Alternative control of the varroosis

Colin M.E.

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Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 21

1997
pages 87-98

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M.-E. COLIN
INRA, PHYTOPHARMACIE
DOMAINE SAINT-PAUL, SITE AGROPARC
84914 AVIGNON CEDEX 9
FRANCE

SUMMARY - In order to avoid the permanent and long use of synthesis acaricides, different alternatives have been proposed. Among plant or animal products, and among some molecules authorised in organic agriculture, organic acids and aromatic plants extracts are already developed in beehives. Formic acid, present naturally in the honey in high contents, is used in evaporation for one or two days or in slow evaporation for several days. If the quantity of evaporated acid ranges from 7 to 10 gr per day, the average efficiency of the acaricide overpasses 95%, without serious disturbances in the colony. However, nowadays no evaporation system guarantees this result, regardless of weather conditions or hive structure. Essential aromatic oils, containing thymol, menthol or camphor, or these molecules themselves, are good acaricides when they are used under correct conditions. These vary depending on the region and climate, something that explains the need to have complementary experiments in Mediterranean hives in order to optimize the use of such substances. Today these alternatives to chemical control can only be used if we can estimate the level of parasitic infestation of colonies at the end of the treatment.

Key words: Varroosis, alternative treatment, organic acids, aromatic products, formic acid, thymol, thyme essential oil.

RESUME *- "Contrôle alternatif de la varroose". Pour éviter de traiter trop souvent ou trop longtemps avec des acaricides de synthèse, différentes alternatives ont été proposées. Parmi les produits d'origine animale ou végétale, ainsi que parmi certaines molécules autorisées en agriculture biologique, les acides organiques et les extraits de plantes aromatiques font déjà l'objet d'un développement dans les ruchers. L'acide formique, présent naturellement dans le miel à des teneurs élevées, est utilisé en évaporation sur une journée ou deux ou en évaporation lente sur plusieurs jours. Si la quantité d'acide évaporée varie entre 7 et 10 g par jour, l'efficacité acaricide moyenne dépasse 95%, sans perturbations graves de la colonie. Cependant aucun système d'évaporation ne garantit actuellement ce débit, quel que soit le climat ou le modèle de ruche. Les huiles essentielles aromatiques, contenant du thymol, du menthol ou du camphre, ou ces molécules elles-mêmes, sont de bons acaricides lorsqu'elles sont utilisées dans des bonnes conditions. Celles-ci sont souvent très variables selon les régions et les climats, ce qui explique la nécessité d'expériences complémentaires dans les ruchers méditerranéens pour optimiser l'emploi de ces substances. Aujourd'hui, ces alternatives à la lutte chimique ne peuvent être utilisées que si on peut estimer le niveau d'infestation parasitaire des colonies à la fin du traitement.*

Mots-clés *: Varroose, traitement alternatif, acides organiques, produits aromatiques, acide formique, thymol, huile essentielle de thym.*

Introduction

To avoid some consequences of a permanent chemical control, such as presence of post-therapeutic residues in honey, accumulation of the residues in the hive (wax, propolis) and emergence of mite resistance, the use of natural products is helpful,

even if it is more time-consuming. Three categories of these products can be distinguished in the field of the varroosis: (i) the molecules produced by animal metabolism; (ii) the extracts of edible plants; and (iii) methods or products allowed in biological agriculture.

Products of the animal metabolism

The organic acids

Formic acid, lactic acid are naturally present in the hive products or in the animal organisms. The study of Sabatini *et al.* (1994) gives the different levels of the lactic and formic acids in monofloral honeys harvested in Italy (Table 1). The maximum level of lactic acid is 686 mg/kg in honeydew honey and 273 mg/kg of formic acid in chestnut tree honey. Thus, if the treatment is undertaken outside a nectar flow, no residue problems can occur. Because the basis of the treatment are given by Imdorf *et al.* (1996) in Swiss conditions, some adaptations of the method are needed in Mediterranean conditions to obtain the quality of their results. Whatever the country, the corrosive property of the acids forced the beekeeper to be well protected with rubber gloves, mask and glasses when preparing the acid solutions or treating with it.

