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Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants

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Abstract: Bioassays of *Rosmarinus officinalis* L. essential oil and blends of its major constituents were conducted using host-specific strains of the two-spotted spider mite, *Tetranychus urticae* Koch, on bean and tomato plants. Two constituents tested individually against a bean host strain and five constituents tested individually against a tomato host strain accounted for most of the toxicity of the natural oil. Other constituents were relatively inactive when tested individually. Toxicity of blends of selected constituents indicated a synergistic effect among the active and inactive constituents, with the presence of all constituents necessary to equal the toxicity of the natural oil.

Keywords: plant essential oils; *Rosmarinus officinalis*; *Tetranychus urticae*

1 INTRODUCTION

1.1 Two-spotted spider mite

The two-spotted spider mite, *Tetranychus urticae* Koch, is one of the most important pests of fruit, vegetable and ornamental plants worldwide.[1] The mite has been reported to attack about 1200 species of plants,[2] of which more than 150 are economically important.[3] The economic threat posed by these mites is constantly increasing because of the development of pesticide resistance and the resurgence of mite populations following the use of non-selective synthetic pesticides that eliminate natural enemies such as predaceous mites and spiders.[4] Spider mites have evolved resistance to more than 80 acaricides to date, and resistance has been reported from more than 60 countries.[5] In the USA in 2001, spider mite control programmes cost approximately US$8 million in cotton alone (National Cotton Council of America). Spider mites impose a great expense on greenhouse growers worldwide in terms of damage and control cost and are therefore considered one of the most important pests of greenhouse production.

1.2 *Rosmarinus officinalis* essential oil

Plant essential oils are obtained through steam distillation of herbs and medicinal plants.[6] These oils have been used traditionally as medicines in many countries, and ancient peoples were also aware of their pesticidal properties; however, only in recent years have these oils been commercialised as pest control products.[7] Most of these oils are environmentally non-persistent and non-toxic to humans (with some exceptions),[8–10] while being effective against several pest species.[11–15] Rosemary (*Rosmarinus officinalis* L.) oil has been traditionally used as a medicine for colic, nervous disorders and painful menstruation. Recent studies revealed that rosemary oil is an effective antibacterial agent which can control many food micro-organisms such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli* O157:H7, *Shigella dysenteria, Bacillus cereus* and *Staphylococcus aureus*.[16] It can also inhibit the activity of food spoilage bacteria and yeast strains.[17]

Rosemary oil is relatively effective against insect and mite pests. It has been shown that the aromatic vapour of rosemary oil has ovicidal and larvicidal effects on several stored product pests[18,19] and the two-spotted spider mite[15] as a fumigant. The oil can have sublethal effects as well, for example acting as a repellent to onion thrips, *Thrips tabaci* Lind.[20]

Synthetic acaricides usually contain a single active compound; however, botanical pesticides such as

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plant essential oils are complex mixtures of several constituents. In the present study we characterise the toxicity of rosemary oil and its major constituents as residual acaricides against *T. urticae*.

2 MATERIALS AND METHODS

2.1 *Rosmarinus officinalis* essential oil

Pure *R. officinalis* essential oil (Intarome TO, Lot #0 213 142MB-100%) was obtained from EcoSMART Technologies Inc. (Franklin, TN, USA). Major constituents of the essential oil were identified by gas chromatography/mass spectrometry (GC/MS) using a Varian 3900 system with a Saturn 2100T ion trap mass-selective detector (Varian Inc., Walnut Creek, CA). We used a WCOT fused silica $30\,m \times 0.25\,mm$ i.d. column with a CP-Sil 8 CB low-bleed MS coating (Varian Inc.), a 1 µl injection volume and pure helium as the carrier at $1.0\,ml\,min^{-1}$. The temperature programme used was 80 °C for 0.5 min, an increase of $8.0\,°C\,min^{-1}$ for 8.0 min, followed by an increase of $50\,°C\,min^{-1}$ for 3.2 min. Cinnamic alcohol (Sigma, St Louis, MO, USA) was used as an internal standard.

