Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

Assessment of Selected Acaricides for Controlling *Varroa destructor* in Hybrid Carniolan Honeybee Colonies

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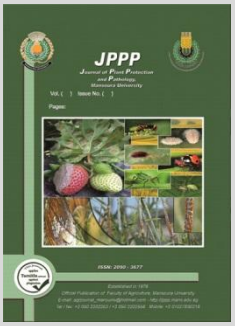

Article Information
Received 14 / 7 / 2025
Accepted 4 / 8 / 2025

ABSTRACT

This study evaluated the efficacy of selected acaricides against *Varroa destructor* and their safety to hybrid Carniolan honeybee workers under laboratory and field conditions. In residual contact bioassays, flumethrin, abamectin, and amitraz showed the highest toxicity to *V. destructor*, with LC_{50} values around 1–2 µg/mL after 24–48 hrs, while oxalic acid and thymol exhibited substantially higher LC_{50} values (50–80 µg/mL), indicating lower but meaningful efficacy aligned with organic beekeeping practices. Honeybee worker assays revealed that abamectin and alumethrin posed significant risks (LC_{50} < 20 µg/mL at 48 h), whereas amitraz demonstrated lower bee toxicity (LC_{50} in the range of 190–200 µg/mL), and oxalic acid and thymol were safest (> 300 µg/mL). Field trials conducted during March–April 2025 in Metoubes district, Kafr El Sheikh province, confirmed these patterns after four weekly treatments, infestation levels in adult workers fell from 10.83% (control) to 3.33% (amitraz), 3.84% (oxalic acid), and 5.17% (thymol). Brood infestation dropped similarly from 11.92% (control) to 3.75–5.92% across treatments. Mean weekly mite fall also increased markedly in treated colonies. These results emphasize the importance of integrating highly effective miticides like amitraz with safer organic options (oxalic acid, thymol) for sustainable varroa management that reduces mite pressure while safeguarding colony health.

Keywords: *Varroa destructor*; *Apis mellifera*; acaricide toxicity; integrated pest management; bee safety

INTRODUCTION

Honeybees (*Apis mellifera* L.) are among the most important pollinators globally, playing a vital role in maintaining biodiversity and supporting the productivity of numerous crops of economic significance (Klein *et al*., 2007; Potts *et al*., 2010; Taha *et al*., 2016; Al-Kahtani *et al*., 2017). Their ecological services are valued in billions of dollars annually, and the stability of agroecosystems is intimately tied to healthy bee populations (Gallai *et al*., 2009). However, in recent decades, global declines in honeybee colonies have been widely documented and are attributed to multiple stressors including habitat loss, nutritional deficits, pesticide exposure, pathogens, and notably, parasitic mites (Goulson *et al*., 2015; van der Zee *et al*., 2012). Among the biotic threats, *V. destructor* has emerged as the single most damaging ectoparasite of honeybees worldwide (Rosenkranz *et al*., 2010; Al-Kahtani and Taha, 2022). Originally a parasite of *Apis cerana*, this mite switched hosts to *A. mellifera* and has since spread nearly worldwide (Traynor *et al*., 2020). *Varroa destructor* feeds on the fat bodies and hemolymph of both developing brood and adult bees, compromising their immune function, reducing lifespan, and serving as a vector for multiple viral pathogens such as deformed wing virus (Ramsey *et al*., 2019; Nazzi and Le Conte, 2016). Heavy infestations can lead to colony collapse within a single season if left unmanaged (Rosenkranz *et al*., 2010). Consequently, effective varroa control is widely recognized as a critical component of sustainable beekeeping. Chemical acaricides have long been the primary tool for varroa management, offering quick and reliable population suppression when properly applied (Rosenkranz *et al*., 2010; Tihelka, 2018). Commercially available acaricides encompass synthetic chemicals such as amitraz, fluvalinate, flumethrin, and organosilicon compounds like spiromesifen, as well as naturally derived options including thymol and oxalic acid (Bogdanov, 2006; Rademacher and Harz, 2006). These treatments differ in their modes of action, residual persistence, volatility, and temperature dependence (Rosenkranz *et al*., 2010). Importantly, the success of an acaricide in practical beekeeping depends not only on its efficacy against varroa mites but also on its safety margin for honeybees, brood, and hive products. Nevertheless, intensive and repeated use of acaricides has led to widespread issues of resistance development in varroa populations, compromising the efficacy of previously reliable treatments (Milani, 1999; González-Cabrera *et al*., 2018). Resistance management strategies recommend rotation among compounds with different modes of action and integrating non-chemical control measures such as brood interruption or drone brood removal (Rosenkranz *et al*., 2010; Tihelka, 2018). However, in many regions, beekeepers continue to rely heavily on chemical treatments, underscoring the need for continued evaluation of both established and alternative acaricides. Additionally, sublethal and lethal effects of acaricides on honeybee workers are a major concern for colony health and productivity. Even compounds marketed as "bee-safe" can cause increased mortality, impaired foraging, or behavioral disruption at certain doses or under certain application conditions (Johnson *et al*., 2013; Berry *et al*., 2013).

Therefore, comprehensive evaluation of acaricides requires testing not only for their efficacy against varroa mites

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DOI: 10.21608/jppp.2025.403682.1359

but also for their relative safety to adult honeybees under realistic exposure scenarios.

