

# EFFECTS OF SYNTHETIC AND ORGANIC ACARICIDES ON HONEY BEE HEALTH: A REVIEW

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**Abstract:** The honey bee is a crucial pollinator of agricultural crops and also an economically important producer of commodities such as honey and beeswax that find diverse uses in the food industry, cosmetics and medicine. At present, the ectoparasitic mite *Varroa destructor* is viewed as the most damaging pest of the honey bee worldwide. Without treatment, colonies generally collapse within a few years. To keep the population of the Varroa mites low, beekeepers rely on the use of synthetic and organic acaricides, the most popular commercially available ones include amitraz, coumaphos, flumethrin, fluvalinate, formic acid, oxalic acid and thymol. These conventional acaricides are cheap and easy to apply, but prolonged use causes Varroa mites to rapidly develop resistance and bee products can become contaminated. Residues of acaricides are present in high concentrations throughout the hive and bees are exposed to them all year around. The present review summarises the current knowledge of the deleterious effects of conventional acaricides on honey bee health. Numerous commercially available acaricides and their active substances have been shown to have negative effects on honey bee brood development, queen and drone reproductive health, learning, longevity and colony strength. Acaricides do not only act alone, but also in synergic combinations to affect bee health. Since some drugs cause substantial weakening of bee colonies, they can make them more susceptible to other diseases such as nosematosis or to extreme climatic events. As wax combs are contaminated with high concentrations of acaricide residues and Varroa mites are chronically exposed to them, the parasite may develop resistance faster. In combination with other stressors, acaricides could be a contributing factor to colony collapses.

**Key words:** synthetic acaricides; varroacides; honey bee; *Apis mellifera*; synergy; organic beekeeping;

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## Introduction

The honey bee (*Apis mellifera* L. 1758) ranks among the most economically important pollinators in the world, providing key ecosystem services in both artificial and natural landscapes (1). It has been estimated that the worldwide value of pollination is about €153 billion annually (2), making honey bees key contributors to global economy. Also important is the role of honey bees

in collecting and manufacturing products including honey, beeswax, propolis, royal jelly or bee venom that today find wide uses as foods, natural medicines and cosmetics. Beekeeping is the source of income for many around the world and a key poverty alleviation measure (3). However, there are a number of pests and parasites of the honey bee that require control. As of present, the Varroa mite (*Varroa destructor*) is considered to be the most damaging parasite of the honey bee worldwide (4). Without intervention most, but not all, colonies collapse within several years. Today, Varroa treatments consist primarily of chemotherapy (5).

In Europe, the most frequently used chemicals to control *V. destructor* are synthetic compounds such as coumaphos (active ingredient in medicaments such as Check-Mite™ or Perizin®), fluvalinate (Apistan®, Gabon®), flumethrin (Bayvarol®) and amitraz (Apivar®, Varidol®) (6). In attempt to find more “natural” cures to varroasis, oxalic acid, formic acid (Formidol®) and thymol (Thymovar®) have been introduced and are becoming increasingly popular among organic beekeepers (7). Organic acids and thymol occur naturally in low concentrations in honey, thereby their organic reputation. An overview of the most popular acaricidal chemicals used globally is given in Tab. 1. Today, many of the medicaments originally developed to control *V. destructor* are also used to control other widespread bee parasites such as *Tropilaelaps* mites or bee lice (*Braula coeca*) (8, 9).

bee products (13). Residues of some acaricides have been shown to be very persistent and present a concern for human health (14). Equally importantly, the residues of acaricides can also have serious consequences for the health of the colony.

As of present, much research concentrated on the quantification of acaricide residues in hive products but relatively few studies addressed the impact of residues on honey bee health. This question is also highly controversial given the financial interest of a number of bee research organisations in manufacturing and selling bee medicaments. This review presents a compilation of the published effects of common commercially available synthetic and organic acaricides on honey bee health.

A review of the negative side effects of acaricides is listed separately for every active ingredient

**Table 1:** Examples of popular acaricides used across the world (10)

Compound	Examples of Commercial Products	Residues	Resistance
Amitraz	Apivar® Varidol®	wax, pollen, honey	described
Coumaphos	Checkmite™ Perizin®	wax, pollen, honey	described
Flumethrin	Bayvarol®	wax, pollen, honey	described
Fluvalinate	Apistan®	wax, pollen, honey	described
Formic acid	MAQS®	not known	not known
Oxalic acid	Bienenwohl® Oxuvar®	not known	not known
Thymol	Apilife VAR® Apiguard® Thymovar®	not known	not known

No pesticide used in honey bee medicine is 100% efficient. “Easy to use” drugs were initially seen as a simple, cheap and fast solution to honey bee disease problems, but their widespread use and loose legislation control in some countries resulted into Varroa populations rapidly developing resistance. As such, beekeepers are made reliant on more and more chemical products (11).

Following the application of these medicaments, residues can be detected throughout the beehive in products such as royal jelly but also in adult bees and brood (12). Acaricide residues constitute a significant portion of chemical contaminants of

in Tables 2-7. The impacts of these chemicals on honey bee colony health are discussed systematically in the sections below.