2.2 Spider mites

Two colonies of *T. urticae* were used in this study. The first colony was collected from the UBC Horticulture greenhouse and reared on 3-week-old green bush bean (*Phaseolus vulgaris* L. cv. Speculator #24A Stokes) plants. The second colony originated from a research colony maintained on tomato plants for more than 5 years without any pesticide exposure at Agriculture and Agri-Food Canada (Agassiz, BC, Canada). These mites were reared on 3-week-old vine tomato (*Lycopersicon esculentum* Mill cv. Clarence) plants provided by Houwelings Nurseries (Delta, BC, Canada).

2.3 General growing conditions for plants and mites

Plants contaminated with mites were kept inside an isolated greenhouse section at $24 \pm 3\,°C$ and 45–60% relative humidity (RH) under natural daylight. Plants were irrigated three times per week, twice with water and once with water-soluble fertiliser (Peters EXCEL 15-5-15 Cal-Mag, The Scotts Co., Marysville, OH). Adult female mites were transferred to clean plants, allowed to oviposit for 48 h and then removed from the plants. Development of these eggs resulted in a cohort of evenly aged mites that were used for all bioassays.

2.4 Calculating LC_{50} values of the oil

A leaf disc painting method was used for calculating the LC_{50} of the rosemary oil. Tests were conducted in disposable plastic petri dishes (3 cm diameter). The bean colony of mites was treated with six nominal concentrations (2.5, 5, 10, 20, 40 and $80\,ml\,litre^{-1}$) of the essential oil in methanol + water (70 + 30 by volume). The tomato colony was treated with the same six concentrations, using water plus a spreader sticker adjuvant (Latron B-1956, $60\,mg\,litre^{-1}$) as the carrier solvent (Rohm and Haas, Philadelphia, PA). Leaf discs (3 cm diameter) were cut from leaves of greenhouse-grown plants using a cork borer. A 20 µl aliquot of each concentration was painted on the underside of the leaf disc with a micropipette. After drying at room temperature for 5 min, each disc was placed in the bottom of a petri dish atop a 3 cm diameter disc of Whatman No 1 filter paper wetted with 50 µl of distilled water. Five adult female spider mites were introduced into each petri dish and the covered dishes were placed in a growth chamber at $26 \pm 2\,°C$ and 55–60% RH with a 16/8 h light/dark photoperiod. Mortality was determined under a dissecting microscope 24 h after treatment. Mites were considered dead if their appendages did not move when prodded with a fine paintbrush. Control mites were held on leaf discs painted with the carrier solvent alone (methanol + water, 70 + 30, or Latron B-1956, $60\,mg\,litre^{-1}$). All treatments were replicated five times.

2.5 Comparative toxicities

Based on the 100% lethal concentration and following the natural composition of the oil indicated by GC/MS (see Table 1), individual constituents were tested at levels equivalent to those found in the LC_{100} of the oil ($20\,ml\,litre^{-1}$ for beans and $40\,ml\,litre^{-1}$ for tomatoes) (see Table 2). Individual constituents (α-pinene 98%, β-pinene 99%, 1,8-cineole 99%, *p*-cymene 99%, α-terpineol 97%, bornyl acetate 97%, borneol 99%, camphor 96%, D-limonene 97% and camphene 95%) were obtained from Sigma-Aldrich (St Louis, MO, USA). In order to identify the contribution of each constituent to the toxicity of the oil, we made a blend of all major constituents as well as blends each lacking one of the ten major constituents (see Fig. 1). We compared the toxicity of the complete and incomplete blends with that of pure rosemary oil. In the next step we made blends of those constituents which contributed to the toxicity of the oil (active constituents) and compared them with those which did not affect the toxicity (inactive constituents) (see Fig. 2). The leaf disc painting method described in Section 2.4 was used for all bioassays.

2.6 Data analysis

Mortality data were analysed using the SPSS program version 11.5 for analysis of variance (ANOVA). Tukey's test was used to compare means. Probit analysis was used to determine LC_{50}, using the EPA probit analysis program version 1.5.