Filter paper residual contact bioassays are widely used for standardized laboratory assessment of acaricide toxicity. This method simulates contact exposure on treated hive surfaces and enables dose-response modeling under controlled conditions (Milani, 1995; Aliano *et al*., 2006). Results from such bioassays allow for estimation of lethal concentrations (e.g., LC_{25}, LC_{50}, LC_{90}) and the calculation of safety margins, informing the selection of appropriate treatments and rotation plans in integrated varroa management. Moreover, field validation remains essential to confirm laboratory results under practical beekeeping conditions, accounting for colony-level factors such as brood presence, temperature fluctuations, and behavioral dynamics. Given the diverse acaricides available on the market in Egypt and elsewhere (including synthetic chemicals and naturally derived options) it is important to conduct regionally relevant evaluations to identify compounds with the best balance of high efficacy against *V. destructor* and minimal adverse effects on honeybee workers. Differences in formulation quality, application methods, environmental conditions, and local varroa susceptibility profiles can significantly influence treatment outcomes (Rosenkranz *et al*., 2010; Tihelka, 2018).

This study was designed to assess the comparative toxicity of six commercially available acaricides belonging to different chemical groups on both *V. destructor* mites and adult honeybee workers (*A. mellifera* L.) using laboratory-based residual contact bioassays. The selected compounds included synthetic miticides such as amitraz, abamectin, spiromesifen, and flumethrin, along with organic acids and plant-derived products such as oxalic acid and thymol. Both in vitro bioassays and a field validation trial were conducted in order to determine lethal concentration values, evaluate relative safety to bees, and identify promising candidates for varroa management in local beekeeping operations. The ultimate objective of this research is to support evidence-based recommendations for integrated varroa control that safeguard colony health while maintaining effective mite suppression.

MATERIALS AND METHODS

Six acaricidal compounds representing different chemical groups (and control) were selected to evaluate their efficacy against *V. destructor* and their relative safety to honeybee workers (*A. mellifera* L.). The tested compounds were as follows: metake (amitraz, 20%), which was commercially available and obtained from Al-Motaheda Company, Egypt; abalon (abamectin, 1.8%), purchased from the International Company for Chemicals and Commercial Agencies – Acta, Egypt; and kofex (Spiromesifen, 24%), obtained from agrimar Commercial Agencies Company. In addition, fumethrin (1%) (imported by Cairo Chemicals Company) and oxalic acid were procured from licensed local distributors supplying beekeeping treatments in Egypt, while thymol (pure crystalline form) was purchased from a certified supplier specializing in natural essential oils for agricultural use. All acaricide formulations were stored according to manufacturer recommendations prior to each assay.

Honeybee colonies used in this study were maintained at the experimental apiary located in Metoubes district, Kafr El Sheikh province. Colonies were regularly inspected to ensure overall health and standardized conditions. Selected colonies were nearly equal in strength and stored food, each headed by a young sister mated queen aged 5–6 months, with 5 brood combs and approximately equal adult bee populations covering 7 frames. Importantly, all experimental colonies had not received any acaricide treatments for at least 12 months prior to the study to prevent potential resistance effects or residual contamination.

For the collection of *V. destructor* mites used in vitro assays, heavily infested donor colonies were identified within the apiary. Adult female mites were carefully removed from worker bees using fine paintbrushes and transferred immediately to sterile Petri dishes lined with moist filter paper to maintain humidity. Collection and transfer were performed under laboratory conditions at 28 ± 1 °C and 65 ± 5% relative humidity to minimize handling stress and mortality prior to testing.

Laboratory biological tests were conducted at the Plant Protection Research Institute, Agricultural Research Center to determine the acute toxicity of selected insecticides to both varroa mites and worker bees. Five serial concentrations were prepared for each tested compound. Filter papers were cut to fit the bottoms of sterile Petri dishes and immersed in the test solutions for 1 minute to ensure uniform saturation. After brief air-drying to remove excess solvent, treated leaves were placed in Petri dishes designated for the treatment groups. Control dishes were prepared similarly but using only the solvent treatments. For the varroa mite experiments, groups of 20 live adult female mites were carefully placed on the treated leaves in each replicate, with four replicates prepared for each concentration. Petri dishes containing the mites were maintained at 28 ± 1 °C and a relative humidity of 65 ± 5%, and mite mortality was assessed one and two days after exposure. Mites were considered dead if they failed to respond to gentle examination with a fine brush under a stereo microscope.

For honeybee worker assays, foragers were collected at 10:00 -11:00 am from hives at the same apiary. Bees were gently shaken from brood frames into ventilated wooden cages constructed with mesh sides and a feeding hole at the top. For laboratory testing, groups of twenty bees were introduced into each treated Petri dish replicate (four replicates per concentration). Bees were held under controlled conditions (28 ± 1 °C, 65 ± 5% relative humidity) and provided with 50% (w/v) sucrose solution. Mortality was recorded after 1 and 2 days of continuous exposure on treated papers.

Five serial concentrations were prepared for each tested acaricide to evaluate their toxicity against both *V. destructor* mites and *A. mellifera* workers, with specific ranges selected to reflect their differing sensitivities. These concentration series were chosen based on preliminary sensitivity assays designed to identify appropriate testing ranges for each target organism. For varroa assays, concentrations were: Amitraz, abamectin, and flumethrin at 0.5, 1, 2, 4, and 6 µg/mL; spiromesifen at 30, 20, 10, 5, and 2.5 µg/mL; and oxalic acid and thymol at 25, 50, 100, 200, and 300 µg/mL. For honeybee worker assays, adjusted concentration ranges were used to better reflect their higher tolerance levels: flumethrin at 50, 40, 30, 20, and 10 µg/mL; Abamectin at 20, 15, 10, 5, and 2.5 µg/mL; spiromesifen and amitraz at 500, 400, 300, 200, and 100 µg/mL; and oxalic acid and thymol at 1200, 800, 600, 400, and 200 µg/mL. All solutions were freshly prepared using distilled water with a

small amount of surfactant to ensure even distribution on the filter paper used in the residual contact bioassay.