### *Negative Effects of Acaricides on Honey Bees*

#### Effects on Adult Worker Bees

A mounting body of research (reviewed in Tab. 2-7) agrees that among others, exposure of bees to some acaricides at recommended doses can lead to high bee mortality and shortened lifespan. But

what affects the resistance of individual bees to acaricide intoxication? There seem to be several factors. Coumaphos and fluvalinate are more toxic to older bees than young bees (15, 16 cited in 17). Workers that were subjected to less stress appear to be more resistant to fluvalinate and coumaphos poisoning (17, 18). Bee mortality can also increase with higher outdoor temperature. Thymol is practically harmless at outdoor temperatures ranging from 5°C to 9°C (19), but was linked with high bee mortality at temperatures above 27°C (20, 21). The tolerability of the oxalic acid treatment depends strongly on the method of administration used. Colonies treated with the trickling method had a significantly higher adult bee mortality than those treated with the vaporizer method (22). Quite interestingly, it has been shown that queen bees can tolerate higher doses of acaricides than workers, suggesting that the physiological differences between the two can affect pesticide sensitivity (23). Other factors that may affect the mortality of workers that contacted acaricides include their pathogen load (24), mobility (25) and the acaricide dose received (26, 27). On the other hand, the strength of the colony seems to not affect the resistance of bees to acaricide application (28).

Acaricide exposure also affects the bee's behaviour. Bees fed with coumaphos had reduced trophallaxis which could severely affect the food

transfer and energetic distribution of the whole colony (29). Organic acaricides such as formic acid or thymol are irritant to bees and cause an increase in fanning (30 cited in 31, 32, 33), probably to rid their hive of unwanted acaricides vapours.

Negative effects of acaricides on bees also include impaired metabolism. Bolli et al. showed that under laboratory conditions, high concentrations of formic acid in the air inhibit oxygen intake in young adult bees (34). Application of amitraz and flumethrin leads to a reduction of proteins, carbohydrates and lipids in the hemolymph of 0-, 7- and 21-day old worker bees (35). Adult bees as well as bee brood from colonies treated with amitraz (Apivar®), flumethrin (Bayvarol®) and thymol (Apiguard®) had lower levels of Glutathione S-Transferase activity (36).

Some acaricides have been shown to affect the physiology of adult worker bees. Formic acid treatment had a significant effect on the number of sensilla found in the bee's antenna. Non-significant differences were recorded for the mean length and surface area of the sensilla (37). Topically administered oxalic acid is known to penetrate keratin and can subsequently be detected in the bee's internal organs including the digestive tract, rectum and hemolymph (38), which it can damage and cause elevated mortality (39).

**Table 2:** The effects of amitraz on honey bees

Formulation	Exposure	Effects	Reference
Apivar®	2 strips per hive, 6 weeks	↓ proteins, ↓ carbohydrates, ↓ lipids in haemolymph of adults	(31)
Taktic®	aerosol	associated with weakening of treated colonies	(40)
in larval diet	25-440 ppb in larval diet	↓ chance of pupation ↓ chance of survival to adulthood ↓ defecation	(41)
	200 ppm	affects expression of some proteins	(42)
topical application to adults, 24-hour exposure	283 ppm per treatment group	↓ glucose dehydrogenase expression may compromise cellular immunity	(43)
topical application to adult queen bees	1.0µg/µl in acetone	↓ brood survival	(4)
		shortly after application breaks down into 2,4-dimethylaniline which is very persistent, mutagenic, oncogenic and genotoxic	(44, 45-48)

**Table 3:** The effects of coumaphos on honey bees

Formulation	Exposure	Effects	Reference
Check Mite+®	two strips per colony	↓ sperm viability in drones ↓ sperm numbers in drones ↓ viability of stored sperm queen bees fail to develop	(49, 50)
		in extreme cases, acute colony positioning (abnormal bee behaviour, loss of about 2/3 of the adult population)	(18)
		↑ pkac gene (detoxification) ↓ CYP306 gene (detoxification ) ↓ VGMC gene (development) ↓ DSC37 gene (immunity)	(51)
		alters bee gut microbiome	(52)
		↑ emergency queen cell construction ↑ adult bee mortality ↓ brood survivorship	(53)
		↓ queen acceptance ↓ queen mating success ↓ drone production	(54)
Perizin®	10 µl solution	↑ volume of haemolymph	(15)
	oral administration to adult worker bees	3-day old worker bees are three-fold less susceptible to coumaphos than 8- and 13-day old bees	
	colonies treated according to manufacturer's instructions	↑ bee mortality if colonies were exposed simultaneously to some organophosphorus pesticides (parathionethyl, dimethoate, dialifos)	(55)
	20ml per colony	alters expression of immunoregulatory genes	(56)
10% coumaphos strips	one to four strips per colony	queen cells torn down queen bees fail to develop	(57, 58)
	¼ to ½ strips per colony	↓ queen weight ↓ ovary weight ↓ number of sperm	
beeswax cups treated with coumaphos	1 to 100 mg/kg coumaphos in beeswax	↓ egg laying in queens	(59, 60, 61)
	10 to 100 mg/kg coumaphos in beeswax	↑ queen rejection ↓ queen pupal weight ↓ queen adult weight skews the relationship between queen weight and spermatheca size probably longer queen development time	
	100 to 1000 mg/kg coumaphos in beeswax	↑ queen rejection ↓ queen cell acceptance ↓ queen pupal weight ↓ queen adult weight queens fail to develop probably longer queen development time	

caged bees fed coumaphos	2 µg of coumaphos per cage	↑ worker mortality	(29)
	5µg of coumaphos per cage	↑ worker mortality ↓ trophallaxis	
	100 ppm per treatment group, dissolved in sucrose solution	alters the expression of some genes related to detoxification, behavioral maturation, immunity	(62)
	in queen candy	↑ larvae mortality in combination with fluvalinate, ↓ intestinal stem cell proliferation	(63, 64)
coumaphos in acetone	1-3µg dissolved in 1 µl of acetone, topical application to adult worker bees	↑ adult worker mortality toxicity of coumaphos ↑ by up to 3.4-fold when fluvalinate is present	(65, 66)
	5ppm coumaphos in acetone, 2µl topical application to queens	alters the expression of P450 subfamily genes, antioxidant, immunity and development genes	(67)
acaricide-laden combs from a beekeeping operation	bees reared in combs containing 281 to 6311000 ng/g coumaphos	↑ <i>Nosema ceranae</i> infection rate ↑ brood mortality ↓ lower longevity delayed larval development delayed adult emergence	(68, 69, 70)
in larval diet	50 ppm	affects expression of some proteins	(42)
topical application to adults, 24-hour exposure	751 ppm per treatment group	↓ hymenoptaecin and ↓ abaecin expression may compromise cellular immunity	(43)