3 RESULTS

3.1 Essential oil constituents

GC/MS analysis indicated that there are ten major constituents in the oil, comprising 92.8% of the total weight. 1,8-Cineole was the most abundant compound

Table 1. Major constituents of *Rosmarinus officinalis* essential oil and their relative proportions in the pure oil

Constituent	% v/v
Camphene	8.0
1,8-Cineole	31.5
β-Pinene	6.8
Camphor	20.0
p-Cymene	0.9
Borneol	1.2
D-Limonene	3.7
α-Terpineol	1.1
Bornyl acetate	2.2
α-Pinene	17.5
Other compounds	7.2

(31.5%), followed by camphor (20.0%) and α-pinene (17.5%) (Table 1). There are more than 40 other compounds in the rosemary oil, mostly monoterpenes, but their concentration in the oil is very low.[21]

3.2 LC_{50} of the oil

The LC_{50} of rosemary oil was 10.0 ml litre^{-1} (95% confidence interval (CI) = 6.95–13.11) for adult female spider mites reared on bean plants and 13.0 ml litre^{-1} (95% CI = 10.05–17.78) for those reared on tomato plants. Complete mortality (100%) of mites was obtained with a 20 ml litre^{-1} concentration of the oil on bean plants and 40 ml litre^{-1} on tomato plants. No mortality was observed in the controls.

3.3 Comparative toxicities of individual constituents and blends thereof

For the bean host strain of mites, bioassays of single constituents revealed that two (1,8-cineole and α-pinene) were significantly toxic at the tested concentration ($P < 0.05$), one (β-pinene) was slightly but not significantly toxic and the remaining seven (*p*-cymene, borneol, bornyl acetate, camphor, D-limonene, camphene and α-terpineol) were non-toxic to mites (Table 2).

For the tomato host strain of mites, bioassays indicated that three constituents (camphene, camphor and *p*-cymene) were non-toxic to mites, five (bornyl acetate, β-pinene, D-limonene, borneol and α-terpineol) were moderately toxic and two (α-pinene and 1,8-cineole) were highly toxic (Table 2).

Bioassays with artificial mixtures showed that the greatest mortality was obtained when all ten constituents were present (full mixture). The mortality caused by the artificial mixture of all ten constituents did not differ significantly from that caused by pure rosemary oil for either strain of mites ($P > 0.05$; Fig. 1).

In the bean host strain, component elimination assays indicated that the absence of 1,8-cineole or α-pinene caused the largest decrease in toxicity of the blend. Removal of *p*-cymene, α-terpineol or bornyl acetate also had a significant effect ($P < 0.05$) on the toxicity of the blend, but less so than for 1,8-cineole or α-pinene. Excluding the five remaining constituents (camphor, camphene, borneol, D-limonene and β-pinene) from the mixtures did not significantly affect the toxicity of the blends. In the tomato host strain, α-pinene, 1,8-cineole, α-terpineol and bornyl acetate were found to contribute to the toxicity of the oil, whereas β-pinene, *p*-cymene and borneol had only a moderate influence on toxicity. Camphor, camphene and D-limonene were found to be inactive when tested individually, and their absence did not have any effect on the toxicity of the mixture (Fig. 1). Our comparison between the toxicity of a mixture of effective constituents and that of a mixture of non-effective constituents showed that the effective constituents alone are not as toxic as the full mixture of all constituents (both actives and inactives). The inactive constituent blend did not cause any mortality in either strain, but, when added to the active constituent blend, toxicity became equivalent to the natural oil (Fig. 2).

Table 2. Mortality caused by individual constituents of rosemary oil to *Tetranychus urticae* when applied at levels equivalent to those found in the 100% lethal concentration of the pure oil (LC_{100} = 20 ml litre^{-1} for *T. urticae* on beans and 40 ml litre^{-1} on tomato)[a]

Bean		Tomato	
Constituent	Mortality (%)	Constituent	Mortality (%)
Camphene	4 (±4) c	Camphene	0 c
1,8-Cineole	88 (±4.8) a	1,8-Cineole	80 (±6.2) a
β-Pinene	16 (±7.4) bc	β-Pinene	32 (±4.8) b
Camphor	4 (±4) c	Camphor	4 (±4) c
p-Cymene	0 c	*p*-Cymene	4 (±4) c
Borneol	0 c	Borneol	36 (±7.4) b
D-Limonene	8 (±4.8) c	D-Limonene	24 (±4) bc
α-Terpineol	4 (±4) c	α-Terpineol	12 (±8) bc
Bornyl acetate	0 c	Bornyl acetate	40 (±8.8) b
α-Pinene	32 (±4.8) b	α-Pinene	72 (±4.8) a

[a] Values are mean (±SE) of $n = 5$ replicates with five adult female mites per replicate. Means in each column followed by the same letter are not significantly different (Tukey test, $P < 0.05$).

