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Original article

Determination of the LC_{50} in the mite *Varroa jacobsoni* of the active substances in Perizin® and Cekafix®

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Summary — A bioassay has been developed to measure the toxicity of the organophosphorus acaricides, the active ingredients of Perizin® and Cekafix®, to the mite *Varroa jacobsoni* Oud. The distribution of the lethal concentrations was rather narrow (LC_{90}/LC_{10}~4). Slight, but significant and consistent differences (up to three times) were found among groups of mites taken from different brood stages; at 26 °C the toxicity was 1.2–2.7 times lower than at 32.5 °C; at a given concentration coumaphos was about twice as active as the active ingredient of Cekafix®. No increase in the LC_{50} to coumaphos of mites resistant to pyrethroids was noted. *Varroa* strains resistant to these acaricides were not studied.

***Varroa jacobsoni* / toxicity / resistance / coumaphos / organophosphorus acaricide**

INTRODUCTION

The recent occurrence of strains of *Varroa jacobsoni* Oudemans resistant to pyrethroids has drawn the attention of researchers and beekeepers to the limitations of the chemical control of this parasitic mite. Acaricide resistance is going to be an increasing problem in the near future, and is made even worse by the low number of alternatives and by the fact that the increase of the resistant populations is often unnoticed and can lead to the unexpected collapse of the colonies and to heavy bee losses (Astuti et al, 1996).

Prompt detection of *Varroa* populations resistant to an acaricide could reduce bee losses and, at least in part, prevent further spread of resistant strains by changing as soon as possible to other effective control techniques. The availability of reliable methods for testing the susceptibility of the *Varroa* mite to different acaricides is also important for basic research dealing with the resistance mechanisms in this mite. Recently, bioassays for pyrethroids (Milani, 1995; Faucon et al, 1996) and amitraz (Faucon et al, 1996) have been developed.

We thought it useful to develop a bioassay to evaluate the toxicity of two

organophosphorus acaricides widely used against *V jacobsoni*, the active ingredients (ai) of the products Perizin® and Cekafix® (Schäbitz et al, 1991) even though the ineffectivess of products containing these ai has not been reported so far. In fact, populations of mites never treated with these acaricides are readily available, making it easier to establish a 'baseline level' for the susceptibility of unselected strains.

A modification of the technique used to assess resistance to pyrethroids (Milani, 1995) was chosen; this may seem inappropriate, since these ai and especially coumaphos have been considered 'systemic' acaricides (Ritter, 1985), however, some doubt has been cast on this mode of action (van Buren et al, 1992) and other formulations that rely upon contact action have been used (Ho and An, 1980; Gajadusková et al, 1990).

MATERIALS AND METHODS

A technique similar to that described by Milani (1995) was used, with some modifications suggested by previous experience.

Acaricides tested

Investigations were carried out with the ai of Perizin® (coumaphos, phosphorothioic acid *O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl)-*O,O*-diethyl ester; Bayer), using the ai supplied by the manufacturer (standard 910701dor02, 97% purity), and with the ai of Cekafix® (phosphorothioic acid *O*-(3-bromo-4-methyl-2-oxo-2H-1-benzopyran-7-yl)-*O,O*-diethyl ester, AG Chemie; referred to as Cekafix-AI) (standard A7009, purity ≥ 97%).

Origin of mites

Most mites were sampled from an experimental apiary, kept in Udine (Friuli, north-eastern Italy) which had not received any acaricide treatment for 3 years, except for some colonies, which had been treated with Apilife Var (tablets impregnated with essential oils; Chemicals Laif). The population of the mite was susceptible to fluvalinate, but towards the end of the experiments, a low proportion (~10%) of mites was found to be resistant to fluvalinate in some colonies, due to the spread of the resistant strains also in Friuli (Trouiller, 1995). In that region organophosphoous acaricides had very seldom been used against *V jacobsoni* until autumn 1995.

A sample of mites which had survived an Apistan treatment was taken from an apiary near Manzano, a village about 15 km from Udine; large populations of pyrethroid-resistant mites were detected there during 1995, probably following their introduction in 1993 or earlier; that mite population had not been treated with organophosphorus acaricides.

