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TOXICOLOGICAL ASSESSMENT OF CERTAIN ACARICIDES ON *VARROA DESTRUCTOR* AND HONEY BEES, *APIS MELLIFERA* UNDER LABORATORY AND FIELD CONDITIONS

Konper. Hanan M. A.[(1)]; Seddik, M. A.[(1)]; Amer, S. M.[(1)] and Anter, M. A. [(2)]

[(1)] Department of Apiculture, Plant Protection Research Institute, Agricultural Research Center, Dokki, 12619, Giza, Egypt.

[(2)] Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Cairo

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ABSTRACT: The present study evaluated the acute contact toxicity and field efficacy of four acaricides: coumaphos, tau-fluvalinate, oxalic acid, and the thymol-based formulation Varoviga against *Varroa destructor* and honey bee workers (*Apis mellifera*) at varying concentrations. Laboratory bioassays demonstrated clear differences among the tested compounds. After 12 and 24 hours of exposure, coumaphos consistently exhibited the highest potency against mites, with LC_{50} values of 2.28 and 1.34 µg/mL, respectively, while maintaining selectivity toward honey bees (LC_{50} = 27.33 and 18.24 µg/mL). Oxalic acid and tau-fluvalinate showed moderate toxicities to *V. destructor* (LC_{50} = 25.89–21.26 µg/mL and 32.96–24.69 µg/mL, respectively), whereas Varoviga® was the least effective (LC_{50} = 157.95–124.67 µg/mL). For honey bees, the toxicity ranking differed: coumaphos was most toxic (LC_{50} = 27.33–18.24 µg/mL), followed by oxalic acid (86.81–71.42 µg/mL), tau-fluvalinate (334.71–296.07 µg/mL), and Varoviga® (526.26–443.16 µg/mL). These findings confirm that although synthetic acaricides provide strong control of mites, they may also pose significant risks to bee health if misused or applied in excessive amounts. Field trials corroborated laboratory findings, with coumaphos producing the highest mite drop, closely followed by oxalic acid. Tau-fluvalinate and Varoviga were less effective, but they still significantly reduced infestations compared to untreated controls. The combined results emphasize the importance of balancing acaricidal potency with honey bee safety and highlight the potential of integrating natural products, such as oxalic acid and thymol formulations, into honey bee treatment programs to enhance sustainability and delay the development of resistance.

Keywords: *Varroa destructor*, honey bees, coumaphos, oxalic acid, tau-fluvalinate, Varoviga, toxicity, resistance management.

INTRODUCTION

The western honey bee, *Apis mellifera*, is a cornerstone of agriculture and ecosystems, providing vital pollination services in addition to hive products such as honey, beeswax, and propolis. More than one-third of agricultural production depends directly or indirectly on insect pollination, with honey bees recognized as the most efficient and economically valuable pollinators (Klein *et al*., 2007; Calderone, 2012). Thus, any factor undermining colony health poses serious risks to food security and ecosystem stability. In recent decades, honey bee populations have faced increasing pressures from habitat loss, climate change, pesticide exposure, and pathogens (Goulson *et al*., 2015). Among these, the ectoparasitic mite *Varroa destructor* remains the most persistent and damaging threat to honey bees. Originally a parasite of *Apis cerana*, this mite shifted to *A. mellifera* and spread globally, where it feeds on hemolymph and fat body tissue, weakens bees, and vectors harmful viruses such as Deformed Wing Virus (DWV) and Acute Bee Paralysis Virus (ABPV) (Rosenkranz *et al*., 2010; Ramsey *et al*., 2019). Uncontrolled infestations often lead to rapid colony collapse, making Varroa one of the most destructive pests of managed bees worldwide. Varroa control has relied mainly on chemical acaricides, including tau-fluvalinate, coumaphos, and amitraz, as well as alternatives such as oxalic acid and essential oils (Rosenkranz *et al*., 2010; Rinkevich, 2020). However, a heavy reliance on

*Corresponding author: drhananm29@yahoo.com

these treatments has accelerated the development of resistance (Elzen *et al*., 1999; Milani, 1999), caused residue accumulation in hive products, and raised concerns about sublethal effects on bee health (Johnson *et al*., 2010). These challenges underscore the need to continuously evaluate current and alternative control strategies for sustainable Varroa management.

Laboratory bioassays remain essential for assessing the relative toxicity of acaricides. Contact exposure methods provide reliable data on acute toxicity to both mites and bees, allowing estimation of lethal concentrations (LC_{25}, LC_{50}, LC_{90}) that are central to toxicological evaluations. These assays identify intrinsic efficacy and safe dosage ranges, minimizing adverse effects on honey bee workers (Dietemann *et al*., 2013). Probit analysis and related statistics enable comparison of compounds through toxicity indices, giving insight into potency and selectivity (Finney, 1971; Sun, 1950). This approach ensures that candidate acaricides are objectively evaluated before field application. Field trials complement laboratory assays by testing acaricides under colony conditions. Treatments applied via impregnated strips, evaporation devices, or liquid formulations simulate practical use, allowing for the monitoring of mite mortality, colony strength, brood area, and potential side effects. Sticky boards are commonly used to quantify mite fall, while brood and adult samples provide indices of infestation (Rosenkranz *et al*., 2010). Field studies also account for factors such as colony size, brood presence, and environmental conditions, which may influence treatment outcomes. Efficacy thresholds are critical, with resistance generally suspected when treatment efficacy falls below 60% (Thompson *et al*., 2002). Given the economic and ecological significance of honey bees and the persistent threat of V. destructor, it is vital to evaluate acaricides that combine efficacy with safety to bees and their hive products. Accordingly, this study evaluated the acute toxicity of tau-fluvalinate, coumaphos, oxalic acid, and the natural thymol-based formulation Varoviga against *V. destructor* and *A. mellifera* honeybees in both laboratory and field studies.

