Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Antifungal activity of thyme oil against *Geotrichum citri-aurantii in vitro* and *in vivo*

X. Liu[1], L.P. Wang[2], Y.C. Li[1], H.Y. Li[3], T. Yu[1] and X.D. Zheng[1]

1 Department of Food Science and Nutrition, Zhejiang University, Hangzhou, Zhejiang, China
2 Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China
3 Biotechnology Institute, Zhejiang University, Hangzhou, Zhejiang, China

Keywords
citrus fruit, *Galactomyces citri-aurantii,* *Geotrichum citri-aurantii,* sour rot, thyme oil.

Correspondence
Xiadong Zheng, Department of Food Science and Nutrition, Zhejiang University, Hangzhou, Zhejiang, China. E-mail: xdzheng@zju.edu.cn

2009/0246: received 7 February 2009, revised 11 March 2009 and accepted 12 March 2009

doi:10.1111/j.1365-2672.2009.04328.x

Abstract

Aims: To investigate antifungal effect of thyme oil on *Geotrichum citri-aurantii* arthroconidia germination and germ tube elongation, to reveal effects of thyme oil on morphological structures on fungal hyphae and arthroconidia and to assess potential bio-control capacities of thyme oil against disease suppression *in vivo* conditions.

Methods and Results: Thyme oil controlled the growth of *G. citri-aurantii* effectively. Arthroconidia germination and germ tube elongation in potato dextrose broth was greatly inhibited by thyme oil. At 600 μl l^{-1}, it inhibited the germination of about 94% of the arthroconidia and the germ tube length was only 4·32 ± 0·28 μm. Observations using light microscope, scanning electron microscope and transmission electron microscope revealed ultrastructural modifications caused by thyme oil that included markedly shrivelled and crinkled hyphae and arthroconidia, plasma membrane disruption and mitochondrial disorganization. Thyme oil applied to 'Satsuma' mandarin oranges that had been artificially wounded and inoculated with *G. citri-aurantii* reduced sour rot from 78·1% among untreated control fruit to 14·1% after 5 days at 26°C. Thyme oil applied to intact fruits reduced the decay from 76% among untreated control fruit to 35% after 30 days at 20°C. Thyme oil treatment did not harm 'Satsuma' mandarin oranges when they were examined after treatment and storage at 20°C for 30 days.

Conclusions: Thyme oil may provide an alternative means of controlling postharvest sour rot on citrus fruit.

Significance and Impact of the Study: The use of such essential oil may constitute an important alternative to synthetic fungicides. They can be exploited in commercial production and applied under storage and greenhouse conditions.

Introduction

Worldwide citrus sour rot caused by *Geotrichum citri-aurantii* has been reported as an important postharvest disease of citrus fruit (Butler *et al.* 1965; Hershenhorn *et al.* 1992; Dewa *et al.* 1997). Although less common than blue mould and green mould, it can cause significant losses during periods of high rainfall. Sour rot is not controlled with the currently registered fungicides imazalil and thiabendazole, and was partially controlled with sodium *o*-phenylphenate. However, use of sodium *o*-phenylphenate was limited due to risk of fruit injury (Droby *et al.* 1998). Sour rot could be partially prevented by sanitation control and low-temperature storage. But, chilling injury and temperature fluctuation during transport and marketing were still major bottlenecks.

Essential oils are natural products of vegetal origin, which can be used as natural additives in many foods due to their antibacterial, antifungal, antioxidant and anticarcinogenic properties (Conner and Beuchat 1984; Teissedre and Waterhouse 2000; Smith-Palmer *et al.* 2001; Ultee *et al.* 2002; Fisher *et al.* 2007; Omidbeygi

et al. 2007). Essential oils played an important role in plant defence mechanism against phytopathogenic micro-organisms (Palhano *et al*. 2004; Tzortzakis 2007). Nychas (1995) reported antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic and onion against bacteria and moulds.

Numerous studies have been carried out on antimicrobial efficacy of plant essential oils against pathogens in fruits, i.e. treatment of oranges with essential oils of *Mentha arvensis*, *Ocimum* and *Zingiber officinale* had been found to control blue mould, thereby enhancing shelf life (Tripathi and Dubey 2004). The objectives of this study were (i) to investigate antifungal effect of thyme oil on arthroconidia germination and germ tube elongation, (ii) to reveal effects of thyme oil on morphological structures on fungal hyphae and arthroconidia and (iii) to assess potential bio-control capacities of thyme oil against disease suppression *in vivo* conditions.