**Table 4:** The effects of flumethrin on honey bees

Formulation	Exposure	Effects	Reference
Bayvarol®	4 strips per colony for six weeks	↓ proteins, ↓ carbohydrates, ↓ lipids in haemolymph of adults	(35)
	12 strips per colony	↑ glutathione S-transferase activity (biomarker of toxic stress) in larvae, pupae and nurse bees ↓ lower protein content in treated bees	(71)
topical application to adults	751 ppm per treatment group, 17-hour exposure	↑ hymenoptaecin expression may compromise cellular immunity	(43)

**Table 5:** The effects of fluvalinate on honey bees

Formulation	Exposure	Effects	Reference
Apistan®	two strips per colony	↓ drones survive the first day of their adult life ↓ drone longevity ↓ drone weight ↓ drone mucus gland weight	(72)
	two strips per colony for 24-28 days three times during the season	suspected to negatively affect queen bee survivorship	(73)
	two strips per colony for four weeks	↑ worker mortality ↑ glutathione S-transferases activity ↓ acetylcholinesterase activity	(74, 75)

**Table 5:** continuation

<b>Formulation</b>	<b>Exposure</b>	<b>Effects</b>	<b>Reference</b>
Apistan®		↓ sperm numbers in drones	(54)
	two strips per colony for six weeks	alters bee gut microbiome	(52)
	two strips per colony for 42 days	↑ emergency queen cell construction ↓ brood survivorship	(53)
	according to manufacturer's instructions, for three and seven days	in combination with coumaphos, ↓ intestinal stem cell proliferation	(64)
Apistan Queen Tab®	caged bees exposed to one strip for five days	↑ worker bee mortality	(24)
Fluwarol™	two strips for six months	associated with weaker colony development, drone laying and queen death contaminated combs probably induce laying of unfertilized eggs by queens	(76)
10% fluvalinate strips	eight strips per colony for 10 days	↓ queen weight	(57, 58)
1% fluvalinate strips	caged bees exposed for 3 days	↑ queen supersedure ↑ worker bee mortality	(17)
	caged bees exposed for 7 days	↑ queen mortality	
fluvalinate in acetone	10 mg fluvalinate in acetone per dish, caged workers exposed for 24-hours by contact method	↓ rate of odour learning	(77)
	dissolved in 1-1.25 µl of acetone, topical application to adult workers	↑ adult worker mortality toxicity of fluvalinate ↑ if other pesticides are present	(65, 66)
	0.125µg-1.25µg, dermal application	affects learning, memory and responsiveness to sucrose	(78)
	0.125µg-1.25µg, oral application		
	0.3µg-3µg topical application	↓ worker mobility	(79)
beeswax cups treated with fluvalinate	100-1000 mg/kg coumaphos in beeswax	↑ queen rejection ↓ queen production probably longer queen development time	(60)
caged bees fed fluvalinate	100 ppm per treatment group, dissolved in sucrose solution	alters the expression of some genes related to detoxification, behavioral maturation, immunity	(62)
	in queen candy	↑ larvae mortality	(63)
in larval diet	100 ppm	affects expression of some proteins	(42)

acaricide-laden combs from a beekeeping operation	bees reared in combs containing 68000-104000 ng/g fluvalinate	<ul style="list-style-type: none"> <li>↑ brood mortality</li> <li>↑ <i>Nosema ceranae</i> infection rate</li> <li>↓ lower longevity</li> <li>delayed larval development</li> <li>delayed adult emergence</li> </ul>	(68, 70)
		suspected to ↑ larvae mortality	(80)

**Table 6:** The effects of formic acid on honey bees

Formulation	Exposure	Effects	Reference
Apivarol®	three fumigations one tablet each, then 40 ml of 60% formic acid per colony in evaporators	↓ pH of honey stores throughout the following season	(81)
Bee Var	one gel packet per hive, two treatments in two week intervals	interrupts brood rearing	(82)
Miteaway™	according to manufacturer's instructions	↑ pkac gene (detoxification)	(51)
Varterminator®	6% formic acid gel for 20 days	↑ egg mortality by about 77%	(83)
85% formic acid	10 ml per colony for 30 days	↑ heat shock proteins in bee brains, a molecular indicator of stress	(84)
	in deionized water, applied by evaporation	cell death in gut	(85)
65% formic acid	22g of formic acid per absorbent pad, colonies treated four times at four day intervals	application can drive bees out of their hive and irritate them	(30)
	40ml per absorbent pad three times in weekly intervals	application can drive bees out of their hive, ↑ fanning behaviour and irritate them	(32)
	200ml per Ziploc bag for 40 days	<ul style="list-style-type: none"> <li>↓ worker population</li> <li>↓ worker brood area</li> </ul>	(86)
	200ml per absorbent pad for 6 weeks	↓ brood survival	(87)
	cotton strips saturated with formic acid and black cumin oil suspension, replaced weekly for 32 weeks	negative effects on sensory organs in the antennae	(37)
	one gel pack per colony for 21 days	<ul style="list-style-type: none"> <li>↓ adult drone survival</li> <li>delays drone production</li> <li>drone eggs removed from combs</li> </ul>	(88)