MATERIALS AND METHODS

Acaricides

The acaricides employed in this study included tau-fluvalinate, coumaphos, oxalic acid, and Varoviga (a natural product consisting of thymol, menthol, organic oil, and camphor). Stock solutions were serially diluted with ethanol or distilled water to achieve a wide range of test concentrations, based on their solubility properties. Control groups included untreated samples and solvent-only treatments to account for background mortality.

Varroa mite acute toxicity assays

Adult female of *V. destructor* mites was collected from infested colonies of *A. mellifera* (Carniolan hybrid) using the powdered sugar roll method (Dietemann *et al*., 2013). Mites were isolated directly prior to each assay to ensure viability and then transferred to the laboratory, where they were kept in a dark incubator maintained at 34 °C until use. Acute contact toxicity bioassays were conducted by placing mites in glass vials lined with filter paper impregnated with defined concentrations of each acaricide based on preliminary assays. For coumaphos, the tested concentrations were 0.5, 1, 5, 10, and 25 µg/mL, while oxalic acid was examined at concentrations of 10, 20, 30, 40, and 50 µg/mL. Varoviga (a thymol-based natural blend) was tested at concentrations of 50, 100, 150, 200, and 400 µg/mL, and tau-fluvalinate was tested at concentrations of 10, 20, 30, 50, and 100 µg/mL. These ranges were selected to encompass low, intermediate, and high doses, thereby ensuring the inclusion of sublethal, median lethal, and near-maximum response levels suitable for probit analysis. Groups of twenty mites were exposed per replicate, and each treatment was repeated four times, alongside solvent and untreated controls. Mortality was recorded at 12 and 24 h using a stereomicroscope, and individuals were classified as alive (coordinated or uncoordinated movement) or dead (no response to stimulation).

Honey bee acute toxicity assays

Worker bees (*A. mellifera*) were collected from brood combs of healthy colonies in the

Nubaria district (Behiera Governorate). Individuals were maintained overnight in laboratory cages, provided with a 50% sucrose solution at 34 °C, prior to testing. Contact toxicity was assessed using glass arenas lined with filter paper treated with graded concentrations of each acaricide, which were allowed to dry before introducing bees. Groups of twenty bees were used per concentration, and each treatment was replicated four times, including solvent and untreated controls. Mortality was recorded after 12 and 24 hours, and bees were considered dead when they were unable to right themselves following gentle stimulation. In the honey bee bioassays, a separate series of graded concentrations was employed to evaluate the acute contact toxicity of the tested acaricides to adult worker bees. For coumaphos, the concentrations applied were 10, 25, 50, 100, and 200 µg/mL, while tau-fluvalinate was tested at 100, 200, 300, 400, and 500 µg/mL. Oxalic acid was examined at 10, 50, 100, 150, and 200 µg/mL. For the natural formulation Varoviga, the concentration series consisted of 1000, 800, 600, 400, and 200 µg/mL. These ranges were selected to reflect the generally higher tolerance of honey bees compared to *Varroa destructor*, thus allowing for an accurate assessment of the compounds' selectivity and safety profile in relation to the host.

Mortality values obtained for honeybee workers and *Varroa* mites were adjusted using Abbott's correction formula (Abbott, 1925) to eliminate the influence of background mortality observed in the controls. Dose–response relationships were analyzed using probit regression, following the procedure outlined by Finney (1971), which provided estimates of LC25, LC50, and LC90, along with their corresponding 95% confidence intervals. Calculations were performed using the LdP-Line program (Ehab Software, http://www.ehabsoft.com/ldpline/), which enables the accurate modeling of probit-based mortality curves. To facilitate comparison among tested compounds, a toxicity index (TI) was derived according to Sun (1950), where the LC_{50} of the most potent acaricide was used as a reference and expressed relative to the LC_{50} of each compound using the equation:

TI (%) = [LC_{50} (reference) / LC_{50} (test compound)] × 100 (Sun, 1950)

Field evaluation of efficacy in honeybee colonies

Field experiments were conducted in May 2025 at a private apiary located in the Nubaria region, Beheira Governorate, using naturally infested colonies of *A. mellifera*. A total of twenty colonies of equal strength were selected and randomly distributed into five experimental groups, consisting of four treatment groups and one group of untreated control (each group consisted of four replicate colonies). Corrugated cardboard strips (20 × 20 cm) were placed in solutions of each acaricide for 5 minutes, adjusted to the LC_{90} values obtained from laboratory bioassays. After draining the excess liquid, strips were placed horizontally on top of the bars inside the brood frames of the hives. Applications were repeated once per week over a four-week treatment period. Colony performance, including brood area and adult bee population, as well as Varroa infestation levels, was monitored prior to treatment initiation, throughout the experimental period, and at the end of the trial.