Materials and methods

Essential oil

Pure-grade thyme oil was purchased from International Flavors and Fragrances Inc., Shanghai, China, and stored at 4°C.

Fungi and culture

Geotrichum citri-aurantii was isolated from decayed Satsuma mandarin citrus fruit. The fungus was maintained on potato dextrose agar plates (PDA: extract of boiled potatoes, 200 ml; dextrose, 20 g and distilled water, 800 ml) at 4°C. Arthroconidium suspension was obtained by flooding 7- to 14-day-old PDA cultures of pathogen with sterile distilled water. An arthroconidium suspension was determined by a haemocytometer and adjusted to 1×10^6 arthroconidia per millilitre.

Antifungal activity of thyme oil *in vitro*

Arthroconidium germination assay

The effect of thyme oil on arthroconidium germination and germ tube elongation was tested in potato dextrose broth (PDB). Thyme oil was added to a 10 ml glass tube containing 5 ml PDB to final concentration 0, 200, 400 and 600 μl l^{-1}. At the same time, aliquot (100 μl) of arthroconidium suspension (1×10^7 arthroconidia per millilitre) of *G. citri-aurantii* was added to each tube. After 8 h of incubation at 26°C on a rotary shaker (200 rev min^{-1}), the samples were examined with a BH-2 light microscope (Olympus, Japan). Then, 2 h later, at least 200 arthroconidia per replicate were observed microscopically to determine germination rate and germ tube length.

Scanning electron microscopy

A mycelia disk (2 mm diameter) was taken from the periphery of the colony grown on the cellophane disk placed on the PDA media with thyme oil of 0, 200, 400, or 600 μl l^{-1} after 5 days' incubation. Samples were fixed in 2·5% glutaraldehyde for 24 h at room temperature. They were washed for 15 min by 0·1 mol l^{-1} phosphate buffer for three times, followed another 1 h of fixation in 1% OsO_4 solution. The specimens were then dehydrated in a graded ethanol series (30%, 50%, 70%, 10 min for each alcohol dilution and 30 min in 100% ethanol). Then the samples were coated with gold platinum using an ion coater (Eiko IB-5, Hitachi, Japan). The samples were observed using a Phillips XL 30 microscope at 20 KV (Phillips, Holland).

Transmission electron microscopy

Small pieces of mycelium (2 mm diameter) grown for 5 days on the cellophane disk placed on the PDA media with thyme oil of 0, 200, 400 and 600 μl l^{-1} were fixed with 2·5% glutaraldehyde for 30 min at room temperature. They were washed for 15 min by 0·1 mol l^{-1} phosphate buffer for three times, followed another 1 h of fixation in 1% OsO_4 solution, The specimens were then dehydrated in a graded ethanol series (50%, 70%, 90%, 95% 15 min for each alcohol dilution and 20 min in 100% ethanol). They were passed through three changes of acetone ethanol (1 : 1 and 1 : 3) for 1 h each and embedded in epoxy media. Blocks were sectioned with diamond knife into ultrathin sections of about 70 nm. The ultrathin sections were contrasted with uranyl acetate followed by lead citrate each for 30 min and examined on a Jeol 1230 Ex TEM.

Effects of thyme oil on sour rot development in artificially inoculated and wounded fruit

Citrus fruit cultivar 'Satsuma mandarin' was harvested at commercial maturity from Chun'an of Zhejiang province. Fruits were selected for freedom of infections and placed in 1·5 l plastic boxes. Fruits were dipped in 1% sodium hypochlorite for 2 min, rinsed with tap water and air-dried before wounding.

Citrus fruits were wounded with a sterile puncher to make one uniform 2 mm deep by 5 mm wide wound on their peels at the equatorial region. Aliquot of 30 μl of 400, 800, 1600 and 3200 μl l^{-1} thyme oil and sterile distilled water (control) was pipetted into each wound. Then, 20 μl of arthroconidium suspension of *G. citri-aurantii* (1×10^6 arthroconidia per millilitre) was pipetted

into it. Fruits were sealed in polyethylene-lined plastic boxes to maintain high humidity (approx. 95%) and incubated at 26°C (Wang *et al.* 2008). The number of the infected fruit lesion and diameter of lesion were recorded after 5 days' incubation. Fruit with no infection was not counted for lesion size measurements. Each treatment was recorded three times with 20 fruits per replicate.

Effects of thyme oil on natural infection development in intact citrus fruit

The concentration of thyme oil was prepared by dissolving the requisite amounts (1600 μl) in 25 ml of 0·05% Tween-20 and then mixed with 475 ml of sterilized water. Similarly, control was prepared using only sterilized water.

