Effectiveness of organic acids in *Varroa* (Acarina: Varroidae) mite control.

Higes, M.[1]**; Martín Hernández, R.**[1] **& Meana. A.**[2]
[1] Regional Apicultural Center. Consejería de Agricultura. Junta de Comunidades de Castilla - La Mancha. Camino de San Martin s/n. 19180 Marchamalo. Spain.
[2] Department of Animal Health. Faculty of Veterinary. Complutense University of Madrid. Avda. Puerta de Hierro s/n. 28040 Madrid. Spain.

Received: 04.07.2005

Hay hombres que luchan un día y son buenos,
los hay que luchan un año y son mejores,
pero hay los que luchan toda una vida,
esos son los imprescindibles, como Ignacio.

Accepted: 20.09.05

Abstract: The effectiveness of some organic acids in Varroa (*Varroidae*) mite control during the broodless period was tested on honeybee colonies. Autumn field assays were done on ninety Langstroth hives with propionic, malic, citric, acetic and oxalic acids. Hives trickled with 50% sugar solution, Perizin®, with Apivar® strips or untreated were the four control groups. Laboratory toxicity tests to determine the field doses to be used were carried out prior to the field trial. Only oxalic acid showed rates of success, similar to commercial control products, and no satisfactory acaricide effectiveness occurred with the other organic acids used. All the treatments, including control sugar solution caused some honeybee mortality higher than the natural rate. The higher mortality caused by propionic and acetic acids advises caution when increasing the dose to enhance the efficacy.

Key words: Organic acid, *Apis mellifera*, Varroa, field test, acaricide.

Resumen: Se presentan los resultados de los ensayos de campo realizados para evaluar la eficacia acaricida de los ácidos propiónico, málico, cítrico, acético y oxálico para el control de *Varroa destructor* en abejas melíferas durante periodos sin cría. Se utilizaron 90 colmenas Langstroth y los tratamientos con los diferentes ácidos orgánicos se administraron mediante goteo de forma aleatoria cada uno a 10 colmenas. Como testigos se utilizaron 40 colmenas; dos grupos de 10 actuaron como testigos negativos (sin tratamiento o goteado con una solución de agua y azúcar al 50% respectivamente) y los otros dos como testigos positivos, tratados con Perizin® y Apivar® respectivamente. La determinación de las dosis de los ácidos orgánicos se realizó en base a estudios de toxicidad realizados previamente en el laboratorio. Tan solo el ácido oxálico mostró una eficacia acaricida similar a la obtenida en los grupos control tratados con los productos comerciales, siendo muy inferior con el resto de los ácidos orgánicos. Todos los tratamientos, incluido el grupo testigo en el que se administró la solución de azúcar, produjeron mortalidad de abejas superior a la mortalidad natural.

Palabras clave: Ácidos orgánicos, *Apis mellifera*, Varroa, Test de campo, acaricidas.

1. Introduction.

The main parasitic disease affecting *Apis mellifera* Linneus is caused by the acari V*arroa destructor*, Anderson and Trueman 2000. Although many efforts have been carried out to find new substances to control this acari, nowadays serious restrictions limit the products available, especially for those beekeepers who want to follow ecological beekeeping practices.

Organic acids, many of them natural constituents of honey, can play an important role in achieving natural and safe hive products (Colin *et al.,* 1989). Oxalic, formic and lactic acids are the most studied organic acids for Varroa control (Fries, 1991; Bolli *et al.,* 1993; Mutinelli *et al.,* 1997), but results so far have been unsatisfactory. Limitations imposed by toxicity (Higes *et al.,* 1999) or low effectiveness have been reported (Greatti *et al*., 1992; Nanetti *et al.,* 1995, Gregorc and Planic, 2002). Therefore, in an attempt to develop new acaricide substances to control Varroa infections acceptable

Corresponding author:
Dr. Aránzazu Meana
Dpto. Patología Animal I (Sanidad Animal). Facultad de Veterinaria.
Universidad Complutense de Madrid.
Avda Puerta de Hierro s/n. 28040 Madrid. España.
FAX: 34-1-3943908
E-mail: ameana@vet.ucm.es

for ecological beekeeping, we studied the efficacy of citric, acetic, oxalic, malic and propionic acids, some of which (acetic acid, for example) are included in European Ecological Beekeeping Regulation (1999).