To assess treatment effectiveness, sticky boards coated with petroleum jelly were placed under each hive to collect fallen mites. These boards were replaced weekly, and all mites counted. Additional assessments included infestation rates in adult bees and brood samples, as well as recording dead bees from hive floors to evaluate possible adverse effects. The efficacy of each acaricide treatment was expressed as the percentage reduction in mite infestation compared to control colonies. According to Thompson *et al*. (2002).

RESULTS AND DISCUSSION

Acute contact toxicity of acaricides against *V. destructor* after 12 hours of exposure

The bioassay results obtained after 12 hours of exposure revealed marked differences in the

acute contact toxicity of the five evaluated acaricides against *V. destructor* (Table 1 and Fig. 1). Among the tested compounds, coumaphos exhibited the highest potency, with an LC_{50} of 2.284 µg/mL and a toxicity index (TI) of 100, highlighting its relatively high toxicity within short exposure periods, which is attributable to its inhibition of acetylcholinesterase and subsequent cholinergic overstimulation. These values are in close agreement with data reported by Milani (1999) and Spreafico *et al*. (2001), which indicate that organophosphate-based products exhibit fast-acting toxicity against mites, although resistance development has frequently been documented.

In contrast, pyrethroid-based tau-fluvalinate and oxalic acid displayed moderate toxicities, with LC50 values of 32.963 µg/mL and 25.89 µg/mL, respectively, resulting in much lower TI values (6.93 and 8.28). The relatively lower toxicity of tau-fluvalinate within 12 h may be explained by slower cuticular penetration and possible pre-existing resistance in field mite populations, which has been widely reported in Europe and North America (Martín-Hernández *et al*., 2012; Kamler *et al*., 2016). Similarly, the acid salt oxalic acid, despite its strong efficacy in field applications through sublimation or trickling methods, requires more prolonged exposure and higher doses to elicit significant contact mortality (Rademacher & Harz, 2006).

Table 1. Probit parameters and toxicity index (TI) of acaricides against *V. destructor* after 12 h contact exposure

Acaricides	LC_{25} (95% confidence limits)	LC_{50} (95% confidence limits)	LC_{90} (95% confidence limits)	Regression Slope ± SE	Chi-square	Toxicity Index
Coumaphos	0.615 (0.377-0.883)	2.284 (1.708-2.979)	27.631 (17.993-50.221)	1.184±0.117	1.536	100
Tau-fluvalinate	15.308 (11.45-18.49)	32.963 (28.27-38.58)	141.57 (105.4-218.44)	2.025±0.217	1.355	6.93
Oxalic acid	16.426 (13.99-18.54)	25.89 (23.46-28.42)	61.44 (52.73-75.93)	3.415±0.33	6.734	8.28
Varoviga	83.46 (67.97-97.37)	157.945 (138.96-180.48)	530.782 (414.83-759.98)	2.435±0.256	5.159	1.44

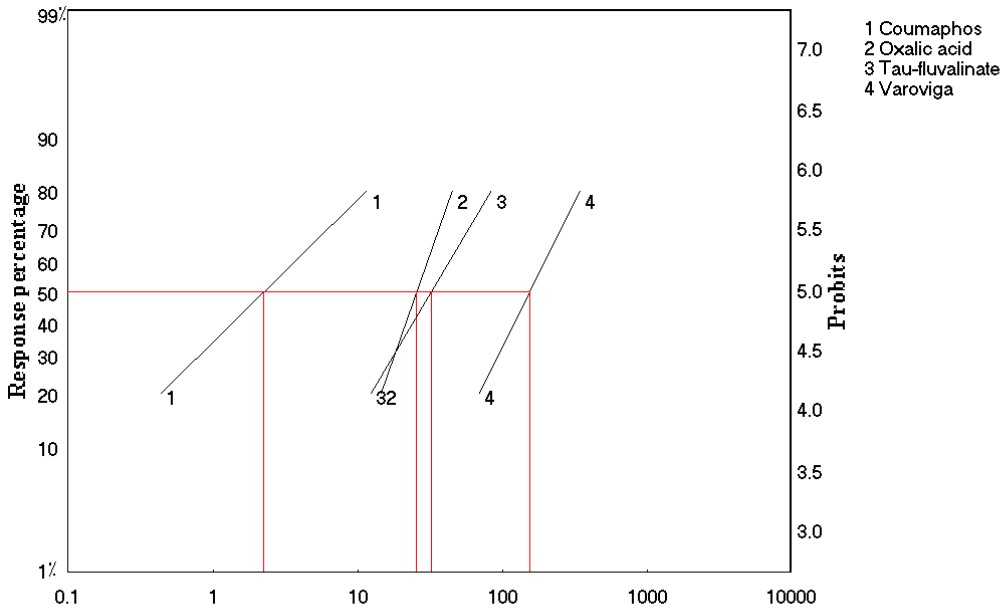

Fig. 1. Toxicity lines of acaricides against *V. destructor* after 12 h contact exposure.

The least effective treatment after 12 hours was Varoviga, a thymol-based natural blend, with an LC_{50} of 157.945 µg/mL and a TI of only 1.44. Essential oils such as thymol and menthol are known to exert fumigant action and behavioral repellency rather than acute contact toxicity (Imdorf *et al*., 1999; Emsen *et al*., 2007). Therefore, the relatively poor performance of Varoviga® in short-term contact assays is not unexpected, since its biological activity is primarily achieved via vapor-phase exposure within colonies. Taken together, the 12-hour data underscore the clear superiority of coumaphos in providing rapid knockdown effects, while other compounds may require extended exposure periods or alternative application methods to demonstrate comparable efficacy.

