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ORIGINAL RESEARCH ARTICLE



# Acute toxicity of essential oils and other natural compounds to the parasitic mite, *Varroa destructor*, and to larval and adult worker honey bees (*Apis mellifera* L.)

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## Summary

Essential oils and other natural compounds were evaluated for their toxicity to the parasitic mite *Varroa destructor* and to larval and adult worker honey bees (*Apis mellifera* L.). A glass-vial residual bioassay was used to fast-screen the relative toxicity of 22 natural products to *V. destructor*. Results showed that menthol, clove oil, organum oil, and thymol were the most toxic products, causing 87, 96, 100, and 100% mite mortality, respectively, at a dose of 0.75 mg / vial. The acute toxicity of these four products to the mites was further evaluated by estimating LC<sub>50</sub> values. Thymol and organum oil had the lowest LC<sub>50</sub> (56.1 µg / vial in both cases). In a subsequent experiment, topical applications of eight dilutions of the above four selected products were carried out on adult worker bees and larvae, using a newly-developed larval assay, to estimate their LC<sub>50</sub>s. Thymol had the lowest LC<sub>50</sub> for adult bees (210.3 µg / bee) and menthol had the highest (523.5 µg / bee). Furthermore, thymol was the most toxic product to larvae, with a LC<sub>50</sub> of 150.7 µg / larva, while menthol was the least toxic with a LC<sub>50</sub> of 382.8 µg / larva. Tau-fluvalinate used as a control was significantly less toxic to mites and equally or more toxic to workers and larvae than the above four natural compounds. Results of this study suggest that besides thymol, which is already a registered varroacide, organum oil, clove oil, and menthol, are potential candidate products to be further tested for the control of *V. destructor*.

## Toxicidad aguda a aceites esenciales y otros compuestos naturales del ácaro parásito *Varroa destructor* y de larvas y abejas obreras de abejas (*Apis mellifera* L.)

### Resumen

Se evaluó la toxicidad a aceites esenciales y compuestos naturales del ácaro parásito *Varroa destructor* y de larvas y abejas obreras de abejas (*Apis mellifera* L.). Se usó un ensayo con un vial de vidrio residual para revisar de forma rápida la toxicidad relativa a 22 productos naturales de *V. destructor*. Los resultados mostraron que el mentol, los aceites de clavo y orégano y el timol son los productos más tóxicos, causando una mortalidad de ácaros del 87, 96, 100 y 100% respectivamente en una dosis de 0,75 mg / vial. La toxicidad más aguda de estos cuatro productos para los ácaros se evaluó más tarde estimando los valores LC<sub>50</sub>. Los aceites de timol y orégano tuvieron los valores LC<sub>50</sub> más bajos (56,1 µg / vial en ambos casos). En otro experimento, se aplicaron tópicamente ocho diluciones de los cuatro productos seleccionados en larvas y abejas obreras adultas, usando un ensayo novedoso sobre larvas para estimar sus LC<sub>50</sub>s. El timol mostró el valor LC<sub>50</sub> más bajo en abejas adultas (210,3 µg / abeja) y el mentol el más alto (523,5 µg / abeja). Es más, el timol fue el producto más tóxico para las larvas con un LC<sub>50</sub> de 150,7 µg / larva, mientras que el mentol fue el menos tóxico con un LC<sub>50</sub> de 382,8 µg / larva. El fluvalinato-tau usado como control fue significativamente menos tóxico a los ácaros, e igual o más tóxico a las obreras y las larvas que los otros cuatro compuestos naturales.

Los resultados de este estudio sugieren que aparte del timol, que ya es un conocido varroacida, los aceites de orégano y clavo y el mentol son productos candidatos potenciales para ser testados para el control de *V. destructor*.