**Table 6:** continuation

Formulation	Exposure	Effects	Reference
60% formic acid	20-40ml per colony	↓ larval feeding	
	200ml per colony for five weeks	↑ H+ and H- natural protease inhibitor activities in workers ↑ H+ protease activities in pupae ↓ antifungal and antibacterial activities of adult and larvae worker's cuticle ↓ H+ protease activities in workers and larvae ↓ H+ and H- natural protease inhibitor activities in larvae and pupae activates thiolic proteases	(89)
30% formic acid	10 ml per colony for 30 days	↑ heat shock proteins in bee brains, a molecular indicator of stress	(84)
40ml in absorbent pad	five applications in 4-day intervals	↓ area of sealed brood bees clear brood cells close to the absorbent pads associated with queen loss	(90)
formic acid odour	applied by the fumigation method	induces aversion in adult worker bees	(91)
fumigation	60 ppm formic acid for 9 days per overwintering room	↑ worker bee mortality associated with queen losses	(92)
	45 ppm formic acid for 11 days per overwintering room	associated with queen losses	

**Table 7:** The effects of oxalic acid on honey bees

Formulation	Exposure	Effects	Reference
Api-Bioxal®	35 g of Api-Bioxal® dissolved in 500 ml	↑ adult bee mortality	(22)
dissolved in sucrose solution	dissolved in 50% sucrose solution, applied twice by trickling to hives	↑ brood removal ↓ amount of brood	(93)
	50 mL of 3% oxalic acid in 32% sucrose solution (w/w), applied once	↑ capped brood removal	(94)
	50 ml of 1.5g oxalic acid in sucrose solution, applied by trickling twice in a 3-week interval	↑ bee mortality	(39)
	2% solution of oxalic acid in an aqueous 50% sucrose solution by oral administration	can penetrate keratin and reach the bee's internal organs	(38)



Formulation	Exposure	Effects	Reference
dissolved in sucrose solution	30-45g of oxalic acid per 1l of sucrose solution, trickling 5-6ml per bee space	higher bee mortality with increasing doses	(95)
	3.2% solution of oxalic acid in 50% sucrose solution by trickling 5ml per bee space	↓ pH of honey stores throughout the following season ↑ heat shock proteins in bee brains, a molecular indicator of stress	(81, 84)
	4.2% solution of oxalic acid in 50% sucrose solution by trickling 5ml per bee space	↓ colony strength	(96)
in aqueous solution	submersing bees in 1%-2% oxalic acid in aqueous solution	↑ adult bee mortality	(27, 97)
	spraying bees with 1%-1.5% oxalic acid in aqueous solution, 25ml per frame	↑ adult bee mortality colony weakening	
	spraying bees with 0.56%-2.25% oxalic acid in aqueous solution, 50 ml per frame	↑ colony mortality ↑ adult bee mortality ↓ brood	(28)
	spraying bees with 3% oxalic acid in aqueous solution, 8ml per frame	↓ brood associated with queen death	(98)
	10% (w/w) oxalic acid in water and 1% Tween® 20, 6 µL by topical application per bee	permanent damage to the bee's digestive and excretory organs	(39)
	10%-20% oxalic acid in water, 6 µL by topical application per bee	can penetrate keratin and reach the bee's internal organs	(38)
topical application to larvae	0.121 mg per larva	cell death in gut and salivary glands	(85, 99)
sublimation	3.6g per hive	↑ heat shock proteins in bee brains, a molecular indicator of stress	(84)

**Table 8:** The effects of thymol on honey bees

Formulation	Exposure	Effects	Reference
Apilife VAR®	two wafers per colony applied three times in 51 days	↓ amount of brood	(100)
	applied for 42 days, replaced at 21 days	slows down colony development (measured by adult bee population, brood, stored pollen) associated with ↓ brood	(101)
	one tablet per colony	↓ food intake in treated colonies ↓ sealed brood area bees empty cells next to the tablets bees remove most of the product in 10 days	(102, 103)

**Table 8:** continuation

<b>Formulation</b>	<b>Exposure</b>	<b>Effects</b>	<b>Reference</b>
Apilife VAR®	one tablet per colony, replaced in 10-day intervals	↓ sperm numbers in drones ↓ percentage of live spermatozoa in queens	(49)
	one tablet per colony, three applications in 7-day intervals	↓ phototaxis	(104)
Apiguard®	one gel per colony	↑ larval and pupal mortality ↓ sealed brood area bees remove most of the product in 10 days	(103, 67)
	one gel per arena	avoided by older workers contact via bee antennae induces fanning behaviour	(33)
	according to manufacturer's instructions	↑ CYP6a514 gene (detoxification) ↑ pkar gene (detoxification) ↑ pkac gene (detoxification) ↓ CYP306 gene (detoxification) ↓ VGMC gene (development) ↓ DSC37 gene (immunogen) ↓ BASK gene (immunogen)	(51)
	20 days	when applied in conjunction with queen caging ↓ the colony population	(105)
Ecostop®	30 days	↑ heat shock proteins in bee brains, a molecular indicator of stress	(84)
Thymix	20-40ml per colony	brood damage	(106)
Thymovar®		if the temperature exceeds 30°C brood dies off	(Thymovar® manufacturer instructions)
thymol in acetone	3-10 µg by topical application per bee	probably ↓ drone flight activity	(107)
	50 mg of thymol per kg of food by oral administration to larvae	altered vitellogenin expression patterns	(108)
thymol in acetone	500-100 mg of thymol per kg of food by oral administration to larvae	↓ larval survival ↓ larval weight	(108)
thymol-oil spray (4.8 thymol mol/l in 20% canola oil)	400 ml sprayed per colony	associated with queen deaths	(109)
thymol in vermiculture (3.6g per block)	one block per colony replaced every four days for 24 days	↑ adult bee population	
thymol powder	0.5g per comb	application disturbed bees	(19)