Acute contact toxicity of acaricides against *V. destructor* after 24 hours of exposure

After 24 hours, the relative toxicity profiles of the acaricides remained broadly similar, though LC_{50} values decreased for most compounds, reflecting the cumulative effects of extended contact exposure (Table 2 and Fig.2). Coumaphos retained its status as the most potent acaricide with an LC_{50} of, with an LC_{50} reduced to 1.343 µg/mL and TI of 100, confirming its strong contact toxicity and supporting its historical use in strip formulations.

On the other hand, tau-fluvalinate and oxalic acid exhibited further decreases in LC50 values (24.692 and 21.26 µg/mL, respectively), with TIs of 5.44 and 6.32, respectively. These findings reinforce their classification as moderately toxic in contact bioassays but are still significantly less effective than coumaphos. Tau-fluvalinate's relatively low TI, despite being a pyrethroid, may again be associated with widespread resistance phenomena in *Varroa* populations (Milani, 1995; González-Cabrera *et al*., 2013). Oxalic acid, meanwhile, is acknowledged for its higher efficacy in field applications, particularly through sublimation, rather than via direct surface contact as simulated in this assay (Nanetti *et al*., 2003).

Varoviga® maintained the lowest efficacy after 24 h, with an LC50 of 124.665 µg/mL and a TI of only 1.08. Although its toxicity improved slightly compared to 12-h exposure, its overall potency remained substantially inferior to that of the synthetic acaricides. Nevertheless, its role in integrated pest management cannot be discounted, as essential oils offer advantages such as reduced risk of residues in honey and wax, lower probability of resistance selection, and greater acceptance in organic beekeeping systems (Mattila & Otis, 2000).

The extended exposure data thus emphasize that coumaphos remains the most effective contact toxicant for short-term control of V. destructor, while tau-fluvalinate and oxalic acid provide moderate activity, and Varoviga acts primarily through alternative mechanisms not well captured by acute residual contact assays. The observed differences in LC values and TI rankings across 12-h and 24-h assays are consistent with previous reports, which highlight not only the intrinsic potency of each active ingredient but also the significant influence of exposure duration, resistance status, and mode of delivery on observed outcomes. From a management perspective, these findings support the continued use of amitraz as a first-line treatment, while also cautioning against overreliance on single chemistries, given the well-documented risk of resistance development. Integrating natural products, such as Varoviga, alongside the rotation of synthetic acaricides may thus represent a more sustainable approach for long-term Varroa management.

Table 2. Probit parameters and TI of acaricides against *V. destructor* after 24 h contact exposure

Acaricides	LC_{25} (95% confidence limits)	LC_{50} (95% confidence limits)	LC_{90} (95% confidence limits)	Regression Slope ± SE	Chi-square	Toxicity Index
Coumaphos	0.355 (0.227-0.586)	1.343 (0.984-1.762)	13.484 (8.645-26.33)	1.279±0.152	2.314	100
Tau-fluvalinate	11.886 (8.969-14.56)	24.692 (21.04-28.59)	99.06 (77.11-142.03)	2.124±0.224	1.188	5.44
Oxalic acid	13.46 (5.76-15.344)	21.26 (12.88-28.55)	50.69 (44.97-122.68)	3.397±0.318	2.112	6.32
Varoviga	65.763 (51.835-78.17)	124.665 (108.74-141.68)	420.255 (336.03-580.02)	2.428±0.255	6.233	1.08

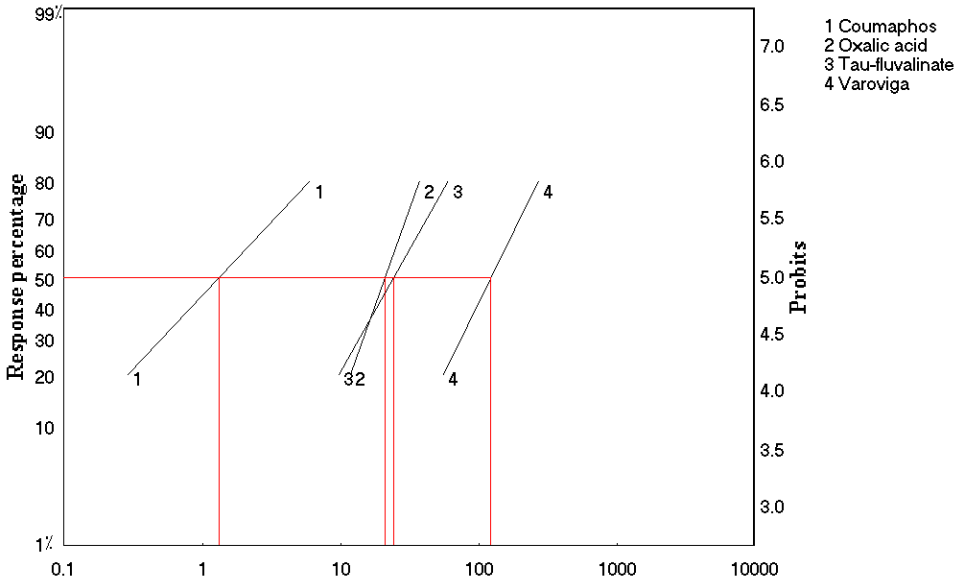

Fig. 2. Toxicity lines of acaricides against *V. destructor* after 24 h contact exposure

Acute contact toxicity of acaricides against *A. mellifera* after 12 hours of exposure

The acute contact bioassays performed on honey bees (*A. mellifera*) after 12 hours of exposure to different acaricides revealed substantial variation in toxicological responses among the tested compounds (Table 3 and Fig. 3). The LC values demonstrated a wide spectrum of sensitivity, with coumaphos showing the highest toxicity to honey bees, followed by oxalic acid, tau-fluvalinate, and finally Varoviga.

