the essential oils on sclerotial viability were also tested in steam-sterilized natural sandy soil. For volatile effect, different concentrations of essential oils were added to sterile filter papers and placed on the inner surface of the inverted lid of Petri dishes as described for fungal mycelium. For contact effect, 10 g of steam-sterilized natural sandy soil, placed in the bottom dish of a Petri plate, was mixed with the different concentrations of essential oil. Fungal sclerotia produced on PDA after 30 days of growth were gently removed from the PDA plate surface. Twenty sclerotia were placed either at 0.5 cm depth in soil (contact effect) or over the soil surface (volatile effect) in sterile glass Petri plates. The plates were sealed together with parafilm and incubated at 20°C for 10 days. Sclerotia in soil treated with ethanol (0.5%) and Tween 20 (0.1%) were used as control. The treated sclerotia were removed after 10 days, rinsed in sterile water, surface disinfected in 70% ethanol for 2 min and finally washed with sterile distilled water. Sclerotia were, then, dried on sterile filter paper, bisected and placed on a PDA in Petri dishes with the freshly cut surface towards to agar. The number of sclerotia showing mycelial growth of *S. sclerotiorum* (viable) was assessed after 10–14 days incubation at 20°C. For each concentration, 20 sclerotia were used. There were three replicates for each treatment and the experiments were repeated twice.

Determination of *in vivo* antifungal effects of the essential oils

For *in vivo* effects of essential oil, *S. sclerotiorum* was grown on petri plates containing PDA. Plates were incubated in the light for 4 weeks at 20°C and the sclerotia

which formed were dislodged from the surface of the plates and used for inoculum source. This inoculum was produced in sterile polythene bags containing steam-sterilized peat-soil mixture inoculated with pathogen sclerotia (3%, w/w) and incubated for 20 days at 24–28°C in the darkness. The *S. sclerotiorum* inoculum contained $\log 4.9$ CFU g^{-1} soil (as determined by spread plates).

Different concentrations of essential oil were prepared by dissolving the requisite amounts in sterile Tween 20 (0.1%, v/v) solution, and mixed in the flasks with inoculated soils to obtain final concentrations of 0.4, 0.8, 1.6, 2.4 and 3.2 $\mu g\ ml^{-1}$. After treatment, inoculated and uninoculated soil samples were distributed on seedling trays. The tomato seeds (*Lycopersicum esculentum* cv F-144) were then sown into treated soils. The seedling trays were incubated on a bench in a growth chamber (20°C with the photoperiod of 16 h) for 3 weeks. The trays were kept covered with plastic lids throughout the experiment. The boxes were watered regularly to maintain soil moisture content at $\approx 75\%$ water holding capacity. Percentages of surviving plants were recorded. The experiment was performed twice with three replicates per treatment (oil concentration) and each treatment with 50 seeds per replication. Controls consisted of pathogen-infested soil non-amended with the essential oil or uninfested soil with the essential oil.

Determination of effects of the essential oils on hyphal and sclerotial morphology

Determination of volatile and contact phase effects of essential oils on hyphal morphology was described in our earlier study (Soyulu *et al.* 2006). For the determination of volatile phase effect of essential oils on hyphal morphology, a mycelial agar disc from a 7-days-old culture was first placed in the centre of PDA plate and incubated at 20°C for 2 days to allow mycelium to grow into the medium. After 2 days of pre-incubation, different concentrations of essential oils used *in vitro* studies were dropped (onto covers of Petri dishes), sealed by parafilm and incubated at 20°C for 3 days. Determination of contact phase effect of essential oils on hyphal morphology was as described in an earlier paper. Thin layers (1 mm) of agar blocks (3–4 cm^2) containing mycelium were removed at one-day intervals for examination by light microscopy. The blocks cut from growing edges were placed in a drop of 50% glycerol on microscope glass slides, covered with glass cover slip and examined using a phase contrast light microscope (Olympus BX50, Tokyo, Japan).

