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Research Article

Toxicity of Anethole and the Essential Oils of Lemongrass and Sweet Marigold to the Parasitic Mite *Varroa destructor* and Their Selectivity for Honey Bee *(Apis mellifera)* Workers and Larvae

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This study examined the toxicity of anethole and that of the essential oils of lemongrass *(Cymbopogon citratus)* and sweet marigold *(Tagetes lucida)* to the mite *Varroa destructor* and to honey bee workers and larvae. Anethole was the most toxic compound to *V. destructor* (LC_{50}: 304.9 μg/ml), whereas *Tagetes* oil was the least toxic (LC_{50}: 1256.27 μg/ml). The most and least toxic compounds to worker bees were anethole and *Tagetes* oil with LD_{50}s of 35942 and 85381 μg/ml, respectively. For larvae, *Tagetes* oil was the most toxic compound (LD_{50}: 9580.7 μg/ml) and anethole the least toxic (LD_{50}: 14518.0 μg/ml). Anethole and *Cymbopogon* oil had the highest selectivity ratios. The expression of *AChE*, a gene that regulates the production of acetyl cholinesterase, a detoxifying enzyme, was not altered in bees treated with the plant compounds at 48 h post-treatment. This study showed that anethole and *Cymbopogon* oil have potential for controlling *Varroa* mites and seem to be relatively safe for larvae and adult honey bees.

1. Introduction

The mite *Varroa destructor* is responsible for widespread losses of honey bee colonies, particularly in North America [1–4]. *V. destructor* feeds on the hemolymph and fat body of its host and can transmit deadly viruses [5, 6]. Colonies infested with *V. destructor* that are treated with miticides produce more honey than untreated colonies [7], but the frequent use of synthetic miticides has resulted in the development of resistance to many of these products in mite populations [8]. Synthetic miticides may also contaminate hive products [9]. Therefore, there is demand for new compounds with high miticidal activity that at the same time are safe for the bees, the environment, and nontarget species (including bees and humans). Plant essential oils and the components of these substances are attractive miticide candidates for controlling pests and diseases in hives because they meet most of these criteria [10–12]. Furthermore, many essential oils and their components exhibit considerable miticidal activity against *V. destructor* [10].

Miticides are also toxic to bees to some degree [13]. Consequently, bees activate detoxification mechanisms when a pesticide is too toxic to them. One of these mechanisms results in the increased production of acetyl cholinesterase (AChE), an enzyme that regulates the concentration of acetylcholine at the synapses level of individuals exposed to pesticides such as carbamates and organophosphates [14], as well as to pyrethroids [15] and neonicotinoids [16–18]. Therefore, AChE activity is widely used as neurotoxicity biomarker. However, the activation of these mechanisms and the production of AChE are not well known for arthropods exposed to essential oils [19, 20]. Additionally, the activation

of detoxification mechanisms is energy demanding and costly to the bees, which may compromise their physiology and health. Thus, it is important that miticides are minimally toxic to bees and that they do not need to invest considerable resources in detoxification processes when exposed to miticides during *Varroa* mite control treatments.

This study examined the toxicity of anethole, an aromatic compound of the essential oil of anise *(Pimpinella anisum)*, as well as that of the essential oils of lemongrass *(Cymbopogon citratus)* and sweet scented marigold *(Tagetes lucida)* to *Varroa* mites and honey bees. The main component of *Cymbopogon citratus* is the monoterpenoid geraniol, whereas that of *Tagetes lucida* is the aromatic phenylpropene estragole. Other researchers have reported on the pesticide properties of the plant compounds we tested. For example, anethole has shown toxicity to several insects [21, 22], nematodes [23], and mites [24]. *Cymbopogon* oil can kill mosquitoes [25] and mites [26]. *Tagetes* oil has evidenced pesticide activity against insects such as bedbugs [27] and against ticks [28]. Moreover, *Tagetes* oil has been tested against several pathogens of the honey bee, including the bacterium *Paenibacillus larvae*, the fungus *Ascosphaera apis,* and the mite *V. destructor* [29, 30]. However, this study is the first to evaluate the relative toxicity and selectivity of anethole and lemongrass oil on *Varroa* mites and on larvae and adult honey bees. The expression of *AChE*, the gene that regulates the production of the detoxifying enzyme AChE, was also analyzed in bees treated with these compounds.