At 12 hours, the LC_{50} of coumaphos ranked first in toxicity, with an LC_{50} of 27.33 µg/mL, suggesting that although this organophosphate compound is more selective to *V. destructor*, it still poses a non-negligible risk to bees at higher concentrations. Oxalic acid, widely used in beekeeping as a natural miticide, exhibited a relatively moderate toxicity with an LC_{50} of 86.81 µg/mL. This aligns with earlier findings by Rademacher and Harz (2006), who emphasized that oxalic acid could harm honey bees at elevated doses or under certain application methods, despite being considered "bee-friendly" compared to synthetic acaricides.

Tau-fluvalinate exhibited a significantly lower LC50 (334.71 µg/mL), indicating a much higher acute toxicity to honey bees compared to coumaphos. This result is consistent with previous reports (Elzen *et al*., 1999; Johnson *et*

al., 2013), highlighting that pyrethroids, such as tau-fluvalinate, are tolerated by bees at moderate levels; however, their residues can accumulate in wax and cause sublethal stress over time. Varoviga displayed the lowest acute toxicity to bees with an LC_{50} of 526.26 µg/mL. This observation aligns with previous studies (Imdorf et al., 1999; Emsen et al., 2020), which have demonstrated that thymol-based formulations, although less effective against Varroa, generally provide a broader safety margin for honey bee colonies.

The toxicity index (TI) values provided further clarity: coumaphos was set as the reference compound (TI = 100), and oxalic acid followed closely (TI = 31.48). Varoviga (TI = 5.19) displayed substantially reduced toxicity to bees. This ranking pattern differs from that observed for V. destructor in Tables 1 and 2, where coumaphos exhibited superior acaricidal action, albeit with a significantly lower LC_{50} value. Importantly, the LC values for honey bees were consistently higher than those reported for *Varroa*, confirming that these compounds, when applied at recommended field doses, maintain a level of selectivity that favors mite mortality over bee mortality. Comparing both hosts, coumaphos showed an LC_{50} of 2.28 µg/mL against Varroa versus 27.33 µg/mL in bees, again illustrating stronger efficacy on mites relative to bees. Tau-fluvalinate and oxalic acid followed similar patterns, though with smaller margins of selectivity, suggesting that overdosing or repeated treatments may jeopardize bee health.

Overall, the 12-hour contact toxicity results emphasize the critical balance between acaricidal efficacy and honey bee safety. The distinct ranking patterns observed between *Varroa* and *A. mellifera* highlight the importance of dose optimization: coumaphos is highly potent against mites but needs careful application to avoid bee toxicity, whereas natural products like Varoviga and oxalic acid, although safer for bees, require higher concentrations or repeated applications for effective mite control. These findings corroborate earlier field observations where thymol-based and oxalic acid treatments were valued for their safety profiles but criticized for variable efficacy (Gregorc & Planinc, 2001; Rosenkranz *et al*., 2010).

Table 3. Probit parameters and TI of acaricides against *A. mellifera* after 12 h contact exposure

Acaricides	LC_{25} (95% confidence limits)	LC_{50} (95% confidence limits)	LC_{90} (95% confidence limits)	Regression Slope ± SE	Chi-square	Toxicity Index
Coumaphos	11.218 (7.805-14.623)	27.331 (22.02-32.96)	148.414 (112.95-215.88)	1.744±0.171	1.915	100
Tau-fluvalinate	198.217 (168.25-224)	334.709 (301.32-376.31)	905.702 (724.8-1268.4)	2.964±0.334	3.773	8.16
Oxalic acid	32.688 (23.01-42.004)	86.813 (71.192-105.98)	555.39 (378.76-988.92)	1.59±0.18	4.893	31.48
Varoviga	303.302 (99.03-334.87)	526.256 (314.65-816.36)	1499.511 (1594-7875)	2.818±0.305	1.695	5.19

