



Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

The use of Citri-V™® — An antimicrobial citrus essential oil vapour for the control of *Penicillium chrysogenum*, *Aspergillus niger* and *Alternaria alternata* in vitro and on food

C.A. Phillips*, K. Laird¹, S.C. Allen

School of Health, University of Northampton, Northampton, NN2 7AL, UK

ARTICLE INFO

Article history:

Received 29 October 2010

Accepted 21 July 2011

Available online xxxxx

Keywords:

Citrus essential oils

Fungi

Food spoilage

ABSTRACT

Spoilage and poisoning of food by fungi are a major problem for the food industry and consumers. Decay may increase post harvest losses up to 50% without fungicide treatment. However the use of synthetic fungicides is becoming more restrictive and thus alternative treatments need to be developed to reduce environmental risk and satisfy the demands of consumer groups. Essential oils (EOs) have been shown to be effective against a range of fungi but their use may lead to changes in organoleptic properties. However the use of EO vapours may address this issue whilst still reducing contamination.

As an initial screen, the effect of a citrus EO based antimicrobial vapour was tested against *Penicillium chrysogenum*, *Aspergillus niger* and *Alternaria alternata* using the disc diffusion method. Mycelial growth of all three species was completely inhibited and spore germination was reduced.

The effect of the citrus EO vapour on mycelial growth and spore germination of the three fungi in culture and on the growth of *A. alternata* on tomatoes and *P. chrysogenum* and *A. niger* on grain was also determined. When exposed in culture mycelial growth was reduced by 44%, 34% and 67% for *P. chrysogenum*, *A. niger* and *A. alternata* respectively and, although the citrus EO vapour was not an effective treatment to reduce spoilage of tomatoes by *A. alternata*, it reduced the growth of *A. niger* and *P. chrysogenum* by 50–60% on grain over 10 days, suggesting its possible use in reducing spoilage in grain by these two species, especially as this treatment has previously been shown not to affect the organoleptic properties of raw vegetables.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The growth of fungi and subsequent spoilage and poisoning of foods are a major problem for the food industry. Although decay can be reduced by 90–95% by the use of post harvest fungicides, post harvest losses due to decay may increase by up to 50% without fungicide treatment (Magan & Aldred, 2007). These post-harvest losses are more significant for highly perishable fresh fruit and vegetables than field crops with *Alternaria* and *Penicillium* being two of the most common pathogenic fungi (Agris, 1997). Contamination of cereals by filamentous spoilage fungal organisms and by mycotoxins results in losses in dry matter, nutritional content and hence quality. Mycotoxins can be carcinogenic or produce feed refusal and emesis (Magan & Aldred, 2007).

As well as the fact that many synthetic chemicals are becoming ineffective (Reimann & Deising, 2000; Spotts & Cervantes, 1986), the use of synthetic fungicides is becoming more restrictive due to health and environmental concerns. Therefore it is necessary to develop alternative treatments to replace these in order to reduce environ-

mental risk and satisfy the demands of consumer groups (Trienekens & Zuurbier, 2008).

Essential oils (EOs) are mainly obtained from steam distillation of various plants sources and their anti-microbial activity has been reported extensively over the last few decades, although in terms of their effect on fungi their action has been less well studied. However a number of EOs have been reported as active against *A. niger* in vitro such as lemongrass (Tzortzakos & Economakis, 2007) and *Matricaria chamomilla* flower (Tolouee et al., 2010). Tea tree (*Melaleuca alternifolia*) EO (TTO) is effective against *Fusarium* spp. in vitro and when used at 2 g seed kg⁻¹ TTO reduces growth on barley seeds by up to 90%, dependant on species. A single spray of TTO as low as 0.5% completely eliminates *Blumeria graminis* growth on barley leaves (Terzi et al., 2007). *A. alternata* is a saprophytic pathogen of tomato causing post harvest losses at high frequency. Its growth in culture is reduced by 500 ppm thyme and cassia EOs by 62% and 100% respectively and cassia EO at 500 ppm also reduces the decay of tomatoes caused by *A. alternata* by 80%. However treatment with sage, nutmeg or eucalyptus EOs has no effect (Feng & Zheng, 2007). Thyme EO at a concentration of 250 ppm also eliminates aflatoxin production by *Aspergillus parasiticus* (Rasooli, Rezaei, & Allameh, 2006).