Mortality data for both honeybee workers and varroa mites were corrected using Abbott's formula (Abbott, 1925) to account for natural mortality in controls. Probit analysis following Finney's method (Finney, 1971) was performed to estimate the lethal concentrations (LC_{25}, LC_{50}, and LC_{90}) along with their 95% confidence intervals. These values were calculated using LdP-Line software (Ehab Software, *http://www.ehabsoft.com/ldpline/*), which enables precise probit regression modeling of dose–response data. To compare relative toxicity among treatments, the toxicity index (TI) was determined by dividing the LC_{50} of the reference (most toxic) compound by the LC_{50} of the tested compound and multiplying by 100, as follows:

TI (%) =

(LC_{50} of reference compound / LC_{50} of test compound) × 100

(Sun, 1950)

Field evaluation of amitraz, thymol, and oxalic acid in honeybee colonies

Amitraz, thymol, and oxalic acid were used in the field experiment, as they showed the highest efficacy against *Varroa destructor* with relatively low toxicity to honeybee workers under laboratory conditions. The experiment was conducted from March to April 2025 at a privet apiary in Metoubes district, Kafr El Sheikh province. Twelve standardized colonies of hybrid Carniolan honeybee (*Apis mellifera lamarkii* Cockerell × *A. m. carnica* Pollmann) colonies were equaled and randomly assigned into four equal groups (three treatments and one control). Varroa infestation levels in the experimental colonies were recorded before and relatively equaled to be approximately 12 %. Corrugated cardboard strips (15 × 15 cm) were immersed for 5 minutes in each acaricide solution at LC_{90} concentrations (for varroa) obtained from in vitro assays. After draining the excess solution, the strips were placed horizontally across the top bars of brood frames inside the hives. Treatments were applied once a week for four consecutive weeks. The percentages of infestation were redetermined after the treatment period to evaluate both efficacy and safety of the compounds.

In addition to assessing infestation rates in adult worker bees and brood samples, the number of fallen varroa mites and bee workers were monitored using screened sticky boards placed on the bottom of each hive. The sticky boards were coated with a thin layer of petroleum jelly to trap mites that fell naturally or due to treatment. Boards were collected and replaced with clean ones weekly interval, and all fallen mites and workers were counted. These counts were used alongside the fallen varroa mites adult bees to evaluate the overall efficacy of the acaricide treatments.

Statistical analysis

The obtained data were analyzed using one-way ANOVA using PROC GLM ver. 9.1.3 SAS® software computer program (SAS Institute, 2003).

RESULTS AND DISCUSSION

Toxicity of selected acaricides against *V. destructor* in residual contact bioassays

After 24 hours

The residual contact bioassay results after 24 hours of exposure revealed clear differences in the toxicological profiles of the tested acaricides against *V. destructor* (Table 1 and Fig. 1). Flumethrin demonstrated the highest efficacy, achieving the lowest LC_{50} value of 1.421 µg/mL and serving as the reference for relative toxicity (100% T.I.). Abamectin and Amitraz also showed substantial activity, with LC_{50} values of 1.671 µg/mL (T.I. 85.04) and 1.943 µg/mL (T.I. 73.13) respectively. In contrast, spiromesifen exhibited markedly reduced potency, with an LC_{50} of 10.137 µg/mL (T.I. 14.02), while oxalic acid (78.25 µg/mL, T.I. 4.12) and Thymol (108.22 µg/mL, T.I. 1.31) were the least toxic in this assay.

These results are broadly consistent with prior studies indicating the superior performance of synthetic pyrethroids and macrocyclic lactones in varroa management. For instance, Elzen *et al*. (1999) and Sammataro *et al*. (2005) reported high in vitro efficacy for flumethrin and amitraz via contact exposure, underscoring their suitability for hive treatments. The relatively high toxicity of abamectin observed here aligns with findings by Milani (1995), who demonstrated that avermectins disrupt mite neurophysiology at low concentrations. By contrast, the significantly higher LC_{50} values for thymol and oxalic acid reflect their known requirement for higher application rates to achieve effective mite control. These organic acids and essential oils are valued for their lower risk of resistance and residue accumulation (Rosenkranz *et al*., 2010), but their lower acute toxicity in short-term assays highlights the need for repeated or prolonged exposure in practical settings.

The data underscore the importance of selecting acaricides not only for their immediate lethality but also for their mode of action, residual activity, and safety profile within the hive environment. While flumethrin, abamectin, and amitraz achieved potent knock down within 24 hours, their chemical nature raises concerns about potential resistance development, as documented by Gracia-Salinas *et al*. (2006) and Rinkevich (2020). Conversely, oxalic acid and thymol, despite their weaker acute toxicity here, remain essential tools in integrated mite management programs precisely because of their compatibility with organic beekeeping practices and lower risk of resistance selection.

After 48 hours

After 48 hours of continuous contact exposure, all tested acaricides demonstrated improved toxicity against *V. destructor*, reflected in lower LC_{50} values compared to the 24-hour assessment (Table 2 and Fig. 2). Flumethrin maintained its status as the most effective compound with the lowest LC_{50} of 1.026 µg/mL, serving again as the benchmark for relative toxicity (100% T.I.). Abamectin and amitraz followed closely with LC_{50} values of 1.336 µg/mL (T.I. 76.8) and 1.426 µg/mL (T.I. 71.95), respectively, indicating sustained potency over time. Spiromesifen showed moderate improvement with an LC_{50} of 7.233 µg/mL (T.I. 14.18), while oxalic acid (57.12 µg/mL, T.I. 1.79) and thymol (79.278 µg/mL, T.I. 1.29) continued to exhibit the lowest toxicity.