For SEM analysis, fungal hyphae and sclerotia were processed as described before (Soyulu *et al.* 2006). Mycelial discs (1 cm in diameter) or sclerotia exposed to the most effective concentration of oregano or fennel essential oil

were fixed with 2.5% glutaraldehyde in 0.1 mol l⁻¹ phosphate-buffer (pH = 7.2) for 2 h at room temperature. They were washed twice, each time for 10 min, in the same buffer. After fixation, the samples were dehydrated in a graded ethanol series (70%, 80%, 90% and three times at 100%) for a period of 30 min in each series. The samples were critical-point dried in a drying apparatus (Polaron CPD 7501, East Sussex, UK) up to the critical point with CO₂. The fixed material was then mounted on stubs using double-sided carbon tape and coated with gold/palladium in a sputter coater system in a high-vacuum chamber (Polaron SC7620, UK) for 150 s at 9 mA. The samples were examined and digital images captured using a JEOL JSM 5500 SEM at an accelerating voltage of 5 kV.

Statistical analysis

All experiments were performed twice with at least three replications of each oil concentration. SPSS statistic program (version 11.5, USA) was performed for all calculations. Where necessary, arcsine transformation was performed on data before statistical analysis. Analysis of variance was performed at the significance level of $P < 0.05$. When appropriate, means were separated by using Tukey's test ($P \leq 0.05$). The data from two independent experiments were analysed separately but were not significantly different ($P > 0.05$).

Results

Antifungal effects of the essential oils *in vitro* conditions

The volatile and contact phase effects of different concentrations of essential oils on the mycelial growth of *S. sclerotiorum* are shown in Fig. 1. Both essential oils were found to inhibit the mycelial growth of *S. sclerotiorum* in a dose-dependent manner. Essential oil of fennel was more inhibitory to *S. sclerotiorum* than oregano oil in both volatile and contact phase effect studies.

Volatile inhibitory effects of essential oils were greater on mycelial growth than contact inhibitory effect (Fig. 1a). Mycelial growth of *S. sclerotiorum* was totally inhibited by fennel oil at a relatively low concentration of 0.2 $\mu\text{g ml}^{-1}$ air. Mycelial growth was completely inhibited by oregano essential oil at concentrations of 0.3 $\mu\text{g ml}^{-1}$ air.

Results of contact phase effects of essential oils are shown in Fig. 1b. As seen in Fig. 1b, relatively higher concentrations were required to inhibit mycelial growth. Although oregano oil at the concentration of 1.6 $\mu\text{g ml}^{-1}$ caused significant reduction in mycelial growth of *S. sclerotiorum*, mycelial growth was completely inhibited at the relatively higher concentration (3.2 $\mu\text{g ml}^{-1}$). Unlike