EOs can have a fungicidal or fungistatic effect and this may vary according to the concentration used. For example, at low concentrations the effect of summer savoury EO on *Alternaria mali* is fungistatic

* Corresponding author. Tel.: +44 1604892309.

E-mail address: carol.phillips@northampton.ac.uk (C.A. Phillips).

¹ Present address: The Leicester School of Pharmacy, Faculty of Health and Life Sciences, Hawthorn Building, De Montfort University, Leicester, LE1 9BH, UK.

effect whereas at higher concentrations it is fungicidal (Boyraz & Özcan, 2006).

One problem with using EOs in liquid phase is that generally EOs are less effective in food than in vitro resulting in higher concentrations having to be used to bring about the same effect (Al-Burtamani, Fatope, Marwah, Onifade, & Al-Saidi, 2005). The use of an EO in vapour phase may overcome this issue (Goni et al., 2009). Cinnamon, clove, basil and ginger EO vapours have been shown to be effective against *Aspergillus flavus* and *Penicillium islandicum* (Lopez, Sanchez, Batlle, & Nerin, 2005) and thyme, sage and nutmeg EO vapours are active against a range of clinical isolates of *Aspergillus* sp. and environmental isolates of *Penicillium* sp. (Tullio et al., 2007) in vitro.

Although there have been reports of the effect of citrus EOs and their vapours on bacterial strains (Fisher & Phillips, 2006) there have been limited reports regarding the effect of citrus EOs on fungal species. Orange oil was suggested by Subba, Soumithri, and Suryanarayana (1967) as a possible preservative in food because of its effectiveness against a range of food pathogens and food spoilage organisms including *Aspergillus* spp. More recently Sharma and Tripathi (2008) also demonstrated that *Citrus sinensis* (L.) Osbeck epicarp EO exerted a fungicidal effect on *A. niger*.

Since citrus EO vapours are GRAS (Generally Recognised as Safe) they lend themselves to use in food (Fisher & Phillips, 2008). The citrus EO vapour (Citri-V™®) used in this present study has been shown not to affect the organoleptic properties of food (Fisher, Phillips, & McWatt, 2009) and so this study investigated its effect on *P. chrysogenum*, *A. niger* and *A. alternata* in culture and, *P. chrysogenum* and *A. niger* in grain and *A. alternata* on tomatoes.

2. Materials and methods

2.1. Species and culture

A. niger, *P. chrysogenum* and *A. alternata* were obtained from the Health Protection Agency (Colindale UK) and cultured on potato dextrose agar (PDA).

2.2. Essential oil vapour

The EO vapour under test was Citri-V™®, a 50:50 mix of orange: bergamot essential oils (AMPHORA, Bristol, UK) vapourised to give a final concentration of 15 mg/L air. The composition in the headspace that results is shown in Table 1.

2.3. Screening of fungal species

2.3.1. Vegetative cells

A 1 cm plug of a seven day culture of either *A. niger*, *P. chrysogenum* and *A. alternata* was taken from the outer edge of the mycelium growth and placed in the centre of a PDA plate. A 2 cm filter disc impregnated with Citri-V™® and placed in the lid of the plate approximately 8 mm from fungi as previously described for bacterial cultures (Edwards-Jones, Buck, Shawcross, Dawson, & Dunn, 2004; Fisher & Phillips, 2006).

2.3.2. Spores

The surface of a seven day culture of *A. niger*, *P. chrysogenum* and *A. alternata* grown on PDA was washed with 10 mL of sterile water and then decanted. Aliquots of 0.1 mL of the decanted water were spread plated onto PDA and then a 2 cm filter disc impregnated with Citri-V™® placed in the lid of the plate as described for vegetative cells. Spore counts were conducted using the cotton blue staining method and a haemocytometer.

All plates were incubated at 37 °C for 24 h and the zones of inhibition measured using Verner callipers.

Table 1
GC–MS analysis of Citri-V™®.