### *Effects on Queen Bees and Drones*

In recent years, beekeepers have reported mounting problems with queen failures. As a result, a large body of research investigated the effects of acaricides on the reproductive biology of queen bees as well as drones. In general, insecticides can lower the percentage of matings, female fecundity and reduce egg hatch in arthropods (110). In *A. mellifera*, where a single queen is the only reproductive individual, this could have dramatic effects. Acaricides can lower queen bee reproductive potential throughout hindering comb construction (and thus giving the queen bees less space to lay eggs), reducing the egg production, the ability to requeen and supersede queens successfully (111).

Several studies were devised to test whether queen bees reared in wax cups contaminated with acaricides are negatively affected. Indeed, it was shown that coumaphos treatment drastically decreases the acceptance rate of grafted queen cells (54). In addition, queen bees reared in queen cups containing 1000mg/kg of coumaphos failed to develop. A concentration of 100mg/kg causes over 50% queen rejection; the surviving queens weigh significantly less than the untreated controls (59). Queen bees exposed to coumaphos or fluvalinate show high mortality as well as lower ovary weight, lower sperm numbers, lower body mass, physical abnormalities, atypical behaviour and other characteristics that make them unsuitable for commercial use (49, 57, 58, 60, 61, 112). Fluvalinate contamination of the queen cells also reduced the number of queens reared (60). Collins et al. further underlined that the adverse effects of acaricide wax contamination on queen rearing may be further deepened by stressful conditions such as excessively hot, cool, dry or humid weather, high initial *V. destructor* levels or migratory beekeeping practices (60). Under field conditions, the pesticides beekeepers apply in their hives can combine and their synergistic interactions can amplify the negative effects on honey bee reproductive characteristics. Honey bee queens raised in wax cups containing high concentrations of both coumaphos and fluvalinate, at 94 ppm and 204 ppm respectively, had significantly lower sperm counts and sperm viability (112).

Haarmann et al. proposed two ways via which coumaphos can contaminate queen cells. Firstly,

the worker bees that secrete the wax used to construct queen cells could have physically contacted coumaphos residues in the brood nest. Secondly, the developing queens may contact coumaphos via feeding by nurse bees (58). The second of the two seems less likely, since trophallaxis plays only a minor role in coumaphos distribution among bees (113). This suggests that in order to mitigate the negative impacts of acaricides on queen bees, beekeepers should frequently change old pesticide-laden combs for new uncontaminated ones.

Some acaricides have also been linked with acute mortality of queen bees. In mite-free colonies, fluvalinate application decreased queen bee survivorship by 22% after 13 months, although this difference was not statistically significant (73). According to Sokol, in colonies treated with 250 mg strips of fluvalinate for six months, the queen ceases to lay eggs and dies. Combs contaminated with fluvalinate caused new queens to lay unfertilised eggs (76 but see 114). It has been reported that 50% of colonies treated with a thymol-oil spray as a means of Varroa control lost their queens (109). Oxalic acid and formic acid have been associated with queen losses (90, 98).

Less research focused on how acaricide application affects the reproductive characteristics of drones. Significantly less drones infested with Varroa mites and treated with Apistan® (fluvalinate) survive the first day of their adult life. Like this, many drones die before reaching sexual maturity. Acaricide treatment also has negative effects on drone weight, mucus glands, number of spermatozoa, sperm viability and seminal vesicles weight (50, 54, 60, 72, but see 107). Exposing drones to recommended label concentrations of coumaphos throughout their development and maturity caused a 50% reduction in spermatozoal production (49).

Even organic treatment methods can leave negative imprints on drone reproductive fitness. Formic acid causes bees to remove their drone brood, delay their drone production and reduces drone survival (88). Likewise, it was hypothesised that thymol treatment may cause a reduction in drone flight activity (107). Acaricide treatment decreases the protein content of drone seminal fluids, probably leading to lower sperm survival (50). In effect, acaricides may cause queens to

mate with an insufficient number of drones of a poor sperm quality. This probably results in an earlier than normal supersedure of the queen bee (115) and adversely impacts colony survivorship and productivity.

### *Effects on Brood Development*

The widespread application of synthetic acaricides, organic acids and homemade acaricidal drugs creates a potentially deadly cocktail of chemicals that can negatively affect bee brood (116). Acaricide residues are ever-present in the hive and the brood can contact them directly in the walls of the wax combs, or can be fed dangerous doses by nurse bees (117). It has been shown that brood raised in wax combs contaminated with coumaphos and fluvalinate have higher mortality, lower longevity, delayed larval development and delayed adult emergence (68, 69). Laboratory studies have shown that acaricides applied at field doses can significantly increase larvae mortality (63). This could severely affect job division in the colony and make the colony less able to respond to abrupt changes in workforce demand such as in times of nectar dearth or when a large portion of the workers is lost for some reason, for example after pesticide poisoning.