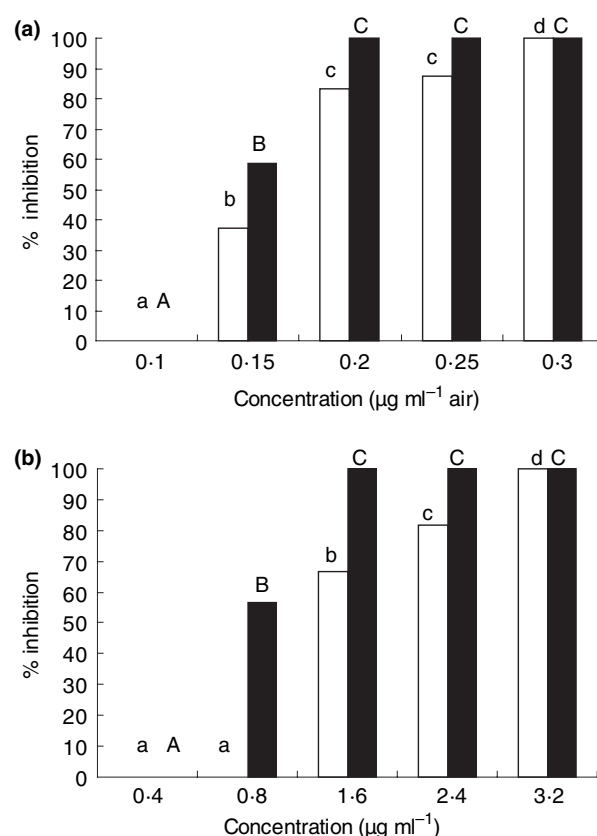


Figure 1 The effects of different concentrations of volatile (a) and contact (b) phases of essential oils of oregano (□) and fennel (■) on the mycelial growth of *S. sclerotiorum*. Arcsine transformation was performed prior to statistical analysis. Bars, for each essential oil, with the same small or large letters represent values that are not significantly different according to Tukey Test ($P < 0.05$).

oregano essential oil, fennel essential oil-inhibited growth of *S. sclerotiorum* completely at the concentration of 1.6 $\mu\text{g ml}^{-1}$ (Fig. 1b).

Antifungal effects of the essential oils on sclerotial viability

In another approach to reduce the pathogen development, over-wintering sclerotia were exposed to different concentrations of essential oils. The antifungal effects of essential oils on the viability of sclerotia were evaluated after 10 days of incubation in soil amended with different concentration of essential oils. Soil amendment with essential oils has significant effect on reducing sclerotial viability compared to the control treatments. Volatile inhibitory effects of essential oils were greater on sclerotial viability than contact inhibitory effects (Fig. 2). Sclerotial viability was totally affected by fennel and oregano essential oils at concentration of 1.5 and 2.0 $\mu\text{g ml}^{-1}$ air, respectively (Fig. 2a).

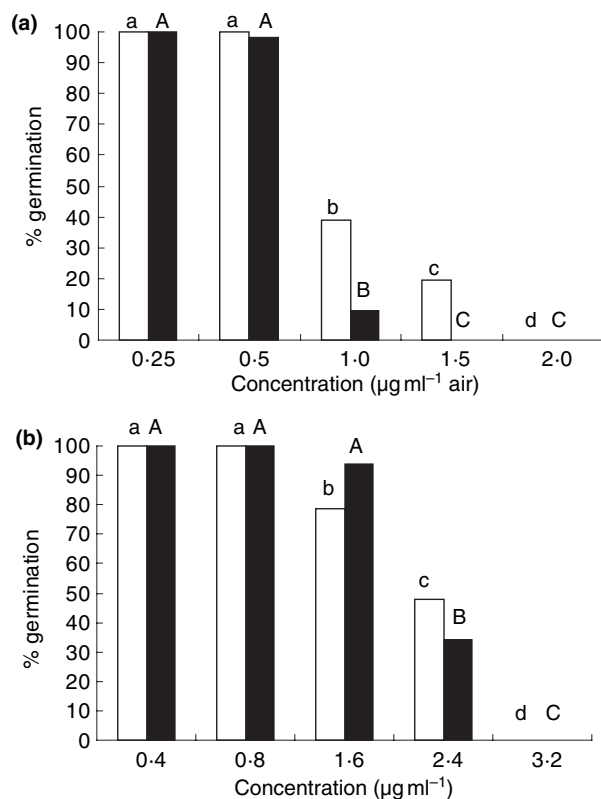


Figure 2 The effects of different concentrations of volatile (a) and contact (b) phases of essential oils of oregano (□) and fennel (■) on the sclerotial germination. Arcsine transformation was performed prior to statistical analysis. Bars, for each oil, with the same small or large letters represent values that are not significantly different according to Tukey Test ($P < 0.05$).

Contact phase effects of different concentrations of essential oils on the sclerotial viability are shown in Fig. 2b. Both essential oils completely inhibited sclerotial germination at the concentration of $3.2 \mu\text{g ml}^{-1}$.