Compound	LRI	mg/mL	%CV
1R-alpha-pinene	1042		
β-pinene	1137		
Thujene isomer	1149		
(–) β-pinene	1186	28.12	1.6
Limonene	1234	603.99	0.4
Unknown	1244		
Gamma terpinene	1279		
Cymene isomer	1308		
Terpinolene	1320		
Citronellal	1514		
Decanal	1531		
Linalool	1563	60.21	0.2
Linalyl acetate	1580	137.17	0.6
Alpha-bergamotene	1622		
Lilac alcohol C	1633		
Terpineol isomer	1646		
Caryophyllene	1655		
Alpha-terpineol	1737		
Neryl acetate	1753	0.32	5.4
β-Bisabolene	1765		
Geranial (citral equivalent)	1779	0.86* (1.44)	4.6
Geranyl acetate	1784	0.1	4.1

LRI: linear retention indices of the compounds relative to an alkane series.

* Estimated actual Geranial content based on citral standard with 60:40 ratio of geranial to neral isomers.

2.3.3. Growth in a larger space

The effect of the citrus EO vapour against *A. niger*, *P. chrysogenum* and *A. alternata* in culture was tested in a 600 L sealed chamber as previously described (Fisher & Phillips, 2009).

2.4. Growth of *A. alternata* on tomatoes

Wounded tomatoes were inoculated with 10^7 Log₍₁₀₎ *A. alternata* spores into the wound and either exposed to the citrus EO vapour for 15 min once or for 15 min daily for 5 days. Controls were inoculated tomatoes without exposure to the citrus EO vapour. The tomatoes were incubated for 7 days at 25 °C before growth of *A. alternata* was assessed.

2.5. Effect of citrus EO vapour against the growth of *A. niger* and *P. chrysogenum* in grain

Grain (Hereward Class 1 Milling grain; Heygates UK, Bugbrooke, Northants) was sterilised by washing in 5% hypochlorite solution for 5 min. The grain was washed three times in sterile distilled water and dried in a sterile hood before the moisture content was established. Triplicate samples were ground and weighed before heating at 110 °C for 24 h. The moisture content was calculated as a percentage of dry weight compared to wet weight and was found to be 12%, which was then adjusted to 18.8% by adding sterile distilled water at 7 mL per 100 g and leaving for 24 h at 4 °C, shaking periodically to disperse the water.

Sterilised, moisture adjusted grain was inoculated with a 1 cm² plug of vegetative growing hyphae and conidiophores from *A. niger* or *P. chrysogenum* taken from 2 week old solid phase cultures and incubated at 25 °C for 10 days in 9 cm petri dishes sealed to prevent moisture loss. The controls were either untreated or un-inoculated grain samples. All samples were treated with Citri-V™® for 15 min at 24 hour intervals for 5 days. At each time point non-treated samples were also removed from the incubator and replaced to simulate the movement that the treated samples experienced. After the 5 days of vapour treatment all samples were incubated for a further 5 days, when the vegetative and conidiophore growth of the fungi on the grain was monitored and measured.

Table 2

Mean increase in diameter of mycelial growth when treated with citrus EO vapour ($\pm$ SD, $n=6$).

Organism	Mean diameter ($\pm$ SD)	
	Untreated	Treated
<i>Penicillium chrysogenum</i>	4.52 $\pm$ 0.08	0
<i>Aspergillus niger</i>	6.65 $\pm$ 0.07	0
<i>Alternaria alternate</i>	6.18 $\pm$ 0.06	0

2.6. Statistical analysis

All experiments were carried out in triplicate on two separate occasions. Statistical analysis was carried out using SPSS Version 15.0 for Windows.

3. Results

3.1. Screening experiments

Treatment with Citri-V™ completely inhibits mycelial growth of *P. chrysogenum*, *A. niger* and *A. alternata* when they are exposed to the vapour using the disc diffusion method (Table 2). Using the same method spore germination of all three species is inhibited by up to 6 cm directly below the source of the vapour (Table 3).

When the cultures were exposed to Citri-V™ over 24 h (results not shown) the greatest reduction in mycelial growth compared with untreated samples occurred after 15 min exposure in a 600 L space in vitro with a 67% reduction for *A. niger*, 43.8% for *P. chrysogenum* and 34% for *A. alternata* (Fig. 1).