Organic acaricides can be equally damaging to the brood. Apilife VAR<sup>®</sup>, a thymol-based medicament, decreases brood production and causes brood removal (100, 102, 103), especially at high in-hive temperatures (106). Thymol is in particular toxic to larvae (118). This toxicity was attributed to the strong and aromatic vapours the acaricide produces (101). Bee brood in the immediate vicinity of thymol gauze bags is readily removed by the bees (40). Gregorc et al. reported increased cell death in honey bee larvae following the application of oxalic and formic acid (85). By five days, 82% of epithelial cells of the treated larvae were affected, this was then followed by cell death in the entire larval body. Silva-Zacarin et al. further confirmed increased cell death in bee larvae treated with oxalic acid (99). A total of 18.7% of capped brood treated with oxalic acid is removed compared to 13.3% for the controls, and this difference was statistically significant (94). In addition, laboratory studies have shown that a high concentration of formic acid in the hive air (about 2500 ppm) halted oxygen consumption in bee brood. Subsequent *in vivo* studies have shown

that treating bee colonies with 20-40ml of 60% oxalic acid reduces the rate of respiration and larval feeding (34). This may explain why colonies treated with oxalic and formic acid tend to have less brood (86, 90, 93, 98) and why bees clear cells close to absorbent pads with formic acid (90).

### *Effect on Honey Bee Learning*

Responses to odour are key for the survival of honey bee colonies, since they play a key role in communication and foraging (111). Fluvalinate and coumaphos were found to impair bee odour learning and discriminatory abilities (77, 119). Whether chronic exposure to fluvalinate or coumaphos under field conditions could affect bee learning and foraging remains unknown.

### *Effects on Disease Susceptibility*

Although acaricidal drugs are meant to alleviate parasite pressure on honey bees, they could also have the opposite effect. Short term, acaricides can efficiently control bee mites and contribute to stronger and healthier bee colonies (112). However, chronically applied sublethal doses that weaken bees could also make colonies more susceptible to other diseases.

Wu et al. (2012) found that bees that developed in combs that contained both coumaphos and fluvalinate residues subsequently suffered from higher infection with *Nosema ceranae*. This may be because bee brood developing in acaricide-laden combs utilize more energy for detoxification purposes which results in lowered immunity (43, 51, 120). The effects may be drastic, since nosematosis often results into colony collapse.

Previous research has shown that high levels of acaricides residue in brood combs prolong the developmental times of brood. Since bees take longer to develop, this provides more time for Varroa mites to reproduce possibly leading to a steeper population growth (69). Although no clear consensus on the effects of *A. mellifera* development time on Varroa population has been reached, it is possible that sublethal acaricide concentrations can paradoxically lead to higher mite population build up over a longer period of time.

Organic acids have been shown to negatively affect the immunity of adult worker bees. For example, formic acid was shown to impact the

proteolytic system in the bee cuticle. Adult bees treated with formic acid had a higher H<sup>+</sup> protease and H<sup>-</sup> protease activity. The cuticle of treated bees showed lower antimicrobial activity. This may have critical implications for bee metabolism and body defence. It is widely believed that as a result of impaired metabolism and body defence, bees treated with formic acid are more susceptible to other serious diseases, namely fungal diseases (89).

Locke et al. showed that shortly after winter treatment with fluvalinate, the titres of the Deformed Wings Virus (DWV) increased for a certain period of time (121). The authors suggested that this may be because the acaricides application weakens the bees and makes them more susceptible to viral infections.

### *Effects on Colony Strength*

The adverse sublethal effects of chemical residues on the queen, drones, adult workers and brood impair colony strength (111). Some acaricides induce aversion in worker bees, retard colony build-up and slow down food intake (91, 102, 122), leading to lower honey yields. For example, the application of thymol and formic acid causes up to 30% decrease of honey production in treated colonies, although the effect was not statistically significant (31, 90). Following the application of thymol or coumaphos, colonies experience a temporary period of stagnation when the amount of brood, adult honey bees and pollen does not increase or increases less than in untreated colonies (100, 101). These temporary stagnations and reductions in honey production may leave a lasting impact and lead to a poorer overwintering and even to colony collapses during the following winter (76, 95, 97). Figure 1 shows how a combination of adverse side effects of acaricides could lead to elevated bee colony losses.

Past research found that the effects of acaricides vary considerably between different colonies. Firstly, the infestation rate of the colony will affect its chances of survival (86). Secondly, Rademacher and Harz pointed out that the tolerability of oxalic acid varies throughout different climates (70). While most researchers in Southern Europe find their colonies to tolerate doses of oxalic acid as high as 7% applied throughout trickling without outstanding negative effects on colony strength, researchers in Northern Europe report that their