Intact citrus fruits were treated by dipping them in the suspension of thyme oil for 15 or 30 s, respectively at room temperature and air dried. Similarly, intact citrus fruits as controls were dipped in sterile distilled water for 15 or 30 s, respectively. Treated citrus fruits were stored at 20°C for 30 days. The percentage of infected fruits was recorded regular interval till the end of storage. Each treatment was replicated thrice with 20 fruits per replicate.

Statistical analyses

All data were analysed using SAS software ver. 8.0. Mean separations were performed by Duncan's multiple range tests. Differences at $P < 0·05$ were considered as significant. The percentage of *G. citri-aurantii* hyphal growth inhibition was assessed using two-factor ANOVA and means were separated using the LSD test, at $P < 0·05$.

Results

Inhibition of thyme oil to fungus growth *in vitro*

Inhibition of arthroconidium germination

Table 1 and Fig. 1a showed the effect of thyme oil on arthroconidium germination and germ tube length. The concentration of thyme oil strongly influenced the arthroconidium germination of *G. citri-aurantii.* The correlations between arthroconidium germination and inhibition concentration was 0·90, showing that the inhibition ratio was proportional to concentration. The germ tubes were too long to be determined in control after 10 h. Thyme oil at 400 μl l^{-1} inhibited the germination of about 70% arthroconidia and the germ tube length was only 6·78 ± 0·09 μm.

Observations by scanning electron microscopy

The effect of thyme oil on the morphology of *G. citri-aurantii* hypha and arthroconidia examined by SEM were shown in Fig. 1b. The growing noncontacted healthy mycelium grown on PDA medium presented a normal morphology with linear, regular, homogeneous cell wall hypha. The growing apexes of the hypha were tapered with a smooth surface. The arthroconidium was columned and smooth. These normal morphological structures varied in the presence of the essential oil in the culture medium. The hypha was modified and evident craters appeared on the cell wall. Arthroconidia were distorted and had conspicuous depressions on their surface. Greater damages were observed with concentration of 600 μl l^{-1}. It caused cell wall disruption and, consequently, cell death.

Table 1 Effects of thyme oil on arthroconidia germination and germ tube elongation of *G. citri-aurantii*

Thyme essential oil (μl l^{-1})	Spore germination (%)*	Germ tube length (μm)*
Control	100 a†	ND
200	100 a†	86·6 ± 0·37 a
400	31·0 ± 0·35 b	6·8 ± 0·09 b
600	6·4 ± 0·53 c	4·3 ± 0·28 c

*Germination rate and germ tube length were measured after 10 h incubation at 26 °C in PDB.

†Value was the mean of three replicates ± SD. All the values were means of three replicates ± SD. Different letters meant significant differences between different concentrations for each time point ($P < 0·05$).

ND, not done.

Transmission electron microscopy

The main change concerned the alteration of the hypha and arthroconidium wall thickness. On control transversal sections, the wall of hypha had a thickness of 1250 Å, and on control longitudinal section, the wall of arthroconidium had a thickness of 2000 Å. Moreover, the wall of them was composed of a single, uniform layer, which was weakly electron dense after the Thiery reaction (Fig. 2a,f).

The wall thickness in filaments and arthroconidia treated with thyme oil became bigger with increased concentration of oil: filaments cell wall was 2500 Å at 200 μl l^{-1} (Fig. 2b) and increased to 3750 Å at 600 μl l^{-1} (Fig. 2c). Whilst arthroconidia cell wall increased to 3200 Å at 600 μl l^{-1} (Fig. 2g) from 2000 Å in control (Fig. 2f). The cell wall became very thick and strong electron density. The general cells shape was also modified and lost its regularity compared to the control.

At 200 μl l^{-1} of thyme oil, the plasmalemma became irregular and festooned with numerous lomasomes (membranous structure, often containing internal membranes, located between the plasma membrane and cell wall). At 600 μl l^{-1}, discrete cytoplasmic alterations were also observed (Fig. 2c,g). The cytoplasm appeared very