4 DISCUSSION

4.1 Rosemary oil as an acaricide

Our results clearly indicate that rosemary oil can be considered an acaricide against the two-spotted spider mite, causing complete mortality in the laboratory at concentrations that cause no phytotoxicity to host plants (unpublished data). Rosemary oil and most other plant essential oils are environmentally non-persistent, as they readily volatilise from plants and other surfaces. Some essential oils are not toxic to non-target organisms and can be used in conjunction with biological control. Furthermore, most plant essential oils, including rosemary oil, are safe for humans and other mammals, and many are used as flavourings in foods, beverages and medicines. In some rare cases,

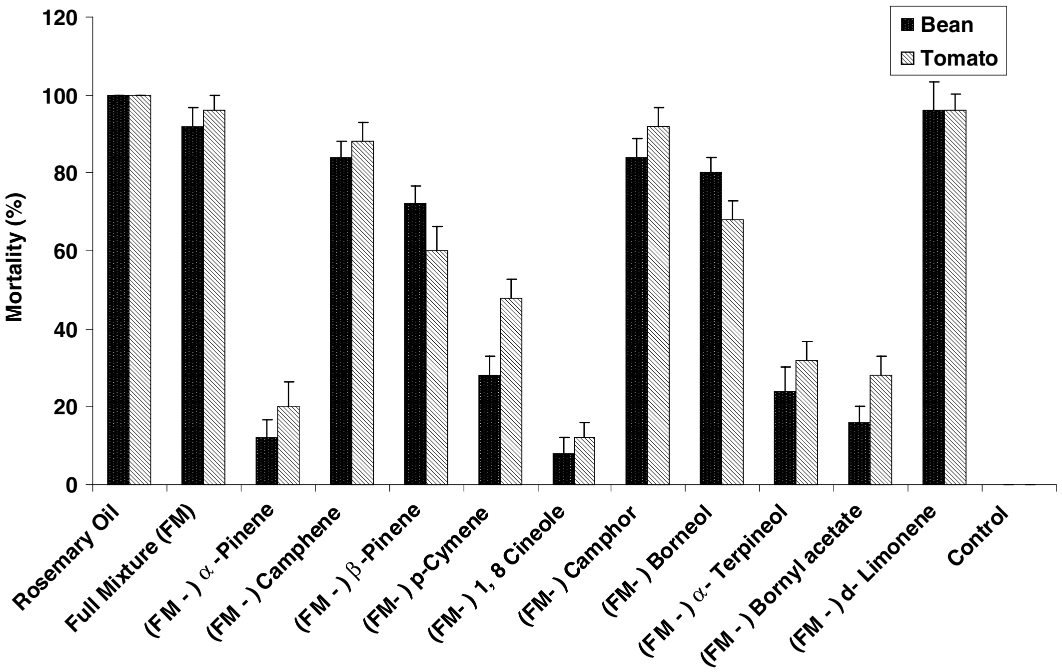

Figure 1. Mortality caused by selected blends of constituents of rosemary oil to *Tetranychus urticae* when applied at levels equivalent to those found in the 100% lethal concentration of the pure oil (LC_{100} = 20 ml litre^{-1} for *T. urticae* on beans and 40 ml litre^{-1} on tomato). Error bars represent the standard error of the mean of five replicates. (FM-) indicates a blend of ten constituents minus the compound noted.