Antifungal effects of the essential oils *in vivo* conditions

Protection of tomato seedlings against *S. sclerotiorum* by essential oils were also investigated *in vivo* conditions and results are given in Fig. 3. None of the oils showed harmful effects on the germination and emergence of tomato seeds. In control, the pathogen inoculum significantly reduced emergence of the tomato seedlings. Percentage number of seed germination and seedling emergence increased significantly in infested soil amended with either essential oil. Amendment of infested soil with oregano and fennel essential oils significantly improved plant survival compared to control treatment. The presence of oregano oil in infested soil at the concentration of $3.2 \mu\text{g ml}^{-1}$ increased the number of surviving seedlings to 69.8% from 26.6% in treatment with pathogen alone

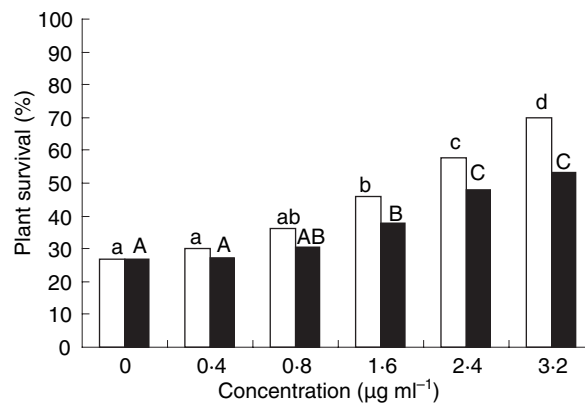


Figure 3 Protection of tomato seedlings against *S. sclerotiorum* by essential oils *in vivo* conditions. Values are percentage of surviving seedlings over initial number of seeds sown into soil with *S. sclerotiorum*. Arcsine transformation was performed prior to statistical analysis. Bars, for each oil, with the same small or large letters represent values that are not significantly different according to Tukey Test ($P < 0.05$). □, oregano; ■, fennel.

(control). Percentage number of surviving seedlings also increased significantly in the presence of fennel oil at the concentration of $3.2 \mu\text{g ml}^{-1}$ in infested soil (from 26.6% to 53.3%). The number of surviving seedlings in infested soil amended with fennel oil at the concentration of $3.2 \mu\text{g ml}^{-1}$ is significantly lower than those observed in soil amended with oregano oil ($P < 0.05$).

Effects of the essential oils on hyphal and sclerotial morphologies

Microscopic observation of *S. sclerotiorum* hyphae exposed to the most effective concentrations of both essential oils vapour (volatile phase) or grown on PDA amended with the different concentrations of essential oils (contact phase) showed similar degenerative changes in the hyphal morphology in comparison to hyphae in control plates (Fig. 4a). After exposure to the most effective oregano and fennel oil concentrations determined *in vitro* studies, [contact ($3.2 \mu\text{g ml}^{-1}$) or volatile phases ($0.3 \mu\text{g ml}^{-1}$ air)], hyphae appeared degraded (Fig. 4b), large vesicles are also visible within the cell walls. Shrivelled hyphal cells had either no cytoplasm or the cytoplasm was depleted of organelles (Fig. 4c). Under the influence of both oils, the growth of the fungus was suppressed and the hyphal structure has undergone several morphological changes when viewed by SEM (Fig. 5).

Unusual pattern of hyphal growth, as well as alterations in cell shape and size are also demonstrated by SEM (Fig. 5). Shrivelled hyphal aggregates (Fig. 5b), reduced hyphal diameters and lyses of hyphal wall (Fig. 5c) were commonly observed on oregano- or fennel oil-treated

