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Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*

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Abstract

The aim of this study was to find an alternative to synthetic fungicides currently used in the control of devastating oomycete pathogen *Phytophthora infestans*, causal agent of late blight disease of tomato. Antifungal activities of essential oils obtained from aerial parts of aromatic plants such as oregano (*Origanum syriacum* var. *bevanii*), thyme (*Thymbra spicata* subsp. *spicata*), lavender (*Lavandula stoechas* subsp. *stoechas*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*), and laurel (*Laurus nobilis*), were investigated against *P. infestans*. Both contact and volatile phase effects of different concentrations of the essential oils used were determined by using two *in vitro* methods. Chemical compositions of the essential oils were also determined by GC-MS analysis. Major compounds found in essential oils of thyme, oregano, rosemary, lavender, fennel and laurel were carvacrol (37.9%), carvacrol (79.8), borneol (20.4%), camphor (20.2%), anethole (82.8%) and 1,8-cineole (35.5%), respectively. All essential oils were found to inhibit the growth of *P. infestans* in a dose-dependent manner. Volatile phase effect of oregano and thyme oils at 0.3 μg/ml air was found to completely inhibit the growth of *P. infestans*. Complete growth inhibition of pathogen by essential oil of fennel, rosemary, lavender and laurel was, however, observed at 0.4–2.0 μg/ml air concentrations. For the determination of the contact phase effects of the tested essential oils, oregano, thyme and fennel oils at 6.4 μg/ml were found to inhibit the growth of *P. infestans* completely. Essential oils of rosemary, lavender and laurel were inhibitory at relatively higher concentrations (12.8, 25.6, 51.2 μg/ml respectively). Volatile phase effects of essential oils were consistently found to be more effective on fungal growth than contact phase effect. Sporangial production was also inhibited by the essential oil tested. Light and scanning electron microscopic (SEM) observation on pathogen hyphae, exposed to both volatile and contact phase of oil, revealed considerable morphological alterations in hyphae such as cytoplasmic coagulation, vacuolations, hyphal shrivelling and protoplast leakage.

Key words: antimicrobial activity, essential oil, *Phytophthora infestans*, SEM, tomato

Introduction

Phytophthora infestans (Mont.) de Bary, the causal agent of late blight is the most devastating disease of tomato and potato plants world-wide. Unlike most *Phytophthora* species, which causes soil-borne root-rotting diseases, *P. infestans* is a specialized pathogen, primarily causing disease on the foliage, stems, potato tubers and tomato fruits, with most infection spread by airborne asexual sporangia during the growing season [1]. *Phytophthora infestans* can be very serious on tomato particularly when the weather is consistently cool and rainy and it is responsible for a large proportion of total monetary losses sustained by growers each growing season. If disease incidence is high the pathogen may cause extensive defoliation leading to a reduction of economic fruit yield.

Control of this disease is still heavily reliant on multiple applications of chemical fungicides during flowering and fruiting [1]. A combination of high inoculum pressure, humid conditions that favour pathogen growth and frequent pesticides applications have resulted in emergence of resistant pathogen strains in several country [2]. Further, the use of some synthetic chemicals to control fungal disease of food commodities is restricted due to their possible carcinogenicity, high and acute toxicity, long degradation periods, and environmental pollution. There are concerns over increasing loss of efficacy of conventional fungicides due to pathogen resistance and general unacceptability of fungicides usage because of and environmental risks.

The use of biologically based compounds in plant extracts may be an alternative to currently used fungicides to control phytopathogenic fungi and bacteria, because they virtually constitute a rich source of bioactive chemicals such as phenols, flavonoids, quinons, tannins, alkaloids, saponins and sterols [3, 4]. Since these extracts can be active against fungal and bacterial pathogens, are biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management programs, they could become a new class of safer disease control agents. Some phytochemicals of plant origin (i.e. azadirachtin, carvone, pyrethroids) have been formulated as botanical pesticides and are used successfully in integrated pest management programme [5]. Most of the work on antimicrobial effects of essential oils has been conducted on human or food pathogens. To our knowledge, there is no information available on the antifungal activity of essential oils from aromatic plants used in this study against *P. infestans*. Therefore, efforts have focused on the antimicrobial activities of essential oils, obtained from aromatic plants growing in the Eastern Mediterranean Region of Turkey, against *P. infestans*.