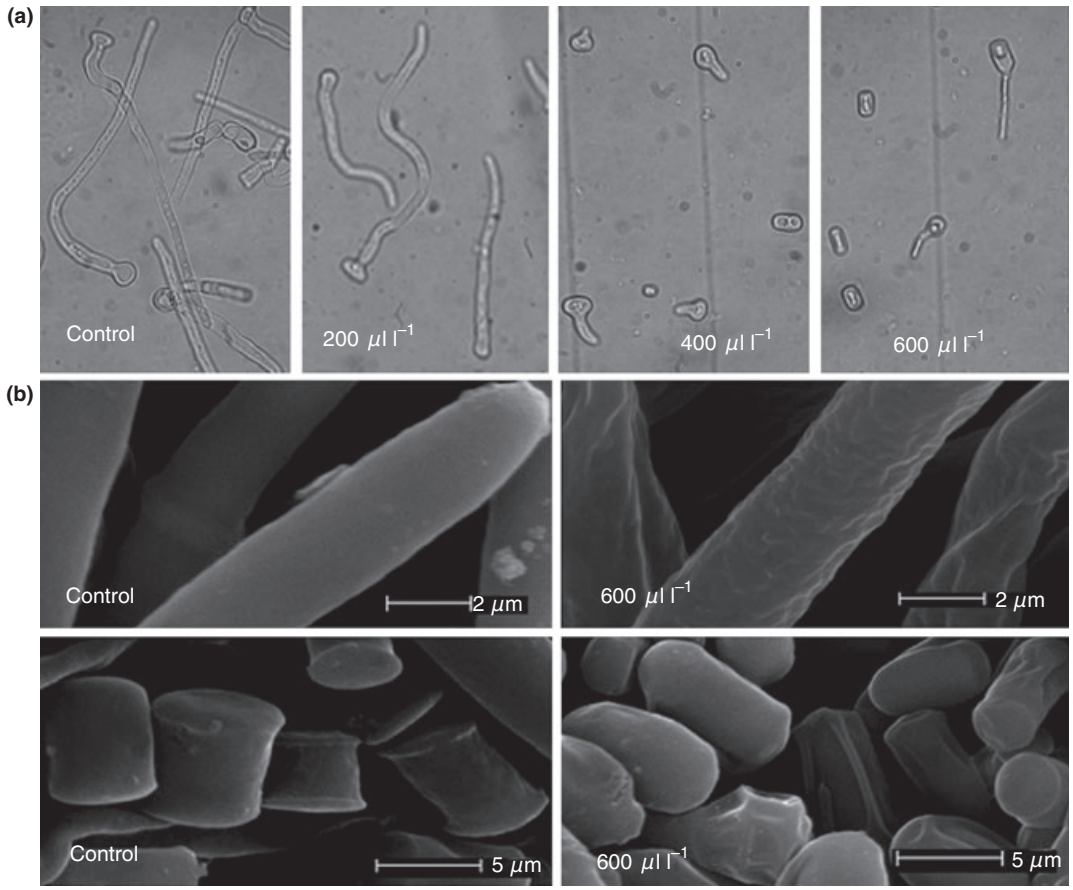

Figure 1 Light and scanning electron micrographs of *G. cirti-aurantii* mycelium and arthroconidia with or without thyme oil. (a) Displayed light micrographs of arthroconidia germination and germ tube length, cultured in PDB and measured after 8 h incubation in controlled environment chambers maintained at 26°C. (b) Showed scanning electron micrographs hypha and arthroconidia of *G. citriaurantii* grown on PDA during 5 days of incubation at 26°C.

dense, showing wide vacuoles, without uniform glycogen granules and containing irregular and unknown bodies.

Efficacy of thyme oil for reducing sour rot development in artificially inoculated citrus fruit

The results in Fig. 3 showed that when wounded citrus were treated with thyme oil, the higher the concentration of thyme oil, the lower the disease incidence exhibited. Incidence of sour rot and lesion diameter on control citrus was 78·1% and 16·8 mm, whereas the treatment with 3200 μl l^{-1} thyme oil suppressed sour rot incidence and lesion diameter to 14·1% and 1·8 mm, respectively.

Efficacy of thyme oil for reducing natural decay development in intact citrus fruit

Our experiments evaluated the effect of thyme oil on reducing the development of natural decay. The result presented in Fig. 4 indicated that the application of thyme oil resulted in low-average decay incidence in fruit 35%, compared with 76% in the water-treated control fruit.

Discussion

The results of the present study indicated that thyme oil inhibited the growth of *G. citri-aurantii.* Arras and Usai (2001) reported that *Thymus capitatus* essential oil inhibited the growth of the four fungi (*Penicillium digitatum*, *Penicillium italicum*, *Botrytis cinerea* and *Alternaria citri*) at a concentration of 250 ppm (v/v). Yahyazadeh *et al.* (2008) also showed thyme oil completely inhibited *P. digitatum* growth when added into the medium 600 μl l^{-1}.