Assessment of the effect of Citri-V™ exposure against the spores of *P. chrysogenum* in a 600 L space shows that after one hour exposure the resulting spore outgrowth is reduced for *P. chrysogenum* and *A. niger* but not significantly for *A. alternata* (Fig. 2). Fig. 3 illustrates the white colouring of the *P. chrysogenum* cultures after one hour exposure compared to the other time points which are green. The initial stages of *P. chrysogenum* vegetative growth are white and then the conidia turn green over time, thus the white colouring after one hour exposure illustrates the delay in forming conidia produced by the citrus EO vapour treatment. The delay only occurred in 50% of the *A. niger* cultures exposed to the citrus EO vapour therefore, the results were not significant (Fig. 4).

3.2. Growth of *A. alternata* on tomatoes

After 7 days, all wounds of both the control and treated tomatoes were found to be infected regardless of the number of times they had been treated (results not shown) demonstrating that exposure to Citri-V™ is not an effective antifungal treatment against *A. alternata* on tomatoes.

3.3. Grain infectivity with *A. niger* and *P. chrysogenum*

Each region of conidiophore production was measured as the infectivity of the fungus, whilst the vegetative hyphal growth was

Table 3

Mean diameter (cm) in zones of inhibition for spore outgrowth when treated with citrus EO vapour ($\pm$ SD, $n=6$).

Organism	Mean diameter ($\pm$ SD)	
	Untreated	Treated
<i>Penicillium chrysogenum</i>	0*	5.46 $\pm$ 0.05
<i>Aspergillus niger</i>	0*	5.97 $\pm$ 0.1
<i>Alternaria alternate</i>	0*	6.22 $\pm$ 0.07

* No zones measurable – plates covered with fungal growth.

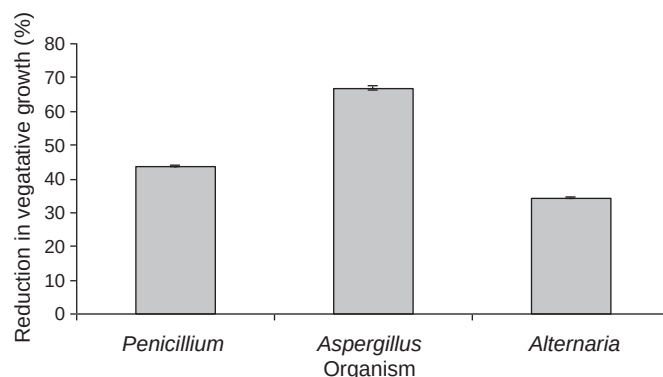


Fig. 1. Percentage inhibition (compared with untreated cultures) of *P. chrysogenum*, *A. niger* and *A. alternata* mycelium growth in the presence of Citri-V™ in a 600 L space for 15 min ($n=6$, $\pm$ SE).

observed. The un-infected sterilised control grain, with or without exposure to Citri-V™, showed no growth. The grain infected with either *A. niger* or *P. chrysogenum* showed 9 cm of infectivity and conidiophore growth in nearly all the non-vapour treated samples. In each case the hyphal growth had reached all parts of the dish.

The EO vapour treated samples showed a visual difference in infectivity and conidiophore growth compared with the untreated samples and there was statistically significant ($p \leq 0.5$) reduction in both *A. niger* (70.8%) and *P. chrysogenum* (57.8%) growth on grain in the EO vapour treated samples (Fig. 5).

4. Discussion

Although there have been many reports of the antibacterial and antifungal effects of essential oils per se (Al-Burtamani et al., 2005; Holley & Patel, 2005) there are fewer on EO vapours and particularly on citrus EO vapours. Vapours are generally more effective than the oils against fungi (Lopez et al., 2005; Tullio et al., 2007). Also, the results of this study demonstrate, yet again, that in vitro studies do not necessarily give an indication of how effective an EO treatment will be in/on food systems as the citrus EO vapour is more effective on agar than it is in food systems as has been previously shown for citrus oils on a range of bacterial species (Fisher & Phillips, 2006) and also for cassia and thyme EOs against *A. alternata* (Feng & Zheng, 2007).

