



## EVALUATION OF *TAGETES MINUTA* L. ESSENTIAL OILS TO CONTROL *VARROA DESTRUCTOR* (ACARI: VARROIDAE).

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### Abstract

The *Varroa destructor*, an ectoparasitary mite of the *Apis mellifera*, causes serious damage to beekeeping. Synthetic acaricides have been used to combat the *V. destructor* but mites have developed resistance against them, moreover, it has been reported that synthetic acaricides contaminate honey and wax. Natural products, such as essential oils, might be an option to control *V. destructor*. Acaricidal effects of *Tagetes minuta* L. essential oils on *V. destructor* mites were studied. The essential oils were extracted by steam distillation from different parts of the plant in diverse stages of their vital cycle. Live *V. destructor* mites were sampled from an infested experimental apiary to which no previous acaricide treatments had been applied. Groups of ten mites were kept in

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Petri dishes with small oiled wooden sticks under controlled atmosphere. After three hours of exposure, the essential oil obtained from flowers showed the highest toxicity for the mites, causing 85 % of mortality, while mortality counts corresponding to the oil from leaves of bloomed plants and oil from leaves of plants that had not yet bloomed were 57 % and 38 % respectively. After six hours, most mites exposed to any of the oils were dead. Oils toxicity for bees was also evaluated. Groups of ten bees each were enclosed with wooden sticks that had been treated with the essential oil extracted from flowers and no toxic effect was found after three and six hours of exposure. These results opens the possibility of developing new alternative methods to control *V. destructor*.

**Keywords:** *Tagetes minuta* L; essential oil; varroicide power

### Resumen

La *Varroa destructor*, ácaro ectoparasitario de la *Apis mellifera*, ocasiona serios daños a la apicultura. Para combatirla se han usado acaricidas sintéticos, pero se ha reportado que los ácaros han desarrollado resistencia a sus principios activos y se han encontrado residuos de los acaricidas en miel y cera. El uso de productos naturales, tales como aceites esenciales, podría ser una alternativa para el control de la *V. destructor*. Se estudiaron los efectos de aceites esenciales de *Tagetes minuta* L. sobre los ácaros de *V. destructor*. Se extrajeron aceites esenciales de diferentes partes de la planta en distintas etapas de su ciclo vital mediante destilación por arrastre de vapor. Se tomaron muestras de ácaros vivos de *V. destructor* de un apiario experimental infectado, al cual no se le había aplicado ningún tratamiento acaricida previo. Se colocaron, bajo condiciones controladas, grupos de diez ácaros en cajas de Petri agregando a cada una de ellas un disco de madera tratado con aceite. Después de tres horas de exposición, el aceite obtenido de flores mostró ser el más tóxico para los ácaros registrando 85 % de mortalidad, mientras que los conteos de mortalidad correspondientes a aceites de hojas de plantas florecidas y de plantas no florecidas fueron 57 % y 38 % respectivamente. Después de seis horas, casi todos los ácaros expuestos a cualquiera de los aceites estaban muertos. Además, se evaluó la toxicidad de los aceites para las abejas. Se repitió la experiencia realizada con los ácaros pero utilizando grupos de diez abejas sanas y sólo el aceite esencial de flores. No se detectaron efectos tóxicos significativos después de tres y seis horas de exposición. Estos resultados estimulan el desarrollo de nuevos métodos alternativos para controlar la *V. destructor*.

**Palabras Claves:** *Tagetes minuta* L, aceite esencial, acción varroicida

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## Introduction

*V. destructor* is a parasitic mite that infests its natural host, the honey bee. The disease caused by the varroa mites is called varroasis and it is one of the main causes of economic damages to the beekeeping industry.

Varroa eggs are laid on bee larva for hatching. The young mites attach to the body of bee pupae and feed from its hemolymph. When the bee emerges from the cell, some mites remain attached while others may spread to other bees and larvae, weakening them by sucking hemolymph and spreading viruses.

Varroasis is especially harmful in areas where winters are not very rigorous and bee activity is maintained year round, facilitating an uninterrupted reproduction of the mites and the consequent gradual deterioration of bee population.

In Argentina, this disease has become endemic and has been responsible for massive death of honey bee colonies, particularly in the Province of Chaco, where severe outbreaks are frequent.

The use of synthetic acaricides has been the most effective method for controlling this pest, but mites resistance to traditional acaricides, such as fluvalinate and flumethrin, has been reported (Elzen et al. [1], Milani [2], Imdorf et al. [3]). Another shortcoming of traditional acaricides is that they can contaminate hive products, especially honey and wax. So, the development of different and safer ways to control *V. destructor* would help reduce the use of such conventional chemical mite-

control methods in beekeeping. Natural products, such as essential oils, represent an alternative to synthetic acaricides for controlling *V. destructor*.