Fungal growth inhibition was associated with the degeneration of fungal hypha after treatment with *T. capitatus*, *Thymus vulgaris* L., *Lavandula* R.C., and *Mentha piperita* essential oils using SEM (Arras *et al.* 1995; Zambonelli *et al.* 1996). Major thickening and disruption of the cell wall, together with increased roughness and

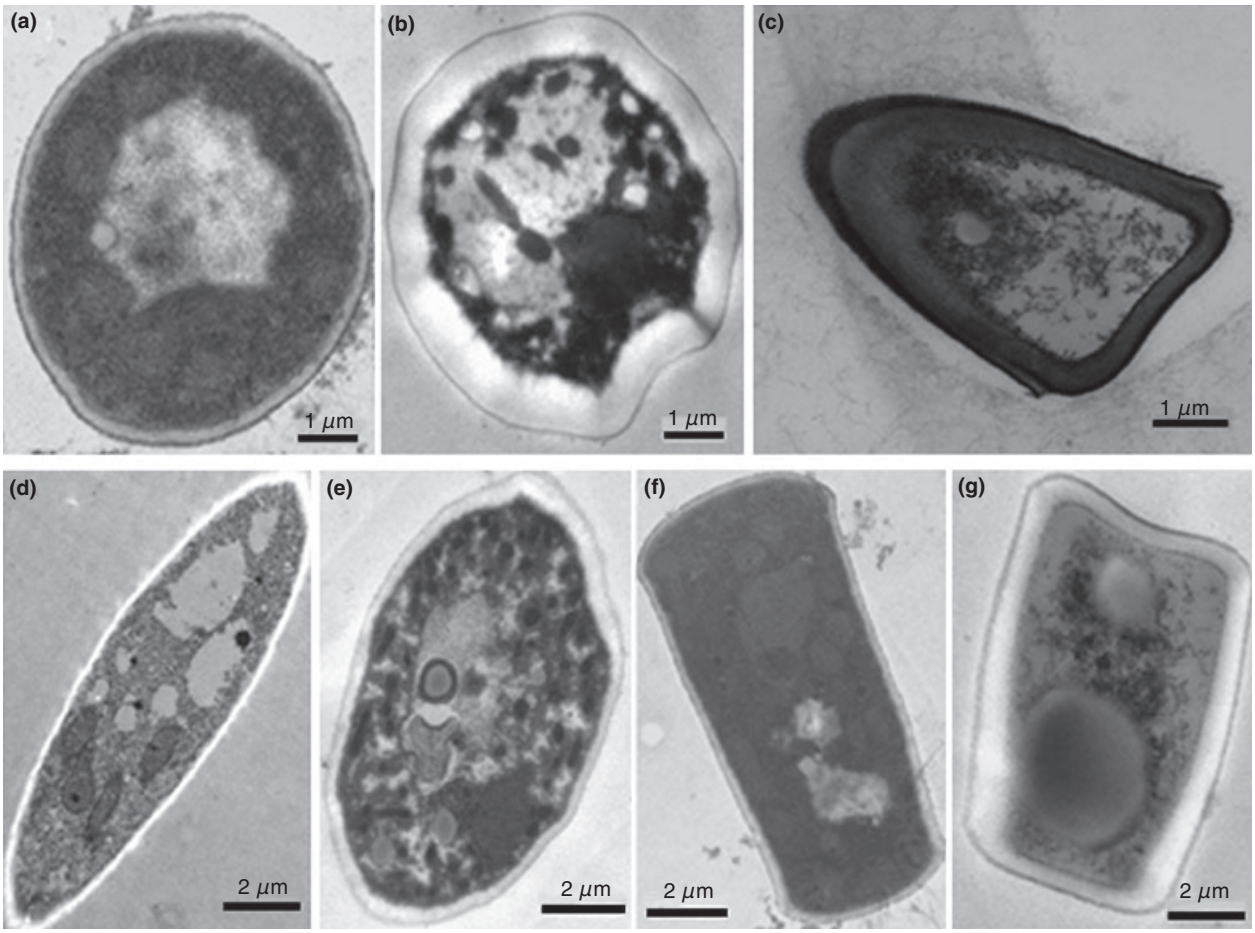

Figure 2 Transmission electron micrographs for the hypha (a–e) and arthroconidia (f–g) with or without thyme oil on agar plates. (a) Without thyme oil (×20 K); (b) Treatment at 200 μl of oil/l (×20 K); (c) Treatment at 600 μl l^{-1} oil (×20 K); (d)Tangential section through the control hypha of *G. citri-aurantii* (×12 K); (e) Tangential section through the hypha treated with 600 μl of oil/l (×12 K). (f)Longitudinal section through the arthroconidia treated without thyme oil (×12 K); (g) Longitudinal section through the arthroconidia treated with 600 μl l^{-1} oil (×12 K).