Collection of mites

Mites were collected from bee brood as described in Milani (1995). *Varroa* mites from brood of different ages (spinning larvae, *l5*; pupae with white eyes, *pw*; pupae with dark eyes and white or pale body, *pd*) were assayed separately. Mites from pupae with pigmented body and from adult bees were not used, since their response might not be uniform and the mortality in the controls higher (Milani, 1995).

The bioassay

Shallow capsules, made of two glass disks (62 mm diameter) and two stainless steel rings (56 mm inner diameter, 5 mm total height) were prepared. The interior of these capsules (including the rings) was coated with paraffin wax (Merck 7151, melting point 46–48 °C) containing a known concentration of the ai. Ten grams of paraffin wax was melted in a Petri dish kept in a water bath heated to 60 °C; then the required amount of active ingredient, dissolved in 2.5 mL acetone (Prolabo 20066.321), was added. Acetone only was added to the control. The mixture was stirred for 30 min and then the steel rings were immersed in the molten paraffin wax and one side of the glass disks was coated by lowering the disk onto

the molten paraffin. Glass disks and iron rings were weighed before and after coating; capsules in which the total amount of coating paraffin was outside the range 1.6–2.0 g were discarded. The capsules were then kept open for at least 24 h at room temperature to allow possible acetone residues to evaporate and then were stored at 32.5 °C. The concentration used was determined on the basis of a series of preliminary tests as 0 (control), 2, 5, 10, 20, 50 and 100 ppm.

Ten or fifteen *Varroa* females were introduced into each capsule; after 4 h they were transferred to a clean glass Petri dish (60 mm diameter) with respectively two or three worker larvae taken from cells 0–24 h after capping.

The mites were observed under a dissecting microscope 4 h (when transferred to the Petri dish), 8, 24 and 48 h after the introduction into the capsule and classified as mobile, paralysed or dead as in Milani (1995). Mites lost or accidentally killed were not included into the counts.

Experiments carried out

Assays were carried out on mites from *l5, pw* and *pd* at 32.5 °C and 70% RH and at 26 °C, 70% RH, with both ai, using capsules prepared 1–12 days earlier. All the experiments could not be carried out at the same time (as required in a true factorial experiment), but part of the assays under different conditions or with mites from different brood stages were carried out simultaneously to rule out the possibility that the observed differences were the effect of some uncontrolled factor. Mites from the population resistant to pyrethroids, taken from *pw* and *pd*, were assayed at 32.5 °C.

The effect of ageing of the capsules was assessed with coumaphos, by comparing freshly prepared capsules with those prepared 30 days (on mites from *pw* and *pd*) and 60 days beforehand (on mites from *pw*).

As a rule, the assay was repeated two to four times per brood stage and temperature, until 30–60 mites per concentration were assayed (about 15 mites per concentration and each bee stage in the case of the mites resistant to pyrethroids); when the number of mites was limited, more mites were assayed at concentrations around the median lethal concentration (Finney, 1971), and assays at 2 and 100 ppm were not replicated when respectively a mortality not exceeding that of the controls or 100% mortality were expected.

The assays were carried out in summer and early autumn 1994.

Statistical analysis

The data were analysed using the probit transformation; the natural mortality rate was taken into account using the iterative approach, according to Finney (1949). The concentrations which kill a given proportion z of mites (LC_z) and their fiducial limits were computed according to Finney (1971).

Sets of data obtained with mites from different brood stages, at different temperatures or times, or with the two ai were compared to obtain the 'relative potency' of the treatment (Finney, 1971), when they could be fitted with parallel lines, by maximising the total likelihood function; calculations were carried out using an Excel 4.0 spreadsheet. The relative potency (eg, at different temperatures) was calculated as the weighted average of results obtained under different conditions (eg, at different times); when this was not possible because of the heterogeneity of respective estimates or because of some departure from parallelism of one or two sets of data, it was preferred to indicate the range rather than resort to a more complex and somewhat artificial handling of the data.

