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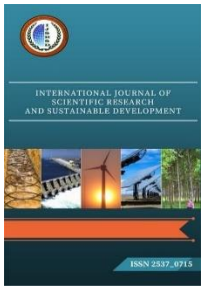

Evaluation of acaricide toxicity to the parasitic mite (*Varroa destructor*), honeybee workers (*Apis mellifera* L.) and their residues in honey and beeswax

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Abstract

Evaluate the toxic effects of five acaricides on the parasitic mite (*Varroa destructor*) and honeybee workers (*Apis mellifera* L.): Denfer 15% (Spirodiclofen), Kofex 24% (Spiromesifen), Metake 20% (Amitraz), Koloum super 24% SC (chlorfenapyr), and Abalon 1.8% (Abamactine). These conventional acaricides are cheap and easy to apply, but prolonged use causes Varroa mites to rapidly develop resistance, and bee products can become contaminated with residues of acaricides. The bioassay test was used to detect acaricides toxic effects. Spirodiclofen and Spiromesifen were the most toxic compounds to honeybee worker with LC_{50} values of 0.923 and 1.195 ug/ml, respectively, after 5 days, but Amitraz, Chlorfenapyr, and Abamactine were the least toxic compounds to honeybees with LC_{50} values of 1.752, 42.72, and 56.868 ug/ml, respectively, after 5 days. Amitraz, Spiromesifen and Spirodiclofen were the most toxic compounds on *V. destructor* with LC_{50} values of 0.497, 0.888 and 2.014 ug/ml, respectively, after 4 h, but Abamactine and chlorfenapyr were the least toxic compounds on *V. destructor* with LC_{50} values of 70.972 and 76.62 ug/ml, respectively, after 3 h. Varroa mite infestation rates decreased in brood (75.5% vs 67%) and adult honeybee workers (77.16% vs 100%) of colonies treated with Spiromesifen and Amitraz, respectively, when compared to only non-treated colonies. Detecting residuals of acaricides by HPLC in honey and beeswax revealed that they were contaminated with Amitraz and Spiromesifen residues, but that they did not exceed the MRLs established by EPA and European Commission guidelines.

Keywords: acaricides, *Varroa destructor*, *Apis mellifera*, Wax, Honey, HPLC

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Introduction

Honeybees provide highly valued pollination services for a wide variety of agricultural crops (Khalifa *et al*., 2021). However, over the last few years, a lot of studies have been published about the losses of honeybee colonies and the declining population of native and wild bees. Several factors are implicated behind these losses including pathogens and parasites, poor nutrition, beekeeping management, climate change, and pesticides (Flores *et al*., 2021; Hristov *et al*., 2020).

The parasitic mite, *Varroa destructor*, became the single greatest threat to honeybee health, the apiculture, and pollination industries, since it spread from its native host, the Asian honeybee (*Apis cerana*) to the naive European honeybee (*A. mellifera*). Traditionally, Varroa mites has been controlled with synthetic pesticides, but residues in honeybee products and recently arisen resistances to chemicals call for new control methods (Hernandez *et al*., 2022).

Varroa infestation occur mainly during winter and causes significant impacts on honeybee colony health because of its transmission of a cocktail of viruses while feeding on honeybee fat bodies (Ramsey *et al*., 2019). Highly infested of weak colonies facilitate mite dispersal and disease transmission to stronger and healthier colonies (Gisder & Genersch, 2021). In untreated honeybee colonies, heavy Varroa infestation causes 100% mortality in a few weeks.

Synthetic acaricides such as fluvalinate, flumethrin, amitraz, coumaphos, and cymiazole have been successfully used to control *V. destructor*. However, the use of acaricides inside beehives implies a risk of contamination of honey and other hive products (beeswax for example). Acaricides mostly contaminate beeswax due to its non-polar nature, while honey remains relatively free of contaminants (Gisder & Genersch, 2021). In addition, the mite has already developed resistances to chemicals that limit their use (Higes *et al*., 2020).

Amitraz is a nonsystemic acaricide and insecticide which was less harmful to mammals. It is also widely used in the beekeeping industry as a control for the *V. destructor* mite, although there are recent reports of resistance (Hernández-Rodríguez *et al*., 2021). Abamectin, spirodiclofen (registered for mite control in 2007), spiromesifen , and chlorfenapyr were used to control acaricide (miticide) (Bahreini *et al*., 2020, 2022).

The purposes of the study were (1) evaluate the efficacy of acaricides as a potential chemical control agent of *V. destructor* (2) assess potential drawbacks for honeybee colonies specially to brood and adult (3) detecting residuals of tested acaricides by HPLC in honey and beeswax.