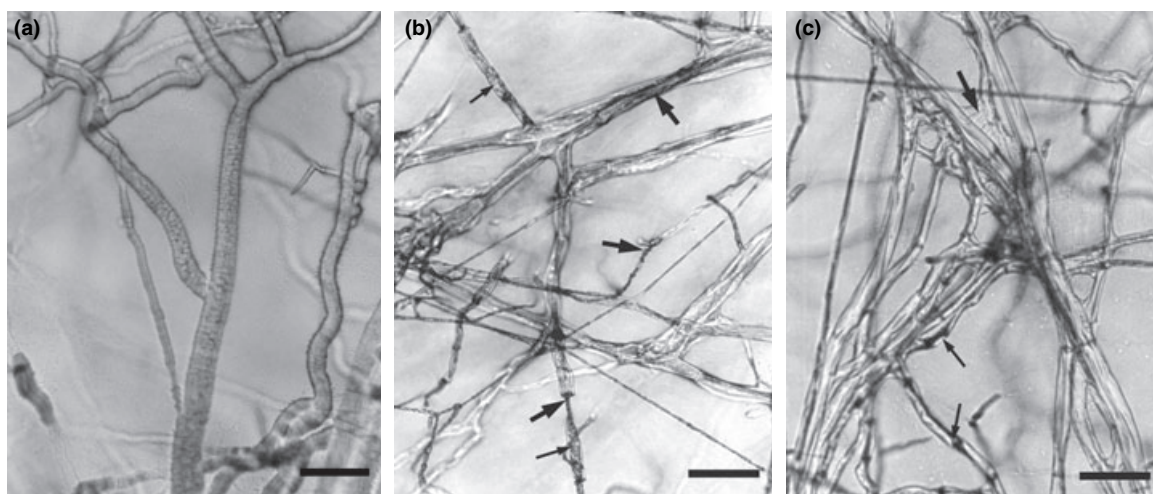


Figure 4 Effect of essential oils on hyphal morphology of *S. sclerotiorum* under light microscope. (a) Hyphae growing on control medium. (b) and (c) Contact and volatile phase effects of fennel and oregano essential oils, respectively, on hyphal morphology. Note marked deformations and cytoplasmic coagulations (small arrows) and necrosis (large arrows) on hyphae in oregano oil containing plate (b) and cytoplasmic coagulations (small arrow) and hyphal lysis (large arrow) in fennel oil containing plate (c). Bar = 50 μ m.

mycelium, compared with thick, elongated, normal mycelial growth in control Petri plates (Fig. 5a). Similar degenerative changes were also observed in the sclerotial morphology (Fig. 6d–e) compared to those developed in control treatments (Fig. 6a–c). The surfaces of oil-treated sclerotia were appeared as crinkled and desiccated (Fig. 6d). Both essential oils used at the highest concentrations also caused shrivelling and lysis on rind globular cells inside the sclerotia (Fig. 6e,f).

Discussion

Plant extracts and especially volatile essential oils from medicinal plants, have been reported to possess antimicrobial activity against a variety of food-borne, human and plant pathogens and pest (Isman 2000; Kalembe and Kunicka 2003; Burt 2004). In this study, we have tested the antifungal activities of essential oils from oregano and fennel on fungal structures of *S. sclerotiorum* such as mycelium and sclerotia *in vitro* and *in vivo*. We also investigated the effect of essential oils on morphologies of fungal hyphae and sclerotia under light and SEM. The results of this study confirm that essential oils from oregano and fennel possess antifungal activity against *S. sclerotiorum*. Although *in vitro* antifungal activities of plant extract and essential oils of different plant species were previously reported against *S. sclerotiorum* (Edris and Farrag 2003; Pitarokili *et al.* 2003; Soyulu *et al.* 2005a), to our knowledge, this is the first study showing antifungal activities of essential oils of oregano and fennel against *S. sclerotiorum*. The chemical compositions of the essential oils used in this study were previously determined by gas