Lindberg et al. [4], Abbadi and Nazer [5], Neira et al. [6] and González-Gómez et al. [7] report that these oils might be useful in reducing both mite infestation rates and hive contamination.

Essential oils are volatile secondary metabolites that plants produce for their own needs other than nutrition (i.e. protection or attraction). In general, they are complex mixtures of organic compounds that give the plants their characteristic odour and flavour.

The *T. minuta* L. is a plant specie native to South America, which is vulgarly called "chinchilla". It grows wild in the spring and practically disappears with the beginning of the winter after its complete life cycle.

Bees usually visit the flowers of this species (Figure 1). This plant is well known among beekeepers and constitutes the last source of pollen and nectar in autumn. We corroborated, by means of a melissopalynological analysis, the presence of *T. minuta* L. pollen in the honey of the hives used for this work.



**Figure 1.** Bees visiting flowers of *Tagetes minuta* L.

Studies on *T. minuta* L. essential oils reported diverse biocidal activity on several organisms (Zygadlo et al. [8], Vasudevan et al. [9]).

For all these reasons we consider the use of *T. minuta* L. essential oils as a possible organic alternative for *V. destructor* control.

The aim of the present work is to evaluate the biocide power of the essential oils of *T. minuta* L. by means of laboratory bioassays.

Some authors have reported that the composition of essential oils varied according to the harvesting location (Zygadlo et al. [8]), the growth stage (Ybarra et al. [10]) and the different parts of the plant (Weaver et al [11]). In order to evaluate varroicidal power of the *T. minuta* L. essential oils, it is necessary to consider such variations.

## Materials and methods

### *Essential oils extraction*

Three different oils were tested, all of them obtained by steam distillation: oil from leaves of plants that had not yet bloomed, oil from leaves of bloomed plants and oil from flowers. (Chamorro, et al [12])

The leaves and flowers of *T. minuta* L. used in this work were collected in the Province of Chaco (Argentina). The collected material was kept in the shadow for 24-48 hours, and then it was cut into small pieces, loaded to a 100 L hydro-distiller and compacted.

Water steam was forced into the distiller, heating the vegetable material and dragging its essential oils to a condenser, where an emulsion was formed. The oils were separated from the emulsion in a dynamic decanter, collected in closed glass containers and stored in the dark at 4°C until their use.

### *Mites collecting*

Live *V. destructor* mites were sampled from an infested experimental apiary located in the Province of Chaco (Argentina) in which no acaricide treatments had been previously applied. Infested adult bees were placed in a wooden box with two of the parallel sides made of wire netting. Mites were forced to abandon the bees by sprinkling confectioners' sugar onto the bees through the wire netting. Then the mites were collected, placed in a Petri dish and immediately taken to the laboratory.

### *Mite mortality assay*

Using a syringe, 4 µL of oil was applied to a small wooden disk (7 mm diameter, 2 mm height approximately) located in the center of a Petri dish. Immediately afterwards ten live mites were placed in the dish and a top was put on. The dish was kept in a controlled atmosphere at 32°C and 40-50 % relative humidity. The number of dead mites was counted after 3 and 6 hours. Groups of three dishes were used for each test and each test was replicated several times. Tests were carried out with three different oils. Reference tests were carried out under the same conditions but without using oils.

### *Bee tolerance assay*

Ten healthy bees and a wooden disk (20 mm diameter, 2 mm height approximately) with 200 µL of oil obtained from *T. minuta* L. flowers were placed inside a 1 L flask with a mesh fabric cap. The flask was kept in a controlled atmosphere at 32°C and 40-50 % relative humidity. The number of dead bees was counted after 3 and 6 hours. Groups of three flasks were used for each test and each test was replicated three times. Reference tests were carried out under the same conditions but without using the oil.

### *Statistical analysis*

The nonparametric test of Kruskal-Wallis was used to identify differences among the effects of different oils on mites and to evaluate the bee tolerance to the flowers oil. A significance level of 0.05 was selected for discrimination criteria.

The Kruskal-Wallis test tests the null hypothesis that the medians of the response within each of the levels of the variable factor are the same. The data from all the levels is first combined and ranked from smallest to largest and then, the average rank is computed for the data at each level.