lack of cytoplasm have recently been reported in *Listeria monocytogenes* on treatment with thyme essential oil. Except that, cells of *L. monocytogenes* exposed to the essential oil from *Thymus x-porlock* also exhibited decreased size, closer together and budding scars in cell wall (Rasooli *et al.* 2006). This was probably different from fungi treated by essential oils. Large alterations in hyphal morphology, abnormal branching of hypha in the apical region and loss of linearity with the appearance of barrel-like formations were observed in *B. cinerea* via electron microscopy under the effect of 1·85 μg ml^{-1} of *Tagetes patula* L. essential oil (Romagnoli *et al.* 2005). In our study, *G. citri-aurantii* treated with thyme oil showed alteration in the morphology, which appeared collapsed in mycelium and arthroconidia structure.

A marked alteration of hypha wall thickness was observed in our study in the presence of thyme oil. The wall became much thicker than control. This result differed entirely from others. For examples, De Billerbeck *et al.* (2001) reported that the wall of *Aspergillus niger* hypha was disappeared in some regions after treated with *Cymbopogon nardus* essential oil. Similar results were also found on *A. flavus* when treated with *Cymbopogon citratus* (Helal *et al.* 2007). However, Maisnier-Patin and Richard (1996) reported that exposure of *Listeria innocua* to nisin concentrations of 500 and 4000 IU ml^{-1} induced cell wall thickening as well as irregularities. Krzesłowska and Woźny (1996) founded that 48 h-treatment 4 μmol l^{-1} $PbCl_2$ resulted in cell wall thickening at the tip of the apical cell.

In recent years, more and more essential oils have been studied and most of them have been reported to inhibit postharvest fungi *in vitro* and *in vivo* conditions (Amezi-ane *et al.* 2007; Helal *et al.* 2007; Nikos and Costas 2007; Nurhayat *et al.* 2007; Szczerbanik *et al.* 2007; Thierry *et al.* 2008). Our results indicated thyme oil could reduce

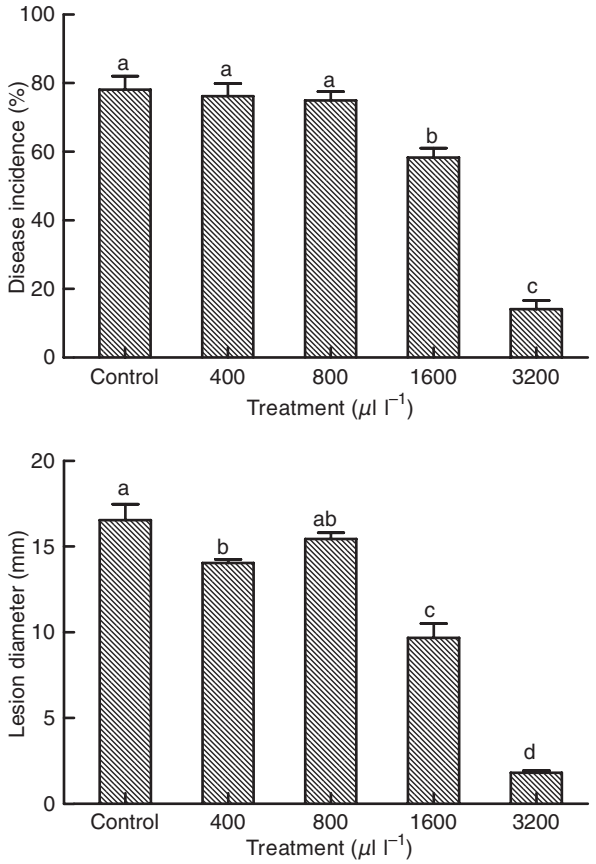

Figure 3 Inhibition of *Geotrichum citri-aurantii* on artificially inoculated and wounded citrus fruits by thyme oil. Significant differences ($P < 0{\cdot}05$) between means were indicated by different letters above histogram bars.