2. Materials and Methods

2.1. Compounds. Anethole (technical grade) was obtained from Sigma-Aldrich (St. Louis, MO, USA), whereas the essential oils of *Cymbopogon citratus* and *Tagetes lucida* were obtained from plant tissues by steam distillation as per Ames and Matthews [31]. Tau-fluvalinate, used as positive control, was also obtained from Sigma-Aldrich.

2.2. Varroa Mite and Honey Bee Sources. The trials were conducted at the University of Guelph's Honey Bee Research Centre in Guelph, ON, Canada. Colonies headed by Buckfast queens were used as sources of mites and bees. The mites were obtained from adult bees [32] of three colonies that were heavily infested with *V. destructor* and that had not been treated with miticides for at least one year. Three other colonies provided adult bees and larvae. For adult bees, combs with emerging brood were caged and incubated (33°C and 60% RH) overnight to obtain newly emerged workers, and the same colonies provided the larvae for the experiments as described below.

2.3. Toxicity to V. destructor. After preliminary tests, six logarithmic interval concentrations of each compound (Table 1) were prepared in 1.5 ml of HPLC grade acetone. Then, as per Gashout and Guzmán-Novoa [33], 0.5 ml of each solution was placed into a 20 ml glass scintillation vial (Fisher Scientific Ltd., Ottawa, ON, Canada) with a pipette, including acetone and tau-fluvalinate as controls. The vials were rolled out to distribute the solution on their inner walls. After the acetone evaporated (about 10 min), the vials were capped. Five female mites were introduced into each vial with a fine paintbrush and left in the dark at room temperature (26°C and 65% RH). A total of 280 mites were used for each compound in eight replications. To determine the acute toxicity of each compound, the number of dead mites was recorded at 4 h post-treatment (hpt). A needle was used to touch the body of each mite. If the parasite did not move, it was assumed to be dead.

2.4. Toxicity to Honey Bee Workers and Larvae. The plant compounds and tau-fluvalinate were dissolved in 96% ethanol to obtain the same concentrations as above and a topical assay was used to treat newly emerged workers as follows. Each bee received 5 μl of solution of one treatment concentration on her prothorax with the aid of a micropipette. A total of 25 bees were treated per concentration for each compound and control bees were treated with ethanol. Groups of 25 treated bees were introduced into hoarding cages (12.7 × 8.5 × 14.5 cm) equipped with two 20 ml gravity feeders, one containing 50% (w : v) sugar : distilled water solution and the other containing distilled water. Then, the cages were held in an incubator at 33°C and 60% RH. Dead bees were counted at 24 and 48 hpt. Three trial replications were conducted.

For honey bee larvae, combs containing abundant third or fourth instar larvae were retrieved from the source colonies to treat them as per Gashout and Guzmán-Novoa [33]. Briefly, groups of 25 larvae were individually treated with 5 μl of solution for each concentration per compound by placing the solution at the bottom of each cell with a micropipette. The outer edges of cells containing experimental larvae were painted with different colors with nontoxic markers (L551P2, Hunt Int., Mississauga, ON, Canada) to identify the treatments. The combs containing the larvae were returned to their respective hives after treatment. Mortality was determined at 24 and 48 hpt. Pearl white larvae were assumed to be alive whereas larvae with a brownish color or missing from cells due to the hygienic behavior of bees were assumed to be dead. Three trial replications were conducted.