RESULTS

Conditions of the mites at different times

The mortality in the controls ('natural mortality') was very low (overall mean 0.2, 0.3, 1.4 and 1.8% respectively at 4, 8, 24, 48 h in experiments with coumaphos; 0.5, 0.5, 0.5, 2% with the Cekafix-AI); the numbers of mites dead in the controls were so small that any difference between mites from different brood stages or experiments at different temperatures could not be detected.

In mites treated with coumaphos, at 4 h, symptoms of intoxication were evident only

at the highest concentrations tested (50, 100 ppm; also 20 ppm for mites taken from larvae), at which 50% or more mites were paralysed; even at these concentrations, only 3% were dead. At 8 h about one third of the mites had died at 50 and 100 ppm, while paralysed (around 20%) and dead mites (8%) were observed at 10 and 20 ppm. Some repeated observations between 4 and 10 h showed a fast evolution of the condition of the mites during this period. At 24 h the proportion of paralysed mites was very low (2%) and it fell further at 48 h (< 1%), in assays at both 32.5 and 26 °C. Paralysed mites never recovered. In contrast, many mites that had not shown any apparent sign of intoxication later died, usually after having regurgitated a droplet of fluid.

The number of dead mites increased further between 24 and 48 h; the increase in the potency of coumaphos during that period under the various conditions was 1.4 times.

With the Cekafix-Al, similar effects were observed, but at somewhat higher concentrations. The proportion of paralysed mites was 9, 16, 0.2, 0.2% at 4, 8, 24, 48 h respectively. The increase in the potency between 24 and 48 h was 1.1 at 32.5 °C and 1.4 at 26 °C.

Although the effects on the *Varroa* mite were clearly concentration-dependent at 4 and 8 h, the observations at 24 and 48 h depict a more stable situation and thus they are the results reported and discussed the most in the next sections.

General observations

The results of the replications were consistent and a good fit using the probit regression was found in nearly all the cases; the results of the χ^2 test for heterogeneity approached significance ($0.05 < P(\chi^2) < 0.1$) in three cases out of 30. The distribution of the lethal concentrations was rather narrow, resulting in steep regression lines; the LC_{90}/LC_{10} ratio was about four.

Comparison of Varroa *mites from different brood stages*

In experiments using coumaphos, at both temperatures and times the susceptibility of mites from different brood stages (larva just after capping, pupa with white eyes, pupa with dark eyes and white or pale body) was slightly, but significantly and consistently different; if data from *l5, pw* and *pd* are pooled, very highly significant heterogeneity χ^2 arise ($P < 10^{-8}$). The fiducial intervals of the median lethal concentration (LC_{50}) do not overlap (table I); the mites from *l5* were more susceptible than those from *pd* and the latter were more susceptible than mites from *pw*. The regression lines for the three groups of mites (fig 1) do not deviate significantly from parallelism, indicating that the variance of the lethal concentration was the same; the relative potency depended on the temperature and was 1.5:0.7:1 at 32.5 °C and 2.4:0.6:1 at 26 °C on mites from *l5, pw, pd* respectively.

With the Cekafix-Al (table II), similar variations in the susceptibility of mites taken from different brood stages were observed at both temperatures and times; again, the χ^2 test shows highly significant heterogeneity among groups of mites from brood of different age, the mites from *l5* being the most susceptible and those from *pw* the least susceptible; the relative potency on mites from *l5, pw, pd* was 2.0:0.7:1 at 32.5 °C and 1.2: 0.5:1 at 26 °C.

Effect of temperature

The toxicity of coumaphos was lower at 26 °C than at 32.5 °C (table I); the decrease

Table I. Median lethal concentration (LC_{50}) and its fiducial limits in assays with coumaphos using capsules prepared 1–12 days beforehand.