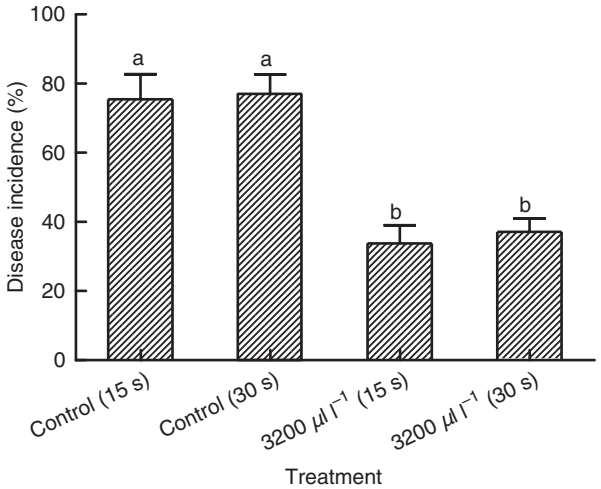

Figure 4 Effects of thyme oil on naturally infected development in unwounded citrus fruits. Intact citrus fruits were dipped in the 3200 μl l^{-1} suspension of thyme oil for 15 and 30 s, respectively. Similarly, intact citrus fruits as controls were dipped in the sterilized water for 15 and 30 s, respectively. Significant differences ($P < 0{\cdot}05$) between means were indicated by different letters above histogram bars.

postharvest diseases on citrus fruit caused by *G. citri-aurantii*. The disease incidence of artificially inoculated citrus fruit reduced about 82% at 3200 μl l^{-1} as compared to control. However, the inhibition by thyme oil in citrus was not as dramatic as that in plates. In general, levels of essential oils and their compounds necessary to inhibit microbial growth were higher in foods than in culture media. This was due to interactions between phenolic compounds and the food matrix (Nychas and Tassou 2000). Because essential oils are made up of many different volatile compounds, the residual levels would be low after storage.

Thyme oil possessed antifungal activity inhibiting the growth of *G. citri-aurantii* and leading to deleterious cellular morphological modifications. Moreover, it did not affect the flavour or the appearance of the citrus fruits. Although this subject needs further study to fully understand the mechanism of action of thyme oil, it can be concluded that plant essential oils used in this study can serve as possible alternative to synthetic fungicides.

Acknowledgements

This research was supported by grants from the National Key Technology Support Program (2006BAD22B02) and the National High-Tech Research and Development Program (863 Program) of China (2006AA10Z348) and the National Natural Science Foundation of China (30771514) and the Zhejiang Provincial Natural Science Foundation of China (Z307440).

References

Ameziane, N., Boubaker, H., Boudyach, H., Msanda, F., Jilal, A. and Benaoumar, A.I.T. (2007) Antigungal activity of Moroccan plants against citrus fruit pathogens. *Agron Sustain Dev* **27**, 273–277.

Arras, G., Agabbio, M., Piga, A. and D'hallewin, G. (1995) Fungicide effect of volatile compounds of *Thymus capitatus* essential oil. *Acta Hortic* **379**, 593–600.

Arras, G. and Usai, M. (2001) Fungitoxic activity of 12 essential oils against four postharvest Citrus pathogens: chemical analysis of *Thymus capitatus* oil and its effect in subatmospheric pressure conditions. *J Food Prot* **64**, 1025–1029.

Butler, E.E., Webster, R.K. and Eckert, J.W. (1965) Taxonomy, pathogenecity and physiological properties of the fungus causing sour rot of citrus. *Phytopathology* **55**, 1262–1268.

Conner, D.E. and Beuchat, L.R. (1984) Effects of essential oils from plants on growth of food spoilage yeasts. *J Food Sci* **49**, 429–434.

De Billerbeck, V.G., Roques, C.G., Bessière, J.M., Fonvieille, J.L. and Dargent, R. (2001) Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of Aspergillus niger. *Can J Microbiol* **47**, 9–17.

Dewa, N.S., Kei, A. and Hisashi, I. (1997) Effects of volatile compounds on arthrospore germination and mycelial growth of *Geotrichum candidum* citrus race. *Mycoscience* **38**, 31–35.

Droby, S., Cohen, L., Daus, A., Weiss, B., Horev, B., Chalutz, E. and Katz, H. (1998) Commercial testing of aspire: a yeast preparation for the biological control of postharvest decay of citrus. *Biol Control* **12**, 97–101.

Fisher, K., Rowe, C. and Phillips, C. (2007) The survival of three strains of *Arcobacter butzleri* in the presence of lemon, orange and bergamot essential oils and their components in vitro and on food. *Lett Appl Microbiol* **44**, 495–499.

Helal, G.A., Sarhan, M.M., Abu Shahla, A.N.K. and Abou, E.K. (2007) Effects of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *Aspergillus flavus* ML2-strain. *J Basic Microbiol* **47**, 5–15.