2.5. Relative Selectivity Ratios. To assess safety margins of the natural compounds for the honey bee, relative selectivity ratios [34] were calculated by dividing the toxicity value of the host (LD_{50} of worker or larvae) by that of the pest (LC_{50} of *V. destructor*). These ratios permitted comparisons of the tested compounds relative to each other and to tau-fluvalinate.

2.6. Acetyl Cholinesterase Gene Expression. Real-time quantitative PCR (RT-qPCR) was used to measure the expression of *AChE* in samples of worker bees. About 15 bees were collected per treatment and per replicate at 0 and 48 hpt. The bees were placed in plastic bags and kept frozen at −70°C for later RNA extraction.

After analyzing the toxicity of the compounds, control bees and the bees treated with the concentration closest to and within the 95% confidence limits of the LD_{50} value at 48 hpt were used to measure the expression of *AChE* (the bees treated with 110 μg/ml of tau-fluvalinate, 34,000 μg/ml of anethole, and 60,000 μg/ml of *Cymbopogon* or *Tagetes* oil).

Table 1: Logarithmic interval concentrations of compounds were used for determining the lethal concentration (LC_{50}) or dose (LD_{50}) for *Varroa destructor* female mites and for worker or larvae of *Apis mellifera*, respectively. Actual concentrations are shown in the table.

Products	Concentrations (μg/ml)		
	Mite	Worker	Larvae
Anethole	100, 150, 230, 350, 530, 800	25000, 29400, 34000,40000, 47000, 55000	5000, 7200, 10200, 14600, 21000, 30000
Cymbopogon oil	160, 230, 340, 500, 720, 1050	7000, 11600, 20000, 34000, 60000, 100000	4000, 7000, 12000, 20000, 35000, 60000
Tagetes oil	200, 320, 500, 800, 1300, 2000	10000, 18000, 34000, 60000, 110000, 200000	3000, 4000, 5500, 7700, 10300, 14000
Tau-fluvalinate	20, 40, 70, 140, 260, 500	20, 50, 110, 220, 460, 960	50, 110, 220, 460, 960, 2000

Table 2: Lethal concentration (LC_{50}) and 95% confidence limits (CL) estimated for *Varroa destructor* female mites at 4 h after exposure to 0.5 ml/vial of six concentrations of essential oil products and tau-fluvalinate.

Compounds	N	LC_{50} (μg/ml)	95% CL	Slope ± SE	Intercept	X^2	df
Anethole	280	304.94	225.24–357.87	3.35 ± 0.86	−3.31	26.70	46
Cymbopogon oil	280	474.13	349.86–592.17	2.98 ± 0.39	−2.97	59.65	46
Tagetes oil	280	1256.27	1018.54–1578.25	1.92 ± 0.27	−0.97	32.89	46
tau-fluvalinate	280	80.43	55.76–106.42	1.71 ± 0.24	1.75	28.23	46

Total RNA was extracted by homogenizing each composite sample of 15 bees per treatment in extraction buffer as per Chen et al. [35]. The amount and concentration of RNA were determined with a spectrophotometer (Nanovue GE Healthcare, Cambridge, UK) and cDNA was synthesized from the RNA using a cDNA synthesis kit (Revert Aid, Fermentas Life Sciences, Burlington, ON, Canada) according to the manufacturers protocol.

Each PCR reaction of 20 μL consisted of 2 μL of cDNA template, 10 μL of SYBR Green/ROX/qPCR Super Mix (Thermo Scientific, Mississauga, ON, Canada), 0.6 μL of each specific primer at a concentration of 300 nM, and 6.8 μL of nuclease-free H_2O. The qRT-PCR reactions were performed in 96-well plates (Diamed®, Mississauga, ON, Canada) with a BioRad CFX96 (Bio-Rad Laboratories, Mississauga, ON, Canada) thermocycler. The target gene primers used in this study were designed with the Primer-BLAST tool and then verified for hairpin loops and dimers with Gene Runner (Hasting Software Inc., New York, USA) after obtaining and aligning *AChE* sequences from the NCBI (Accession Number NC_007077.3). The forward and reverse primers used were GGACATAATGGCGGCTACGA and CTCCTCGCTGTTCGTGAAGT, respectively. The reference gene used was *RpS5* [36]. The primers were ordered from Laboratory Services of the University of Guelph (Guelph, ON, Canada).