In the study described herein, we assessed *in vitro* antifungal activities of essential oils derived from locally grown aromatic plants *Origanum syriacum* L. var. *bevanii*, *Thymbra spicata* L subsp. *spicata*, *Lavandula stoechas* L. subsp. *stoechas*, *Rosmarinus officinalis* L., *Foeniculum vulgare* Mill., and *Laurus nobilis* L., against late blight pathogen *P. infestans*. Both contact and volatile phase effect of the essential oils on hyphal growth, sporangium formation were determined. Morphological changes in hyphae were also investigated by using light and scanning electron microscopes (SEM).

Materials and methods

Plant material and isolation of essential oils

Locally available plants of different families were collected from the region of Hatay, Turkey for the extraction of essential oils (Table 1). They were identified by Dr. I. Üremis (University of Mustafa Kemal, Department of Plant Protection, Laboratory of Herbology). A voucher specimen of each sample was deposited in the herbarium of the department. Leaves of oregano, thyme, lavender, rosemary and laurel were used for extraction of the essential oils and in the case of fennel, seeds were used for essential oil extraction. Air-dried plant samples were placed in a 1 l round-bottom distillation flask and 300 ml distilled water added. The essential oils were obtained by steam distillation for 3 h with Clevenger's apparatus, according to European Pharmacopoeia method [6]. The oils

Table 1. Major chemical compounds detected in the essential oils used in this study

Essential oil	No. of compound identified	Major compounds[a] identified
Oregano	24	Carvacrol (79.8%), *p*-cymene (8.2%)
Thyme	16	Carvacrol (37.9%), *p*-cymene (18.3%), linalool (15.9%), γ-terpinene (14.7%),
French lavander	21	Camphor (20.2%), 1,8-cineole (35.5%), α-thujone (15.9%), fenchone (13.5%)
Rosemary	30	Borneol (20.4%), camphor (19.5%), 1,8-cineole (17.4%), linalool (6.1%)
Fennel	17	*trans*-Anethole (82.8%), 4-allyl-anisole (6.5%), limonene (5.8%)
Laurel	39	1,8-Cineole (35.5%), sabinene (15.0%), α-terpineyl acetate (14.2%), α-pinene (7.5%)

[a]Components showing a peak area of more than 5% relative to the total peak area on gas chromatography (GC) are listed in order of their highest relative peak area. Numbers are percentage of compound relative to total essential oil.

were separated, dried over anhydrous sodium sulphate and stored in an amber bottle at 4 °C until used.

GC-MS analysis of essential oil

Analysis of the essential oils used in these experiments was performed using a Hewlett–Packard 6890 gas chromatography (GC) linked to a Hewlett–Packard 5973 mass selective detector equipped with a HP-5 MS (Crosslinked 5% Phenyl Methyl Siloxane) capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium, at a rate of 1.3 ml/min. The oven temperature was initially 45 °C, then increased at 2 °C/min to 130 °C, 3 °C/min to 170 °C, then to 10 °C/min to 220 °C and finally isothermal for 5 min. The ionization energy was 70 eV. The interface temperature was 250 °C. The essential oil components were tentatively identified by comparing their relative retention times and mass spectra with those of Wiley Registry of Mass Spectral Data [7] and publication of Adams [8].

Test microorganism

The *P. infestans* isolate used in the study was isolated from infected leaves of tomato. Tissue was cut from the margins of lesions, surface sterilized and transferred to Petri plates containing V8 juice agar medium amended with antibiotics (streptomycin sulphate 50 μg/ml, rifampicin 50 μg/ml). 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 V8 juice agar for growth and sporulation at 20 °C. Stock cultures were maintained on V8 juice agar slants and kept at 4 °C and sub-cultured once a month.

Determination of antimicrobial activities of the essential oils

The antifungal properties of essential oils were evaluated for assessing its contact and volatile phase effects towards mycelial growth of *P. infestans*. Contact phase effect of essential oils was tested by the poisoned food technique. V8 juice agar medium was autoclaved and cooled in a water bath at 40 °C. Different concentrations of essential oils were prepared by dissolving various amounts in 1 ml of ethanol (0.5%) and Tween 20 (0.1%) sterilized with disposable membrane filters (0.2 μm pore size) and added to flasks with sterile molten medium. The V8 juice agar with essential oil was poured into sterile 90 mm glass Petri plates (≈20 ml/plate). V8 agar discs (7 mm diameter) from the edge of a 7-days old *P. infestans* culture were placed at the centre of the each Petri plate and incubated at 20 °C. Growth inhibition was determined by daily periodic measurement of colony diameter.