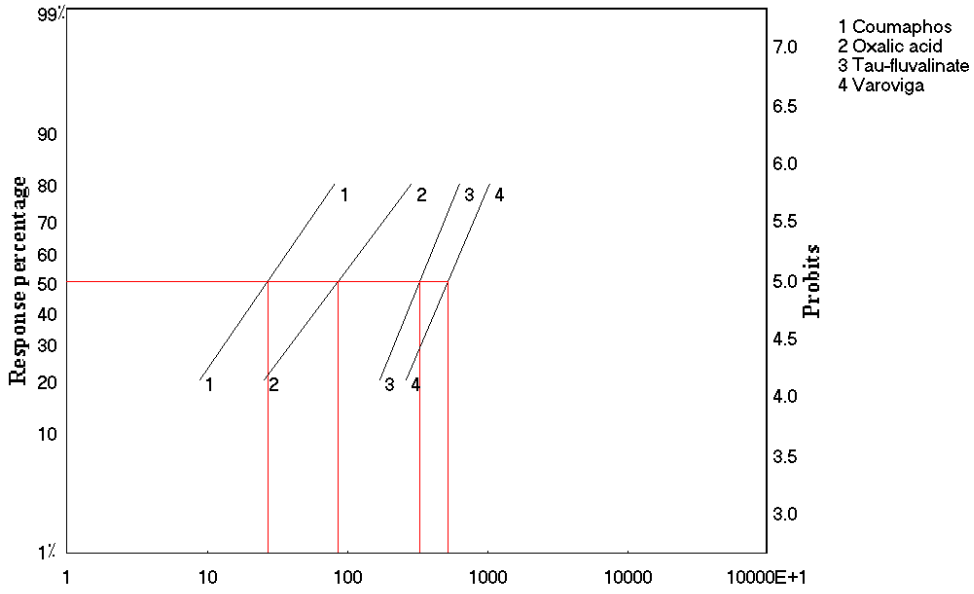

Fig. 3. Toxicity lines of acaricides against *A. mellifera* after 12 h contact exposure

Acute contact toxicity of acaricides against *A. mellifera* after 24 hours of exposure

After 24 hours of exposure, honey bees exhibited notable shifts in their sensitivity to the tested acaricides (Table 4 and Fig. 4). Similar to the 12-hour bioassay, the ranking of toxicity among compounds remained broadly consistent; however, the LC values decreased across treatments, indicating cumulative toxic effects with prolonged exposure time.

Coumaphos exhibited an LC_{50} of 18.24 µg/mL at 24 hours, representing a marked reduction from the 12-hour LC_{50} of 27.33 µg/mL. This highlights its progressive impact on honey bee survival. Despite this, coumaphos remained more selective against *V. destructor* (LC_{50} = 1.34 µg/mL at 24 h, Table 2), showing a roughly 13-fold higher safety margin in bees. Such findings support previous conclusions that coumaphos can be effective in mite management, but misuse or over-application may lead to elevated bee mortality and even residue contamination in hive products (Wallner, 1999).

Oxalic acid displayed moderate toxicity, with an LC_{50} of 71.42 µg/mL, a decline from the 12-hour value of 86.81 µg/mL. This supports its classification as relatively bee-safe acaricide when applied under controlled doses, consistent with prior studies (Rademacher & Harz, 2006; Gregorc & Planinc, 2001). Importantly, its LC_{50} against *Varroa* was 21.26 µg/mL (Table 2), which indicates only a threefold difference in selectivity between mites and bees. This narrower margin suggests that oxalic acid, while natural and popular among beekeepers, must be carefully dosed to minimize bee mortality, especially under stressful colony conditions.

Tau-fluvalinate showed an LC_{50} of 296.07 µg/mL at 24 hours, slightly reduced from its 12-hour LC_{50} (334.71 µg/mL). This continues to indicate low acute toxicity to honey bees relative to coumaphos. However, compared to mites (LC_{50} = 24.69 µg/mL, Table 2), the selectivity ratio narrows to about 12-fold. While tau-fluvalinate appears relatively safe for bees in acute assays, residue accumulation in wax and subsequent chronic exposure have been widely reported as long-term risks (Pettis *et al.*, 2004; Mullin *et al.*, 2010). These residues can compromise queen health and brood development, suggesting that laboratory LC_{50} values may underestimate the risks in the field.

Varoviga® again exhibited the lowest toxicity, with an LC_{50} of 443.16 µg/mL at 24 hours. This reinforces its safety for bees; however, when compared with *Varroa* LC_{50} values (124.66 µg/mL, Table 2), the selectivity ratio is reversed, indicating that the product is less effective against mites than against bees. This finding explains why thymol-based treatments are generally recommended as part of integrated pest management (IPM) strategies rather than as stand-alone acaricides, offering safer long-term options with reduced chemical residues (Imdorf *et al*., 1999; Rosenkranz *et al.*, 2010). When comparing the toxicity results obtained for honey bees (Tables 3 and 4) with those for *V. destructor* (Tables 1 and 2), striking differences emerge. In both hosts, coumaphos was the most toxic agent, but the relative sensitivity was far greater in mites than in bees. For example, the LC_{50} of coumaphos after 24 hours was 1.34 µg/mL in mites versus 18.24 µg/mL in bees. This demonstrates clear selectivity, enabling their practical use in mite control. In contrast, oxalic acid and tau-fluvalinate showed narrower selectivity margins, which highlights potential risks to bees if overdosing occurs. Interestingly, Varoviga exhibited the least toxicity to bees but was also the least effective against mites, suggesting a trade-off between colony safety and mite control efficacy. These findings align with earlier reports (Rosenkranz et al., 2010; Johnson et al., 2013) that emphasize the importance of balancing acaricidal potency with honeybee safety. Synthetic acaricides, such as coumaphos, remain highly effective, but the development of resistance in *Varroa* populations is a growing challenge. Meanwhile, natural products such as oxalic acid and thymol formulations (Varoviga) offer safer alternatives for bees but may require integrated application strategies to achieve sufficient mite control.