T (°C)	*Bee stage*	*Approximate No of mites per concentration*	*24 h*		*48 h*	
			LC_{50}	*Fiducial limits*	LC_{50}	*Fiducial limits*
26	*l5*	30	8.1	6.6, 9.6	5.9	4.6, 6.9
26	*pw*	35	34.7	29.1, 41.5	20.6	17.2, 24.7
26	*pd*	30	21.3	16, 29.2 [a]	14.6	12.5, 17.3
32.5	*l5*	60	5.7	4.9, 6.5	4.5	3.7, 5.2
32.5	*pw*	45	12.6	11.0, 14.3	9.8	8.7, 11.1
32.5	*pd*	30	8.5	6.9, 10.4	6.4	3.4, 9.2[a]

[a] $P(\chi^2) \approx 0.07$ (in both cases) and so a heterogeneity factor was used.

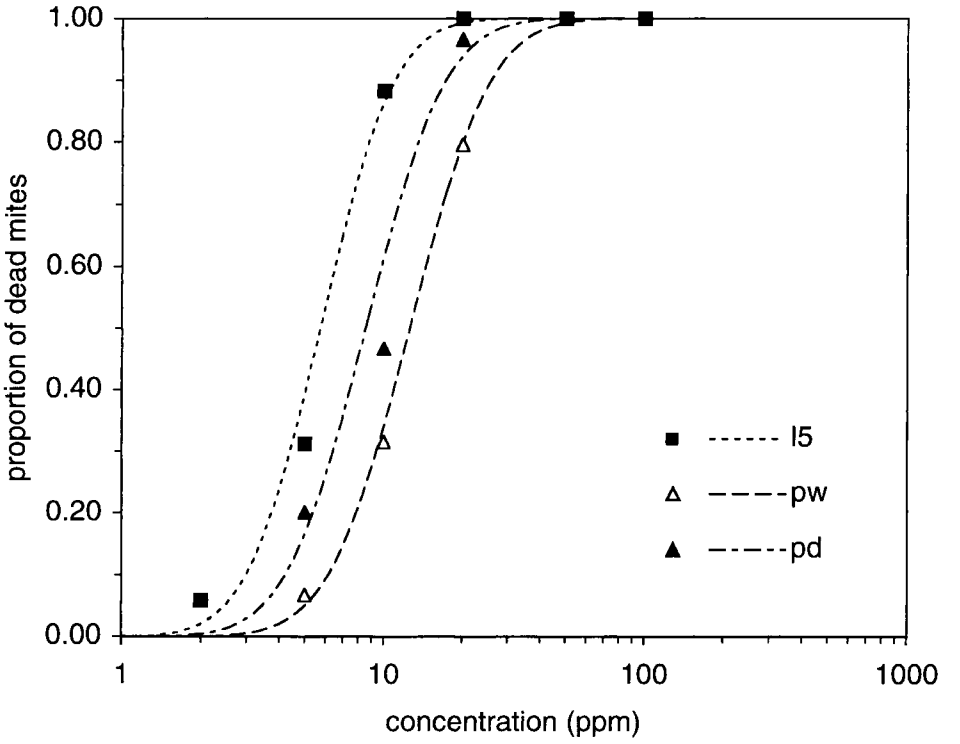

Fig 1. Proportion of dead *Varroa* mites at 24 h and 32.5 °C in the assays carried out with coumaphos on mites taken from brood of different age in the Udine apiary, and regression lines obtained using the probit transformation. No mites died in the controls, and thus no correction for natural mortality was made.

Table II. Median lethal concentration (LC_{50}) and its fiducial limits in assays with the Cekafix-Al using capsules prepared 1–10 days beforehand.

T (°C)	*Bee stage*	*Approximate No of mites per concentration*	*24 h*		*48 h*	
			LC_{50}	*Fiducial limits*	*LC_{50}*	*Fiducial limits*
26	*l5*	30	19.6	15.8, 24.4	14.5	11.4, 18.1
26	*pw*	30	48.1	39.8, 57.9	35.3	20.7, 51.8[a]
26	*pd*	30	23.1	19.0, 28.4	16.3	12.9, 20.3
32.5	*l5*	45	9.8	8.5, 11.2	9.4	8.2, 10.6
32.5	*pw*	45	29.2	25.3, 33.9	24.6	21.1, 28.7
32.5	*pd*	30	19.2	16.4, 22.0	18.9	16.1, 21.8

[a] $P(\chi^2) \approx 0.09$ and so a heterogenity factor was used.

was more evident for mites from pupae (2.7 times) than for those from larvae (1.3 times); except for mites from *l5* at 48 h, the fiducial intervals of the LC_{50} do not overlap and significant heterogeneity exists between data obtained at different temperatures.