Glass Petri plates (90 mm×20 mm) were used for the determination of volatile phase effect of essential oils on growth of *P. infestans*. Different concentrations of essential oils 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. The petri plates were inoculated with *P. infestans* as described above, and the plate sealed immediately with parafilm to prevent loss of essential oils from the plates and incubated at 20 °C, and inhibition concentrations of volatile phase effect of essential oils were determined by daily periodic measurement of colony diameter.

In the control, equal amounts of sterilized ethanol and Tween 20 was 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 when the plate surface of the control Petri was covered by fungus 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 in relation to the control treatment by using the formula, MGI (%) = ((dc−dt)/dc)×100, where dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively. The experiments were conducted twice.

Determination of antimicrobial properties of essential oils

The minimum concentration of essential oils (expressed as μg/ml or μg/ml air) required to give complete control or the minimum inhibitory con-

centration (MIC) for pathogen growth was calculated. The MIC of each of the essential oil was classified as fungicidal or fungistatic in its effect. A fungicidal effect was where there was no growth, whereas a fungistatic effect was where temporary inhibition of microbial growth occurred. The agar discs of *P. infestans*, which failed to grow were either transferred onto agar media without oils (for contact phase effect of oils) or onto lids of the plate containing Tween 20 without oil (for volatile phase effect of oils). Petri plates were incubated for 5 days. Activity of the MIC of the various oils was considered fungicidal if the pathogen did not grow or fungistatic if the pathogen growth occurred.

Effect of essential oils on sporangium formation

The contact phase effects of essential oils on sporangium formation of *P. infestans* were determined according to method described by Simpfendorfer et al. [9]. Mycelial agar discs (7 mm diameter) were aseptically cut from the margin of a 3-days old non-sporulating *P. infestans* culture on V8 juice agar. Five mycelial discs were placed into Petri plates containing 20 ml V8 juice liquid amended with the different concentrations of essential oils. Oil solubility was enhanced by using ethanol and Tween 20 as described before. For control treatments, equal amounts of ethanol and Tween 20 were added to V8 juice liquid. Three replicate plates of each oil concentration were prepared and incubated in the dark at 20 °C. Sporangium production at the mycelial fringe of each disc was observed at 40× magnification using light microscope and the number of sporangia was recorded in five randomly selected fields of view for each disc after 7 days of incubation.

Determination of effect of essential oils on hyphal morphology

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 V8 juice agar plate and incubated at 20 °C for 2 days to allow mycelia to grow into the medium. After 2 days of pre-incubation, different concentrations of essential oils 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 earlier.

Thin layers (1 mm) of agar blocks (3–4 cm^2) containing mycelia were removed at 1-day intervals for examination by light microscopy. The blocks cut from growing edges were placed in a drop of 50% glycerol on a microscope glass slides, covered with glass cover and examined using a phase contrast light microscope (Olympus BX50, Tokyo, Japan).

For SEM analysis, fungal mycelia were processed as follow: mycelial discs (1 cm in diameter) were fixed with 2.5% glutaraldehyde in 0.1 M phosphate-buffer (pH = 7.2) for 2 h at room temperature. They were washed twice, each time for 10 min, in 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, UK) up to the critical point with CO_2. The fixed material 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, and the significance was determined by means of Duncan's Multiple Range Test ($P < 0.01$).

Results

Major compounds found in essential oils used in experiments

The chemical compositions of the essential oils used in this study were determined by GC-MS analysis. The number of compounds and their relative amount found in essential oils varied according to plant species and the particular compound. The major compounds found in essential oils of thyme, oregano, rosemary, lavender, fennel and laurel were

carvacrol (37.9%), carvacrol (79.8), borneol (20.4%), camphor (20.2%), anethole (82.8%) and 1,8-cineole (35.5%), respectively (Table 1).

Antifungal properties of essential oils

The volatile and contact phase effects of different concentrations of essential oils on the mycelial growth of *P. infestans* are shown in Tables 2 and 3. All essential oils were found to inhibit the growth of *P. infestans* in a dose-dependent manner. The results indicate that of the plant species tested, essential oils of thyme-like species (Syrian oregano and thyme) were more inhibitory to *P. infestans* than rosemary and lavender oils in both the contact and volatile studies.