All reactions were set up in triplicate for each of three biological replicates. A two-step cycling protocol was followed, using a Uracil-DNA glycosylase pretreatment at 50°C for 2 min, an initial denaturation at 95°C for 10 min, and 40 cycles of 15 s at 95°C and 60 s at 60°C. A negative control using nuclease-free H_20 instead of cDNA and a positive control were included in each qRT-PCR run. The efficiency of the primers to optimize qPCR conditions was near 100% and was determined with standard curves from 10-fold serial dilutions using 300 bp synthetic gene fragments (gBlocks, Integrated DNA technologies, Iowa, USA), which included the sequence of the forward primer, the amplicon sequence, and the reverse primer. *AChE* transcript abundance was quantified relative to the reference gene according to the $2^{-\Delta\Delta}$ (Livak) method [37] using the control as the calibrator. The Bio-Rad CFX Manager® software (Bio-Rad Laboratories, Mississauga, ON, Canada) was used to calculate the expression ratio.

2.7. Statistical Analyses. Data on mite, worker, and larvae mortality were subjected to probit analyses using the PoloPlus software [38] to estimate the 50% lethal concentration (LC_{50}) of mites and the 50% lethal dose (LD_{50}) of workers and larvae, as well as slopes and 95% confidence intervals of the different compounds. The PoloPlus program was also used to compare the slopes of dose-response lines of the compounds. Data on abundance of *AChE* transcripts were log 10 transformed and then subjected to ANOVA and to LSD tests when significance was detected.

3. Results

3.1. Toxicity to V. destructor. Anethole had the lowest LC_{50} (304.9 μg/ml) of the natural compounds tested against *V. destructor*, although its toxicity was not significantly different to that of *Cymbopogon* oil. *Cymbopogon* and *Tagetes* oils were ranked 2nd and 3rd, respectively, and differed in toxicity to *V. destructor* from each other. Tau-fluvalinate was significantly more toxic to *V. destructor* than the three natural compounds with an estimated LD_{50} of 80.44 μg/ml (Table 2).

Parallel comparisons of the slopes of dose-response lines revealed no significant differences between anethole and *Cymbopogon* oil ($X^2 = 0.28$, df = 1, and $p = 0.599$), but anethole had a significantly higher dose-response than *Tagetes* oil ($X^2 = 5.49$, df = 1, and $p = 0.019$) and tau-fluvalinate ($X^2 = 7.78$, df = 1, and $p = 0.005$). No differences in dose-response were found between *Tagetes* oil and tau-fluvalinate ($X^2 = 0.37$, df = 1, and $p = 0.544$; Figure 1(a)).