The observed temporal decrease in LC_{50} values for all compounds is consistent with cumulative exposure effects documented in previous acaricide bioassays (Aliano *et al*., 2006). Such time-dependent increases in mortality highlight the importance of considering exposure duration in evaluating product efficacy. Notably, flumethrin's maintained superiority aligns with prior laboratory and field reports demonstrating its robust knockdown capability and residual efficacy against resistant mite populations (Elzen *et al*., 2000; Pettis *et al*., 2016). Similarly, abamectin and amitraz sustained high relative toxicity at 48 hours, underscoring their

value as industry-standard treatments despite concerns over evolving resistance (Maggi *et al*., 2010).

Meanwhile, the more modest improvements in oxalic acid and thymol toxicity support their established profiles as slower-acting but valuable alternatives in resistance management strategies. As shown by Rosenkranz *et al*. (2010), these organic treatments often require repeated applications or specific application methods (e.g., trickling, vaporization) to achieve practical efficacy. Their comparatively lower acute toxicity in this in vitro design may underestimate their real-world effectiveness when applied using recommended methods over longer periods. Therefore, while synthetic acaricides such as flumethrin, abamectin, and amitraz remain highly effective under controlled short-term exposure, integrating less toxic alternatives like oxalic acid and thymol is essential for sustainable varroa control programs that mitigate the risk of acaricide resistance and preserve colony health.

Table 1. Probit mortality parameters and relative toxicity index (T.I.) of tested acaricides against *V. destructor* after 24 hours of residual contact exposure.

Treatments	LC_{25} (95% CL)	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Abamectin	0.808 (0.633-0.975)	1.671 (1.428-1.941)	6.648 (5.23-9.206)	2.137±0.196	5.433	85.04
Amitraz	0.792 (0.583-0.993)	1.943 (1.621-2.328)	10.674 (7.641-17.381)	1.732±0.183	2.431	73.13
Flumethrin	0.626 (0.459-0.786)	1.421 (1.184-1.678)	6.758 (5.166-9.841)	1.893±0.188	2.432	100
Spiromesifen	4.172 (3.1-5.2)	10.137 (8.48-12.14)	54.768 (39.2-89.12)	1.749±0.184	0.357	14.02
Thymol	47.156 (36.23-57.64)	108.22 (91.52-128.61)	524.53 (384.23-818.65)	1.87±0.189	1.525	1.31
Oxalic acid	34.531 (25.72-42.99)	78.25 (65.61-92.26)	370.24 (281.26-544.18)	1.899±0.188	2.787	4.12

χ^2= Chi^2, CL= Confidence limits, TI= Toxicity index

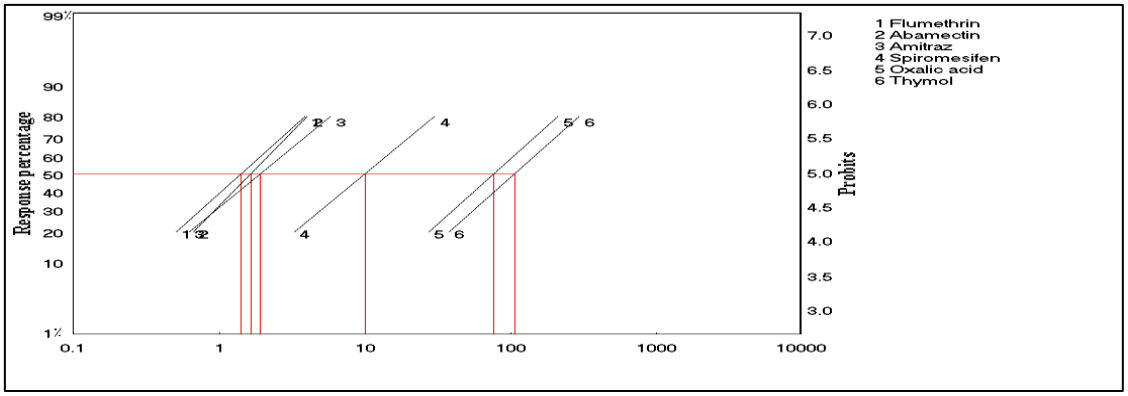

Figure 1. Toxicity lines (probit regression) of tested acaricides against *V. destructor* after 24 hours of residual contact exposure.

Table 2. Probit mortality parameters and relative toxicity index (T.I.) of tested acaricides against *V. destructor* after 48 hours of residual contact exposure.

Treatments	LC_{25} (95% CL)	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Abamectin	0.67 (0.52-0.811)	1.336 (1.139-1.546)	4.963 (4.006-6.603)	2.248±0.203	2.735	76.8
Amitraz	0.591 (0.42-0.757)	1.426 (1.174-1.7)	7.599 (5.652-11.61)	1.764±0.184	3.312	71.95
Flumethrin	0.481 (0.338-0.612)	1.026 (0.844-1.216)	4.328 (3.278-6.579)	2.05±0.244	0.0053	100
Spiromesifen	3.048 (2.19-3.88)	7.233 (5.981-8.6)	37.365 (27.37-56.42)	1.797±0.185	1.53	14.18
Thymol	37.097 (25.1-42.74)	79.278 (66.19-93.87)	393.923 (295.4-592.1)	1.841±0.186	3.708	1.29
Oxalic acid	26.14 (18.98-33.04)	57.12 (47.2-67.39)	252.26 (286.02-350.25)	1.987±0.195	6.058	1.79