Materials and methods

Tested acaricides

Metake (Amitraz, 20%) is commercially available and obtained from Al-Motaheda Company, Egypt.

Abalon (Abamactine, 1.8 %) obtained from International Company for Chemicals and Commercial Agencies – Acta, Egypt.

Denfer (Spidoclofen, 15%) obtained from European group chemicals company, Egypt.

Kofex (Spiromesifen, 24%) obtained from Agrimar Commercial Agencies Company.

Koloum super (chlorfenapyr, 24%) is commercially available and obtained from Match Chem Company for Chemicals and Specialty Vehicles.

Honeybee used for toxicity test (in vitro study)

Foraging honeybee workers were collected between 9.00 AM and 12.00 noon from healthy hives from the Faculty of Agriculture apiary, Benha University, Egypt. The bees were shaken from the frames into wooden cages (with two screen wire sides and a hole in the top of the cage) and then transported to the

laboratory of eco-toxicology in the Department of plant protection, Faculty of Agriculture, Benha University, Egypt.

Mites used for toxicity tests (in vitro study)

Five honeybee colonies that were heavily infested with varroa mites (*Varroa destructor*) and that had not been treated with acaricides for at least a year were used as mite sources. They were gently transferred to the petri dishes with a small paint brush and placed in a room at 28±1°C and 65% ±5R.H. Each one containing five mites as a replicate, four replicates for each concentration, (Gashout & Guzmán-Novoa, 2009).

Toxicity testes

The residual bioassay method (used the paper filtration, which was imbedded for 1 minute in each concentration solution and put in the bottom.) It has been used to detect acaricide toxicity to honeybee workers and Varroa mites (Aliano *et al*., 2006; Hbl *et al*., 2021). Bee workers and Varroa mites were exposed to a variety of acaricide concentrations via serial dilution. For each tested compound, four concentrations were prepared for bioassay on honeybee workers and Varroa mites. A mortality rate was recorded after 1, 2, 3, 5, and 7 days of treatment for honeybee workers.

Mortalities *for V. destructo*r were recorded after 1, 2, 3 and 4 h of 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 when kept for more than 4 h out of their natural habitat and may suffer from starvation and water loss (Bava *et al*., 2021; Milani, 2001). A considerable proportion of controlled, untreated mites can die in less than 6 hours. The parasites were transferred to a petri dish and examined under a stereoscopic microscope. Mites that did not move when probed with a fine paintbrush were dead. The recorded mortalities were corrected according to Abbott's formula (1925).

Field experiment

Based on the results of toxicity testing for acaricides on Varroa mites. Amitraz and spiromesifen showed the greatest acaricidal effect in the screening tests on Varroa mites. They were prepared as LC_{99}s for additional toxicity bioassays. The present experiment was undertaken on the Carniolan hybrid honeybee (*Apis mellifera* L.) at the Faculty of Agriculture apiary, Benha University, Egypt from November to December 2021.Nine honeybee colonies which are approximately similar in their strength (number of brood combs, food stores, number of combs covered with bees, and mated young queen aged 5–6 months old) were prepared for this experiment. These colonies were randomly divided into three equal groups (Amitraz group, spiromesifen group, and control group). Treatments were applied in the form of carton paper (20 × 20 cm), which was impregnated for 5 minutes in each tested acaricide solution and put directly on the top of brood combs. All treatments were applied once a week for six weeks..

Data and sample collection

An initial set of measurements and samples were taken from the colonies prior to treatments. Infestation with *V. destructor* in all tested colonies during the experimental period was determined in worker sealed brood (pupae with pigmented eyes). For pupae, 10 individual cells for each tested colony were inspected. In adult workers (collected from the brood nest), the percentage of infestation (%) was determined in approximately 100 living adult bee workers picked directly from the combs. The infestation percentage was determined before and after acaricide treatments.

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Acaricides residue analysis

Honey and beeswax samples were also sampled for acaricides residue analysis. Honey samples were collected directly from each colony at about (50 g from random locations near and far from the application form of the acaricide. Wax samples (about 50 g each) were randomly cut into 2.5 cm^2 at different locations, including the area of strip application. One composite sample (three colonies) from each application form was made. Samples were taken at the start and end of the experiment procedures (Calatayud-Vernich *et al*., 2018).