In this study it has been shown that the citrus EO vapour under test (Citri-V™) completely eliminates growth of *A. niger* and *P. chrysogenum* on agar plates which corresponds to the results of previous studies testing *C. sinensis* EO on *A. niger* (Sharma & Tripathi, 2008) and citrange EOs on *Penicillium* spp. (Caccioni, Guizzardi, Biondi, Agatino, & Ruberto, 1998). In a study by Chutia, Deka Bhuyan,

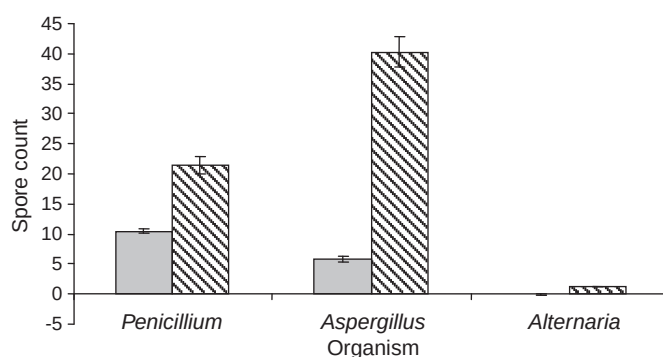


Fig. 2. Spore outgrowth after one hour exposure to Citri-V™ in a 600 L space ($n=6$, $\pm$ SE). Treated (solid bar) and untreated (hatched bar).

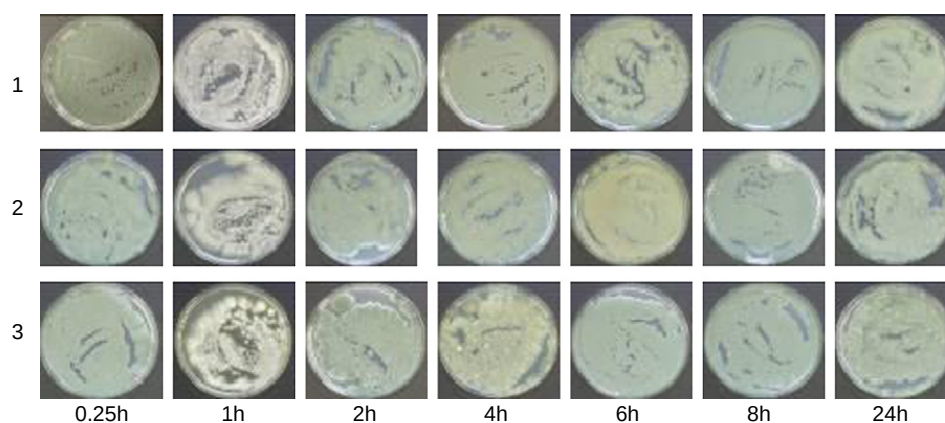


Fig. 3. Inhibition of *P. chrysogenum* conidia formation in the presence of Citri-V™ in a 600 L space.

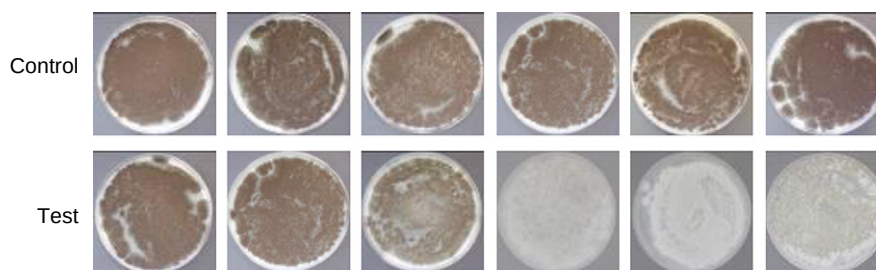


Fig. 4. Inhibition of *A. niger* conidia formation after one hour exposure to Citri-V™.

Pathak, Sarma, and Boruah (2009) *A. alternata* was less susceptible to the effect of *Citrus reticulata* EO than other plant pathogens such as *Fusarium oxysporum* and in this study *A. alternata* was also the most resistant to the activity of the citrus EO vapour compared with either *A. niger* or *P. chrysogenum*. In all these cases the GC–MS analysis of the oils showed a similar composition to the composition of Citri-V™ (Table 1) with limonene being present in the highest quantities and linalool, citral and β -pinene also present and the latter three components having been previously identified as the active antimicrobial compounds (unpublished results).