χ^2= Chi^2, CL= Confidence limits, TI= Toxicity index

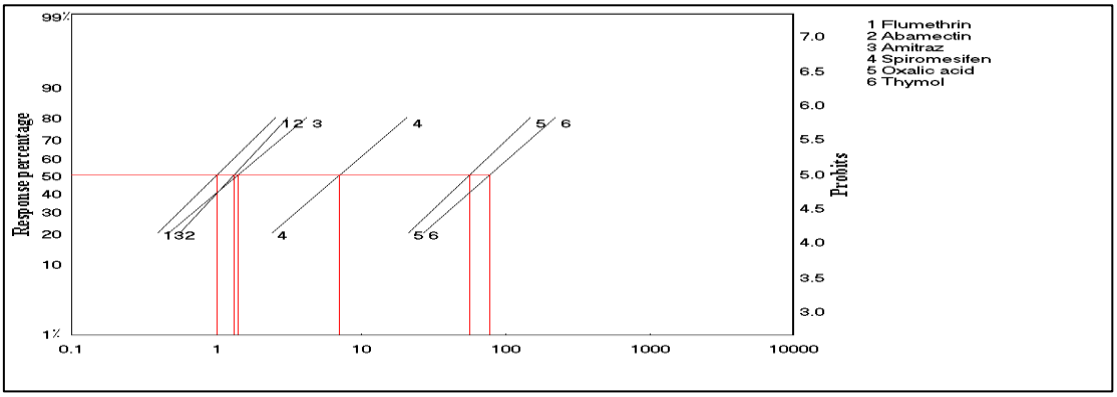

Figure 2. Toxicity lines (probit regression) of tested acaricides against *V. destructor* after 48 hours of residual contact exposure.

Acute contact toxicity of acaricides to honeybee workers, *A. mellifera*

After 24 hours

After 24 hours of exposure in the residual contact bioassay, clear differences in honeybee worker sensitivity to the tested acaricides were observed (Table 3 and Fig. 3). Abamectin exhibited the highest acute toxicity with an LC_{50} of 7.393 µg/mL (95% CL: 6.36–8.51) and served as the reference for relative toxicity (T.I. = 100). Flumethrin also showed considerable toxicity to honeybee workers, with an LC_{50} of

20.185 µg/mL (17.95–22.34) corresponding to a T.I. of 36.63. In contrast, amitraz, spiromesifen, oxalic acid, and Thymol demonstrated substantially lower toxicity levels. Amitraz recorded an LC_{50} of 207.949 µg/mL (186.56–228.76) with a T.I. of just 3.56, indicating a much safer profile for honeybee workers relative to abamectin. Spiromesifen had a slightly higher LC_{50} of 178.03 µg/mL (159.71–195.48), yielding a T.I. of 4.15, while oxalic acid and thymol showed even greater safety margins with LC_{50} values of 445.886 µg/mL (397.03–494.9) and 542.201 µg/mL (481.17–609.05), respectively. Their very low T.I. values (1.66 and 1.36) confirm minimal acute hazard under these conditions. These results suggest that although abamectin and flumethrin are highly effective acaricides, they also pose higher risks to honeybee workers in direct contact exposure assays, while amitraz and the organic acids demonstrate safer profiles for bee health.

After 48 hours

At 48 hours post-treatment, similar relative patterns in honeybee worker sensitivity were maintained, though LC_{50} values generally declined for all compounds, reflecting cumulative toxic effects over time (Table 4 and Fig. 4).

Abamectin remained the most toxic compound with an LC_{50} of 6.246 µg/mL (5.11–7.56) and a T.I. of 100, reinforcing its classification as the most hazardous for bees in this assay. Flumethrin again showed relatively high toxicity with an LC_{50} of 15.951 µg/mL (13.65–18.07), corresponding to a T.I. of 39.16. Amitraz maintained much lower toxicity, with its LC_{50} reducing slightly to 191.98 µg/mL (172.82–210.34), yielding a T.I. of 3.25. Spiromesifen displayed an LC50 of 148.43 µg/mL (127.92–167.16) with a T.I. of 4.21, confirming its comparatively safer profile relative to abamectin. Meanwhile, oxalic acid and thymol again exhibited the highest LC_{50} values (346.977 µg/mL and 462.134 µg/mL, respectively) and the lowest T.I. values (1.8 and 1.35), highlighting their minimal direct-contact hazard to bee workers even after prolonged exposure. These findings confirm that although all compounds become slightly more toxic over time due to accumulation, the relative ranking of their hazard to bees remains consistent.

When comparing these findings with the *V. destructor* bioassay results, a striking divergence emerges in the relative toxicity rankings between honeybee workers and their parasitic mites. For Varroa, compounds such as flumethrin, abamectin, and amitraz demonstrated exceptional acaricidal activity with low LC_{50} values (around 1–2 µg/mL after 24 h) and high relative toxicity indices, indicating their effectiveness as mite control agents. Conversely, the same compounds showed significantly greater hazard to honeybee workers, especially abamectin and flumethrin, whose LC_{50} values for bees remained below 20 µg/mL even at 48 hours, underscoring their potential risk for direct-contact toxicity in treated hives. On the other hand, organic treatments like oxalic acid and thymol presented a markedly different profile: despite showing moderate effectiveness against varroa with LC_{50} values in the approximately 50–80 µg/mL range for mites, they exhibited much higher LC_{50} values for honeybee workers (over 300 µg/mL), indicating a favorable safety margin. Amitraz consistently balanced efficacy against varroa with relatively low toxicity to bees, supporting its historical use in varroa management programs. This contrast in relative sensitivities emphasizes the importance of selecting acaricides not solely on mite control efficacy but also on their safety to honeybee colonies, aligning with recommendations from prior studies (Milani, 2001; Rosenkranz *et al*., 2010; Aliano *et al*., 2006) that highlight the need for integrated varroa management strategies minimizing non-target bee exposure while ensuring effective mite suppression.