Extraction and cleanup of two acaricides

Levels of Amitraz and Spiromesifen in both honey and wax samples were determined according to the method of **Chaimanee *et al.*, 2022** with some modification. Pesticides residues were extracted with 50 ml acetone then mixture was shaken mechanically using electrical shaker for one hour and filtered. The filtered sample was rinsed twice with (25 * 2) acetone and partitioned into n. hexane. The extract was carefully decanted and filtered through filter paper Watman No. 1 then dried through anhydrous sodium sulphate and evaporated on water both of 40 C^o to dryness by using rotary evaporated. The resulting extract of honey and wax were cleaned by C18 cartridge column chromatography. The two acaricides extracts were dissolved in 1 ml methanol and then determined by Agilent HPLC apparatus at the same conditions as described above.

HPLC condition:

High-performance liquid chromatography instruments (Agilent serial 1100) solvent delivery system and quaternary pump with a C18 stainless steel column (2.4 * 250 rm) and UV detector was used under the following conditions as shown in **Table 1**.

Table 1. HPLC conditions for analysis

Acaricides	Solvent system	FLW Rate	Wavelength	Limit of Detection
Amitraz	Acetonitrl 70 % Methanol 30 %	1 ml/min	197	0.05 ng
Spiromesifen	Acetonitrl 70 % Methanol 30 %	1 ml/min	197	0.03 ng

Preparation of standard solution

Weigh 10 mg (related to a purity of 100 %) from both of Amitraz and Spiromesifen reference standard material for each one into a 10 ml volumetric flask and dilute to the mart with methanol LC grade and mixing well.

Calibration

Duplicate injections (5 ul) of Calibration solution and each sample were injected and integrated areas for each peak was recorded. Content of active ingredient in each sample was calculated compared with an external standard

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Results and discussion

Toxicity of tested acaricides on honeybee worker

The effects of the five commercial acaricides were used in the experiments showed variable levels of mortality on honeybee worker mortalities dependent on the time after treatments and concentrations as shown in **Table 2**. After 1, 2, 3, 5, and 7 days with Spirodiclofen at concentrations of 2.4 Ug/m, the mortality percentages were 60, 66, 70, 80, and 90%, respectively, while it was 10, 10, 13, 20, and 40% at concentrations of 0.3 Ug/m. Also, the mortality percentages at concentration 4.8 Ug/m after 1, 2, 3, 5 and 7 days of the treatment with Spiromesifen were, 73.3, 80, 85, 100, and 100 %, respectively, while they were 0, 10, 13.3, 26.7 and 60 % at concentration 0.6 Ug/m at the periods. Amitraz showed the same results with 75, 60, 90, 100 and 100 % mortality after 1, 2, 3, 5 and 7 days of treatment at a concentration of 4.8 Ug/m, respectively, while it was 13.3, 20, 20, 44.6 and 66 % at 0.6 Ug/m.

Table 2. Efficacy of tested acaricides on mortality percentage on honeybee worker at 28 ±1°C and 65±5 RH

Acaricides	Accumulative mortality % after indicated days					
	Conc. Ug/m	1day	2 days	3 days	5 days	7 days
Denfer 15% (Spirodiclofen)	2.4	60	66	70	80	90
	1.2	33	40	46	65	75
	0.6	20	29	33	25	53.3
	0.3	10	10	13	20	40
Kofex 24% (Spiromesifen)	4.8	73.3	80	85	100	100
	2.4	16.7	40	70	80	90
	1.2	0	20	33.3	43.3	66
	0.6	0	10	13.3	26.7	60
Metake 20% (Amitraz)	4.8	75	60	90	100	100
	2.4	33.3	40	50	90	98
	1.2	20	22	35	61	89
	0.6	13.3	20	20	44.6	66
Koloum super 24% SC (chlorfenapyr)	576	90	92	96	100	100
	288	80	88	92	95	100
	144	73	80	83.3	90	90
	72	13.3	33.3	40	65	88
	36	0	13.3	26.7	40	80
	18	0	10	13.3	25	40
Abalon 1.8 % (Abamactine)	230.4	70	80	100	100	100
	115.2	65	50	75	90	100
	75.6	46.6	48	51	75	80
	28.3	10	13.3	13.3	20	52

In the case of treatment with chlorfenapyr, the mortality percentages at concentrations of 576 Ug/m after 1, 2, 3, 5 and 7 days of treatment were 90, 92, 96, 100 and 100 %, respectively, but at the same periods with 18 Ug/m were, 0, 10, 13.3, 25 and 40 % mortality. After 1, 2, 3, 5, and 7 days of treatment with abamactine at concentrations of 230.4 Ug/m, there were 70, 80, 100, 100, and 100% mortalities, respectively,

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but at the same periods with 28.3 Ug/m concentrations, there were 10, 13.3, 31.3, 20, and 52.0% mortality, respectively.

