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Essential oils toxicity related to *Varroa destructor* and *Apis mellifera* under laboratory conditions

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ABSTRACT

Acaricidal effect of *Tagetes minuta*, *Heterotheca latifolia*, and *Eucalyptus sp.* essential oils against *Varroa destructor* and their toxicity for *Apis mellifera* L. were evaluated under laboratory conditions in two assays. In the first experiment, 10 mg of plant active principles were prepared in water solution with an emulsionant at 3, 4, and 5% concentrations. For each component and to each dose, 10 females *V. destructor* were pulverized in a Burgerjon tower and transferred to a Petri dish with 5 bee pupae to an incubation stove at 70% RH and 33-34ºC for 3 days. Dead and alive mites were counted 12, 24, and 48 h after treatment for five replications and their controls. No significant differences were found among doses of the same component and its effectiveness varied between 63 to 84% related to the control groups. In the second assay, 100 adult bees were pulverized with 10 mg of a 5% solution of the components and placed in an incubator stove at 70% RH and 33-34ºC. Four replications and a control treatment for each sample were taken simultaneously. Dead and alive bees for each replication were counted 72 h post-treatments. There were not significant differences in bee mortality among the control groups (P>0.05) and it was relatively low for all treatments except for eucalyptol in which the bee mortality percentage was higher than 58%. It was concluded that *T. minuta* and *H. latifolia* essential oils can play an important role in an integrated pest management program to control Varroosis in honey bee colonies.

Keywords: *Varroa destructor*, *Apis mellifera*, essential oils, *Tagetes minuta*, *Heterotheca latifolia*, eucalyptol.

Toxicidad de aceites esenciales relacionados con *Varroa destructor* y *Apis mellifera* en condiciones de laboratorio

RESUMEN

El efecto acaricida de los aceites esenciales de *Tagetes minuta*, *Heterotheca latifolia* y *Eucaliptus* sp., contra *Varroa destructor* y su toxicidad sobre *Apis mellifera* L. fue evaluado en condiciones de laboratorio en dos ensayos diferentes. Para el primer ensayo, 10 mg del principio activo de las plantas fue disuelto en agua con un emulsionante al 3, 4 y 5%. Para cada componente y cada dosis, 10 hembras de *V. destructor* fueron pulverizadas en una torre de Burgerjon y transferidas a una cápsula de Petri con 5 pupas de abejas. Las cápsulas se mantuvieron en una estufa a 70% HR y 33-34ºC durante tres días. Los ácaros muertos y vivos fueron contados a las 12, 24 y 48 h post-tratamiento para cinco réplicas y sus respectivos controles. No hubo diferencias significativas entre las dosis del mismo componente y su efectividad varió entre 63 y 84% en relación al control.

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En el segundo ensayo, 100 abejas adultas fueron pulverizadas con 10 mg de una solución al 5% de los componentes y colocadas en la estufa a 70% HR y 33-34ºC. Cuatro réplicas y un control de cada muestra fueron tomadas simultáneamente. Las abejas muertas y vivas de cada réplica fueron contadas 72 h post-tratamiento. No hubo diferencias significativas en la mortalidad de las abejas entre los grupos control (P>0,05) y ésta fue relativamente baja para todos los tratamientos excepto para el eucaliptol cuyo porcentaje fue mayor al 58%. Se concluye que los aceites esenciales de *T. minuta y H. latifolia* pueden formar parte de un programa de manejo integrado de la Varroosis.

*Palabras clave***:** *Varroa destructor, Apis mellifera*, aceites esenciales, *Tapetes minuta y Heterotheca latifolia,* eucaliptol.

INTRODUCTION

Varroa destructor (Anderson & Trueman, 2000) is currently one of the most serious parasitic mite that affects *Apis mellifera* L. colonies. This mite completes all its life cycle inside the beehives, sucking the haemolymph of the pupae and adult bees. When this mite colonizes a hives, the beekeepers must conduct different acaricide treatments to control parasite population size and prevent the death of the colonies.