**Keywords:** *Varroa destructor*, *Apis mellifera*, essential oils, toxicity, mite control.

## Introduction

The mite *Varroa destructor* is the most widespread and damaging parasite of the honey bee, *Apis mellifera* L. (Ellis and Munn, 2005). Current methods used to control the mite primarily rely upon the application of synthetic acaricides to honey bee colonies. Two of the most commonly used synthetic acaricides to control *V. destructor* worldwide are the pyrethroid fluvalinate (Apistan®; Wellmark International) and the organophosphate coumaphos (Checkmite®; Bayer). Although initially highly effective, the frequent use of these pesticides has led to the development of mite resistance to their active ingredients within a few years (Milani, 1999). Mite resistance to fluvalinate has been well documented (Lodesani *et al.*, 1995; Elzen *et al.*, 1998; Thompson *et al.*, 2002). Resistance to coumaphos has also been reported (Spreafico *et al.*, 2001; Elzen and Westervelt, 2002; Pettis, 2004). Moreover, the use of synthetic acaricides inside bee hives involves a risk of contamination of honey and other hive products (Ruijter, 1995; Wallner, 1995). These disadvantages of synthetic acaricides have provided a considerable incentive to develop new medicaments for mite control that minimize the potential for acaricide resistance and the accumulation of chemical residues in bee products.

Ideal formulations for the control of *V. destructor* would kill mites at doses that are relatively harmless to bees and leave little or no residue in honey and wax. In the search for naturally occurring and more environmentally friendly acaricides, organic products such as plant extracts, organic acids, and essential oils (EOs), have been investigated as possible control agents for *V. destructor*. EOs have been shown to have antimicrobial, insecticidal and acaricidal effects on various organisms (Deans *et al.*, 1992; Imdorf *et al.*, 1999; Isman, 2000). Much effort has, therefore, been focused on these plant-derived materials as potential sources of pest control agents, particularly against *V. destructor*.

Numerous studies have been conducted to test the varroacidal effect of organic products, including EOs, but very few have reported their toxicity to adult honey bees, and none so far has reported their toxicity to bee larvae. For example, Colin *et al.* (1994) sprayed water emulsions of thyme oil in petri dishes containing mites and observed >90% mite mortality after 24 h. Sammataro *et al.* (1998) reported that EOs of thyme, organum, clove, bay, and tea tree, caused significant mite mortality under laboratory conditions. Lindberg *et al.* (2000) found that thymol, clove oil and methyl salicylate were amongst the most acaricidal of the 22 products they tested. Ali *et al.* (2002) tested seventeen monoterpenoids in laboratory tests for

controlling *V. destructor* and found that some acetates provided the best control. Ariana *et al.* (2002) showed that thyme, savory and spearmint oils cause mite mortality without apparent harmful effects to adult bees, and Fassbinder *et al.* (2002) found that perillyl acetate provided good control of mites in laboratory bioassays apparently without injuring bees. In a more recent study it was found that menthol fed to bees in sugar syrup resulted in bee mortality levels no different from controls only fed syrup (Ebert *et al.*, 2007).

The selectivity of EOs and other natural compounds among invertebrates and particularly between honey bees and *V. destructor* is not well documented, and honey bees appear to be susceptible to certain EOs in some studies (e.g. Lindberg *et al.*, 2000; Ruffinengo *et al.*, 2007), but not in others (e.g. Kevan *et al.*, 1999; Ariana *et al.*, 2002). These differences may reflect differences in components of the EOs tested and / or differences in experimental design. Additionally, no studies with these products have tested their toxicity to bee larvae, so more research in this area is needed.

In our laboratory study, 22 natural products, mainly EOs or EO components, were screened for their varroacidal effect. These products were chosen because most showed acaricidal effects in previous studies (Colin *et al.*, 1994; Sammataro *et al.*, 1998; Lindberg *et al.*, 2000; Ali *et al.*, 2002; Ariana *et al.*, 2002; Fassbinder *et al.*, 2002). The most promising were further tested to estimate their lethal concentration (LC<sub>50</sub>s) on *V. destructor*, as well as to larvae and adult bees of *A. mellifera*.