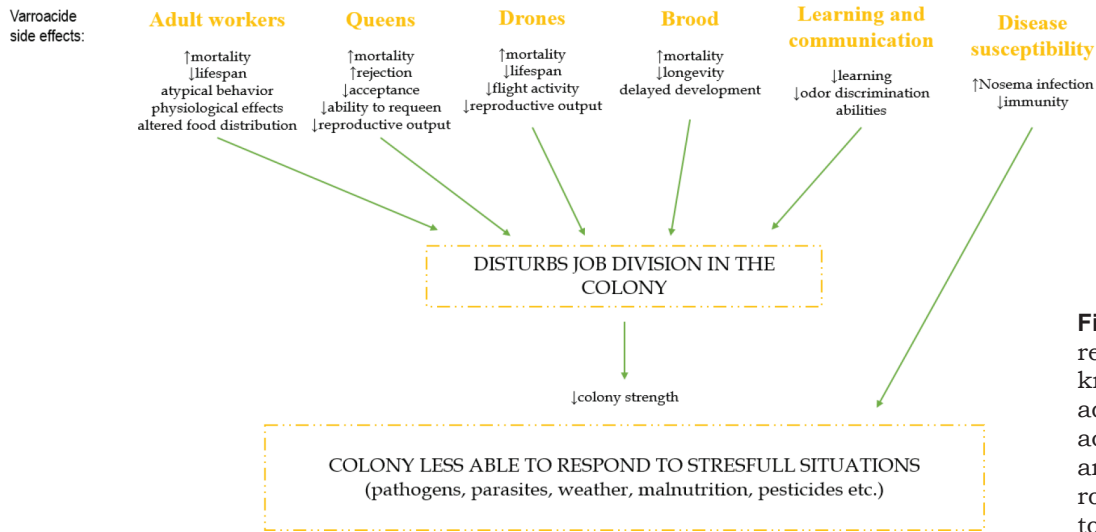
colonies tolerate much lower doses. This trend could be caused by different susceptibility to chemicals by different bee genotypes. Thirdly, the extent to which acaricide application affects colony strength is probably also dependent on the time of application (40). If the acaricides are used during periods when bees cannot fly outside of their hive, they are less able to rid themselves of residues and thus the effect may be much more serious (97). Lastly, the method of application and dose also has a decisive effect on the subsequent strength of the colony. Bee colonies treated by oxalic acid via trickling or spraying had a lower brood area than those treated by sublimation (28).

### **Resistance to Acaricides**

As acaricides are applied routinely on an annual basis, Varroa mites are gradually building up resistance to some of them (123). Beekeepers are forced to switch to other chemicals (124, 125) which further increases the dependency on chemotherapy. It has also been suggested that by developing resistance to coumaphos, *V. destructor* may simultaneously develop resistance to other chemicals such as amitraz (124). This phenomenon is known as “cross resistance” and could mean that the apicultural industry may run out of chemicals effective against *V. destructor* in the foreseeable future.

*V. destructor* is persistently exposed to acaricide residues in bee products throughout its life. For example, Varroa mites continue to die 3 months after fluvalinate is applied. This is because fluvalinate is readily absorbed into beeswax and bee bodies and these residues remain toxic to the mites long after the application (80). Other sources claim that bee combs may show acaricidal activities even one year following the application (126, 127) and acaricide contamination can be detected up to seven years after the last application. As a result of this high contamination of beeswax with acaricides, Varroa mites are chronically exposed to the residues for most of their lives and probably develop resistance easier (128). If this really is the case, it is likely that thanks to acaricide residues in bee products, beekeepers are unknowingly selecting for more and more resistant mites.

The toxic effects of acaricides are not limited just to Varroa mites. Chemicals used in the hives target many other inhabitants of the hive, including



**Figure 1:** A schematic representation of the known and likely adverse effects of acaricides on bees and their possible roles in contributing to colony losses

commensals and parasites. For example, a wide range of popular acaricides including fluvalinate, thymol and coumaphos exert various effects on the development of the small hive beetle (*Aethina tumida*) (129). This could have yet unknown impacts on the small hive beetle's populations. For example, the application of these chemicals may accelerate the beetle's development of cross-resistance to other pesticides. The diverse impacts of acaricides on the development of resistance in other inhabitants of the hive will require large holistic studies in the future.

### Deleterious Interactions of Acaricides

Most acaricides persist in bee products for lengthy periods of time (125, 127). A notable exception is amitraz, that is degraded very quickly and so does not accumulate in wax (125, 130). However, this does not necessarily mean that amitraz doesn't leave any residues behind. Within 3 to 4 weeks after application, amitraz breaks down into 2, 4-dimethyl phenyl formamide and 2, 4-dimethylaniline, both of which are environmentally very stable and furthermore the latter is mutagenic, oncogenic and genotoxic (44, 45-48). This shows that even readily degradable chemicals have a potential to cause significant damage to bees.

The accumulation of residues of acaricides such as coumaphos and fluvalinate can exert a lasting negative pressure on bees. Specifically, Johnson et al. demonstrated toxic interactions among the residues of acaricides as well as antimicrobial

drugs and fungicides found in bee colonies (131). The toxicity of tau-fluvalinate increased when combined with other medicaments. Bees treated with coumaphos are more susceptible to poisoning by organophosphorus pesticides such as parathionethyl, dimethoate, dialifos (55). Likewise, the toxicity of coumaphos increases when applied in conjunction with fluvalinate (65) and bees previously treated with the antibiotic oxytetracycline were more susceptible to coumaphos and fluvalinate (132).

### Discussion

Studies on the negative effects of acaricides on honey bees are relatively scarce, when compared to the total body of literature on Varroa control and many questions require further research. Beekeepers often apply acaricides at the end of the season after the last honey extraction, to minimise the risk of residues in honey. When bee colonies overwinter, which can last as long as 6 months, they are entirely dependent on the stores they produced during the year. If the medications affected bee stores, this could have a crucial effect on bee colony strength as a whole next spring. A study on humans showed that formic acid, oxalic acid and thymol added to honey significantly affected its taste (133). Could residues over sensory levels harm bees? This, among other questions, remain to be resolved with further research. So far, it has been demonstrated that formic acid induces aversion in worker bees (91, 122) and that formic and oxalic acids affect the pH

of honey stores (81). As of present, little is known about the potential effects of pesticide residues on overwintering bees (134).