## Results and discussion

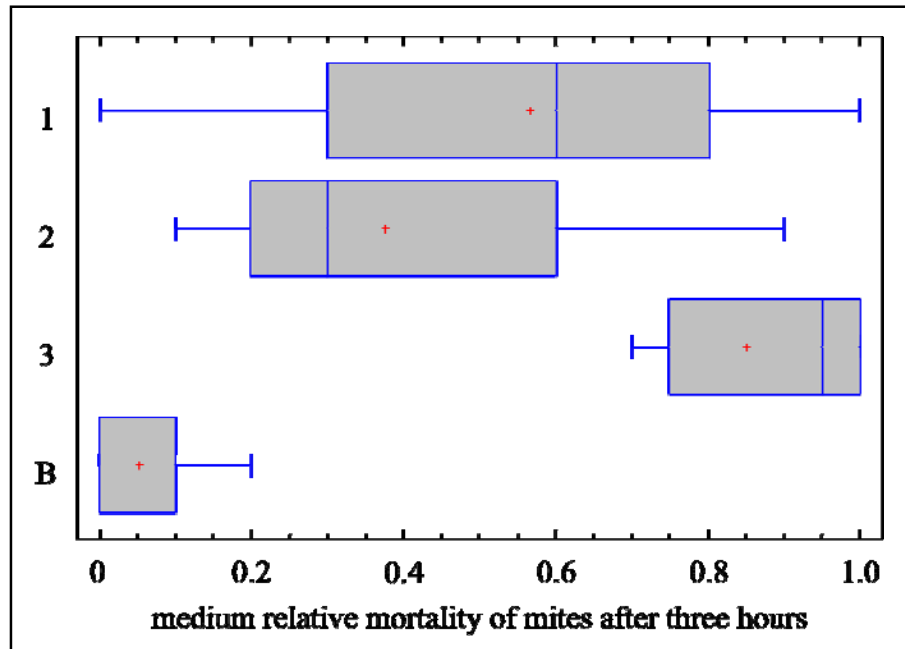
Table 1 shows the medium percentage of dead mites after three and six hours of treatment with each oil and without oil. The corresponding results for Kruskal-Wallis tests are shown in Table 2 and box-whisker plots for three and six hours of treatment are shown in Figures 2 and 3 respectively.

**Table 1.** Medium percentage of dead varroa mites.

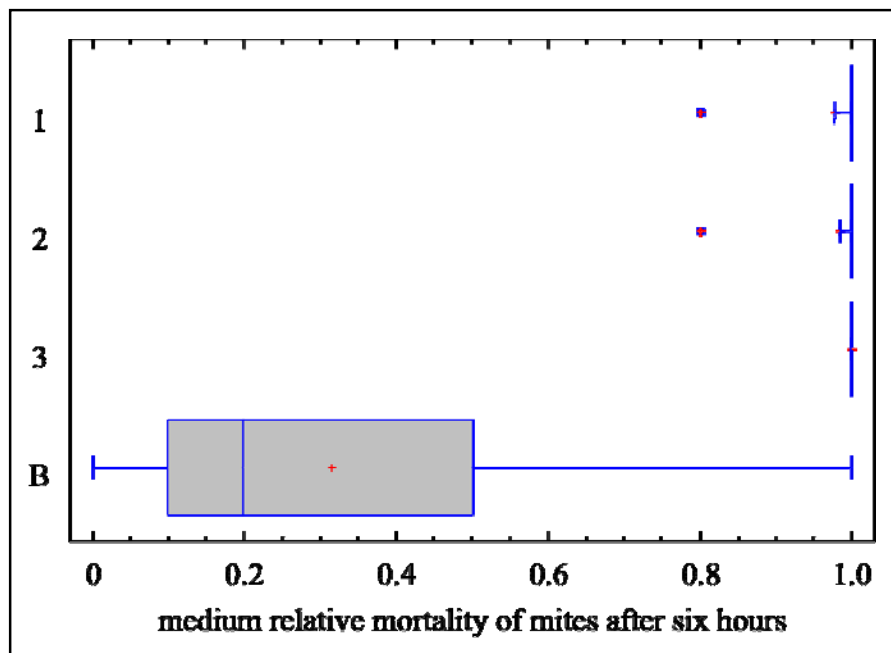
	<b>3 hours</b>	<b>6 hours</b>
Oil from leaves of bloomed plants	56.6	97.7
Oil from leaves of not bloomed plants	37.5	98.3
Oil from flowers	85.0	100
Reference (no oil)	5.2	31.5

**Table 2.** Kruskal-Wallis test for mites mortality by treatment.

	<b>3 hours</b>		<b>6 hours</b>	
Number of observations	60		60	
Number of levels	4		4	
	<b>Sample Size</b>	<b>Average Rank</b>	<b>Sample Size</b>	<b>Average Rank</b>
Oil from leaves of bloomed plants	9	40.2	9	41.9
Oil from leaves of not bloomed plants	12	36.2	12	42.5
Oil from flowers	12	50.8	12	44.0
Reference (no oil)	27	15.7	27	15.4
Test statistic	41.1		44.4	
P-value	6.1 x 10 <sup>-9</sup>		1.2 x 10 <sup>-9</sup>	



**Figure 2.** Box-whisker plot for three hours of treatment. 1: Oil from leaves of bloomed plants. 2: Oil from leaves of not bloomed plants. 3: Oil from flowers. B: Reference (no oil).