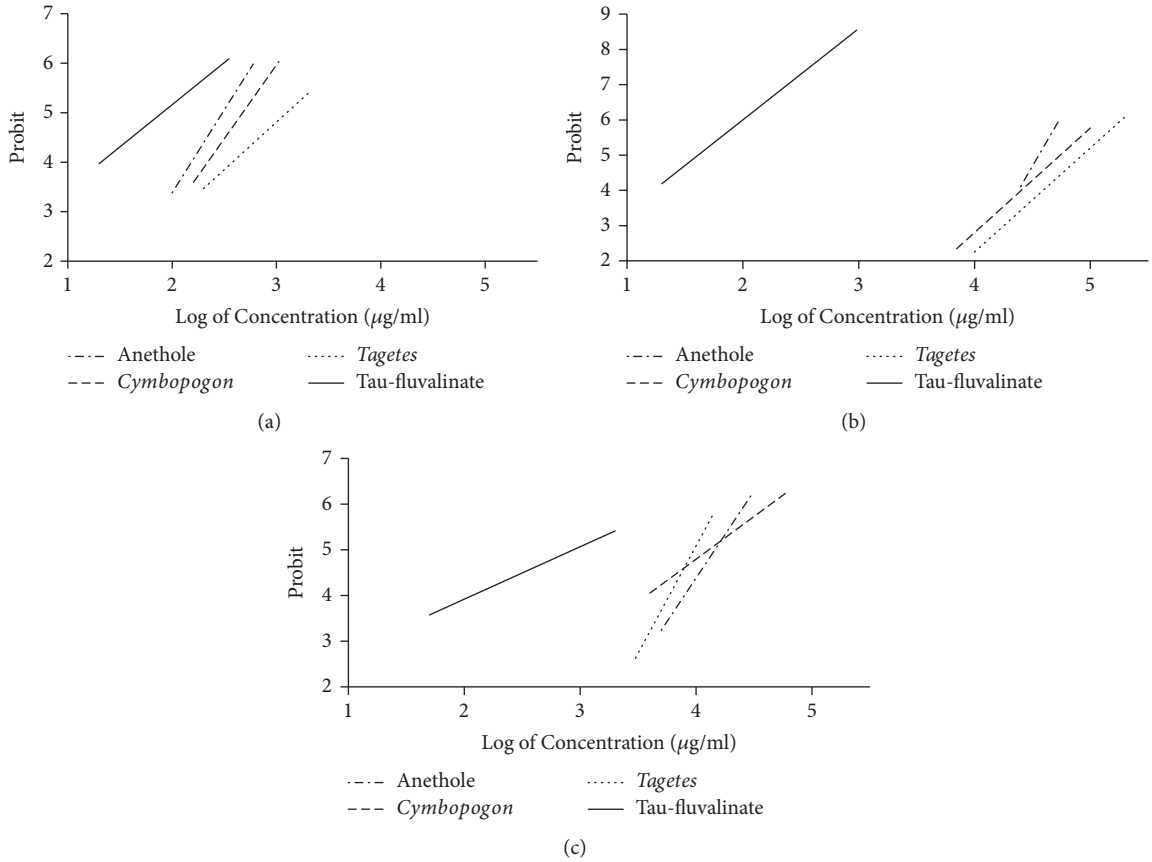

Figure 1: Dose-response lines of plant products or tau-fluvalinate for (a) *Varroa destructor* female mites when exposed for 4 h to 0.5 ml of plant compounds using a vial assay, (b) *Apis mellifera* workers 24 h after a topical treatment with 5 μl of plant compounds, and (c) *Apis mellifera* larvae 24 h after a topical treatment with 5 μl of plant compounds.

3.2. Toxicity to Honey Bee Workers and Larvae. The most and least toxic natural compounds to newly emerged workers were anethole and *Tagetes* oil, respectively, with $LD_{50\,s}$ differing significantly at both 24 and 48 hpt. The toxicity of *Cymbopogon* oil to bees was not significantly different to that of anethole or *Tagetes* oil, whereas tau-fluvalinate was significantly more toxic to worker bees than the three natural compounds by at least 207 times (Table 3). Conversely, for larvae, *Tagetes* oil was the most toxic of the natural compounds, whereas anethole was the least toxic at both 24 and 48 hpt. The LD_{50} values for *Cymbopogon* oil were intermediate between anethole and *Tagetes* oil, but did not differ with any of them in toxicity to honey bee larvae. However, tau-fluvalinate was significantly more toxic to the larvae than the three natural compounds at both time points (Table 3). Its toxicity to honey bee larvae was nearly 11 times higher than that of *Tagetes* oil.