Table 4. Probit parameters and TI of acaricides against *A. mellifera* after 24 h contact exposure

Acaricides	**LC_{25} (95% confidence limits)**	**LC_{50} (95% confidence limits)**	**LC_{90} (95% confidence limits)**	**Regression Slope ± SE**	**Chi-square**	**Toxicity Index**
Coumaphos	8.362 (5.56-11.03)	18.241 (14.45-22.02)	80.287 (61.97-117)	1.991±0.232	5.644	100
Tau-fluvalinate	170.321 (139.46-296.25)	296.074 (264.64-331.99)	846.584 (677.7-1189)	2.809±0.326	3.699	6.16
Oxalic acid	26.604 (18.293-34.75)	71.417 (57.97-86.4)	466.29 (326.02-790.02)	1.573±0.172	5.248	25.54
Varoviga	250.948 (205.41-312.16)	443.16 (362.76-518.87)	1305.678 (1143-1567)	2.731±0.294	3.225	4.12

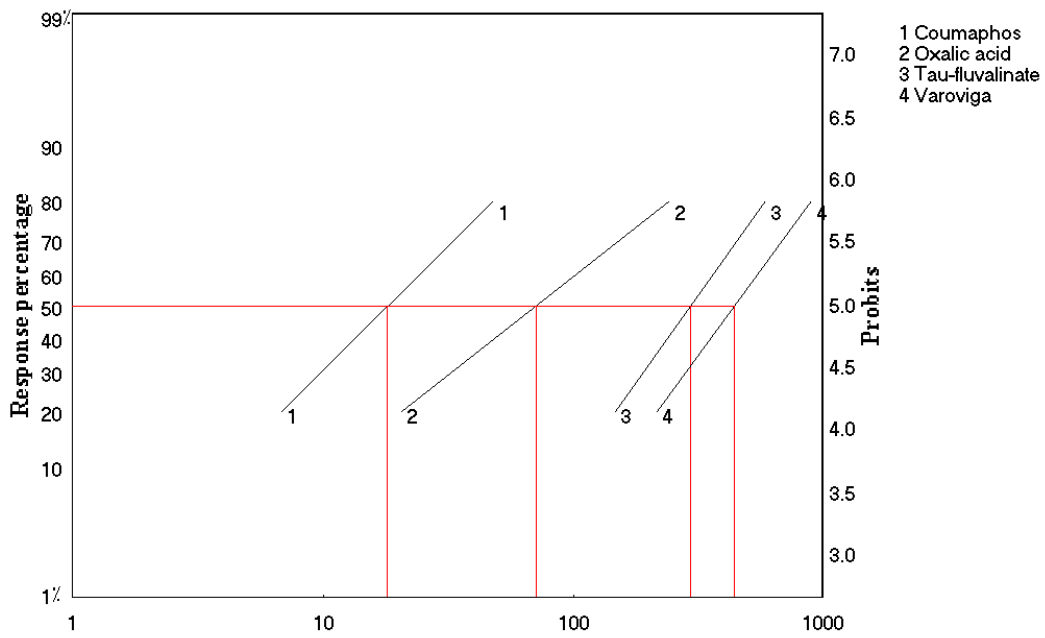

Fig. 4. Toxicity lines of acaricides against *A. mellifera* after 24 h contact exposure.

Field Evaluation of Varroa Mortality after Acaricide Treatments

The field trial data (Fig. 5) present the weekly average number of *V. destructor* mites that fell from honey bee colonies following treatment with different acaricides over a four-week period. The control group showed a very low natural mite drop (25.88 ± 3.67), confirming that untreated colonies had minimal spontaneous mortality and validating the treatment effects. All acaricides significantly increased mite mortality compared to the control. The highest varroa fall was observed in coumaphos (169.13 ± 17.91) followed by oxalic acid (165.56 ± 26.92)**.** Tau-fluvalinate and Varoviga resulted in comparatively lower mite drops (146.63 ± 18.85 and 135.69 ± 18.86, respectively), though still substantially higher than the untreated control. This ranking clearly indicates that coumaphos remains the most potent miticide under field conditions.

When these findings are compared to the laboratory bioassays (Tables 1 and 2), the consistency is evident. In laboratory acute contact assays, coumaphos demonstrated the lowest LC_{50} values against Varroa, reflecting its high toxicity. The field results align well, indicating that these two compounds are the most effective in reducing mite infestation. Conversely, Varoviga, which showed the highest LC_{50} values in lab bioassays (indicating the lowest toxicity to mites), also produced the lowest mite drop in the field. Interestingly, oxalic acid, which exhibited intermediate toxicity levels under laboratory conditions (LC50 ≈ 21–26 µg/mL after 24 h), ranked third in mite reduction in the field, with a level of efficacy comparable to that of coumaphos. This suggests that oxalic acid, although slower-acting or less potent at the individual mite level, achieves strong colony-level efficacy in practical use, possibly due to its mode of action and distribution within the hive. Tau-fluvalinate demonstrated moderate performance in both laboratory and field tests, confirming its role as an effective, though less potent, miticide compared to coumaphos. However, its historical issues with resistance development in mite populations (Elzen *et al.*, 1999; Milani, 1999) must be considered when interpreting its efficacy. Overall, the field results validate the laboratory bioassays, showing that acute toxicity measurements (LC values) can predict field performance trends. Nevertheless,

field trials also capture additional factors such as compound persistence, distribution within colonies, and mite population dynamics, which help explain differences in rank order between laboratory and field performance (e.g., oxalic acid).