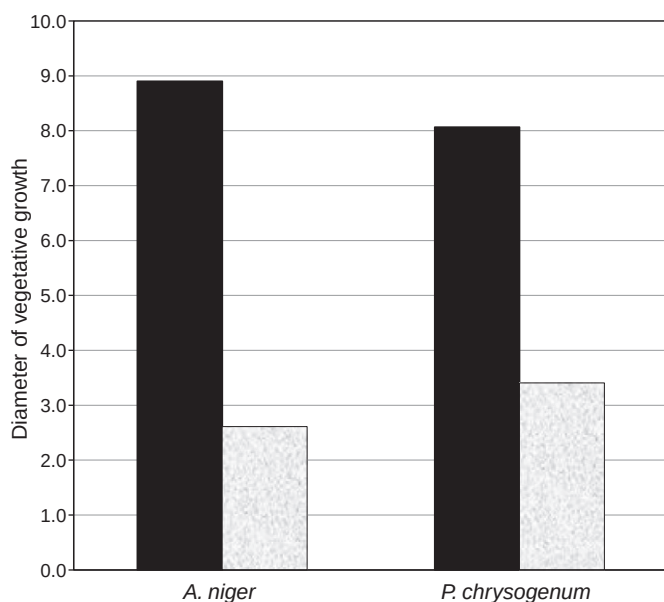


Fig. 5. Reduction in fungal growth on grain with and without EO vapour treatment after 14 days incubation at 25 °C (Control ■ and treated ▨).

Changes in hyphal growth and conidial production similar to those observed in this study have been reported in *A. niger* on exposure to *M. chamomilla* L. flower EO (Tolouee et al., 2010), *C. sinensis* EO (Sharma & Tripathi, 2008) and thyme EO (Rasooli et al., 2006). It is probable that the site of action is at the cell wall as has been shown to be the case in bacteria (Gill & Holley, 2006). Although the delay was not observed in all samples (Figs. 3 and 4) and therefore not significant the fact that some did show delay demonstrates that there is an effect of the citrus EO vapour on production of conidia.

The use of vapours may eliminate to a certain extent the problem of the use of oils, which is that at the concentrations that they are effective, the organoleptic properties of the foodstuff are affected. For example cassia oil reduces the decay of tomatoes very effectively but its effect on taste was not determined (Feng & Zheng, 2007). The citrus EO vapour tested in this study reduced the growth of *A. niger* and *P. chrysogenum* in grain, however in the case of *A. alternata* on tomatoes the samples all decayed at a similar rate whether inoculated or uninoculated or treated which is contrary to that observed for *A. alternata* on tomatoes after treatment with cassia and thyme oils (Feng & Zheng, 2007).

Therefore, in conclusion, a 15 min treatment with the citrus EO vapour used in this study reduces growth of *P. chrysogenum*, *A. niger* and *A. alternata* in culture by 43.8%, 66.9% and 34.4% respectively and spore production is also reduced. Although the EO treatment did not affect the growth of *A. alternata* on tomatoes a 5 × 15 min daily treatment of grain reduces growth of *P. chrysogenum* and *A. niger* on grain.

The fact Citri-V™ has been shown not to affect the organoleptic properties of foodstuffs (Fisher et al., 2009) means that it may be used in industrial situations such as the storage of grain. Although the reduction in *P. chrysogenum* and *A. niger* growth is only approximately 57.8% and 70.8% respectively, Citri-V™, a natural product which is GRAS, could be used combined with other preservative regimes such as chemical fungicides which might mean that the latter could be used at lower concentrations addressing some of the environmental and public perception issues associated with their use.

Acknowledgements

The authors would like to thank East Midlands Development Agency, UK, for funding this project through an Innovation Fellowship grant to CAP and Amphora UK for the supply of essential oils.