Currently, the control of the mite is based, mainly, on the use of synthetic acaricides, especially pyrethroids. Nevertheless, the repeated use and in many cases subletales doses of these substances throughout years has caused problems, such as its accumulation in the different products of the beehive and develop resistance of the mites' population to acaricides (Milani, 1995) due to the fact that most of these compounds have the potential to contaminate bee products. These problems have led to develop investigations on alternative control methods, such as organic acids, essential oils or any of their components. Essential oils are distilled from aromatic plants, they possess intense smell, exhibit low toxicity in mammals and bees and they have less harmful effect over environment and wide public acceptation among producers (Isman, 2000).

About 150 essential oils or any of their components have been tested to control the mite *V. destructor*, with different results. Their toxicity, repellent or attractive effect towards the mite and their influence on the mite's reproduction have been evaluated, when essential oils have been locally administered, or applied in pulverization or in a passive evaporation form (Imdorf *et al*., 1999).

This work evaluates de activity of essential oils of Wild camomile (*Tagetes minuta*), alcanforillo (*Heterotheca latifolia*), and eucalyptol, main component of the essential oil of *Eucalyptus* sp. against the mite *V. destructor* under laboratory conditions. Toxicity in honey bees *Apis mellifera* is also determined.

MATERIALES Y MÉTODOS

Extraction and characterization of essential oils

The extraction and characterization of the essential oils was made at Facultad de Ciencias Económico-Sociales e Ingeniería from Universidad Nacional de San Luis, Argentina. The experiments on toxicity of mites and bees were made at the Laboratory of Arthropods of the Facultad de Ciencias Exactas y Naturales de la Universidad Nacional de Mar del Plata, Argentina, from September 2001 to March 2002.

The essential oils were extracted from leaves and stems of wild chamomile (*Tagetes minuta* L.) and alcanforillo (*Heterotheca latifolia*). On the other hand, the eucalyptol was acquired from Sigma under 100% pure. The vegetable material was distilled for 4 h in a stainless equipment using the method of distillation with steam according to Aldicara (1976). The oil was separated from water by decantation, dried with sodium sulphate and then kept in refrigeration to avoid its deterioration. The oil composition of *T. minuta* and *H. latifolia* aerial parts was analyzed by GC and GC/MS, using a Shimadzu GC-17A chromatograph equipped with a DB-1 fused silica capillary column (60 m x 0.248 mm, film thickness 0.25 µm). The temperature was programmed from 60ºC (5 min) to 220ºC at 3ºC/min and the final temperature was held for 22 min, injector and detector temperatures: 230ºC and 250ºC, respectively, detector FID; carrier gas nitrogen at a flow of 0.9 mL/min. The GC/MS analysis was performed on a Perkin-Elmer, Q-Mass 910 G; separation was accomplished on a 30 m x 0.32 mm fused silica capillary column (Supelco SPB-1), film thickness 1 μm. The injector and detector temperatures 250ºC, oven temperature programmed from 60ºC (5 min), 60-220ºC (3ºC/min) and 220ºC (8 min), carrier gas helium at a flow of 1mL/min, operating at 70 eV. The identification of components was based on comparison of their mass spectra with those reported in literature (Adams, 2001) and by computer search of their 70 eV mass spectra with those stored in the library of the GC/MS data system, as well as by retention indices.

Toxicity in mites

The essences and components were prepared in a distilled water and emulsionant (Tween 20) solution and were pulverized on Varroa females in a Burgerjon tower (Burgerjon, 1956). Ten females of *V. destructor* were used in each assay. They were placed on Petri dishes and pulverized with 10 mg of each essence or component as it follows: Group A: 10 female mites pulverized with 3% solution of active ingredient. Group B: 10 female mites pulverized with 4% solution of active ingredient. Group C: 10 female mites pulverized with 5% solution of active ingredient. Control Group: 10 female mites pulverized with water and emulsionant.