Table 3. Probit mortality parameters and relative toxicity index (T.I.) of tested acaricides against honeybee workers (*A. mellifera* L.) after 24 hours of residual contact exposure.

Treatments	LC_{25} (95% CL)	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Abamectin	3.658 (2.83-4.42)	7.393 (6.36-8.51)	28.148 (22.16-39.51)	2.207±0.223	7.623	100
Amitraz	133.43 (112.13-152.11)	207.949 (186.56-228.76)	483.178 (423.44-575.86)	3.5±0.322	7.294	3.56
Flumethrin	12.536 (10.33-14.46)	20.185 (17.95-22.34)	49.901 (43.27-60.5)	3.26±0.311	6.275	36.63
Spiromesifen	120.021 (101.79-136.14)	178.03 (159.71-195.48)	376.55 (336.79-433.88)	3.939±0.344	7.606	4.15
Thymol	303.372 (247.33-352.92)	542.201 (481.17-609.05)	1630.623 (1319-2217)	2.68±0.284	7.656	1.36
Oxalic acid	269.695 (222.74-311.04)	445.886 (397.03-494.9)	1159.065 (1251-1439)	3.089±0.296	5.161	1.66

χ^2= Chi2, CL= Confidence limits, TI= Toxicity index

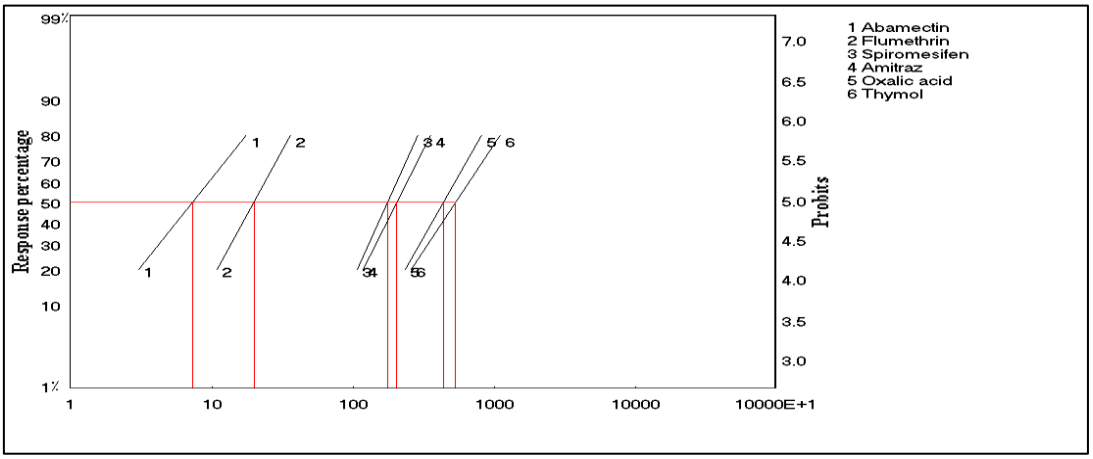

Figure 3. Toxicity lines (probit regression) of tested acaricides against honeybee workers (*A. mellifera* L.) after 24 hours of residual contact exposure.

Table 4. Probit mortality parameters and relative toxicity index (T.I.) of tested acaricides against honeybee workers (*A. mellifera* L.) after 48 hours of residual contact exposure.

Treatments	LC_{25} (95% CL)	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Abamectin	2.607 (1.704-3.398)	6.246 (5.11-7.56)	32.856 (22.25-63.98)	1.778±0.256	0.616	100
Amitraz	126.88 (107.94-143.72)	191.98 (172.82-210.34)	421.728 (376.88-486.4)	3.75±0.316	7.216	3.25
Flumethrin	9.383 (7.08-11.32)	15.951 (13.65-18.07)	43.717 (36.54-57.13)	2.927±0.353	3.807	39.16
Spiromesifen	92.851 (72.14-110.31)	148.43 (127.92-167.16)	361.933 (310.45-450.56)	3.311±0.381	5.242	4.21
Thymol	262.396 (210.79-307.56)	462.134 (407.47-517.82)	1354.645 (1122-1770.27)	2.744±0.283	7.692	1.35
Oxalic acid	214.97 (171.43-251.75)	346.977 (304.35-387.7)	861.73 (732.59-1088.9)	3.244±0.364	3.357	1.8

χ^2= Chi^2, CL= Confidence limits, TI= Toxicity index

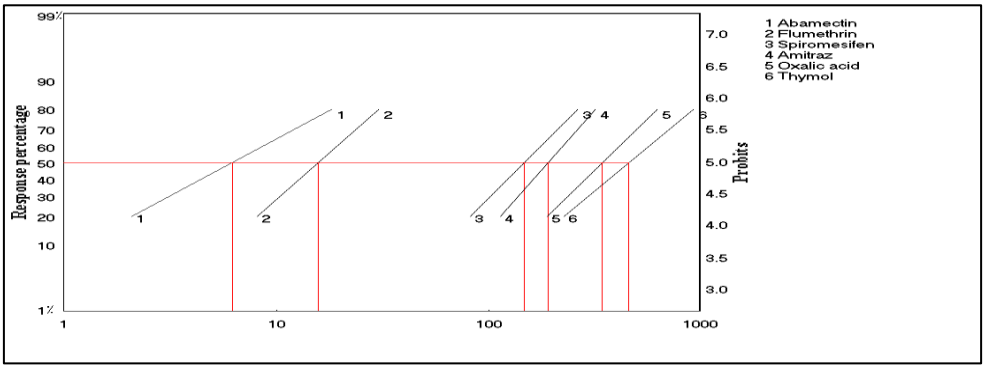

Figure 4. Toxicity lines (probit regression) of tested acaricides against honeybee workers (*A. mellifera* L.) after 48 hours of residual contact exposure.