For worker bees, anethole had a significantly higher dose-response than *Cymbopogon* oil ($X^2 = 28.46$, df = 1, and $p < 0.001$), *Tagetes* oil ($X^2 = 24.89$, df = 1, and $p < 0.001$), and tau-fluvalinate ($X^2 = 44.53$, df = 1, and $p < 0.001$). However, no differences were found between the slopes of the dose-response lines for *Cymbopogon* and *Tagetes* oil ($X^2 = 0.01$, df = 1, and $p = 0.91$) or between those for *Cymbopogon* oil and tau-fluvalinate ($X^2 = 0.86$, df = 1, and $p = 0.354$; Figure 1(b)). For larvae, anethole had a significantly higher dose-response than *Cymbopogon* oil ($X^2 = 32.84$, df = 1, and $p < 0.001$) or tau-fluvalinate ($X^2 = 54.05$, df = 1, and $p < 0.001$), but no significant difference was found between the slopes of the dose-response lines for anethole and *Tagetes* oil ($X^2 = 1.56$, df = 1, and $p < 0.211$; Figure 1(c)).

3.3. Relative Selectivity Ratios. The relative selectivity ratios calculated for anethole, *Cymbopogon* oil, *Tagetes* oil, and tau-fluvalinate were 117.9, 115.7, 68.0, and 2.2, respectively, in the case of adult bees and 47.6, 27.2, 7.6, and 10.9, respectively, in the case of bee larvae.

3.4. Acetyl Cholinesterase Gene Expression. The relative abundance of *AChE* transcripts at 48 hpt was significantly higher in tau-fluvalinate treated bees (27.34 ± 13.4 fold change) than in bees from other treatments. There were no significant differences in the expression level of this gene between bees of the control, anethole, *Cymbopogon*, and *Tagetes* oil treatments at 0 hpt or at 48 hpt, indicating that only tau-fluvalinate was

Table 3: Lethal dose (LD_{50}) and other related parameters estimated for worker and larvae of *A. mellifera*, at 24 h or **48 h** posttreatment with 5 μl/individual of six concentrations of plant products or tau-fluvalinate.

Growth stages	Compounds	Time after treatment (h)	N	LD_{50} (μg/ml)	95% CL	Slope ± SE	Intercept	X^2	df
Worker	Anethole	24	525	35942.0	27984.0–44810.0	5.69 ± 0.52	−20.90	23.91	16
		48	**525**	**35520.0**	**27553.0–44249.0**	**5.74 ± 0.52**	**−21.10**	**24.11**	**16**
	Cymbopogon oil	24	525	54844.0	40701.0–71227.0	2.96 ± 0.43	−9.05	23.88	16
		48	**525**	**53304.0**	**36493.0–72285.0**	**2.92 ± 0.43**	**−8.78**	**22.97**	**16**
	Tagetes oil	24	525	85381.0	56652.0–11129.0	2.96 ± 0.45	−9.58	26.74	16
		48	**525**	**83297.0**	**52116.0–108460.0**	**3.12 ± 0.48**	**−10.35**	**25.27**	**16**
	Tau-fluvalinate	24	525	173.49	120.20–225.60	2.59 ± 0.24	0.81	19.39	16
		48	**525**	**139.70**	**107.73–173.52**	**2.27 ± 0.21**	**0.13**	**18.97**	**16**
Larvae	Anethole	24	525	14518.0	11853.0–17661.0	3.81 ± 0.32	−10.85	19.03	16
		48	**525**	**14143.0**	**11441.0–17085.0**	**3.89 ± 0.34**	**−11.16**	**18.72**	**16**
	Cymbopogon oil	24	525	12900.0	8562.6–18093.0	1.87 ± 0.19	−2.67	15.92	16
		48	**525**	**12359.0**	**8091.7–17389.0**	**1.92 ± 0.19**	**−2.84**	**14.28**	**16**
	Tagetes oil	24	525	9580.7	7024.2–11083.0	4.70 ± 1.01	−13.69	21.99	16
		48	**525**	**9423.0**	**6886.9–10953.0**	**4.58 ± 0.94**	**−13.21**	**20.57**	**16**
	Tau-fluvalinate	24	525	875.20	581.73–1430.21	1.15 ± 0.16	1.63	23.33	16
		48	**525**	**666.21**	**415.76–1046.72**	**1.28 ± 0.18**	**1.39**	**23.18**	**16**