Female mites were removed from brood cells, showing a normal movement after 15 min of their remotion. Afterwards, mites were transferred to a mesh covered dish containing 5 pupae in brown eyes stage for feeding and placed in incubation stove at 70% RH and 33-34ºC for 3 days. Dead and alive mites were counted 12, 24, and 48 after treatment. Five replications for each treatment were made. Data were analyzed by ANOVA for each oil, only considering the oil dose effect and means were compared by Duncan's Test.

Toxicity in adult bees

About 100 adult bees, without age differentiation, were placed inside metallic mesh cages (17 cm length x 10 cm width and 1.5 cm high) and were pulverized with 10 mg of solution 5% of the correspondent oil emulsionated in distilled water. Four replications of each sample were simultaneously taken. A control of distilled water plus emulsionant was used. Ten minutes after the treatments, the cages with bees were placed in stove to 70% RH and 33-34ºC. Receptacles containing candy for bees feeding were placed inside each cage. Dead and alive bees in each cage, 72 hours after the treatments were counted. Data were analyzed by PROC CATMOD (SAS, 1989).

RESULTS AND DISCUSSION

The main chemical components of the evaluated essential oils are presented in Table 1. For *T. minuta* the main compounds were (E)–ocimene y (Z)-ocimenone and for *H. latifolia* those are borneol y camphor.

The values of mite mortality after pulverization, for different treatments at different doses, showed that the effectiveness did not differ significantly between concentrations of 3, 4, and 5%, though there were significant differences ($P<0.05$) when comparing treatments with their respective control (water + emulsionant) (Table 2). The treatments with essential oil of *H. latifolia* and eucalyptol were more effective than those of *T. minuta*. The range of efficacy for those treatments was c.a 63 to 84%.

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Honeybee mortality for different treatments (Table 3) was low and did not significantly differ from the values registered for the control groups ($P>0.05$) varying between 2.05 and 12.17 %, except for eucalyptol in which the percentage of bee mortality reached values up to 70%

Table 1. Composition of the four principal essential oils of *Tagetes minuta* L. (Wild camomille) and *Heterotheca latifolia* (alcanforillo)

Essential oil	Compounds	Composition†
		%
Tagetes minuta L	(E)-ocimene	62.8
	(Z)-ocimenone	10.2
	(E)-ocimenone	6.6
	Limonene	5.8
Heterotheca latifolia	Borneol	31.5
	Camphor	27.2
	Limonene	7.2
	Camphene	6.4

† Relative percentage obtained from GC peak area

Table 2. Average and SD of female dead mites of *Varroa destructor* (5 replications, n=10 individuals each) for four treatments at different doses administered by spraying, at 48 hours.

Treatment, %	Dead Mites ± SD	Effectiveness
		%
Tagetes minuta		
3	6.4 ± 0.17 b†	64
4	7.2 ± 0.36 b	72
5	7.2 ± 0.29 b	72
Control	4.0 ± 0.13 a	40
Heterotheca latifolia		
3	7.6 ± 0.40 b	76
4	8.2 ± 0.38 b	82
5	8.4 ± 0.51 b	84
Control	3.8 ± 0.38 a	38
Eucaliptol		
3	7.8 ± 0.22 b	78
4	6.3 ± 0.14 b	63
5	7.1 ± 0.19 b	71
Control	3.9 ± 0.22 a	39

† Different letters means significant differences (P< 0.05).

Table 3. Bees' mortality for four different treatments after 72 h post-application. Dose was 10 mg of 5%.

Treatment	Dead Bees	Initial n	Mortality %
Tagetes minuta			
Try 1	9	113	7.31 a†
Try 2	8	103	7.76 a
Try 3	14	115	12.17 a
Try 4	6	121	4.95 a
Control	7	123	5.69 a
Heterotheca latifolia			
Try 1	4	151	2.64 a
Try 2	6	126	3.96 a
Try 3	10	138	4.34 a
Try 4	3	146	2.05 a
Control	12	139	8.63 a
Eucalyptol			
Try 1	64	94	68.0 b
Try 2	70	98	71.0 b
Try 3	60	103	58.0 b
Try 4	57	95	60.0 b
Control	5	97	5.15 a

† Different letters means significant differences for the same essential oil ($P<0.05$).