Field efficacy of acaricides in reducing Varroa infestation in honeybee workers

Data illustrated in Fig (5) show that the field results showed significant reductions in average varroa infestation percentages in adult honeybee workers after four weeks of treatment with amitraz, oxalic acid, and thymol compared to the untreated colonies (control). The control colonies maintained a high mean infestation of 10.83%, while amitraz treatment reduced this to 3.33%. Oxalic acid and thymol achieved similar reductions, which lowering infestation to 3.84% and 5.17%, respectively. These average values over the treatment period demonstrate the sustained efficacy of these acaricides under field conditions. Such reductions are consistent with previous reports highlighting Amitraz's strong and reliable performance (Rosenkranz *et al*., 2010; Rinkevich, 2020) and the proven utility of oxalic acid and thymol in integrated pest management programs (Rademacher and Harz, 2006; Milani, 2001). These findings support their role in maintaining colony health by effectively suppressing mite populations on adult bees over extended application periods.

Varroa infestation reduction in brood cells after treatment

Brood cell analysis similarly revealed significant reductions in average varroa infestation percentages after four weeks of treatment (Fig. 5). Control colonies exhibited a mean brood infestation rate of 11.92%, while colonies treated with amitraz showed a marked reduction to 3.75%. oxalic acid and thymol treatments reduced brood infestation to 4.46% and 5.92%, respectively. These average values highlight the importance of sustained treatment for disrupting varroa reproduction within brood cells. The strong efficacy observed for amitraz aligns with its established reputation as a standard miticide for controlling resistant varroa populations (Elzen *et al*., 2000; Sammataro *et al*., 2005). Meanwhile, the effective reductions achieved with oxalic acid and thymol support their value as organic alternatives that can be integrated into resistance management strategies (Aliano *et al*., 2006; Rosenkranz *et al*., 2010). Overall, these results underscore the potential of these compounds to provide long-term varroa control in practical beekeeping operations.

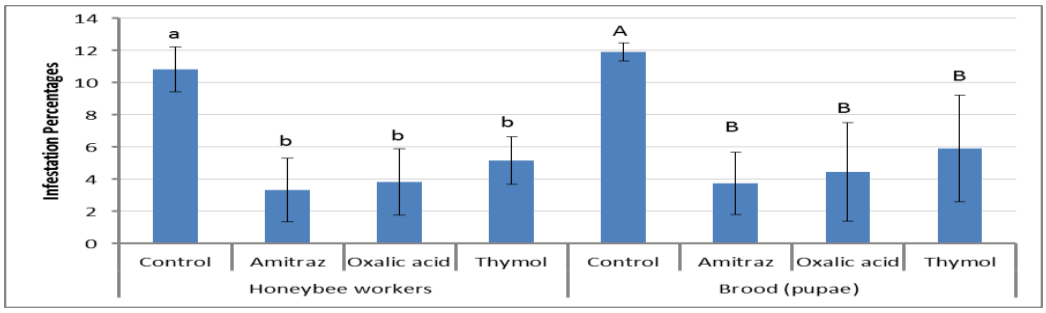

Figure 5. Mean percentage of varroa infestation over four weeks in honeybee workers and brood (pupae) after treatment with selected acaricidal compounds.

Bars labeled with the same letter do not differ significantly (p < 0.05). (F value = ; LSD = for workers and F value = 9.1309; LSD = 3.7886 for pupae).

Impact of acaricide treatments on varroa mite fall over four weeks

As shown in Fig. 6, the mean weekly mite drop over four weeks clearly reflected the efficacy of the tested acaricides compared to the untreated control. Colonies treated with amitraz showed the highest mean number of mite fall (187.84 ± 16.84), followed by oxalic acid (172.17 ± 10.81) and thymol (161.58 ± 12.25), while the control group exhibited minimal natural drop (27.92 ± 2.08). These results strongly support the observed reductions in Varroa infestation percentages in both adult workers and brood (3.33–5.92% in treatments vs. 10.83–11.91% in control), confirming the effectiveness of these

compounds in practical field settings. The increased mite mortality seen in sticky board counts indicates a sustained acaricidal action throughout the treatment period, highlighting the importance of repeated applications (Rosenkranz *et al*., 2010; Rademacher and Harz, 2006). Moreover, integrating mite drop monitoring with infestation rate assessments provides a comprehensive evaluation of treatment success, as emphasized by Sammataro *et al*. (2005) and Aliano *et al*. (2006). Overall, the significant increase in fallen mites under treated conditions demonstrates the value of amitraz, oxalic acid, and thymol as effective components of integrated varroa management strategies, reducing parasite pressure while maintaining colony health.

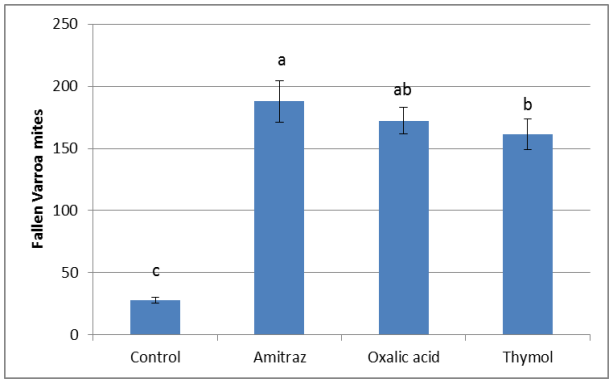

Figure 6. Mean weekly Varroa mite fall over four weeks following treatment with selected acaricides and in the untreated control.