Another research topic that will require more attention in the future are the wider implications of chemotherapy for bee breeding. The heavy use of chemotherapy in apiculture may have much more pronounced effects on bee colonies than just what may seem as minute sublethal effects. Elzen et al. demonstrated that the European honey bee (*A. m. ligustica*) was significantly more tolerant to pyrethroids than African honey bees (*A. m. scutellata*) (135). It was suggested that European bees that are managed much more intensively, face selection pressures because of the routine application of synthetic acaricides. It thus seems that the advent chemotherapy in beekeeping significantly altered *A. mellifera* phenotype and maybe even its gene pool. This may subsequently bring serious problems in bee breeding.

Since all of the acaricides in use today have some kind of a negative side effect on bees, it is difficult to recommend the most bee-friendly product that would leave no negative health effects on colonies. The ability of beekeepers to prevent undesirable side effects of acaricides is also restrained by the fact that the manufacturing process of commercially available veterinary drugs for bees is rather non-transparent. Different formulations of the same acaricide can have very different impacts on bees. In order to overcome pest resistance problems, it is common practice now that pesticide manufacturers frequently change the formulation of their products. For example, earlier formulations of fluvalinate used in the 1980s were only slightly toxic to bees, but modern fluvalinate products are considered highly toxic (136). Furthermore, when acaricides are applied to the hive, the amount of active ingredients released may vary enormously between different commercial formulations. Factors such as nest congestion, bee activity and length of exposure may profoundly affect the amount of substance released per unit of time (137). The actual amount of active ingredient distributed through the colony may therefore be different from the amount given on the label. As such, it is hard for beekeepers and bee researchers to predict what side effect may their acaricides have. Thus, with some exaggeration it can be said that every batch of bee medicaments made has its own, unpredictable effects.

In summary, although acaricides can significantly decrease *V. destructor* infestation, they may exert negative effects if the colony is only lightly infested (86). The scale and extend of the negative impacts of acaricides is strongly influenced by factors such as the climatic conditions, the time of the year, the dose and the method of application (26, 27, 28, 70), so reaching consistent results has long been a problem. The negative effects of acaricides can be so cryptic that they can be hard to recognise by beekeepers and only cause acute poisoning if coupled with other stressors (42). Even methods used in organic beekeeping may leave long-term negative impacts on honey bee health. It is therefore suggested that acaricides are only applied as a last resort when the Varroa population reaches damaging levels. A growing number of beekeepers is turning towards zootechnical and biological methods such as drone comb removal, hyperthermia, selecting for Varroa-resistant and Varroa-tolerant bee stock, powder sugar dusting, using combs with smaller cell size and others that do not require the application of chemicals and so do not leave any residue behind and minimize the risk of the pests developing resistance (5, 130, 138, 139). While often more time consuming and less efficient than conventional drugs, rapid developments are being made that make these methods easier to apply than before (140). Organic beekeepers are organised in a number of societies and are entitled to label their honey as “organic honey”. It has been shown, that the growing interest of beekeepers in organic methods has brought forward a decrease of acaricide residues in bee products (130, 139). Novel organic Varroa control methods will certainly meet more and more popularity in the beekeeping circles in the very near future and contribute to increasing the sustainability of apiculture worldwide.

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## VPLIV UMETNIH IN NARAVNIH AKARICIDOV NA ZDRAVJE MEDONOSNIH ČEBEL

E. Tihelka

**Povzetek:** Čebele so pomemben opraševalec kmetijskih pridelkov in gospodarsko pomemben proizvajalec izdelkov, kot sta med in vosek, ki se uporabljajo v živilski industriji, kozmetiki in medicini. Trenutno je zunanji zajedavec pršica varoja (*Varroa destructor*) eden največjih škodljivcev za čebele v svetu. Brez zdravljenja čebelje družine napadene z varjo večinoma propadejo v nekaj letih. Za ohranjanje nizke populacije pršic varoj čebelarji uporabljajo umetne in naravne akaricide. Najbolj priljubljeni, komercialno dostopni, so amitraz, kumafos, flumetrin, fluvalinat, mravljinčna kislina, oksalna kislina in timol. Ti akaricidi so dokaj poceni in enostavni za uporabo, vendar podaljšana uporaba povzroča hitro razvijanje odpornosti pri pršicah varoja in večjo možnost onesnaženja čebeljih pridelkov. Ostanke akaricidov so po zdravljenju lahko prisotni v visokih koncentracijah v celotnem panju in so jim čebele izpostavljene celo leto. Po uporabi je tudi satje pogosto onesnaženo z visokimi koncentracijami ostankov akaricidov, ki so jim pršice varoja kronično izpostavljene in lahko zaradi tega hitreje razvijejo odpornost. Pregledni članek povzema trenutno znanje o škodljivih učinkih konvencionalnih akaricidov na zdravje čebel. Dokazano je, da imajo številni komercialno dostopni akaricidi in njihove aktivne snovi negativne učinke na razvoj čebel, vplivajo na razmnoževalno sposobnost čebelje matice in zmanjšujejo sposobnost učenja čebel ter dolgoživost in moč kolonije. Akaricidi ne delujejo samo kot posamezne učinkovine, ampak tudi sinergistično, kar lahko dodatno slabo vpliva na zdravje čebelje družine. Nekatera zdravila lahko povzročijo znatno oslabitev čebeljih družin, lahko pa jih tudi naredijo bolj dovzetne za druge bolezni, kot je nozemavost, ali bolj občutljive na slabe vremenske razmere. V kombinaciji z drugimi stresorji lahko akaricidi prispevajo k propadu čebelje družine.

**Ključne besede:** umetni akaricidi; varoicidi; čebela; *Apis mellifera*; sinergija; ekološko čebelarjenje