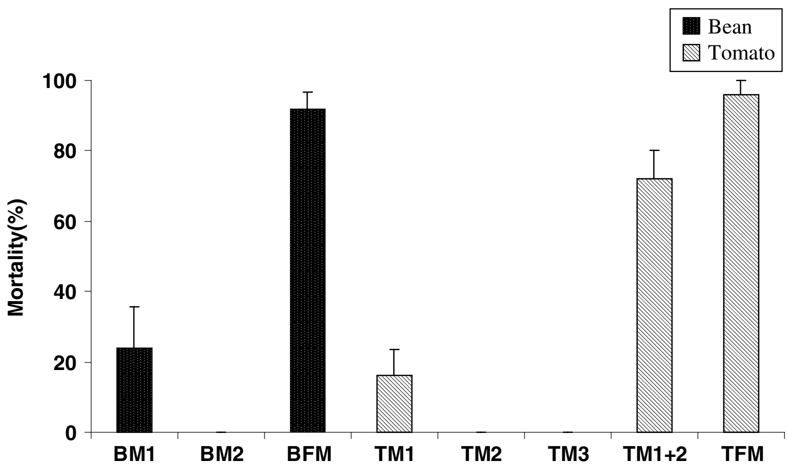

Figure 2. Mortality caused by selected blends of active and inactive constituents of rosemary oil to *Tetranychus urticae* when applied at levels equivalent to those found in the 100% lethal concentration of the pure oil (LC_{100} = 20 ml litre^{-1} for *T. urticae* on beans and 40 ml litre^{-1} on tomato). Error bars represent the standard error of the mean of five replicates. **BM1** ('actives') = α-pinene + 1, 8-cineole + α-terpineol + bornyl acetate + *p*-cymene; **BM2** ('inactives') = β-pinene + borneol + camphor + camphene + D-limonene; **BFM** = full mixture of all constituents; **TM1** ('very active') = α-pinene + 1, 8-cineole + α-terpineol + bornyl acetate; **TM2** ('moderately active') = β-pinene + *p*-cymene + borneol; **TM3** ('inactive') = camphor + camphene + D-limonene; **TM1 + 2** = TM1 + TM2; **TFM** = full mixture of all constituents.

chronic exposure to rosemary oil in high concentration has caused contact dermatitis,[8–10] but acute toxicity of rosemary oil to humans or other mammals has not been reported.

Like other essential oils, natural rosemary oil is a complex mixture of terpenoids. Considering that target site resistance is an important problem for mite control, it is more probable that mites will evolve resistance faster to an acaricide based on a single active ingredient than to one based on a mixture of different active compounds. It has been reported that green peach aphids (*Myzus persicae* Sulzer) developed resistance to pure azadirachtin (the major ingredient of neem insecticide), but not to a refined neem seed extract containing the same absolute amount of azadirachtin but with many other constituents present.[22]

Little is known about the exact site of action of rosemary oil and other plant essential oils in two-spotted spider mites. The octopaminergic nervous system is considered to be the site of action of essential oils in the American cockroach,[23] but this may not be the case for the two-spotted spider mite, and there is the possibility that the essential oils have more than one site of action, since they are complex mixtures. Further studies need to be done to find the exact mechanism(s) of action of the essential oils on spider mites.

4.2 Synergy among constituents

We observed that individual constituents differ in their toxicity to the two host strains of mites, and it seems that they are more toxic to mites that feed on tomato than to those that feed on bean plants. We found that some constituents (i.e. borneol and bornyl acetate) that were not toxic to mites feeding on beans were relatively toxic to mites feeding on tomatoes. Similar results were reported when major constituents of two other essential oils were used alone against two postharvest insect pests.[24] To corroborate the role of individual constituents in the toxicity of rosemary oil to spider mites, we eliminated each individual constituent from a synthetic mixture that simulated natural rosemary oil. We found that the absence of some constituents (1,8-cineol or α-pinene) from the artificial mixture caused a significant decrease in toxicity (84 and 80% respectively), which tempted us to conclude that these constituents are the major contributors to the oil's toxicity. However, when we mixed these active constituents together, we found that their toxicity level was not as high as expected. The toxicity of our artificial mixtures only reached the level of the natural rosemary oil when we mixed the blends of active constituents with inactive ones. This indicates that the 'inactive' constituents have some synergistic effect on active constituents, and, although not active individually, their presence is necessary to achieve full toxicity. Active constituents, on the other hand, might have an antagonistic effect on each other, since their toxicity level is significantly greater when tested individually than in a mixture with other active constituents. The highest mortality rates were obtained in both strains when all the constituents were present in the mixture (96% in tomato mites and 92% in bean mites).

Knowing the role of each constituent in the toxicity of the oil gives us the ability to screen different rosemary oils and choose the most effective one for pest control purposes. It might also be possible to artificially create a blend of different constituents based on their activity and their effect on the pest.