Bars labeled with the same letter do not differ significantly (p < 0.05). (F value = 156.985; LSD = 18.1406)

On the other hand, Figure 7 presents the mean number of dead honey bees collected from bottom board over the four-week post-treatment period for each experimental group. Although worker bees are known to remove dead nestmates from the hive as part of their hygienic behavior, the data represent the remaining individuals found on the hive floor and therefore reflect a partial but informative measure of bee mortality. The highest mortality was recorded in the amitraz-treated colonies (7.75 ± 0.86), followed by oxalic acid (5.25 ± 1.29) and thymol (3.75 ± 1.52), while the untreated control group showed the lowest number of dead bees (3.25 ± 0.83).

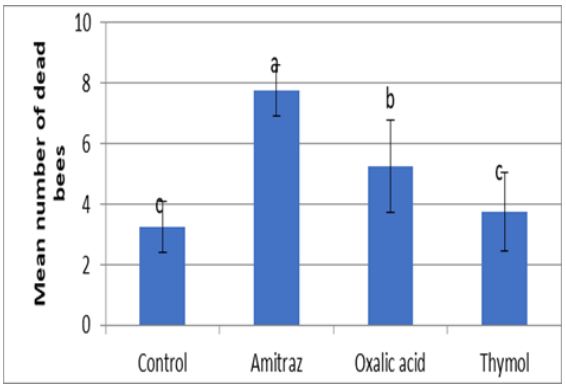

Figure 7. Mean number of dead bees on bottom board in treated and control colonies over four weeks following treatment with selected acaricides.

Bars (columns) labeled with the same letter do not differ significantly (p < 0.05). (F value = 41.897; LSD = 1.7943)

These results suggest that all tested treatments had minimal adverse effects on bee survival, with amitraz showing the highest associated mortality. This pattern aligns with its observed efficacy against *Varroa destructor* and reinforces its status as a commonly used acaricide with relatively low bee toxicity. In contrast, the slightly higher mortality in the thymol group may be attributed to its volatile nature and possible sublethal stress on adult workers, as noted in previous studies.

CONCLUSION

The obtained results demonstrate that amitraz, oxalic acid, and thymol provide effective and sustainable control of *Varroa destructor* in hybrid Carniolan honeybee colonies under both laboratory and field conditions. Amitraz offered a balanced profile of high efficacy and moderate bee safety, while oxalic acid and thymol served as valuable organic alternatives with excellent safety margins for bees. Integrating these treatments into varroa management programs was essential to reduce parasite pressure, mitigate resistance development, and maintain healthy, productive colonies.

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تقييم بعض المبيدات الأكاروسية في مكافحة الفاروا في طوائف هجين نحل العسل الكرينولي

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الملخص

في هذه الدراسة تم تقييم السمية الحادة بطريقة التعرض لمتبقي المبيد لستة مبيدات أكاروسية ضد حلم الفاروا (*Varroa destructor*) وشغالات نحل العسل *Apis mellifera*) والفعالية الحقلية لثلاثة من هذه المركبات. أظهرت مركبات Flumethrin و Abamectin و Amitraz سُمّية عالية وثابتة تجاه الفاروا، محققة قيم LC_{50} تتراوح حول ١–٢ ميكروجرام/مل خلال ٢٤–٤٨ ساعة، مما يؤكد فعاليتها الكبيرة كمبيدات ضد الأكاروس. في المقابل، سجل كل من حمض الأوكساليك و Thymol قيماً أعلى لـ LC_{50} في حدود ٥٠–٨٠ ميكروجرام/مل)، ما يشير إلى فعالية أضعف لكنها ذات أهمية، تتماشى مع استخدامهما في تربية النحل. أما بالنسبة لشغالات نحل العسل ، فقد شكّل كل من Abamectin و Flumethrin الخطر الاكبر، حيث ظلت قيم LC_{50} أقل من ٢٠ ميكروجرام/مل حتى بعد ٤٨ ساعة، مما يشير إلى مستوى مرتفع من المخاطر عند الاستخدام في الخلية. في المقابل، أظهر Amitraz توازناً جيداً من خلال قدرته الفعالة على مكافحة الفاروا عند تركيزات منخفضة وانخفاض واضح في سُميته للنحل (LC_{50} يقارب ١٩٠–٢٠٠ ميكروجرام/مل). وأظهرحمض الاوكساليك و Thymol أعلى معدلات الأمان للنحل، بقيم LC_{50} تتجاوز ٣٠٠ ميكروجرام/مل. وتبرز هذه النتائج أهمية اختيار مبيدات اكاروسية تحقق التوازن بين الفعالية ضد حلم الفاروا وتقليل الضرر على نحل العسل. أكدت التجارب الحقلية (مارس-أبريل ٢٠٢٥، محافظة كفرالشيخ مركز مطوبس) هذه الأنماط: بعد أربع معاملات أسبوعية، انخفضت مستويات الإصابة لدى الشغالات من ١٠,٨٣٪ (الكنترول) إلى ٣,٣٣٪ (Amitraz)، و٣,٨٤٪ (حمض الأكساليك)، و٥,١٧٪ (Thymol). وبالمثل، انخفضت إصابة الحضنة من ١١,٩٢٪ (الكنترول) إلى ٣,٧٥-٥,٩٢٪ في جميع المعاملات. كما زاد متوسط تساقط الفاروا أسبوعيًا بشكل ملحوظ في الخلايا المعاملات. وتؤكد هذه النتائج على أهمية دمج مبيدات القراد عالية الفعالية مثل أميتراز مع خيارات عضوية أكثر أمانًا (حمض الأكساليك، الثيمول) لإدارة الفاروا المستدامة.