LC_{50} and LC_{99} ug/ml values for tested acaricides on honeybee worker*:*

As shown in **Table 3**, the LC_{50} values of tested acaricides and safety indices for *Apis mellifira* L. according to the LC_{50} and T.I. values.

Spirodiclofen and Spiromesifen were the most toxic compounds to worker bees with LC_{50} values of 0.923 and 1.195 ug/ml, respectively, after 5 days, but Amitraz, chlorfenapyr, and Abamactine were the least toxic compounds to honeybees with LC_{50} values of 1.752, 42.72 and 56.868 ug/ml, respectively, after 5 days.

Based on the LC_{50} and toxicity index (T.I.) values, the tested acaricides can be arranged in ascending order from the most to the least toxic as follows: Spirodiclofen, Spiromesifen, Amitraz, chlorfenapyr, and Abamactine, respectively.

Laboratory experiments were carried out to determine the LC_{50} values, after 24 and 48 h of treatment of honeybee workers with five acaricides (Table 2). Based on LC_{50} values, after 24 and 48 h of treatment, Denfer 15% was the most toxic compound, followed by Kofex 24%, Metake 20%, Koloum super 24% SC, and Abalon 1.8 %. The US EPA (2018) classified pesticides based on LD50 values for bees as non-target insects into three categories: non-toxic (> 11g/bee), moderately toxic (21g/bee), and highly toxic (2g/bee). So, Denfer 15%, Kofex 24% and Metake 20% are considered highly toxic to honeybees. In contrast, Abalon 1.8 % and Koloum super 24% SC are considered non-toxic to honeybees.

Table 3. Toxicity data of tested acaricides on honeybee workers.

Acaricides	Exposure period	LC_{50}	LC_{99}	Slope±sE	TI
Denfer 15% (Spirodiclofen)	5days	0.923 (0.643-1.367)	12.425 (5.453-122.05)	1.70±0.96	100
Kofex 24% (Spiromesifen)	5days	1.195 (0.884-1.611)	10.969 (5.177-87.98)	1.362±0.94	77.23
Metake 20% (Amitraz)	3days	1.752 (1.228-2.524)	21.171 (9.33-165.39)	2.253±0.92	52.38
Koloum super 24% SC (chlorfenapyr)	5 days	42.73 (28.416-58.854)	572.522 (288.29-2391.06)	0.962±0.99	2.16
Abalon 1.8 % (Abamactine)	5 days	56.686 (44.038-68.715)	185.206 (129.29-437.04)	2.804±0.90	1.62

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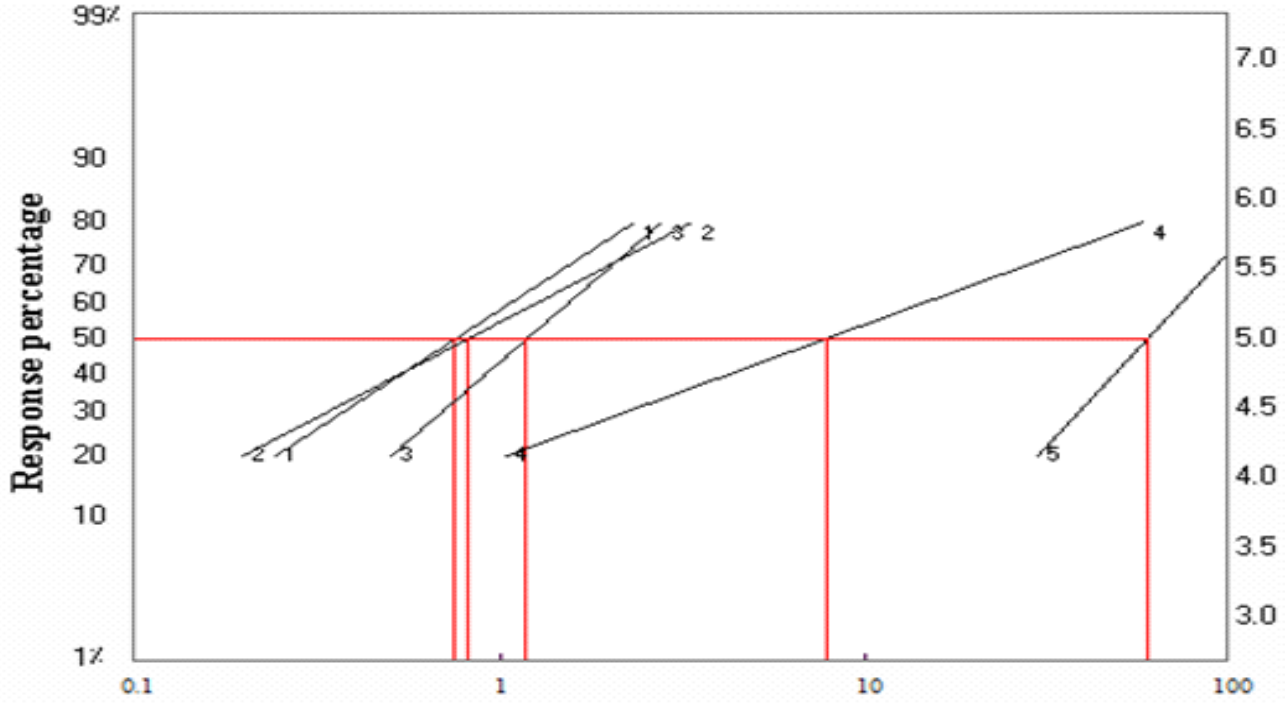