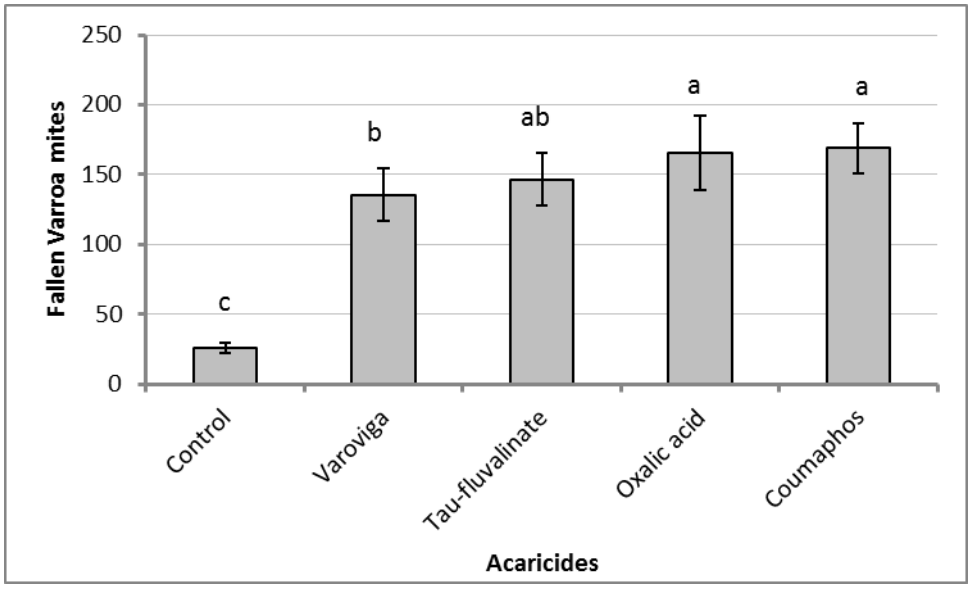

Fig. 5. Mean weekly number of fallen *V. destructor* mites in honey bee colonies following acaricide treatments under field conditions (F = 39.37659; LSD = 28.35287)

Conclusion

This study demonstrates that coumaphos remains the most potent contact toxicant against *Varroa destructor*, providing both rapid and sustained efficacy under both laboratory and field conditions. However, its toxicity to honey bees warrants cautious application. Oxalic acid and tau-fluvalinate offer moderate efficacy, with oxalic acid demonstrating particularly strong field-level performance, despite its lower laboratory potency. Varoviga, while the least effective against mites, proved to be the safest for bees, supporting its use as part of integrated pest management strategies. Overall, the results underscore the importance of using synthetic and natural acaricides in a balanced manner, with rotational treatment programs being essential to enhance sustainability, minimize residue risks, and delay the development of resistance.

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تقدير السمية لبعض المبيدات الأكاروسية علي طفيل الفاروا ونحل العسل تحت الظروف المعملية والحقلية

حنان محمد احمد قنبر[١]، مصطفى عبد النعيم صديق أحمد[١]، سيد محمد عامر على[١]، محمد احمد عنتر[٢]

[١] قسم تربية النحل، معهد بحوث وقاية النبات، مركز البحوث الزراعية، الدقي، ١٢٦١٩، الجيزة، مصر.

[٢] قسم وقاية النبات، كلية الزراعة، جامعة الأزهر، القاهرة

الملخص العربي

تهدف الدراسة إلى تقييم السمية الحادة بالملامسة والكفاءة الحقلية لأربعة مبيدات ضد طفيل الفاروا *Varroa destructor* ونحل العسل *Apis mellifera*، وهي: الكومافوس، التاو- فلوفالينات، حمض الأوكساليك، والمستحضر الطبيعي فاروفيجا® (المعتمد على الثيمول). أظهرت الاختبارات المعملية تبايناً واضحاً بين المركبات. فقد سجل الكومافوس أعلى فاعلية ضد الفاروا بعد ١٢ و٢٤ ساعة من التعرض (LC_{50} = 2.28–1.34 ميكروجرام/مل)، مع بقاء هامش أمان نسبي تجاه نحل العسل (LC_{50} = 27.33–18.24 ميكروجرام/مل). وأظهر حمض الأوكساليك والتاو- فلوفالينات سمية متوسطة (LC_{50} = 25.89–21.26 و٣٢,٩٦–٢٤,٦٩ ميكروجرام/مل على التوالي)، بينما كان فاروفيجا الأقل تأثيراً LC_{50} (157.95–124.67 =ميكروجرام/مل). بالنسبة للنحل، جاء ترتيب السمية كالآتي: الكومافوس الأعلى سمية، يليه الاوكسالك، ثم التاو- فلوفالينات، وأخيراً فاروفيجا الذي كان الأكثر أماناً. وأكدت التجارب الحقلية النتائج المعملية، حيث حقق الكومافوس أعلى معدل سقوط للفاروا، تلاه حمض الأوكساليك، بينما أظهر كل من التاو- فلوفالينات وفاروفيجا تأثيراً أقل لكنه ظل معنوياً مقارنة بالكنترول غير المعامل. تؤكد هذه النتائج أهمية الموازنة بين كفاءة المبيد وسلامة النحل، وتشير إلى إمكانية دمج المنتجات الطبيعية مثل حمض الأوكساليك والمركبات القائمة على الثيمول ضمن برامج المكافحة المتكاملة لتقليل مخاطر المقاومة والحفاظ على صحة الطوائف.