References

- Agrios, G. (1997). *Plant pathology* (4th Edition). San Diego Academic Press.
- Al-Burtamani, S. K. S., Fatope, M. O., Marwah, R. G., Onifade, A. K., & Al-Saidi, S. H. (2005). Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman. *Journal of Ethnopharmacology*, 96(1–2), 107–112.
- Boyras, N., & Özcan, M. (2006). Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey. *International Journal of Food Microbiology*, 107(3), 238–242.
- Caccioni, D. R. L., Guizzardi, M., Biondi, D. M., Agatino, R., & Ruberto, G. (1998). Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. *International Journal of Food Microbiology*, 43(1–2), 73–79.
- Chutia, M., Deka Bhuyan, P., Pathak, M. G., Sarma, T. C., & Boruah, P. (2009). Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India. *LWT – Food Science and Technology*, 42(3), 777–780.
- Edwards-Jones, V., Buck, R., Shawcross, S. G., Dawson, M. M., & Dunn, K. (2004). The effect of essential oils on methicillin-resistant *Staphylococcus aureus*. *Burns*, 30, 772–777.
- Feng, W., & Zheng, X. (2007). Essential oils to control *Alternaria alternata* in vitro and in vivo. *Food Control*, 18(9), 1126–1130.
- Fisher, K., & Phillips, C. A. (2006). The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* 0157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. *Journal of Applied Microbiology*, 101, 1232–1240.
- Fisher, K., & Phillips, C. A. (2008). The use of essential oils as anti-microbials: Is citrus the answer? *Trends in Food Science and Technology*, 19, 156–164.
- Fisher, K., & Phillips, C. A. (2009). The mechanism of action of a citrus oil blend against *Enterococcus faecium* and *Enterococcus faecalis*. *Journal of Applied Microbiology*, 106, 1343–1349.
- Fisher, K., Phillips, C. A., & McWatt, L. (2009). The use of an antimicrobial citrus vapour to reduce *Enterococcus* sp. on salad products. *International Journal of Food Science and Technology*, 44, 1748–1754.
- Gill, A. O., & Holley, R. A. (2006). Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics. *International Journal of Food Microbiology*, 108, 1–9.
- Goni, P., Lopez, P., Sanchez, C., Gomez-Lus, R., Becerril, R., & Nerin, C. (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chemistry*, 116, 982–989.
- Holley, R. A., & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology*, 22, 273–292.
- Lopez, P., Sanchez, C., Battle, R., & Nerin, C. (2005). Solid- and vapour-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. *Journal of Agricultural and Food Chemistry*, 53, 6939–6946.
- Magan, N., & Aldred, D. (2007). Post-harvest control strategies: Minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*, 119(1–2), 131–139.
- Rasooli, I., Rezaei, M. B., & Allameh, A. (2006). Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock*. *Food Control*, 17(5), 359–364.
- Reimann, S., & Deising, H. B. (2000). Fungicides: Risk of resistance development and search for new targets. *Archives of Phytopathology and Plant Protection*, 33, 329–349.
- Sharma, N., & Tripathi, A. (2008). Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiological Research*, 163(3), 337–344.
- Spotts, R. A., & Cervantes, N. K. (1986). Populations, pathogenicity and benomyl resistance of *Botrytis* spp., *Penicillium* spp. and *Mucor piriformis* in Paking houses. *Plant Disease*, 70, 106–108.
- Subba, M. S., Soumithri, R., & Suryanarayana, R. (1967). Antimicrobial action of citrus oils. *Journal of Food Science*, 32, 225–227.
- Terzi, V., Morcia, C., Faccioli, P., Valè, G., Tacconi, G., & Malnati, M. (2007). In vitro antifungal activity of the tea tree (*Melaleuca alternifolia*) essential oil and its major components against plant pathogens. *Letters in Applied Microbiology*, 44(6), 613–618.
- Tolouee, M., Alinezhad, S., Saberi, R., Eslamifar, A., Zad, S. J., Jaimand, K., et al. (2010). Effect of *Matricaria chamomilla* L. flower essential oil on the growth and ultrastructure of *Aspergillus niger* van Tieghem. *International Journal of Food Microbiology*, 139, 127–133.
- Trienekens, J., & Zuurbier, P. (2008). Quality and safety standards in the food industry, developments and challenges. *International Journal of Production Economics*, 113, 107–122.
- Tullio, V., Nostro, A., Mandras, N., Dugo, P., Banche, G., Cannatelli, M. A., et al. (2007). Antifungal activity of essential oils against filamentous fungi determined by broth microdilution and vapour contact methods. *Journal of Applied Microbiology*, 102(6), 1544–1550.
- Tzortzakakis, N. G., & Economakis, C. D. (2007). Antifungal activity of lemongrass (*Cymbopogon citratus* L.) essential oil against key postharvest pathogens. *Innovative Food Science & Emerging Technologies*, 8(2), 253–258.