Hershenhorn, J., Dori, S. and Barash, I. (1992) Association of *Geotrichum citri-aurantii* with citrus groves in Israel. *Phytoparasitica* **20**, 31–36.

Krzesłowska, M. and Woźny, A. (1996) Lead uptake localization and changes in cell ultrastructure of *Funaria hygrometrica* protonemata. *Biologia Plantarum* **38**, 253–259.

Maisnier-Patin, S. and Richard, J. (1996) Cell wall changes in nisin-resistant variants of *Listeria innocua* grown in the presence of high nisin concentrations. *FEMS Microbiol Lett* **140**, 29–35.

Nikos, G.T. and Costas, D.E. (2007) Antifungal activity of lemongrass (*Cympopogon citrates* L.) essential oil against key postharvest pathogens. *Innov Food Sci Emerg Technol* **8**, 253–258.

Nurhayat, T., Betul, D. and Husnu Can Baser, K. (2007) Characterization of volatile constituents of *Scaligeria tripartita* and studies on the antifungal activity against phytopathogenic fungi. *J Chromatogr B* **850**, 221–229.

Nychas, G.D. and Tassou, C.C. (2000) Traditional preservative-oils and spices. In *Encyclopedia of Food Microbiology* ed. Robinson, R.K., Batt, C.A. and Patel, P.D. pp. 1717–1722 London, UK: Academic Press.

Nychas, G.J.E. (1995) Natural antimicrobials from plants. In *New Methods of Foods and Food Preservation* ed. Gould, G.W. pp. 58–60 London: Blackie Academic Professional.

Omidbeygi, M., Barzegar, M., Hamidi, Z. and Nafhdibadi, H. (2007) Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid zmedium and tomato paste. *Food Control* **18**, 1518–1523.

Palhano, L.P., Vilches, T., Santos, R.B., Orlando, M., Ventura, J. and Fernandes, P. (2004) Inactivation of *Colletotrichum gloeosporioides* spores by high hydrostatic pressure combined with citral and lemongrass essential oil. *Int J Food Microbiol* **95**, 61–66.

Rasooli, I., Rezaei, M.B. and Allameh, A. (2006) Ultrastructural studies on antimicrobial efficacy of thyme essential oils on *Listeria monocytogenes*. *Int J Infect Dis* **10**, 236–241.

Romagnoli, C., Bruni, R., Andreotti, E., Rai, M.K., Vicentini, C.B. and Mares, D. (2005) Chemical characterization and antifungal activity of essential oil of capitula from wild Indian *Tagetes patula* L. *Protoplasma* **225**, 57–65.

Smith-Palmer, A., Stewart, J. and Fyfe, L. (2001) The potential application of plant essential oils as natural food preservatives in soft cheese. *Food Microbiology* **18**, 463–470.

Szczerbanik, M., Jobling, J., Morris, S. and Holford, P. (2007) Essential oil vapours control some common postharvest fungal pathogens. *Aust J Exp Agric* **47**, 103–109.

Teissedre, P.L. and Waterhouse, A.L. (2000) Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. *J Agric Food Chem* **48**, 3605–3801.

Thierry, R., Wilmam, D.P., Sandra, C. and Ben, B. (2008) Fungitoxicity of *Lippia scaberrima* essential oil and selected terpenoid components on two mango postharvest spoilage pathogens. *Postharvest Biol Technol* **48**, 254–258.

Tripathi, P. and Dubey, N.K. (2004) Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biol Technol* **32**, 235–245.

Tzortzakis, N.G. (2007) Maintaining postharvest quality of fresh produce with volatile compounds. *Innov Food Sci Emerg Technol* **8**, 111–117.

Ultee, A., Bennik, M.H.J. and Moezelaar, R. (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl Environ Microbiol* **68**, 1561–1568.

Wang, Y.F., Bao, Y.H., Shen, D.H., Feng, W., Yu, T., Zhang, J. and Zheng, X.D. (2008) Biocontrol of Alternaria alternata on cherry tomato fruit by use of marine yeast Rhodosporidium paludigenum Fell & Tallman. *Int J Food Microbiol* **123**, 234–239.

Yahyazadeh, M., Omidbaigi, R.Z. and Taheri, H. (2008) Effect of some essential oils on mycelia growth of *Penicillium digitatum* Sacc. *World J Microbiol Biotechnol* **24**, 1445–1450.

Zambonelli, A., Zechini D'Aulerio, A., Bianchi, A. and Albasini, A. (1996) Effects of essential oils on phytopathogenic fungi *in vitro*. *J Phytopathol* **144**, 491–494.