Volatile inhibitory effects of essential oils were greater on pathogen growth than contact inhibitory effect (Table 2). Mycelial growth of *P. infestans* was totally inhibited by thyme and oregano oils at a relatively low concentration of 0.3 μg/ml air, while complete growth inhibition by essential oils of fennel, rosemary, lavender, and laurel was at concentrations of 0.4, 1.2, 1.6 and 2.0 μg/ml air respectively.

Although oils of oregano and thyme at the concentration of 0.1 μg/ml caused some reduction in mycelial growth of *P. infestans*, mycelial growth

Table 2. Volatile effect of plant essential oils on mycelial growth of *P. infestans*

Conc. (μg/ml air)	Mycelial growth* (cm)					
	Oregano	Thyme	Rosemary	Lavender	Fennel	Laurel
0	9.0e**	9.0e	9.0e	9.0f	9.0d	9.0g
0.05	5.7d	7.8d	8.8e	8.9f	8.7d	8.9g
0.1	4.1c	3.8c	8.8e	8.9f	8.6d	8.9g
0.2	2.7b	2.8b	8.7e	8.8f	3.3c	8.9g
0.3	0a	0a	8.2d	7.3e	1.5b	8.1f
0.4	0a	0a	5.1c	3.3d	0a	4.5e
0.8	0a	0a	4.2b	2.5c	0a	3.9d
1.2	0a	0a	0a	1.4b	0a	2.3c
1.6	0a	0a	0a	0a	0a	0.9b
2.0	0a	0a	0a	0a	0a	0a

* The mean radial mycelial growth of *P. infestans* was determined at 7 days after inoculation. Each number is based on five replicate plates. Experiments repeated twice.
** Mean values followed by different letters within the column are significantly different according to Duncan Multiple Range Test ($P < 0.01$).

Table 3. Contact effect of plant essential oils on mycelial growth of *P. infestans*

Conc. (μg/ml)	Mycelial growth* (cm)					
	Oregano	Thyme	Rosemary	Lavender	Fennel	Laurel
0	9.0g**	9.0f	9.0d	9.0g	9.0c	9.0e
0.1	8.5f	8.3e	9.0d	9.0g	8.9c	9.0e
0.2	6.1e	4.3d	8.9d	7.3f	8.9c	9.0e
0.4	5.5d	3.8c	8.9d	6.2e	8.9c	9.0e
0.8	3.6c	3.6c	8.9d	6.1e	8.9c	9.0e
1.6	3.7c	2.5b	8.7d	5.7de	8.8c	8.9e
3.2	2.4b	1.9b	7.2c	5.4d	6.5b	8.7e
6.4	0a	0a	4.3b	4.4c	0a	6.7d
12.8	0a	0a	0a	3.1b	0a	4.3c
25.6	0a	0a	0a	0a	0a	1.0b
51.2	0a	0a	0a	0a	0a	0a

* The mean radial mycelial growth of *P. infestans* was determined at 7 days after inoculation. Each number is based on five replicate plates. Experiments repeated twice.
** Mean values followed by different letters within the column are significantly different according to Duncan Multiple Range Test ($P < 0.01$).

was substantially reduced by exposure to higher concentrations of all essential oils tested (Table 3). Oregano, thyme and fennel oils at 6.4 μg/ml inhibited growth of *P. infestans* completely whereas mycelial growth was totally inhibited by rosemary and lavender essential oils at 12.8, and 25.6 μg/ml concentrations respectively. Oil of laurel, however, caused complete inhibition at the highest concentration (51.2 μg/ml) used in the study.

Antimicrobial properties of essential oils

The antimicrobial properties of volatile and contact MIC varied according to the essential oils. Both volatile and contact phase of essential oils of oregano, thyme, fennel and rosemary were found to be fungicidal at their respective MIC. Lavender essential oil was fungicidal in its contact phase of 25.6 μg/ml but fungistatic in its volatile phase at 1.6 μg/ml air. The minimum fungicidal activity of lavender essential oil was found to be slightly higher at MIC for volatile phase (2.4 μg/ml air). Both volatile and contact phases of laurel essential oil was however fungistatic since the mycelial discs grew when transferred to medium without essential oil. To be fungicidal, however, the MIC of both volatile and contact phase of laurel oil had to be two fold.