Fig 1. depicts the LC_{50} values and the relationship between the tested acaricides and the honeybee worker.1 =Spirodiclofen2 =Spiromesifen3=Amitraz4= chlorfenapyr5 =Abamactine.

Toxicity of tested acaricides on *V. destructor:*

The effects of the five commercial acaricides that were used in our experiments showed variable levels on *V. destructor* mortalities dependent on the time after treatments and concentrations, as mentioned in **Table 4**. All treatments caused the highest accumulation mortality after 4 h at any concentrations.

The mortality percentages for Spirodiclofen were 20% and reached 80% after 4 h at 8.16, 4.8, 2.4, 1.2, 0.6, and 0.3 Ug/m. While the mortality percentages were 26.6% and reached 86.6% after 4 hours, at concentrations of 4.8, 2.4, 1.2, 0.6, and 0.3 of Spiromesifen. Amitraz caused 80% mortality after 4 h at 2.4 Ug/m concentrations and 33.3% mortality at 0.3 Ug/m concentrations. Chlorfenapyr and Abamactine caused 86.6% and 100% mortality at concentrations of 576 and 230.4 Ug/m after 4 h, respectively.

LC_{50} values of tested acaricides and safety indices for *V. destructor* based on the LC_{50} and toxicity index (T.I.) values were illustrated in **Table 5 and Fig 2**.

Amitraz, Spiromesifen and Spirodiclofen were the most toxic compounds on *V. destructor* with LC_{50} values of 0.497, 0.888 and 2.014 ug/ml, respectively, after 4 h, but Abamactine and chlorfenapyr were the least toxic compounds on *V. destructor* with LC_{50} values of 70.972 and 76.62 ug/ml, respectively, after 3 h. The results also indicated that the T.I. values of the tested acaricides can be arranged in ascending order from the most to the least toxic as follows: Amitraz, Spiromesifen, Spirodiclofen, Abamactine, and chlorfenapyr, respectively. Amitraz and carbamate were the most toxic compounds to *Varroa*, and only differed by 2-fold at the LC_{50} level (**Jack and Ellis, 2021).**

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Table 4. Efficacy of tested acaricides on mortality percentage on *V. destructor* at 28 ±1°C and 65±5 RH

Acaricides	Accumulative mortality % after indicated days				
	Conc (Ug/m)	1h	2h	3h	4 h
Denfer 15% (Spirodiclofen)	8.16	20	40	53.3	80
	4.8	20	33	40	66.6
	2.4	10	20	20	53.3
	1.2	0	10	20	40
	0.6	0	10	33	33
	0.3	0	10	13	20
Kofex 24% (Spiromesifen)	4.8	30	40	60	86.6
	2.4	20	33.3	53.3	66.6
	1.2	13.3	20	33	60
	0.6	10	20	30	40
	0.3	0	10	20	26.6
Metake 20% (Amitraz)	2.4	20	20	50	80
	1.2	10	22	40	73.3
	0.6	10	20	26.6	60
	0.3	0	20	20	33.3
Koloum super 24% SC (chlorfenapyr)	576	33.3	60	73	86.6
	288	20	53.3	66	73.3
	144	10	30	50	60
	72	0	20	40	53.3
	36	0	10	20	33.3
Abalon 1.8 % (Abamactine)	230.4	50	60	80	100
	115.2	30	50	60	100
	75.6	20	30	53.3	62
	28.3	10	13.3	26.6	50

LC_{50} and LC_{90} ug/ml values for tested acaricides on *V. destructor*

Table 5. Toxicity data of tested acaricides on *V. destructor*.