## Materials and methods

### Screening natural compounds for their toxicity to *V. destructor*

A glass-vial residual bioassay that has been extensively used to detect pesticide toxicity and resistance in arthropods (Plapp and Vinson, 1977; Snodgrass, 1996; Elzen *et al.*, 1998; Aliano *et al.*, 2006) was utilized to evaluate the acute toxicity of 22 potential natural varroacides, most of them EOs or EO components. The products tested were: rosemary (*Rosmarinus officinalis*); camphor; tea tree oil (*Melaleuca alternifolia*); lavender (*Lavandula angustifolia*); marjoram (*Origanum majorana*); cineole (*Eucalyptus globulus*); soya oil (*Glycine max*); organum oil (*Origanum vulgare*); methyl salicylate; myrtenyl acetate; spearmint (*Mentha spicata*); carvacrol; potassium diformate (Formi®); wintergreen (*Gaultheria humifusa*); clove oil (*Syzygium aromaticum*); methyl acetate; linalyl acetate; benzyl acetate; citric acid; lactic acid; thymol; and menthol. Tau-fluvalinate was used as a

control. Thymol was obtained from Fisher Scientific Ltd. (Ottawa, Ontario, Canada), whereas the other products were obtained from Sigma-Aldrich Corporation (St. Louis, Missouri, USA). All the products were technical grade (>98% purity).

Each of the 22 products plus tau-fluvalinate was diluted in HPLC-grade acetone (1.5 mg / ml) for a fast screening of their acaricidal potential using the laboratory bioassay. One-half ml of the solution was pipetted into individual 20-ml glass scintillation vials (Fisher Scientific Ltd.; Ottawa, Ontario, Canada) for each product, including tau-fluvalinate and acetone as positive and negative controls, respectively. The vials were rolled on their sides to evaporate the acetone, and to coat their walls with the solution. The vials were closed after the acetone had evaporated (5-10 min). It is unlikely that any significant amount of the products tested had evaporated within this time period, because of their high boiling points, all of which exceed 200°C (CRC, 2007).

Three colonies from the Honey Bee Research Centre at the University of Guelph, Ontario, Canada, that were heavily infested with mites and that had not been treated with acaricides for at least a year, were used as mite sources. Additionally, Pettis tests (Pettis *et al.*, 1998b; BC Ministry of Agriculture and Lands, 2009) were conducted on samples obtained from these colonies, and no apparent mite resistance to tau-fluvalinate was detected. Five live adult female mites, obtained from larvae and non-pigmented pupae of the source colonies were gently transferred to the interior of each vial with a small paint brush, after which the cap was screwed on tightly and the vials were placed in a dark room at 26°C and 65% R.H. Each vial with 0.75 mg of product and containing five mites constituted a replicate and there were 14 replicates for each product tested.

The relative acute toxicity of each product was determined by recording mite mortality 4 h post-exposure to each treatment. This time was selected because it was thought that a fast assessment of the products' effects would be a reliable way of determining their acute toxicity without confounded effects on mite mortality due to other causes. Mites are sensitive to artificial environments when kept for more than 4 h out of their natural habitat and may suffer from starvation and water loss (Milani, 2001). A considerable proportion of control, untreated mites can die in less than 6 h (Sammataro *et al.*, 1998). The parasites were transferred to a petri dish and were examined under a stereoscopic microscope. Mites that did not move when probed with a fine paintbrush were considered to be dead.

The percentage mite mortality caused by the products tested was calculated, arcsine square root transformed, and subjected to analysis of variance using a completely randomized design. Significant differences among means were separated with Fisher's protected LSD tests ( $P < 0.05$ ). The four products that caused the greatest mite mortality were chosen for further experiments.

### LC<sub>50</sub>s of selected EO products to mites

Based on the above results and on preliminary testing, seven dilutions (15.0, 1.5, 1.0, 0.5, 0.3, 0.15, and 0.015 mg / ml) for each of the four products that showed the greatest acaricidal effect in the screening tests (thymol, origanum oil, clove oil and menthol) were prepared as described above for additional acute toxicity bioassays to estimate their LC<sub>50</sub>s. One half ml of each solution was pipetted into individual 20 ml glass scintillation vials for each treatment, including tau-fluvalinate and acetone as controls. These concentrations corresponded to 7500, 750, 500, 250, 150, 75, and 7.5 µg of product per vial. All vials were treated as described above, and a total of 70 mites (5 mites per replicate x 14 replicates) were tested for each of the seven concentrations for all treatments. LC<sub>50</sub> estimates and inverse 95% confidence limits (CL) for the four selected EO products were determined by Probit analysis, using the US Environmental Protection Agency Statistical Program (Version 1.5), which adjusts for control mortality (USEPA, 1992).