chromatography and mass spectroscopy (GC-MS) analysis (Soyulu *et al.* 2006). The number of compounds and their relative amount found in oregano and fennel essential oils varied according to plant species and the particular compound. The major compounds found in the essential oils of oregano and fennel, used in this study, were carvacrol (79.8%) and anethole (82.8%), respectively (Soyulu *et al.* 2006). The antimicrobial properties of essential oils of oregano and fennel and their major constituents, carvacrol and anethole, have been shown to be able to suppress several human and plant pathogenic fungi (Daouk *et al.* 1995; Paster *et al.* 1995; Adam *et al.* 1998; Dorman *et al.* 2000; Lambert *et al.* 2001; Abou-Jawdah *et al.* 2002; Daferera *et al.* 2003; Mimica-Dukic *et al.* 2003; Arcila-Lozano *et al.* 2004; Salgueiro *et al.* 2004; Zambonelli *et al.* 2004; Soyulu *et al.* 2005b; Soyulu *et al.* 2006).

The volatile phases of the essential oils were found to be more effective than the contact phase to the pathogen *in vitro* conditions. Volatile phase of essential oils were also reported to possess more antimicrobial activity against plant pathogenic fungi and bacteria (Edris and Farrag 2003; Soyulu *et al.* 2005a; Soyulu *et al.* 2006). Investigators suggested that the antifungal activity resulted from a direct effect of essential oil vapours on fungal mycelium and postulated that because of their lipophilic nature, the essential oils are absorbed by fungal mycelium (Inouye *et al.* 2000; Edris and Farrag 2003).

The majority of the work initiated so far has concentrated on the effect of essential oils on inhibition of mycelial growth *in vitro* conditions. Plant pathogenic *Sclerotinia* species produce over-wintering structures, called sclerotia (Boland and Hall 1994). Diseases caused by *S.*

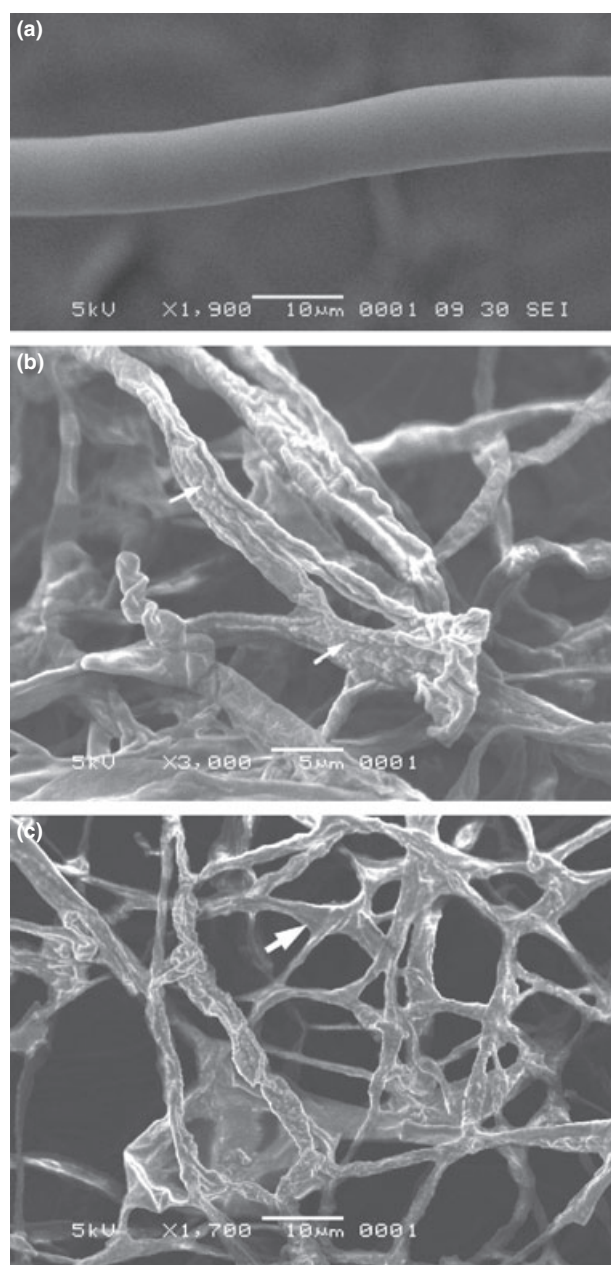


Figure 5 Scanning electron microscopy of hyphae exposed to oregano essential oil volatiles. (a) Healthy hyphae. (b and c) Effects of essential oils on hyphal morphology. Note alterations in hyphal morphology including hyphal shrivelling, blistering (small arrows) in plate (b) and lysis (large arrow) in plate (c).