Acaricides	Exposure period	LC_{50}	LC_{99}	Slope±sE	TI
Metake 20% (Amitraz)	4 h	0.497-	22.13	1.411+0.97	100
Kofex 24% (Spiromesifen)	4 h	0.888 (1.523-0.424)	46.03	1.357+0.98	55.96
Denfer 15% (Spirodiclofen)	4 h	2.014 ((4.542-0.949)	318.95	1.058+0.95	24.677
Abalon 1.8 % (Abamactine)	3 h	70.972 (30.461-30.461	2163.7	1.567+0.53	0.7
Koloum super 24% SC (chlorfenapyr)	3 h	76.62 (137.81-22.22)	6988.9	1.187+0.98	0.649

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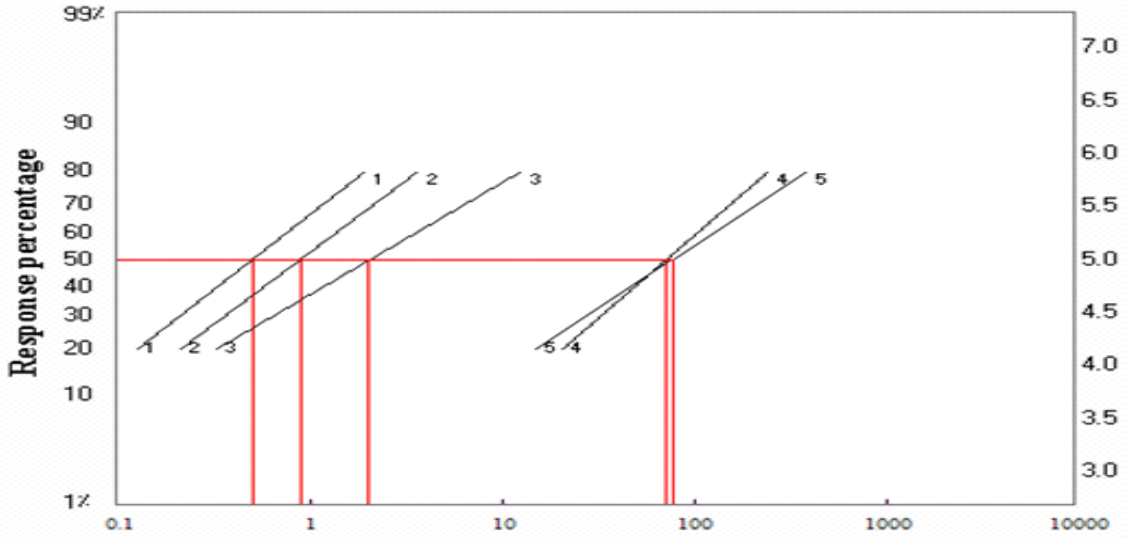

Figure 2. Depicts the LC_{50} values and the relationship between the tested acaricides and the *V. destructor*. 1: (Amitraz), 2: Spiromesifen, 3: Spirodiclofen, 4: Abamactine, 5: chlorfenapyr.

Efficiency of tested acaricides against Varroa infestation

At the beginning of the experiment, mean rates of infestation with *V. destructor* of adult and pupae (brood) of honeybee workers of all experimental colonies were 11% and 10%, respectively. There were no significant differences in rates of infestation with *V. destructor* among replicates assigned to experimental groups prior to treatment. Varroa mite infestation rates decreased significantly in brood (75.5% vs 67%) and adult honeybee workers of colonies treated with Spiromesifen and Amitraz, respectively, when compared to only non-treated (control) colonies, as stated in **Table 6, 7**.

Table 6. Efficiencies (%) of application of Spiromesifen and Amitraz acaricides against varroa mite infesting brood and adult honeybee.

Treatment	Brood	Adult
Spiromesifen	67	100
Amitraz	75.5	77.16
Control	0	18.79

Table 7. Average number of falling mite during the experimental period

Treatment	Weeks					
	1	2	3	4	5	6
Spiromesifen	22	18.3	20.67	29	35	25.67
Amitraz	30.57	24.67	28.67	22	16	9.33
control	12.67	10.00	14.67	16.67	15.33	15.00

Residues of acaricides in honey and beeswax

The residues of amitraz and spiromesifen were detected before and after treatment in honey and wax samples as shown in Table 8. Amitraz residues detected prior to treatment were 0.00191 and 0.00210 for

honey and beeswax, respectively. One week after treatment, the number of residues was 0.0211 and 0.0313 for honey and beeswax, respectively, for Amitraz. The residue values of Spiromesifen were 0.0113 and 0.0211 for honey and beeswax, respectively.