A similar dependence of the toxicity on the temperature was observed with the Cekafix-Al (table II); the LC_{50} was 1.2–2.0 times larger at 26 °C, but in this case the differences and the heterogeneity χ^2 were not always significant.

Effect of ageing of the capsules

The results obtained in successive replications carried out on mites from the same brood stage and the same temperature within 12 days of the preparation of the capsules are not statistically different. The mortality obtained with capsules prepared 30 days earlier was somewhat higher (table III); the LC_{50} was decreased 1.3–1.8 times, but in some cases the fiducial intervals overlap.

Table III. Median lethal concentration (LC_{50}) and its fiducial limits in assays with coumaphos using capsules prepared 30 and 60 days beforehand.

T (°C)	*Capsule age, bee stage*	*Approximate No of mites per concentration*	*24 h*		*48 h*	
			LC_{50}	*Fiducial limits*	*LC_{50}*	*Fiducial limits*
26	30 d, *pw*	30	25.7	19.9, 34.2	16.3	14.0, 18.7
26	30 d, *pd*	30	12.5	10.2, 15.2	8.1	7.0, 9.3
32.5	30 d, *pw*	30	9.6	8.4, 11.0	8.3	6.9, 9.7
32.5	30 d, *pd*	30	6.7	5.3, 7.9	5.4	3.9, 6.5
32.5	60 d, *pw*	40	10.2	8.7, 11.5	8.4	7.2, 9.2

The result of the assay carried out with capsules prepared 2 months earlier was nearly coincident with that obtained with capsules 30 days old.

Comparison between coumaphos and the Cekafix-AI

The pattern of the mortality for the two ai studied was very similar, and the regression lines obtained for coumaphos and Cekafix-AI do not deviate from parallelism at each temperature and time; coumaphos was more active than the Cekafix-AI, at a given concentration and the relative potency was 1.4–2.4, depending on temperature and time.

Mites resistant to fluvalinate

No difference between mites from Udine and from Manzano (resistant to fluvalinate) was found; the LC_{50} at 24 h for the latter was 18.3 (fiducial limit 13.9, 25.9) for mites from *pw,* and 7.2 (5.6, 8.8) for mites from *pd,* with non-significant deviations in opposite directions from those of mites from Udine.

DISCUSSION

The technique described here could highlight even small differences in the susceptibility of the *Varroa* mite to the two organophosphorus acaricides tested; the slope of the curve is higher than that obtained in the case of fluvalinate (Milani, 1995), indicating that the variance of the lethal concentrations is smaller. The distribution of the LC obtained in the present work was narrower than that observed by Abed and Ducos de Lahitte (1993) with Perizin®, using topical application: in their experiment, the LD_{90}/LD_{10} ratio was about 25, six times larger than the LC_{90}/LC_{10} obtained here.

Differences between mites from different brood stages were observed consistently with both ai in all the tests carried out; they could depend on the different feeding condition of the mites and the consequently greater weight of mites from pupae. These differences could make it more complex to estimate possible variations between populations, if samples do not come from the same brood stage. Comparisons could be easily made when the regression lines are parallel; anyway, any differences of practical importance are much larger than those for mites from different brood stages. In this work, slight deviation from parallelism was noted in a few cases; heterogeneity between estimates of the relative potency under different conditions was more often observed, possibly because of interactions between the factors tested. On the other hand, it was not possible to carry out the assays involving thousands of mites all at the same time and thus inevitable, slight variations in the natural susceptibility of mites could have influenced the results to some extent.