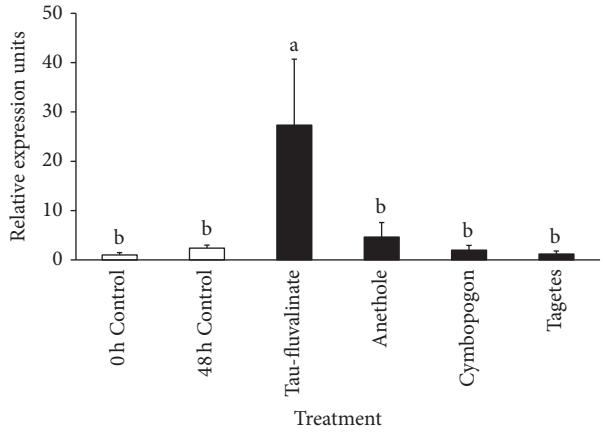

Figure 2: Relative expression of the gene *AChE* using qRT-PCR in *Apis mellifera* workers at 0 and 48 h posttreatment with 5 μl of tau-fluvalinate (110 μg/ml), anethole (34,200 μg/ml), *Cymbopogon* oil (60,000 μg/ml), and *Tagetes* oil (60,000 μg/ml). Control bees were treated with solvent (ethanol). Different letters indicate significant differences of means based on analysis of variance and Fisher protected LSD tests ($n = 45$) performed with log-transformed data.

associated with elevated expression of *AChE* ($F_{5,12} = 4.27$, $p < 0.01$; Figure 2).

4. Discussion

This study was carried out to test the potential of some plant compounds for controlling *Varroa* mites as well as to determine their relative toxicity to honey bee workers and larvae. Both anethole and *Cymbopogon* oil showed significant effects on the mites and at the same time high selectivity and safety margins for the honey bee. The miticidal effect of anethole had been demonstrated before [24], but its relative toxicity and selectivity had never been determined for *V. destructor* and honey bee larvae and adults. In this study, anethole's dose-response and toxicity to *V. destructor* was significantly higher than that of *Tagetes* oil (4.1 times) and similar to that of *Cymbopogon* oil. Its toxicity to worker bees was also significantly higher than that of *Tagetes* oil (2.4 times) but not different to that of *Cymbopogon* oil. Conversely, anethole was less toxic than *Tagetes* oil for honey bee larvae and *Cymbopogon* oil had intermediate toxicity. By comparison, tau-fluvalinate was 3.8 times more toxic to *Varroa* mites than anethole, but it also resulted in being 17 times more toxic to larvae and at least 207 times more toxic to adult bees than anethole. Additionally, the selectivity ratios for anethole were the highest of all compounds tested. From the above results, it can be concluded that anethole has the highest potential as varroacide among the compounds tested and demonstrated large safety margins for honey bees. It thus could be recommended for further testing in hives.

Anethole is currently used as a flavoring or fragrance agent in the food industry and there are no safety concerns for human health [39]. However, its possible effects on odor or taste of honey should be considered. Anethole is isolated from star anise essential oil and is also produced synthetically from sulfate turpentine oils. The annual production of star anise essential oil for isolation of anethole exceeds 3000 tons [40]. Therefore, it can be assumed to be economically available should a new market be created due to its varroacidal activity.