After 6 weeks of treatment, these amounts had reached 0.00128 and 0.00134 for honey and beeswax, respectively, to Amitraze. On the other hand, the residue values of Spiromesifen were 0.00485 and 0.00589 for honey and beeswax, respectively. Although honey and beeswax samples were collected up to 6 weeks after the application of amitraz and spiromesifen, the bee colonies were found contaminated with Amitraz and Spiromesifen. Importantly, residues of both did not exceed the MRLs established by EPA and European Commission guidelines.

The maximum residue limits in honey were established in the No. 2377/90/EC regulation. amitraz 0.2 $mg.kg^{-1}$ respectively. On the other hand, no maximal limits of residues are fixed for the wax even when it is used for pharmaceutical purposes, food packaging or cosmetics, so the honey and beeswax safely to using after treatment with amitraz and spiromesifen**.** These results agree with Jamal et al 2020, who detected on residues by HPLC in each the honey and beeswax samples collected up to 90 days after the application of flumethrin strips in the bee colonies. However, all the samples were found contaminated with flumethrin residues, but they did not exceed the MRLs established by EPA and European Commission guidelines.

Table 8. Amitraz and Spiromesifen residues in honey and beeswax after treatment of honeybee colonies.

Acaricides	Samples	Mean of Residue µg/g			MRL µg/kg [1]
		Pretreatment	After treatment (1week)	After treatment (6 weeks)	
Amitraz	Honey Beeswax	0.00191 0.00210	0.0211 0.0313	0.00128 0.00134	0.2 0.2
Spiromesifen	Honey Beeswax	0.00668 0.00752	0.0113 0.0211	0.00485 0.00589	- -

MRL: Maximum Residue Limit

Conclusion

Five acaricides were evaluated on the Varroa mite. The highest efficiencies were for two acaricides Amitraz and Spiromesifen, which have been tested and their residues in honey and beeswax have been quantified. Both acaricides showed high efficacy against Varroa mites. In comparison to non-treated colonies, Varroa mite infestation rates decreased in brood by 75.5% vs. 67% and adult honeybee workers by 77.16% vs. 100% of colonies treated with Spiromesifen and Amitraz, respectively. The honey and beeswax after 6 weeks of treatment with amitraz and spiromesifen have residues of tested acaricides but did not exceed the MRLs, so they can be used safely.

References

1. Aliano, N. P., Ellis, M. D., & Siegfried, B. D. (2006). Acute contact toxicity of oxalic acid to Varroa destructor (Acari: Varroidae) and their Apis mellifera (Hymenoptera: Apidae) hosts in laboratory bioassays. *Journal of Economic Entomology*, *99*(5), 1579–1582. https://doi.org/10.1093/jee/99.5.1579