Effect of essential oils on sporangium formation

Essential oils used in this study also affected sporangial formation. Sporangia formation by the *P. infestans* hyphae varied according to the essential oil tested (Table 4). Oregano was the most effective essential oil in inhibition of sporangium formation. The remaining oils in decreasing effects on sporangia formation were thyme, fennel, lavender, rosemary and laurel.

Effect of essential oils on hyphal morphology

Microscopic observation of *P. infestans* hyphae exposed to the fungicidal MIC of essential oils vapour (volatile phase) or grown on V8 agar amended with the fungicidal MIC of essential oils (contact phase) showed degenerative changes in the hyphal morphology compared to control hyphae (Figure 1). After exposure to contact or volatile phases, hyphae appeared degraded (Figure 1b), large vesicles are also visible within the cell walls. Shrivelled hyphal cells had either no cytoplasm or the cytoplasm was depleted of organelles (Figure 1c). Under the in?uence of the oils, the growth of the fungus was suppressed and the hyphal structure has undergone several morphological changes when viewed by SEM (Figure 2). Unusual pattern of hyphal growth, as well as alterations in cell shape and size are also demonstrated by SEM (Figure 2).

Discussion

Volatile compounds from plants, especially essential oils, have antimicrobial activity against a variety of food borne, human and plant pathogens and pest [3, 10]. In this study, we tested the effect of essential oils on mycelial growth of *P. infestans in vitro*. We also investigated the effect of essential oils on hyphal morphology under light and scanning electron microscopy. The results of this study confirm that essential oils from aromatic plants such as thyme, oregano, lavender, rosemary, laurel, and fennel possess antimicrobial activity

Table 4. Contact effect of plant essential oils on the number of sporangia produced by *Phytophthora infestans*

Conc. (μg/ml)	Essential oils					
	Oregano	Thyme	Rosemary	Lavender	Fennel	Laurel
0	106.1b	106.1c	106.1d	106.1d	106.1c	106.1c
0.8	0a	33.2b	108.6d	101.8d	104.3c	105.7c
1.6	0a	0a	71.9c	19.2c	81.8b	98.9c
3.2	0a	0a	20.9b	7.8b	0a	94.7c
6.4	0a	0a	0a	0a	0a	14.7b
12.8	0a	0a	0a	0a	0a	0a

Values represent the mean number of sporangia in five randomly selected fields of microscopic view at 40× magnification. Mean values followed by different small letters within the column are significantly different according to Duncan Multiple Range Test ($P < 0.01$).