Table 1. Lactic acid and formic acid contents in honeys from different floral origin (After Sabatini *et al.*, 1994)

Origin	6 samples	D lactic a. ppm	L lactic a. ppm	Total ppm	Formic a. ppm
Citrus	mean	240	201	441	14
	st-dev.	21	18	38	3
	min.	215	175	390	11
	max.	260	220	480	19
Eucalyptus	mean	178	162	340	14
	st-dev.	14	32	45	6
	min.	165	140	310	9
	max.	200	225	425	25
Honey dew	mean	276	410	686	168
	st-dev.	165	212	279	59
	min.	110	151	358	99
	max.	550	675	1026	259
Chestnut	mean	244	235	479	273
	st-dev.	19	19	37	47
	min.	220	205	425	220
	max.	270	260	530	340

Formic acid

The first report of a treatment with formic acid is given by Kunzler *et al.* (1979), but in these field conditions, it was dangerous for the bee brood and for the queen. The main problem is thus to regulate the evaporated acid flow in the hive, independently from the temperature and at the convenient air concentration to kill the mite (Bolli *et al.*, 1993). Today, although no ideal solution is offered to the beekeepers, Imdorf *et al.* (1996) propose two ways of application in the hives: the short-term and the long-term treatment.

(i) The short-term treatment consists in evaporating small amounts of formic acid. A volume of 30 ml is released within 6 to 10 hours. When applied from above, 60% formic acid is used, whereas 85% is necessary from below. Two or three applications within a week, are done two times at an interval of one month. The treatment efficiency of the short term treatment can reach 95%. The value is confirmed in Italy by Mutinelli *et al.* (1996) (Fig. 1), when using 60% formic acid impregnated sponges. However, losses of queens or interruptions of egg-laying are to be feared.

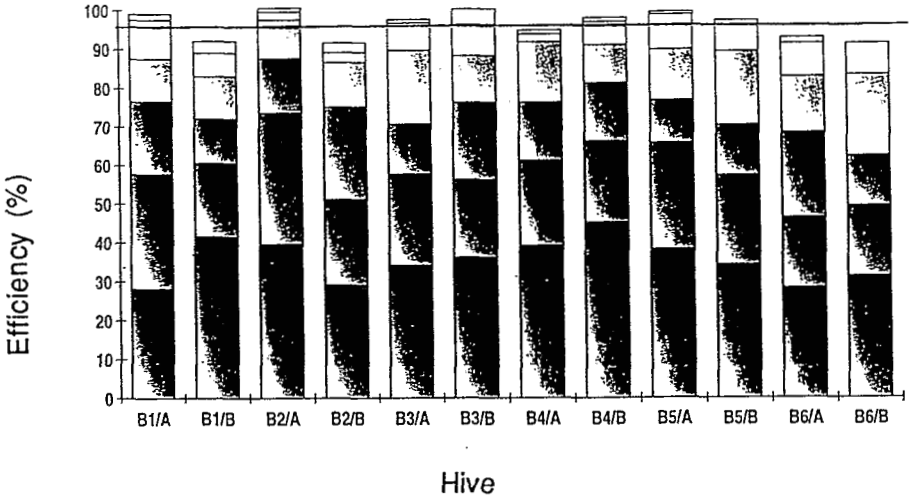

Fig. 1. Efficiency of sponge impregnated with 60% formic acid and placed on top of the frames. The horizontal line indicates the mean efficiency. Each column is the sum of the efficiencies of 6 applications (horizontal bar) in one colony.