**Figure 3.** Box-whisker plot for six hours of treatment. 1: Oil from leaves of bloomed plants. 2: Oil from leaves of not bloomed plants. 3: Oil from flowers. B: Reference (no oil).

Table 3 shows the medium percentage of dead bees after three and six hours when they were enclosed in flasks with and without oil. The corresponding results for Kruskal-Wallis tests are shown in Table 4.

**Table 3.** Medium percentage of dead bees.

	<b>3 h</b>	<b>6 h</b>
Oil from flowers	15.6	24.4
Reference (no oil)	14.4	24.4

**Table 4.** Kruskal-Wallis test for bees mortality by treatment

	<b>3 hours</b>		<b>6 hours</b>	
Number of observations	18		18	
Number of levels	2		2	
	<b>Sample Size</b>	<b>Average Rank</b>	<b>Sample Size</b>	<b>Average Rank</b>
Oil from flowers	9	9.72	9	9.67
Reference (no oil)	9	9.27	9	9.33
Test statistic	0.04		0.02	
P-value	0.84		0.89	

It was found that mites' mortality was much higher when they were exposed to *T. minuta* L. essential oils than when they were not, which demonstrates the acaricidal effect of the oils. This was confirmed by the Kruskal-Wallis tests (Table 2). Due to the very low P-values, much lower than 0.001, statistically significant differences amongst the medians are assured at a confidence level better than 99.9 %.

The essential oil obtained from flowers demonstrated the highest mortality rate and proved to be the most toxic for the mites while the oil from leaves of not bloomed plants was the least toxic. Nevertheless, after enough time of exposure, any of the three oils was lethal for the mites; note that after six hours of exposure to any of the oils, most mites were already dead.

Differences in the acaricidal power of the essential oils can be explained by the differences in their composition, that vary depending on the parts of the plant and its growth stage, so we are considering further studies to determine the active components of the oils.

To evaluate the bees' tolerance we use the oil obtained from flowers, which was the most toxic for mites. In this case, the concentration of oil used, with respect to the volume of the container, was five times greater than the concentration used for the mites' mortality assay.

At a 95 % confidence level, no significant difference in mortality between bees that were exposed to the oil and those that were not was found (Table 3). This was made evident since the P-values obtained from the Kruskal-Wallis tests were greater than 0.05, thus, in this first instance, it can not be said that this oil is toxic for bees.

These results are highly encouraging, nevertheless, given the low predictive value of the laboratory assays referred to in the bibliography (Imdorf et al., 2000 [3]), it is necessary to test the oils effectiveness in apiaries under natural conditions.

Other works, at laboratory scale, have been carried out to evaluate varroicidal power of essential oils. These works involve a great number of variables, such as the kind of oils, the way they are applied, the applied amounts and concentrations, the time of exposure, etc, that make it difficult to compare the results.

Neira et al., (2004) [6] worked with lavender and laurel oils, and evaluated the mites on infected bees, obtaining 42 % and 35 % dead mites after 24 h of exposure to lavender oil and laurel oil respectively.

González-Gómez, R. et al., (2006) [7] worked with crude extracts of neem seeds and evaluated the effects on mites and bees separately. No toxic effect was found for either mites or bees. They also found an important repellency effect. Treating bee pupae with the extracts prevented *V. destructor* mites from settling on pupae, impeding mites' feeding and causing their deaths.

Abadi et al., (2003) [5] tested five volatile oils and three plant materials. They found that worm wood flowers, clove oil and peppermint oil, which seemed to have no adverse effects on bees, were the most effective in controlling *V destructor*.

## Conclusions

*T. minuta* L essential oils are toxic for *V. destructor*, being the oil obtained from flowers the one that showed the highest toxicity for the mites, while no statistically significant toxic effect was found on bees that were exposed up to six hours to this oil. These results opens the possibility of developing new alternative methods to control *V. destructor*.

Although these results are highly encouraging, it is still necessary to test the oils effectiveness in apiaries under natural conditions.

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