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REFERENCES

1 Johnson WT and Lyon HH, *Insects That Feed on Trees and Shrubs* (2nd edn), Comstock Publishing/Cornell University Press, Ithaca, NY, pp. 468–470 (1991).
2 Zhang Z, *Mites of Greenhouses: Identification, Biology and Control*, CABI Publishing, Wallingford, pp. 54–61 (2003).
3 Jeppson LR, Keifer HH and Baker TW, *Mites Injurious to Economic Plants*, University of California Press, Berkeley, CA, pp. 370–376 (1975).
4 Cranham JE and Helle W (editors), Pesticide resistance in Tetranychidae, in *World Crop Pests—Spider Mites: Their Natural Enemies and Control*, Elsevier, Amsterdam, pp. 405–421 (1985).
5 *The Database of Arthropods Resistance to Pesticides* [Online]. Michigan State University, Center for Integrated Plant Systems. Available: http://www.pesticideresistance.org/DB/index.html [28 April 2005].
6 Yatagai M, Miticidal activities of tree terpenes. *Curr Topics Phytochem* **1**:87–97 (1997).
7 Isman MB, Plant essential oils for pest and disease management. *Crop Protect* **19**:603–608 (2000).
8 Roe FJC, Chronic toxicity of essential oils and certain other products of natural origin. *Food Cosmet Toxicol* **33**:311–324 (1965).
9 Cockayne SE and Gawkrodger DJ, Occupational contact dermatitis in an aromatherapist. *Contact Dermatitis* **37**:306–309 (1997).
10 Hjorther AB, Christophersen C, Hausen BM and Menne T, Occupational allergic contact dermatitis from carnosol, a naturally-occurring compound present in rosemary. *Contact Dermatitis* **37**:99–100 (1997).
11 Hummelbrunner LA and Isman MB, Acute, sublethal, antifeedant, and synergistic effects of monoterpinoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep. Noctuidae). *J Agric Food Chem* **49**:715–720 (2001).
12 Obeng-Ofori D, Reichmuth CH, Bekele J and Hassanali A, Biological activity of 1,8-cineole, a major component of essential oil of *Ocimum kenyense* (Ayobangira) against stored product beetles. *J Appl Entomol* **121**:237–243 (1997).
13 Kim EH, Kim HK and Ahn YJ, Acaricidal activity of clove bud oil compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). *J Agric Food Chem* **51**:885–889 (2003).
14 Calderone NW and Spivak M, Plant extracts for control of the parasitic mite *Varroa jacobsoni* (Acari: Varroidae) in colonies of the western honey bee (Hymenoptera: Apidae). *Apicult Social Insects* **88**:1211–1215 (1995).
15 Choi WI, Lee SG, Park HM and Ahn YJ, Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). *J Econ Entomol* **97**:553–558 (2004).
16 Burt S, Essential oils: their antibacterial properties and potential application in food—a review. *Int J Food Microbiol* **94**:223–253 (2004).
17 Managena T and Muyima NYO, Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strain. *Lett Appl Microbiol* **28**:291–296 (1999).
18 Papachristos DP and Stampoulos DC, Fumigant toxicity of three essential oils on the eggs of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). *J Stored Prod Res* **40**:517–525 (2004).
19 Tunc I, Berger BM, Erler F and Dagli F, Ovicidal activity of essential oils from plants against two stored-product insects. *J Stored Prod Res* **36**:161–168 (2000).
20 Koschier EA and Sedy KA, Labiate essential oils affecting host plant selection and acceptance of *Thrips tabaci* Lindeman. *Crop Protect* **22**:929–939 (2003).
21 Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, Chessa M, Cerri R and Casanova J, Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oil from Sardinia and Corsica. *Flav Fragr J* **17**:15–19 (2002).

22 Feng R and Isman MB, Selection for resistance to azadirachtin in the green peach aphid, *Myzus persicae*. *Experientia* **51**:831–834 (1995).

23 Enan E, Insecticidal activity of essential oils: octopaminergic site of action. *Comp Biochem Physiol* **130C**:325–327 (2001).

24 Bekele J and Hassanali A, Blend effects in the toxicity of the essential oil constituents of *Ocimum kilimandascharicum* and *Ocimum kenyense* (Labiatae) on two post- harvest insect pests. *Phytochemistry* **57**:385–391 (2001).