(ii) The long-term treatment was firstly proposed by Krämer (1982). Fibre plates impregnated with formic acid, are sealed in plastic bags (Krämerplatte, Illertissner Milbenplatte). Before application, a calculated number of 1.5 cm diameter holes are opened in the plastic, the number of holes varying according to the hive model, the colony strength and the climate. The plate, isolated from the contact of the cover and of the top bars of the frames is laid during 7 days for the first time and 14 days for the second time one month later. "The treatment efficiency of this method depends on the formic acid concentration in the hive air and on the duration of the treatment .... If there is an evaporation of more than 7 g of formic acid per day, a treatment efficiency of more than 95% may be expected, at 10 g per day it will exceed 97%" (Imdorf *et al.*, 1996) (Table 2). To obtain these good results in Mediterranean conditions, it is

necessary to adapt the Swiss method to regulate the vapour flow of formic acid between 7 and 10 g/day in spite of the temperature variations (Mutinelli *et al.*, 1996) (Fig. 2).

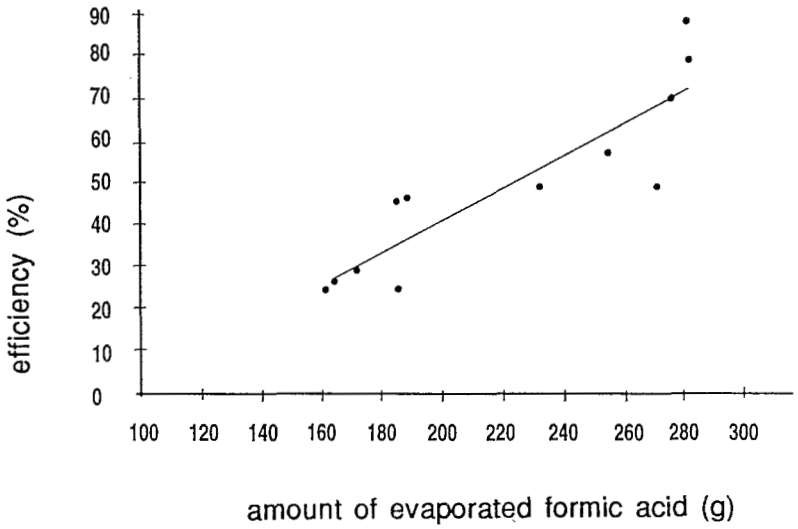

Fig. 2. Relationship between the evaporated formic acid and the efficiency. The highest values of efficiency are only observed when the evaporation amount is higher than 260 g.

Lactic acid

The lactic acid, directly sprayed on the adult bees, crowding on the comb, has an efficiency of approximately 80%, if the colonies are broodless. Considering the brevity of the broodless period around the Mediterranean sea, at least 4 applications per year are needed. All the combs covered with adult bees must be finely sprayed with 5 to 8 ml of 15% lactic acid per side. In case of overdoses, losses of bees can be observed.

Oxalic acid

Although this acid is not from animal origin, oxalic acid also is used in spray at the concentration of 3% (w/v) and at the dosage of 3-4 ml per comb side. In broodless colonies, the efficiency ranges around 98%, with little variations between colonies.

Drone brood extracts

Arculeo *et al.* (1989) tested a commercial formulation of semichemicals involved in the attraction of *Varroa jacobsoni* to the drone larva (Varroutest ®). After spraying defined brood areas, the authors concluded that the mite infestation was of 42% higher in sprayed brood areas than in unsprayed ones. The use of the product in comb trap for the mites, was not successful (Otten and Kraus, 1988).

Table 2. Long term formic acid treatment, Liebefeld method, Dadant (2 x one-week long treatments) (with the permission of Imdorf, A., Federal Dairy Research Institute)

Year	n	FA dosage			Holes			Efficacy % (median)			Queen lost (%)	
		ml	%FA	Place	nb (%)	Diameter (cm)	Surface (cm^2)	1st treat.	2nd treat.	Total	1st treat.	2nd treat.
1993	24	100	60	up	6	3	42	21	18	34	0	8.3
	27	150	85	down	6&9	3	42/64	18	22	34	0	17.2
	10	150	85	down	9&12	3	64/85	47	85	93	0	10
1994	41	180&160	85	down	9&12	3	64/85	88	89	99	7.3	16.7
	21	180&160	85	down	12	3	85	84	81	98	19	0
	15	100	85	up	13	2	41	87	75	96	0	0