Up to the present time, it is unclear what the mode of action is of the essential oils or their components. Previous investigations referred (Ennan *et al*., 1998) that these compounds act on octopaminergic nervous system present in insects. The octopamin receptors are gone in the vertebrates. Therefore, there would be a perfect selective target for a pest control. Many essential oils and their components have biological activity related to insects, mites, bacteria, fungi, and nematods.

The results presented in this investigation showed evidences that the essential oil of *H. latifolia*, *T minuta*, and eucalyptol cause adult mite mortality when these are administered by pulverization. However, the eucalyptol also produces toxicity to adult workers in *A. mellifera* with more than 58% of mortality of the bees. Imdorf *et al.* (1995) studied the toxic effects of volatile compounds on mites and bees. They observed that with a concentration of 240 µg/L of eucalyptol produced a 25% of bee mortality. Furthermore, the use of eucalyptol as a treatment for controlling *Varroa* is uncertain, due to the fact that, there is a little difference in toxicity between bees and mites, even if it is administered by pulverization or evaporation. On the other hand, the oils from *H. latifolia* and *T. minuta* did not show significant differences related to toxicity on bees compared to control group. This phenomenon is very important because of the susceptibility of bees from some essential oils which has been well documented by (Lindberg *et al*., 2000). In relation to other essential oils obtained from this work, the percentage of mortality is low, compared to other studies. Gal *et al.* (1992) reported bee mortality values that ranged between 82 and 91% with the application of origanum oil (*Origanum vulgar*e L.), whereas Kraus *et al.* (1994) reported near 98% of bee mortality with the application of cinnamon oil (*Ocotea spixiana*). Although, in both cases the bee mortality was reached with the oil administration in a 10% solution, exactly the double of those used in this work. It is possible, that a high oil concentration used in this study could be contributed to improve the final efficacy in relation to *Varroa* control, even though it was not observed using concentrations from 3 to 5% (Table 2).

There are little information about the main chemical compounds and their mode of action of the essential oils of *H. latifolia* y *T. minuta.* According to previous studies the camphor affects the mite reproduction, although it also produces serious effects on the bee brood, while the limonene when it is used in high concentrations as pure compound exhibits low toxicity in mites and in bees (Imdorf *et al*., 1999). The borneol, which can be found in low concentrations in essential oil of *Salvia officinalis*, when it is administered simultaneously with the essential oil of *Thymus vulgaris* has been demonstrated an efficacy over 90% against *Varroa* (Colin *et al*., 1994). Similarly, the essential oil of *T. minuta* has not show repellent effects or attraction effects on adult female *Varroa* (Eguaras *et al*., 2005).

The percentage of efficacy and control of the mite presented in this work seem to be low in comparison to those obtained from synthetic acaricides commonly used to control *Varroa*. Although, the development of miticides made from natural substances do not pretend to avoid the use of conventional products to control the mite, but at least reduce the use these chemicals to control mite populations. Therefore, the results presented in this investigation suggest that the essential oils of *H. latifolia* and *T. minuta* could play an important role to use these plants derivates to establish an integrated pest management program to reduce mite populations in *A. mellifera* colonies. Although, this knowledge should be taken into account to control mite based on natural products, further studies need to be done in order to identify some other factors that may be involved in a successful control of Varroosis. Currently, there are very few commercial formulations based on essential oils or their components to be use to control *Varroa* mite.

CONCLUSIONS

These results suggest that *T. minuta* and *H. latifolia* essential oils could play an important role as an integrated program management in controlling the Varroosis in honey bee colonies whereas the eucalyptol exhibited a high mortality to the adult honeybees.

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