### LC<sub>50</sub>s of selected EO products to honey bee workers

A topical bioassay similar to that used by Edwards *et al.* (2003) was utilized to measure the acute toxicity of the four selected EO products and that of tau-fluvalinate on adult honey bees. On the basis of preliminary tests, eight concentrations (150.0, 100.0, 50.0, 25.0, 15.0, 10.0, 0.5, and 0.1 mg / ml) of thymol, origanum oil, clove oil, menthol, and tau-fluvalinate were prepared by dissolving them in HPLC grade acetone as described above, and were applied to worker bees. Each group of acaricide treated bees was paired with another group of bees treated with acetone as a control.

Worker bees were collected directly from lateral combs of three honey bee colonies headed by queens of Carniolan descent, using a 500-ml glass jar. Workers were transported to the laboratory where they were anesthetized with CO<sub>2</sub> to immobilize them temporarily. Immobilized bees were randomly assigned to the various concentration and control treatments for each product. Each treatment consisted of a group of 25 bees / concentration / product and the experiment was repeated on three occasions. The test sub-stances and the solvent used were administered individually as a single topical dose of 5 µl to the dorsal surface of each worker's thorax using a micropipette (Bio-Rad Laboratories Ltd.; Mississauga, Ontario, Canada). The actual doses of products received by the bees corresponded to 750, 500, 250, 125, 75, 50, 2.5, and 0.5 µg per bee. After receiving the topical treatment, each group of 25 bees was placed and maintained in a wooden cage (12.7 x 8.5 x 14.5 cm) with a 3 mesh / cm, wire screened wall. The cages were provided with two gravity feeders, one containing 20 ml of a 50% (w:v) sugar / distilled water solution, and the other contained distilled water, and bees were permitted to feed *ad libitum*. Cages containing the experimental bees were kept in an incubator (32 ± 2°C, 50 ± 10% RH). The number of dead bees was recorded 4 h after

treatment and  $LC_{50}$  estimates were calculated as above. Bees were observed for mortality 4 h after treatment to standardize their mortality assessment with the one used for mites. It has also been shown that this short period of time is sufficient to measure acute toxicity of various pesticides on honey bees (Edwards *et al.*, 2003).

### $LC_{50}$ s of selected EO products to honey bee larvae

A topical bioassay adapted from that used by Pettis *et al.* (1998a) was utilized to document the acute toxicity of the four selected EO products and tau-fluvalinate to honey bee larvae. Serial dilutions of these five products in acetone were prepared as described above, using the same doses as for adult bees. The same three colonies used as the source of workers were also used for the experiments with larvae. These colonies did not have any visible signs of brood disorders and had been given a preventative treatment of oxytetracycline against foulbrood diseases. Combs containing larvae from the selected colonies were taken to the laboratory, where six groups of 25 4<sup>th</sup> to 5<sup>th</sup> instar larvae were chosen to receive the treatments. The outer rim of the hexagonal cells containing the test larvae were coloured with water based, non-toxic, paint markers (L551P2, Hunt Int.; Mississauga Ontario, Canada.) of different colours to identify the different treatment groups.

Groups of larvae were randomly assigned to the various product concentrations and control treatments. Each treatment consisted of a group of larvae per concentration and per product and the experiment was replicated on three occasions. Treatments were applied by a single 5  $\mu$ l injection of test dilutions at the bottom of each test cell, on which