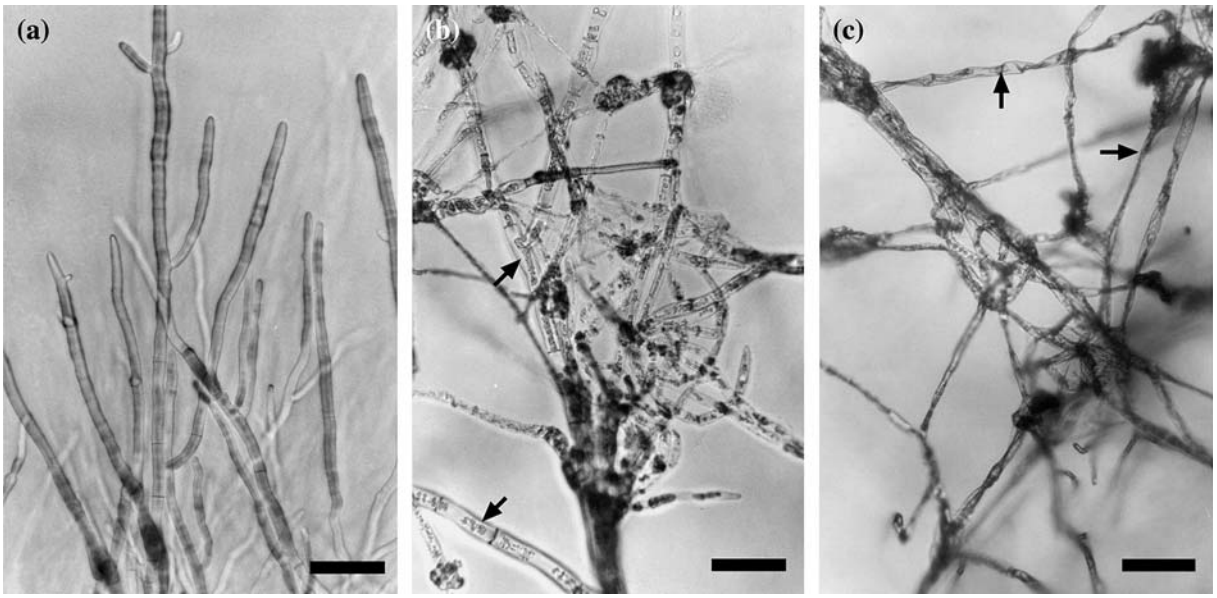

Figure 1. Effect of essential oil of oregano on hyphal morphology of *P. infestans* (at 40× magnification). (a) Hyphae growing on control medium. (b) and (c) Contact and volatile phase effects of essential oil, respectively, on hyphal morphology. Note cytoplasmic coagulation and vesiculation (arrows) in plate (b) and hyphal shrinkage and necrosis (arrows) in plate (c). Bar = 50 μm.

against the *P. infestans*. Of the plants belonging to Lamiacea family, essential oils from thyme and oregano produced highest antifungal activity against *P. infestans*. Rosemary and lavender oils also exhibited antimicrobial activity but were much less than thyme and oregano essential oils. Essential oils from the plants used in this experiment contained as their major components carvacrol, camphor, borneol, 1,8-cineole and anethole. These same compounds have previously been reported to have antimicrobial activity against a variety of human and plant pathogenic fungi and bacteria [11–20]. To our knowledge, this study was the study to show that essential oils of oregano, thyme and lavender have inhibitory to *P. infestans*.

The volatile phases of the essential oils were found to be more toxic than the contact phase to the *P. infestans*. Volatile phase of Artemisia, peppermint, basil and thyme essential oils were also reported to posses more antimicrobial activity against plant pathogenic fungi [19, 20]. Investigators suggested that the antifungal activity resulted from a direct effect of essential oil vapours on fungal mycelium and postulated that the lipophilic nature of essential oils was as possible for them being absorbed by fungal mycelia [19, 21].

Phytophthora species produce sporangia during asexual reproduction [1]. We found that essential oils not only affect the growth rate of mycelia but sporangia production as well which may influence the rate of disease development *in vivo* conditions especially in plants secreting volatile oils.

Although some studies have reported on the antifungal activity of plant extracts including essential oils, the mechanism of action of such oils is poorly understood. Few studies demonstrated effects of essential oils on the morphology and ultrastructure of the human pathogenic fungal structures [22–27]. Light and SEM of untreated *P. infestans* revealed normal mycelia, however hyphae of *P. infestans* grown on media with essential oils revealed alterations in the morphology of the hyphae. Shrivelled hyphal aggregates were commonly observed in oil treated mycelia, compared with thick, elongated, normal mycelial growth in controls. Hyphae grown in media amended with the most inhibiting oil concentrations had reduced hyphal diameters and thinning of hyphal wall. Such modifications may be related to the effect of the essential oil as enzymatic reactions regulating wall synthesis [22]. The lipophilic properties of oil components might have also aided in the ability of the oil to penetrate the plasma membrane [23]. The observations made