sclerotiorum can be effectively controlled if the overwintering sclerotia, the primary inoculum producers, can be destroyed, thus preventing mycelial germination, apothecial formation and ascospore release. No research has been conducted on the antifungal activity of the essential oil towards soil-borne fungal pathogen *in vivo* conditions and inhibition of resting fungal structures such

as sclerotia. This study has clearly showed potential use of essential oils on disease suppression not only *in vitro* but also *in vivo* conditions. Both essential oils not only suppressed *in vitro* mycelial growth but also effectively suppressed sclerotial germination and seedling infection *in vivo* conditions. The significant reduction in the mycelial growth and germination of sclerotia would greatly reduce the pathogen inoculum source which may influence the rate of disease development *in vivo* conditions.

Light and SEM observations of hyphae of *S. sclerotiorum* exposed to essential oils revealed alterations in the hyphal morphology. Shrivelled hyphal aggregates, reduced hyphal diameters and lyses of hyphal wall were commonly observed in oregano- or fennel oil-treated mycelium, compared with thick, elongated, normal mycelial growth in controls. Such modifications may be related to the effect of the essential oil as enzymatic reactions regulating wall synthesis (Rasooli *et al.* 2006). The lipophilic properties of oil components might have also aided in the ability of the oil to penetrate the plasma membrane (Knobloch *et al.* 1989). The observations made with light and electron microscopy are in accordance with previous studies in which essential oils of aromatic plants caused the morphological alterations on the fungal hyphae (Bianchi *et al.* 1997; Fiori *et al.* 2000; de Billerbeck *et al.* 2001; Romagnoli *et al.* 2005; Soylu *et al.* 2005b; Soylu *et al.* 2006). In addition, scanning electron microscopy analyses revealed that the surface of treated sclerotia and rind globular cells were significantly damaged by both essential oils.

Although our results demonstrated the antifungal activities of essential oils, the mechanisms of action are not well documented. Transmission electron microscope observations showed that treatment with *Tagetes patula* essential oil induced alterations in the whole endomembrane system of fungal pathogen *B. cinerea*, such as degeneration of mitochondrial cristae, release and breaking up of the plasma membrane from the cell wall, and partial dissolution of the nuclear envelope and the rough endoplasmic reticulum (Romagnoli *et al.* 2005). They have also concluded that the cell membrane is a very important target of the essential oil components that the terpenoids could interfere with the phospholipid bilayers of membranes as previously observed by Knobloch *et al.* (1989) on isolated bacterial cytoplasmic membrane. The adverse effect of essential oils that we observed on the hyphae of fungal pathogen *S. sclerotiorum* may be responsible for the decrease in the rate of mycelial growth. General change in the morphology of the hyphae and sclerotia could also be due to the loss of integrity of the cell wall. Consequently, plasma membrane permeability might be affected, which could explain the changes in the morphology and size of the internal organelles as suggested earlier (Nakamura *et al.* 2004).