Aromatic or oleaginous plant extracts

Essential oils

Essential oils are obtained by steam distillation of the whole plant or particular part of the plant. Their presence in the hives was evidenced by Lejeune *et al.* (1984), who asserted that the propolis contains 5% to 10% of essential oils. Consequently, the reintroduction into the hive of essential oils extracted from plants intensively visited by the bees seemed to be a natural control of the parasitosis. Colin (1990) tested a mixture of two essential oils: *Thymus vulgaris* L. (thymol type) and *Salvia officinalis* L. The main molecules are para-cymene and thymol for the thyme and camphor, alpha thujone and eucalyptol for the sage (Table 3). Aqueous solutions at 1% of thyme and 0.5% of sage formulated with an emulsifier were administered in the hive by the entrance hole in aerosol form by means of a "Phagogene ®" apparatus. After four 60 s applications of the aerosol at 4 days interval, the effectiveness of the treatment on colonies containing brood was 95.4% (s.d. 3.6%, n=10), but the effectiveness of essential oil treated colonies was not statistically different from colonies treated with amitraz aerosol when the number of mites is lower than 1000 per colony (Table 4). No side effects were observed during and after the experiment. Oils residues in honey were under the detection limit.

Table 3. Main components of essential oils (Colin, 1990)

White thyme		Sage	
Limonene	0.25%	Alfa-pinene	4.53%
Gamma-terpinene	1.32%	Camphene	7.95%
Para-cymene	52.17%	Beta-pinene	1.53%
Camphre	0.22%	Myrcene	1.12%
Caryophyllene	0.43%	Limonene	2.47%
Linalol	0.49%	Cineol 1,8	10.79%
Thymol	19.36%	Alfa-thujone	22.79%
Carvacrol	8.97%	Beta-thujone	3.74%
Unspecified	16.79%	Camphre	21.79%
		Caryophyllene	4.89%
		Bornyle acetate	1.93%
		Humulene	5.78%
		Borneol	2.40%
		Unspecified	8.29%

Vegetable oils

Brodsgaard *et al.* (1994) tested in laboratory conditions, the effectiveness of aqueous solutions of oils of rape or soybean containing an emulsifier approved for foodstuffs. Mite mortality levels higher than 95% were obtained with 50% oil concentrations. However, such concentrations of oils seemed dangerous for the bees.

Table 4. Compared efficiencies of two treatments (4 applications in aerosol form at 4 days interval) (Colin, 1990)

Group	NVj		TA		NVj af tr		N m p	Brood	
	m	s-d	m	s-d	m	s-d		m	s-d
Amitraz	1596	669	99.0	0.6	18.1	8.7	3.8	25.6	11.4
Ess. oil: thyme + sage	1678	1123	95.4	3.6	97.8	89.2	16.6	21.2	13.4

NVj: total number of collected mites; TA: efficiency of the treatment in %; NVj af tr: number of *V. jacobsoni* collected during verification treatment; N m p: number of mutilated pupae collected during control; Brood: brood surface in dm^2 after treatment.

Thymol

Thymol is one of the first effective molecules used in the control of the varroosis in Eastern Europe (Mikitiouk and Grobov, 1978). Administered by evaporation, sublimation when starting from crystals (Lodesani *et al.*, 1990) or dilution in vegetable oil (Gal *et al.*, 1992), the efficacy is irregular about 80% depending on the evaporated amount, which depends itself on the external temperature. Bee mortality also increases when the temperature increases. In order to minimize the drawbacks, Chiesa (1991) uses powdered thymol crystals sprinkled onto the top bars of the combs occupied by the bees in the late fall, when the diurnal temperatures were lower than 12ºC. The doses varied according to the colony strength, that means 0.5 g of thymol per comb completely filled with bees. Treatment consisted of 4 identical applications carried out at 2 days intervals. The effectiveness is higher than 99% in the broodless colonies, the lowest value (i.e., 94.99%) being due to the presence of some brood throughout the treatment period. The data, shown in Table 5 are slightly lower because all the colonies received the same dose. The main recommendations of the author (Chiesa, 1991) are: to choose the dose in relation with the strength of the colony and to treat broodless colonies during the coldest season.