The ageing of the capsules had far less effect than observed with fluvalinate (Milani, 1995) and thus capsules can be used for at least 2 months after their preparation, if large differences in susceptibility are to be investigated. On the contrary, if a very precise estimate of the LC_{50} is needed, the use of capsules of the same age (prepared 1–10 days before) is necessary. The ageing effect could depend on the fact that the paraffin surface is enriched in the ai or that it becomes smoother with ageing.

The *Varroa* strains resistant to pyrethroids did not show any decrease in the susceptibility to coumaphos; this is not unexpected, since the mechanisms that make mites resistant to pyrethroid and to organophosphorus acaricides are usually different (Soderlung and Bloomquist, 1990).

The technique described here, adapted with some modifications from Milani (1995), could be used to measure the toxicity of other contact acaricides to *V jacobsoni,* provided that they are non-volatile, soluble in paraffin wax and stable up to 60 °C.

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Résumé — Détermination de la CL_{50} des substances actives du Perizin® et du Cekafix® chez l'acarien *Varroa jacobsoni.* Un test biologique a été mis au point pour mesurer la toxicité des matières actives des acaricides organosphosphorés Perizin® et Cekafix® pour l'acarien *Varroa jacobsoni* Oud. Les varroas ont été prélevés dans le Frioul (nord-est de l'Italie) où ces insecticides ont été très rarement utilisés. Les essais ont porté à la fois sur des varroas résistant aux pyréthrinoïdes et sur des varroas sensibles. Ils ont été prélevés sur du couvain operculé (jusqu'au stade de nymphe à yeux foncés et corps clair). Les capsules, consistant en deux disques de verre (62 mm de diamètre) et deux écarteurs en acier inox (56 mm de diamètre intérieur, 5 mm de haut), étaient enduites sur la face interne de paraffine renfermant une concentration connue de matière active. Les acariens ont été maintenus dans ces capsules pendant 4 heures, puis transférés dans une boîte de Pétri (60 mm de diamètre), où ils pouvaient se nourrir sur des larves d'abeilles. Ils ont été observés à 4, 8, 24 et 48 heures après leur introduction dans les capsules et classés en trois catégories : mobiles, paralysés (ils pouvaient bouger quelques appendices mais non se déplacer) et morts. Les observations faites à 24 et 48 heures – lorsque la proportion de varroas paralysés est en général négligeable – ont donné des résultats plus fiables. Les varroas paralysés ne se sont jamais rétablis ; en revanche ceux qui n'avaient montré aucun signe d'intoxication sont morts plus tard. Avec le coumaphos, matière active du Perizin®, et la matière active du Cekafix®, la mortalité des acariens à 48 heures a été de 1,4 et respectivement 1,1 à 1,4 fois plus forte qu'à 24 heures. Dans tous les tests réalisés la distribution des concentrations létales a été étroite (CL_{90}/CL_{10} proche de 4). Des différences, faibles mais significatives et régulières, ont été observées parmi les acariens prélevés sur les divers stades de couvain pour les deux acaricides : la CL_{50} du coumaphos à 24 heures et 32,5 °C a varié de 5,7 ppm, pour les acariens prélevés sur les larves juste après l'operculation, à 12,6 ppm pour ceux prélevés sur les nymphes à yeux blancs ; celle du Cekafix® est comprise entre 9,8 et 29,2, respectivement. Dans les deux cas la CL_{50} est intermédiaire pour les nymphes à yeux foncés. La toxicité des deux acaricides décroît avec la température : à 26 °C, elle est 1,2 à 2,7 fois plus faible qu'à 32,5 °C. Pour une concentration donnée le coumaphos est 1,4 à 2,4 fois plus actif que la matière active du Cekafix®. On a observé de légers effets du vieillissement des capsules : avec des capsules de coumaphos âgées de 30 jours la CL_{50} a diminué de 1,3 à 1,8 fois puis s'est stabilisée durant deux mois. On n'a pas noté d'accroissement de la CL_{50} du coumaphos chez les varroas résistant aux pyréthrinoïdes, ni observé de lignées de varroas résistant aux acaricides organophosphorés.