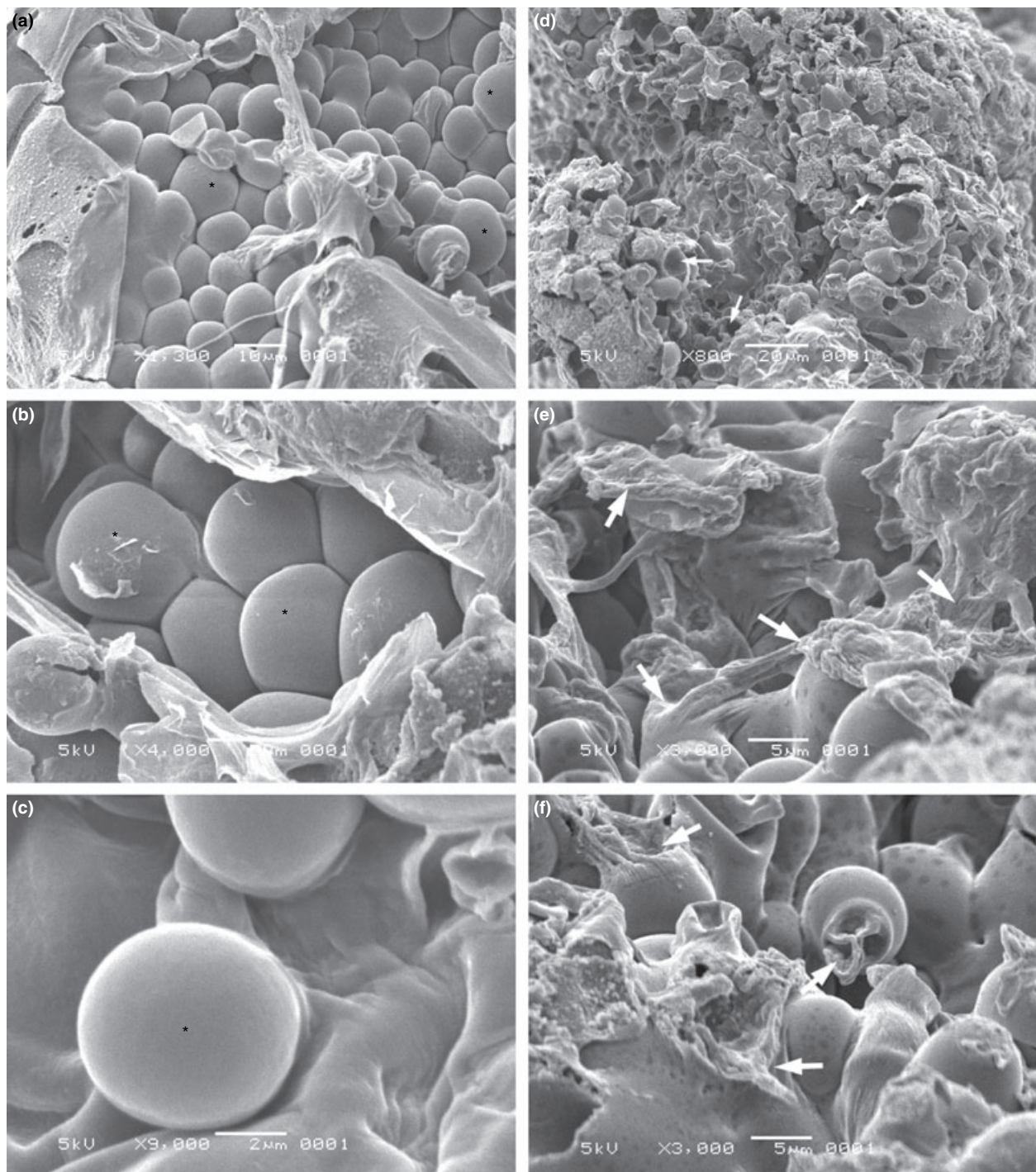


Figure 6 Scanning electron microscopy of sclerotia exposed to oregano essential oil volatiles. (a, b and c) Healthy sclerotia and rind globular cells (asterisk) inside the sclerotium developed in control Petri plates. (d, e and f) Effects of essential oil on surfaces of sclerotia and rind globular cells inside the sclerotium. Note alterations on surfaces of rind globular cells including shrivelling (small arrows) and lysis (large arrows).

Although this work needs further study to fully understand the mechanism of action of both essential oils, considering the reduction in the number of diseased plants

in infested soil amended with essential oil, we concluded that plant essential oils used in this study could become a possible alternative to synthetic fungicides.

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