The association of thymol, menthol, camphor and eucalyptol

Mixtures containing various proportions of the aromatic molecules were first tested in Tunisia by Popa with encouraging results. A few years later, a quaternary mixture (Apilife-Var) was registered in Italy. Approximately 20 g of the mixture (76% thymol, 16% eucalyptol, 4% camphor, 4% menthol) impregnate a small vermiculite tablet. Following the experiments of Imdorf *et al.* (1994, 1995), it is more suitable to lay a tablet on the top of the brood combs and to replace it by another one 3 or 4 weeks later. In good conditions of application, in Switzerland (Table 6) and in the South of France (Jourdan Adapi, personal communication) (Fig. 3), the efficiency of the treatment is greater than 92%. However in Mediterranean climate, it is necessary to take a lot of precautions: (i) the tablet must be above but far from the broodnest; (ii) the diurnal temperature must be over 25ºC; (iii) some bee races are particularly susceptible, that means that the susceptible bees leave the hive. The question of the post-therapeutic residues has to be asked in terms of alteration of the honey taste, which occurs over the threshold of 1.1 mg/kg (Imdorf *et al.*, 1994), not in terms of human toxicology.

Table 5. Number of fallen and surviving *Varroa* mites and percentage of effectiveness achieved by treating honeybee colonies with thymol plus confectioner's sugar (testing year 1988) (Chiesa, 1991)

Active ingredient	Location	Fallen mites per treatment				Total	Surviving	% Eff	Standard error
		1st	2nd	3rd	4th				
Thymol	Moruzzo	1272	469	175	184	2100	56	97.38	1.15
	Udine	384	516	334	387	1621	122	93.56	1.41
Thymol + confectioner's sugar	Moruzzo	1276	415	190	259	2139	62	96.26	2.93
	Udine	1039	508	231	224	2002	59	96.91	0.45

Thymol: n=11, m=94.95%, s.d.=3.77%; Thymol and sugar: n=11, m=96.62%, s.d.=4.23%; Comparison T/T and S: t=-0.983, p=0.34.

Table 6. Mite mortality after treating with "Apilife/Var®" (Control: 2 "Perizin" applications) (Imdorf *et al.*, 1994)

	Apiary 1*			Apiary 2**		
	Mean	Min.	Max.	Mean	Min.	Max.
Apilife/Var®	986	1704	368	2453	696	4509
Control	37	168	3	24	6	59
Efficiency of Apilife/Var (%)	96.4	91.0	99.4	99.0	97.9	99.8

*In the first apiary, the second tablet, placed 15 days after the first one, is removed after 24 days.
**In the second apiary, the second tablet, placed 15 days after the first one, is removed after 65 days.