2. Bahreini, R., Nasr, M., Docherty, C., de Herdt, O., Muirhead, S., & Feindel, D. (2020). Evaluation of potential miticide toxicity to Varroa destructor and honey bees, Apis mellifera, under laboratory conditions. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-78561-2
3. Bahreini, R., Nasr, M., Docherty, C., Muirhead, S., de Herdt, O., & Feindel, D. (2022). Miticidal activity of fenazaquin and fenpyroximate against Varroa destructor, an ectoparasite of Apis mellifera. *Pest Management Science*, *78*(4), 1686–1697. https://doi.org/10.1002/ps.6788
4. Bava, R., Castagna, F., Piras, C., Palma, E., Cringoli, G., Musolino, V., Lupia, C., Perri, M. R., Statti, G., Britti, D., & Musella, V. (2021). In vitro evaluation of acute toxicity of five citrus spp. Essential oils towards the parasitic mite varroa destructor. *Pathogens*, *10*(9), 1–11. https://doi.org/10.3390/pathogens10091182
5. Calatayud-Vernich, P., Calatayud, F., Simó, E., & Picó, Y. (2018). Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. *Environmental Pollution*, *241*, 106–114. https://doi.org/10.1016/j.envpol.2018.05.062
6. Chaimanee, V., Johnson, J., & Pettis, J. S. (2022). Determination of amitraz and its metabolites residue in honey and beeswax after Apivar® treatment in honey bee (Apis mellifera) colonies. *Journal of Apicultural Research*, *61*(2), 213–218. https://doi.org/10.1080/00218839.2021.1918943
7. Flores, J. M., Gámiz, V., Jiménez-Marín, Á., Flores-Cortés, A., Gil-Lebrero, S., Garrido, J. J., & Hernando, M. D. (2021). Impact of Varroa destructor and associated pathologies on the colony collapse disorder affecting honey bees. *Research in Veterinary Science*, *135*(May 2020), 85–95. https://doi.org/10.1016/j.rvsc.2021.01.001
8. Gashout, H. A., & Guzmán-Novoa, E. (2009). Acute toxicity of Essential oils and other natural compounds to the parasitic mite, Varroa destructor, and to larval and adult worker honeybees (Apis mellifera L.). *Journal of Apicultural Research*, *48*(4), 263–269. https://doi.org/10.3896/IBRA.1.48.4.06
9. Gisder, S., & Genersch, E. (2021). Direct Evidence for Infection of Varroa destructor Mites with the Bee-Pathogenic Deformed Wing Virus Variant B, but Not Variant A, via Fluorescence in Situ Hybridization Analysis. *Journal of Virology*, *95*(5), e01786-20. https://doi.org/10.1128/jvi.01786-20
10. Hernandez, J., Hattendorf, J., Aebi, A., & Dietemann, V. (2022). Compliance with recommended Varroa destructor treatment regimens improve the survival of honey bee colonies over winter. *Research in Veterinary Science*, *144*(December 2021), 1–10. https://doi.org/10.1016/j.rvsc.2021.12.025
11. Hernández-Rodríguez, C. S., Moreno-Martí, S., Almecija, G., Christmon, K., Johnson, J. D., Ventelon, M., vanEngelsdorp, D., Cook, S. C., & González-Cabrera, J. (2021). Resistance to amitraz in the parasitic honeybee mite Varroa destructor is associated with mutations in the β-adrenergic-like octopamine receptor. *Journal of Pest Science*, *0123456789*. https://doi.org/10.1007/s10340-021-01471-3
12. Higes, M., Martín-Hernández, R., Hernández-Rodríguez, C. S., & González-Cabrera, J. (2020). Assessing the resistance to acaricides in Varroa destructor from several Spanish locations. *Parasitology Research*, *119*(11), 3595–3601. https://doi.org/10.1007/s00436-020-06879-x
13. Hristov, P., Shumkova, R., Palova, N., & Neov, B. (2020). Factors associated with honeybee colony losses: A mini review. *Veterinary Sciences*, *7*(4), 1–16. https://doi.org/10.3390/vetsci7040166
14. Hýbl, Marian, Andrea Bohatá, Iva Rádsetoulalová, Marek Kopecký, Irena Hoštičková, Alena Vaníčková, and P. M. (2021). Evaluating the Efficacy of 30 Different Essential Oils against Varroa destructor and Honeybee Workers (Apis mellifera). *Insects*, *12*, 1045–1056.

15. Jack ,C.J., and Ellis, J.D.I, (2021). Integrated pest management control of Varroa destructor, the most damaging pest of western honeybee (Apis mellifera L.) colonies. Journal Insect Science .21(5):6.
16. Jamal, M., Aziz, M, A., Naeem ,M., Iqbal, Z., Khalid ,A., Siddique, F., Khan ,K, A., Ghramh ,H, A.(2020). Detection of flumethrin acaricide residues from honey and beeswax using high performance liquid chromatography (HPLC) technique. Journal of King Saud University – Science 32, 2229–2235 www.sciencedirect.com
17. Khalifa, S. A. M., Elshafiey, E. H., Shetaia, A. A., El-Wahed, A. A. A., Algethami, A. F., Musharraf, S. G., Alajmi, M. F., Zhao, C., Masry, S. H. D., Abdel-Daim, M. M., Halabi, M. F., Kai, G., Al Naggar, Y., Bishr, M., Diab, M. A. M., & El-Seedi, H. R. (2021). Overview of bee pollination and its economic value for crop production. *Insects*, *12*(8), 688–703. https://doi.org/10.3390/insects12080688
18. Milani, N. (2001). Activity of oxalic and citric acids on the mite Varroa destructor in laboratory assays. *Apidologie*, *32*(2), 127–138. https://doi.org/10.1051/apido:2001118
19. Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J. D., Cohen, A., Lim, D., Joklik, J., Cicero, J. M., Ellis, J. D., Hawthorne, D., & Van Engelsdorp, D. (2019). Varroa destructor feeds primarily on honeybee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(5), 1792–1801. https://doi.org/10.1073/pnas.1818371116