The toxicity of *Cymbopogon* oil to *V. destructor* and to honey bee workers and larvae was similar to that of anethole. However, it had slightly lower relative selectivity ratios than anethole. Still, it was much higher than the ratios for *Tagetes* oil and tau-fluvalinate, which also makes it a potentially effective and safe varroacidal candidate for testing at the hive level. Further purification of *Cymbopogon* oil and separation of its active ingredients may enhance its varroacidal effect. In previous studies, *Cymbopogon* oil has been found to have miticidal activity [26] and it is known that citral, its main component, may kill *Varroa* mites [41]. However, this is the first study to demonstrate its varroacidal activity and low toxicity to adult bees and larvae.

Tagetes oil was significantly less toxic to *Varroa* mites than tau-fluvalinate and the other two plant compounds tested in this study. It was also the least toxic of the compounds to adult bees, but conversely, second to tau-fluvalinate, *Tagetes* oil was the most toxic of the compounds to larvae. Furthermore, it was the least selective of the plant compounds tested when toxicity results on larvae were used in the calculations. Therefore, it would rank lower than anethole and *Cymbopogon* oil as potential varroacidal compound. It is puzzling why *Tagetes* oil was particularly toxic to honey bee larvae. However, studies in other insects have shown that tissues of *Tagetes* plants contain (5E) ocimenone and α-terthienyl, which have shown potent larvicidal effects against mosquitoes [25]. The mode of action of α-terthienyl is related to its interference with antioxidant defense systems in the larvae of organisms exposed to this plant insecticide [42]. The toxicity of the essential oil of *T. minuta* to *Varroa* mites and adult honey bees had been assessed previously by Eguaras et al. [29] under a potter tower. They estimated a LC_{50} of 4.37 mg/cage for *Varroa* mites and 13.6 mg/cage for worker bees. However, due to different methods of evaluation, it is impossible to

directly compare their result with ours, although both studies revealed the selective property of the oil for adult bees.

We found an elevated expression of *AChE* in bees 48 h after being treated with tau-fluvalinate but not with the plant compounds (Figure 2). This increase in gene expression could be explained as an adaptive mechanism with which bees counteract the AChE inhibitory effect of tau-fluvalinate. Similar results were reported by Badiou et al. [15] when they exposed honey bees to another pyrethroid, deltamethrin. Our results for tau-fluvalinate exposure agree with the notion that *AChE* is likely associated with stress responses. *AChE* increases when worker bees are stressed, for example, under conditions of poor pollen availability, and is inhibited when pollen is available and brood rearing is induced [43]. Therefore, it is possible that only tau-fluvalinate had caused stress on the bees. The low toxicity of the plant compounds to adult honey bees relative to tau-fluvalinate found in this study might have also been related to the lack of changes in *AChE* expression in bees exposed to them and thus only bees exposed to tau-fluvalinate showed higher abundance of *AChE* transcripts. Alternatively, the unresponsiveness of *AChE* expression to the plant compounds may have been due to lack of specificity of these compounds to acetylcholine receptors. It is known that terpenoids such as thymol bind to octopamine or to GABA receptors [44, 45], although other studies have also found that essential oils components such as thymol and linalool alter the expression of *AChE* [19, 20, 27, 46]. Another potential hypothesis to explain the lack of effects of the plant compounds on *AChE* expression is that the compounds could have been quickly metabolized and thus did not show any apparent effect on the expression of *AChE* at 48 hpt. In any case, further investigation is warranted to discover why the plant compounds tested here did not affect *AChE* expression in adult bees.

5. Conclusion

Anethole and *Cymbopogon* oil showed potential for controlling *Varroa* mites and at the same time had low toxicity and considerable safety margins for larvae and adult honey bees. Further studies on the mode of action of these plant compounds and field trials testing them in hives are warranted. Such studies may lead to replacing hazardous chemicals with natural safe compounds in order to maintain healthy honey bee colonies and users of hive products.

Conflicts of Interest

The authors declare that they have no potential conflicts of interest in relation to the study in this paper.

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