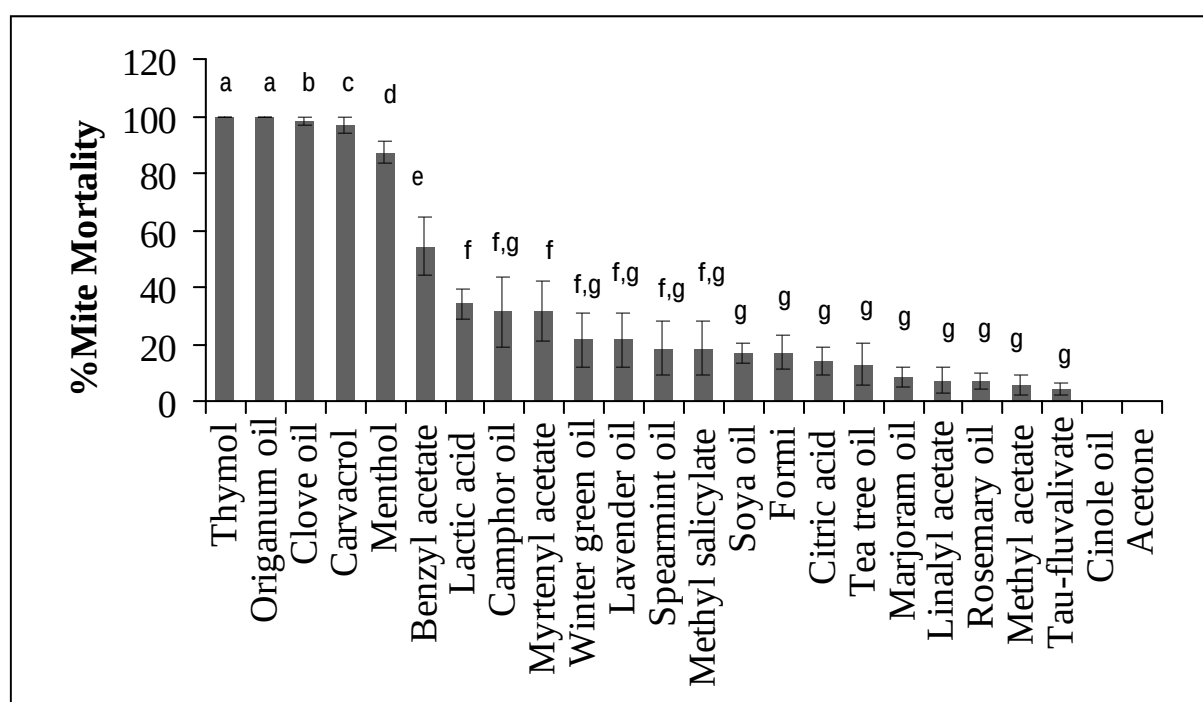
the larvae were laying. Two concurrent controls were used during the tests. Untreated brood served as a negative control, whilst another group of larvae received a solvent (acetone) application. Solvent treated brood received a volume of solvent equal to that administered to the test bees. Control and test larvae were returned to the colonies from which they had been taken.

Combs containing the experimental and control brood were examined for larvae survival 4 h post treatment. Presence of normal (i.e. pearly white) larvae was taken as evidence of survival. Larval mortality was inferred from empty cells or larvae with slight colour changes (i.e., brownish). Empty cells were probably the result of the hygienic behaviour of house-cleaning bees.  $LC_{50}$  estimates were calculated as above.

## Results

### Toxicity of natural products to *V. destructor*

Results of the initial screening of organic products for their varroacidal effect in the laboratory are presented in Fig. 1. All products caused mite mortality except for cineole. There were significant ( $F = 33.9$ ,  $df = 23, 312$ ,  $P < 0.0001$ ) differences in mite mortality rates for the different products tested at a dose of 0.75 mg / vial. Menthol, clove oil, origanum oil, and thymol were the most toxic products, causing 87, 96, 100, and 100% mite mortality, respectively, whilst tau-fluvalinate killed only an average of 4.3% of the mites. No mite mortality was detected in the control vials (acetone). On the basis of these results, thymol, origanum oil, clove oil, and menthol were



**Fig. 1.** Mean percent mortality ( $\pm$  S.E.) of *V. destructor* after 4 h of exposure to 0.75 mg of each of 22 natural compounds, tau-fluvalinate and acetone (control) under laboratory conditions ( $n = 70$  mites per treatment). Different letters above the bars indicate significant differences based on ANOVA and Fisher's protected LSD tests performed on arcsine square root transformed data. Actual non-transformed values are presented.