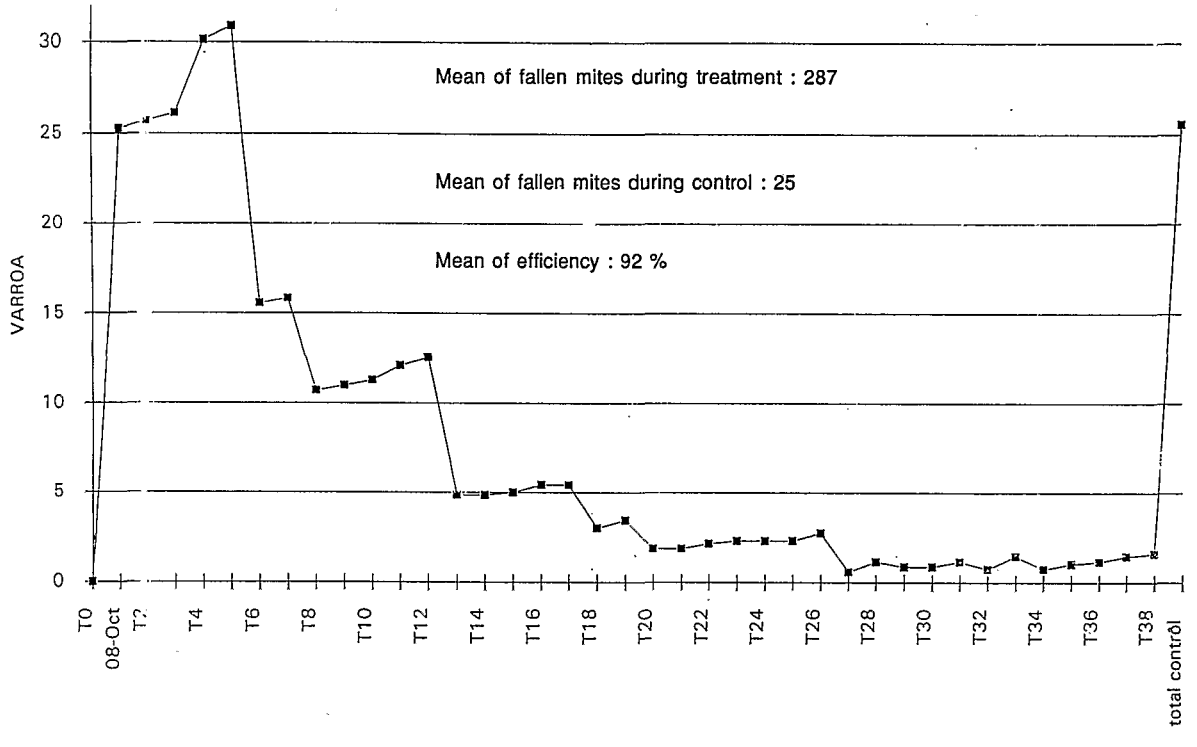

Fig. 3. Daily mean of fallen mites after treatment of seven colonies with Apilife/Var®. With the permission of P. Jourdan (1994).

Methods or products allowed in biological agriculture

Thermotherapy

Grobov (1977) mentions that a temperature of 41°C within 5 minutes kills the mites on the adult bees. According to Le Conte *et al.* (1990), the reproductive success of the mite females in the comb cells is significantly reduced above 36.5°C. Some thermoregulated chambers were conceived to kill the mites either on the adult bees or in the brood.

Copper salts

Considered as natural in biological agriculture, Guiraud *et al.* (1989) hypothesized that the salts directly or indirectly can block the respiratory system of the mite. The cupric gluconate, the less dangerous for the bees was given to the colony in sucrose syrup or in candy sugar at the dose of 2.5 g per colony. In a field experiment, Bruneau (1990) estimated the efficiency on the mite at 47% and consequently advised the beekeepers against treating with it (Table 7).

Table 7. Comparative efficiencies of two treatments: copper salt and Apistan® (Bruneau, 1990)

Treatment	Mean mortality after treatment	Mean mortality after control	Comparative efficiency
Control = syrup fed	1672	2843	41.72
Apistan® & syrup fed	4527	455	89.86
Syrup with copper	1934	1916	45.87
Control = candy fed	1078	2404	32.88
Apistan® & candy fed	6698	142	97.92
Copper & candy	1645	2165	44.13

Concluding remarks

In conclusion, the authors, who have experienced the volatile substances in matter of bee treatment, agree that the efficacy depends on the treatment conditions and that some precautions are needed. The optimal conditions in the Mediterranean region are a high and steady external temperature, the absence of worker brood (the drone brood being cut off in spring). The main precautions consist in avoiding the reinfestations and the robbery (Nazzi *et al.*, 1995), in treating outside the periods of nectar flow or queen rearing and in verifying the efficacy of the treatment (Milani, 1990). The alternative of the chemical control is thus more than the simple use of a vegetable or animal extract in place of a synthetic acaricide. It requires from the beekeeper an additional effort to manage the bee colonies, that means to consume more time in the apiaries. For this price, this type of treatment could alternate with the chemical control every other year.

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