***Varroa jacobsoni* / acaricide organophosphoré / toxicité / résistance / coumaphos**

Zusammenfassung — Bestimmung der LC50 der aktiven Substanzen in Perizin®

und Cekafix® für *Varroa jacobsoni.* Es wurde ein Biotest zur Messung der Toxizität der in Perizin® und Cekafix® als aktive Komponenten enthaltenen organophosphorischen Acarizide für die Milbe *Varroa jacobsoni* Oud entwickelt. Die Varroamilben wurden in Friaul (Nordost-Italien) gesammelt, wo diese Akarizide sehr selten eingesetzt wurden. Die Untersuchungen umfassten gegenüber Pyrethroiden empfindliche und resistente Milben. Die Milben wurden aus verdeckelten Brutzellen mit Puppen bis zum Stadium mit dunklen Augen und blasser Körperfarbe gewonnen. Die verwendeten Testkapseln bestanden aus zwei runden Glasscheiben mit einem Durchmesser von 62 mm mit zwei Abstandshaltern aus rostfreien Stahl (Innendurchmesser von 56 mm, Höhe 5 mm). Die Glasscheiben wurden auf den Innenseiten mit Paraffin bedeckt, das die jeweiligen aktiven Substanzen in bekannten Konzentrationen enthielt. Die Milben wurden vier Stunden lang in diesen Kapseln gehalten und dann in saubere Petrischalen (Durchmesser 60 mm) umgesetzt, in denen sie sich auf Bienenlarven ernährten. Sie wurden dort 4, 8, 24 und 48 h nach Einsetzen in die Kapseln beobachtet und in drei Kategorien eingeteilt: Beweglich, paralysiert (wenn sie zwar einige Glieder bewegen konnten, aber sich nicht mehr fortbewegten und tot. Die Ergebnisse nach 24 h und 48 h waren am zuverlässigsten, zu diesen Zeitpunkten war die Anzahl paralysierter Milben vernachlässigbar. Paralysierte Milben erholten sich nicht, im Gegenteil starben einige Milben später, die zuvor keinerlei Vergiftungserscheinungen gezeigt hatten. Zwischen 24 und 48 h stieg die Mortalität bei Coumaphos 1,4-fach an, bei der aktiven Substanz von Cekafix® 1,1 bis 1,4-fach. In allen ausgeführten Tests verteilten sich die lethalen Konzentrationen in einem sehr engen Bereich (LC90/LC10 etwa 4). Bei beiden Acariziden zeigten sich signifikante und konsistente Unterschiede zwischen Milben aus unterschiedlichen Bienenbrutstadien, allerdings waren diese Unterschiede klein. Die LC50 von Coumaphos bei 32,5 °C und nach 24 h varierte zwischen 7,7 ppm bei Milben von Larven kurz nach der Zellverdeckelung bis zu 12,6 ppm bei Milben von Puppen mit weißen Augen. Die entsprechenden Werte der Aktivsubstanz von Cekafix® betrugen 9,8 bzw 29,2 ppm. In beiden Fällen lagen die Werte für Milben von dunkeläugigen Puppen dazwischen. Die Toxizität beider Substanzen nahm mit fallender Temperatur ab; bei 26 °C war sie 1,2 bis 2,7 mal geringer als bei 32,5 °C. Bei gleicher Konzentration war Coumaphos 1,4 bis 2,4 mal aktiver als die aktive Substanz von Cekafix®. Es konnte eine leichte Altersabhängigkeit der Wirksamkeit der Kapseln festgestellt werden. Bei Coumaphos war die LC50 von 30 Tage alten Kapseln 1,3 bis 1,8 mal höher und blieb danach bis zu zwei Monate konstant. Es konnte keine Erhöhung der LC50 bei pyrethroidresistenten Milben festgestellt werden. Gegenüber organophosphorischen Acariziden resistente Varroalinien konnten nicht beobachtet werden.

***Varroa jacobsoni* / Toxizität / Resistenz / Coumaphos / organophosphorische Acarizide**

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