**Table 1.** Lethal concentration (LC<sub>50</sub>) in µg / vial, µg / bee, or µg / larva and 95% confidence limits (CL), estimated for *V. destructor* and its host *A. mellifera* after 4 h of exposure to eight concentrations of four essential oil products and tau-fluvalinate.

| Products        | Organism            | N   | LC <sub>50</sub> | 95 % CL       | Slope ± SE  | Intercept |
|-----------------|---------------------|-----|------------------|---------------|-------------|-----------|
| Thymol          | Mites <sup>1</sup>  | 560 | 56.1             | 10.1 – 161.1  | 2.18 ± 0.79 | 1.19      |
|                 | Bees <sup>2</sup>   | 675 | 210.3            | 175.7 – 250.1 | 2.01 ± 0.19 | 0.32      |
|                 | Larvae <sup>2</sup> | 675 | 150.7            | 132.8 – 169.6 | 3.18 ± 0.28 | -1.94     |
| Origanum oil    | Mites               | 560 | 56.1             | 10.1 – 161.1  | 2.18 ± 0.79 | 1.19      |
|                 | Bees                | 675 | 331.3            | 270.6 – 411.9 | 1.76 ± 0.19 | 0.56      |
|                 | Larvae              | 675 | 236.4            | 205.6 – 270.3 | 2.74 ± 0.25 | -1.50     |
| Clove oil       | Mites               | 560 | 90.9             | 83.3 – 98.8   | 7.33 ± 0.93 | -9.36     |
|                 | Bees                | 675 | 238.6            | 166.2 – 351.3 | 1.69 ± 0.25 | 0.98      |
|                 | Larvae              | 675 | 281.4            | 247.1 – 321.1 | 2.68 ± 0.23 | -1.57     |
| Menthol         | Mites               | 560 | 173.2            | 152.0 – 196.4 | 3.14 ± 0.33 | -2.04     |
|                 | Bees                | 675 | 523.5            | 357.0 – 948.5 | 2.27 ± 0.52 | -1.17     |
|                 | Larvae              | 675 | 382.8            | 325.6 – 447.9 | 2.53 ± 0.31 | -1.53     |
| Tau-fluvalinate | Mites               | 560 | 696.1            | 375.2 – 784.1 | 1.12 ± 0.17 | 1.82      |
|                 | Bees                | 675 | 194.1            | 161.2 – 257.8 | 1.20 ± 0.30 | 2.26      |
|                 | Larvae              | 675 | 192.3            | 160.2 – 311.9 | 2.25 ± 0.32 | -0.32     |

<sup>1</sup> Each 20-ml vial contained 5 mites.

<sup>2</sup> Bees and larvae were treated individually with the different concentrations of the products tested.

chosen for further testing to estimate their LC<sub>50</sub>s against *V. destructor*. Although carvacrol caused 97.1% mean mite mortality, it was not tested in the second stage of this study because it is also a component of origanum oil.

### Toxicity of selected products to worker bees

Thymol and clove oil had the lowest LC<sub>50</sub>s (210.3 and 238.6 µg / bee, respectively) whereas origanum oil and menthol had the highest (331.3 and 523.5 µg / bee, respectively). Tau-fluvalinate was relatively more toxic to bees (LC<sub>50</sub> = 194.1 µg / bee) than menthol and origanum oil but its confidence limits overlapped with those for thymol and clove oil. Origanum oil and menthol had confidence limits that overlapped with one another but that were higher than those of the other two EO products (Table 1).

### Toxicity of selected products to worker bee larvae

Thymol had the lowest LC<sub>50</sub> value (150.7 µg / larva) and menthol had the highest (382.8 µg / larva). Origanum oil and clove oil had LC<sub>50</sub>s of 236.4 and 281.4 µg / larva, respectively. Tau-fluvalinate was relatively more toxic to the larvae (LC<sub>50</sub> = 192.3 µg / larva) than menthol but not more toxic than the other three compounds. The confidence limits

for tau-fluvalinate were lower than those for menthol but they overlapped with those for thymol, origanum oil, and clove oil (Table 1).

## Discussion

The glass-vial residual bioassay used in this study provided a simple, inexpensive, and rapid way for screening the acaricidal effects of numerous natural products against *V. destructor*. All products except cineole oil caused significantly higher mite mortality than those of acetone treatments at the doses used (Fig. 1).