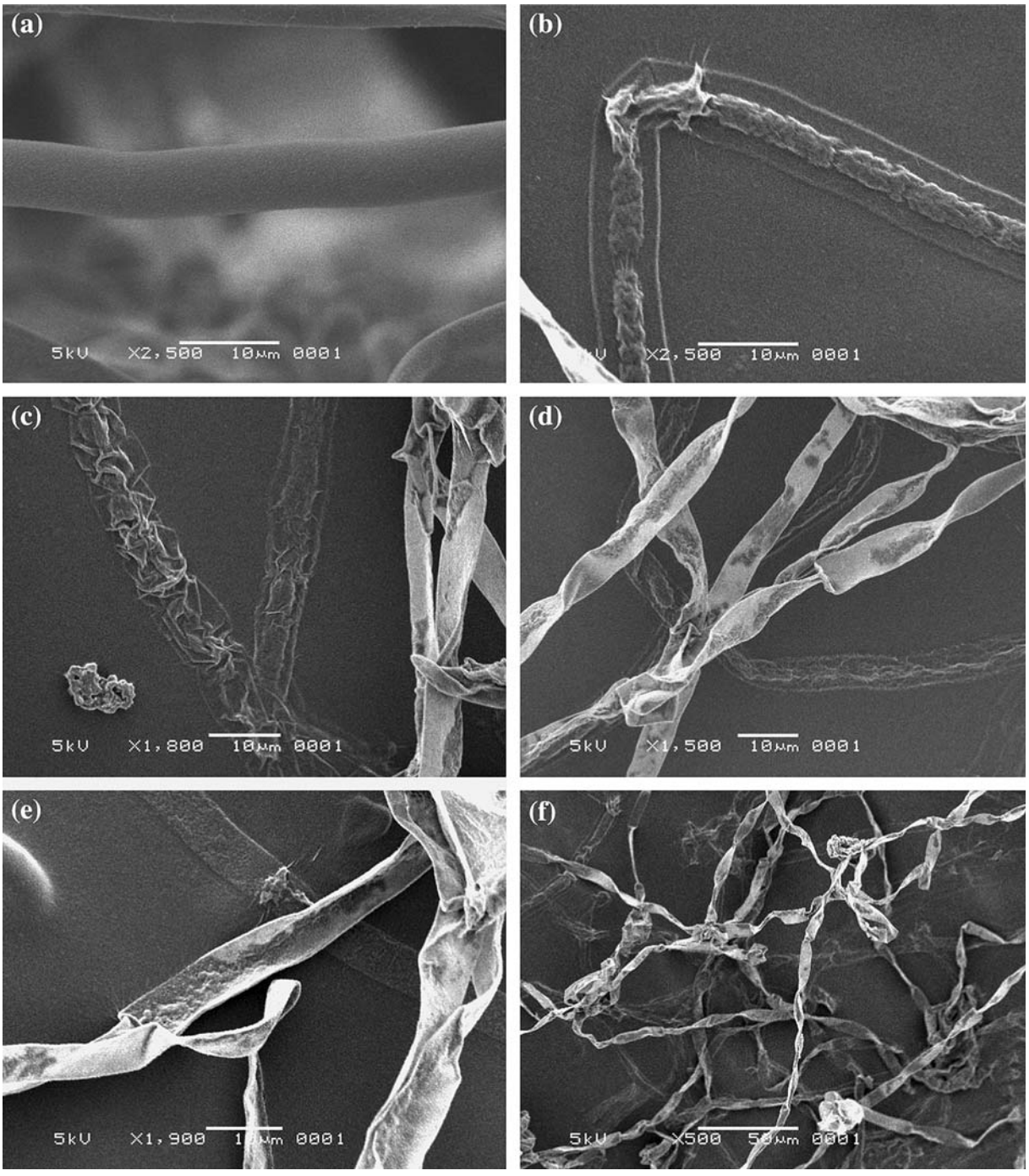

Figure 2. Scanning electron microscopy of hyphae exposed to exogenous essential oil volatiles. (a) Healthy hyphae. (b–f) Effects of essential oil on hyphal morphology. Note alterations in hyphal morphology and cytoplasmic content including cytoplasmic coagulation (b), hyphal shrivelling (c), hyphae without cytoplasm (d), blistering (e) and necrosis (f).

with light microscopy are in accordance with those of Soylu et al. [18], Bianchi et al. [24], and Fiori et al., [25] who verified that essential oils of aromatic plants caused the morphological alterations on the fungal hyphae of *Penicillium digitatum, Didymella bryoniae, Colletotrichum lindemuthia-*

num, *Fusarium solani, Rhizoctonia solani*, and *Pythium ultimum*.

Earlier ultrastructural observations using transmission electron microscopy showed thickening and detachment of the fungal cell wall, an increase in the number of osmiophilic vesicles, lamellar proliferation in the cytoplasm, plasma membrane disruption and mitochondrial destruction in fungal and yeast cells [22, 26, 27]. Although our results demonstrated the antifungal activities of essential oils, the mechanisms of action are not well documented. However the advert effect of essential oils that we observed on the fungal hyphae may be responsible for the decrease in the rate of mycelial growth. General change in the morphology of the hyphae 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 [27].

In conclusion, our results suggest that essential oils have the potential for use in control of *P. infestans* and plant protection. The essential oils tested in this study could be considered as potential alternatives for synthetic fungicides with modification as their structures could lead to the development of new classes of antifungal compounds.

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