Thymol, origanum oil, clove oil, carvacrol, and menthol were the most effective compounds at killing mites in the laboratory assays. These results agree with those of previous researchers who reported that thymol, origanum oil, clove oil and carvacrol were lethal to most mites under laboratory conditions (Sammataro *et al.*, 1998; Lindberg *et al.*, 2000; Ariana *et al.*, 2002). Tau-fluvalinate was at least 20 times less effective as a varroacide than the above EO products. This result is supported by that of Lindberg *et al.* (2000) who found that fluvalinate was five to seven times less toxic than thymol to *V. destructor* in laboratory tests. Furthermore, the LC<sub>50</sub> and confidence limits for tau-fluvalinate found in this study were higher than those of the natural products tested, and did not overlap with them, again, rein-

forcing the conclusion that this synthetic acaricide is less toxic to the mites than the organic compounds selected. The possibility that this result had been a consequence of mite resistance to fluvalinate is unlikely because Pettis tests (Pettis *et al.*, 1998b) conducted on samples collected previously for this study did not detect resistance to this pesticide in mite populations from the University of Guelph's apiaries.

When comparing only the EO products, mortality rates and LC<sub>50</sub> values indicate that menthol was less toxic to *V. destructor* than thymol and origanum oil, similar to results reported by Lindberg *et al.* (2000). However, in both studies, the LC<sub>50</sub> confidence limits for these products overlapped, suggesting that these results must be taken cautiously.

The LC<sub>50</sub> values obtained for adult bees suggest that of the four natural compounds selected, thymol and menthol were the most and least toxic compounds tested, respectively. Tau-fluvalinate had a lower LC<sub>50</sub> than the four EO products, and although its confidence limits overlapped with those of thymol and clove oil, these were lower than those of origanum oil and menthol. Menthol and origanum oil were the least toxic products to worker bees, and neither of the other two natural compounds was more toxic to workers than tau-fluvalinate.

Lindberg *et al.* (2000) also found that thymol was relatively more toxic to adult bees than clove oil, but did not test origanum oil or menthol on workers. In another study, Ellis and Baxendale (1997) found that thymol and menthol were the most toxic compounds to honey bees of seven monoterpenoids tested. The toxicity of thymol from this and the above studies is, however, relative to other EOs tested. Thymol seems to be relatively safe to adult bees when compared with highly toxic pesticides. For example, Albo *et al.* (2003) found that thymol was at least 50 times less toxic to worker bees than dimethoate, whilst Iwasa *et al.* (2004) reported LC<sub>50</sub> values <15 µg / bee for neonicotinoid insecticides. These LC<sub>50</sub> values are at least 14 times lower than those of results reported here (thymol showed LC<sub>50</sub> >210 µg / bee in this study). In another study, Ebert *et al.* (2007) fed bees with various concentrations of several natural products and found that oxalic acid was the most toxic, whilst menthol and clove oil were among the least toxic, and thymol and origanum oil showed intermediate toxicity values. Their results are in agreement with those of this study.

This is the first time that the toxicity of EO compounds to honey bee larvae has been reported. Thymol was again the most toxic of the natural compounds tested and menthol was the least toxic, and their confidence limits did not overlap. Tau-fluvalinate was equally toxic to larvae as thymol, origanum oil, and clove oil.

Of the four natural compounds tested, thymol had the lowest LC<sub>50</sub> values for both adult bees and larvae, while menthol showed the opposite, without overlap between the confidence limits for these two products. It is therefore concluded that thymol was the most toxic of the selected EO products tested on adult bees and larvae, while

menthol was the least toxic. Thymol, was, however, not more toxic to adult bees and larvae than tau-fluvalinate. This result is relevant, because it suggests that the potential use in colonies of the four organic products tested, including thymol, would not result in higher toxicity levels to bees and larvae than those from the most widely used varroacide worldwide.

Not only were the selected EO compounds equally or less toxic to bees and larvae than tau-fluvalinate, but all of them were significantly more toxic to *V. destructor*. Furthermore, the lowest difference between the relative toxicities of the products tested to bees and mites was that of tau-fluvalinate. Thus, apparently, these EO products are more mite-selective and have higher safety margins than tau-fluvalinate.

Overall, the results of this study suggest that thymol, origanum oil, clove oil, and menthol are products that warrant further investigation as agents for the control of *V. destructor*. Thymol is available in commercial varroacidal formulations (Imdorf *et al.*, 1999), but no commercial product has yet been developed for the other three EO products to control *V. destructor*, because further research is still needed on their safety, delivery methods, and efficacy.

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