2. Material and Methods.

2.1. Organic acids dose determination.

The doses for each organic acid were determined on the basis of previous LD_{50} studies on honeybees. Laboratory tests were designed based on the guidelines of the European and Mediterranean Plant Protection Organization (OEPP nº 170). In every trial, we used uniform, adult worker bees (*Apis mellifera*) from an apiary located in central Spain (Marchamalo, Guadalajara). Bees were collected in early morning from frames with no brood, kept in holding cages and immediately transported to the laboratory. Bees were anaesthetized with carbon dioxide and randomly assigned to the different groups. Test substances and chemical data are shown on Table 1. Stock substances were prepared with distilled water and 1% (w/w) of Tween® 20 to reduce solution surface tension and to administer minute quantities of solution to the bees. A range-finding test established the definitive test dosage levels, all of which, in the end, fell within the range selected. The definitive test dosage level and the equivalent active ingredient per bee are shown on Table 1.

Table 1. Test substances, chemical data and definitive test dosage level per bee.

Test substance	Purity	Commercial Supply and Reference no.	Definitive concentration (or range) used	Highest dose used	Highest dose that did not cause bee mortality greater than control
Acetic acid, glacial CH_3COOH	≥99.7%	Panreac 131008	30 % (v/v)	1782 µg/bee	666 µg
Citric acid anhydrous $HCO(COOC)(CH_2COOH)_2$	100%	J.T.Baker Chem. 0090	30% (w/w)	2048 µg/bee	666 µg
Oxalic acid 2-hidrato $(COOH)_2{\cdot}2H_2O$	99%	Panreac 141041	5-50% (w/w)	500 µg/bee	100 µg
Propionic acid $C_3H_6O_2$	≥99%	Fluka 81912	30-50% (w/w)*	3645 µg/bee	1200 µg
DL-Malic acid $C_4H_6O_5$	≥99.5%	Panreac 372051	20% (w/w) **	1320 µg/bee	200 µg

* No higher concentration was obtained.
** Oxalic acid solution was applied at 37ºC.

Groups of 12 bees received increasing doses of the appropriate concentration on the thorax and abdomen using a micropipette (Nichiryo Co Ltd). Solutions were freshly prepared before each test, and control bees were dosed only with the solvent.

The selected doses of every organic acid for the field assay were the highest that did not cause laboratory mortality greater than in the controls (Table 1), multiplied by 15,000, this being the mean number of honeybees estimated in the hives in the moment of the assay.

2.2. Field Assay

Trials were carried out in autumn 2001 on homogeneous honeybee colonies of *Apis mellifera* naturally infected with *V. destructor*. The colonies did not receive acaricide treatment in the eight months prior to the study. Ninety Langstroth hives adapted for mite collection were used (Higes *et al.,* 1999). No brood was present at the moment of the assays. The experimental apiaries were located in Guadalajara, situated in Central Spain (with a mean altitude of 680 meters above sea level and a warm Mediterranean climate). *Thymus* ssp., *Retama sphaerocarpa*, *Lavandula stoechas* and culture plants like sunflower (*Helianthus annuus)* and corn (*Zea mays*) made up the predominant flora of the region.

Previous to the treatments, parasite infection was checked by counting fallen mites at the bottom of the hives. The treatments involved trickling 50 ml of the corresponding solutions, prepared by dissolving each acid in a 50% water and sugar solution (w/w). The fallen mites were collected and counted every two or three days.

Hives were randomly assigned to either receive a treatment or served as controls. We tested the acaricide effectiveness of propionic (PR), malic (MA), citric (CIT), acetic (AC) or oxalic (OX) acids, administered by trickling (Table 2) in ten hives per organic acid. Two applications were done with each acid, fifteen days apart. The first one was applied at the end of October. Twenty control hives were treated with amitraz (Apivar®, APV) and ten with coumaphos (Perizin®, PZ). A group of another ten hives was trickled with a 50% sugar solution (w/w) in distilled water as a trickling control (TC). Finally, other ten hives that no received any treatment served as the Varroa natural mortality control group (NMC). To determine the number of surviving mites after the treatments, all the colonies (including controls) were treated with fluvalinate (Apistan®) thirty days from the first organic acid administration. After one month, the strips were removed, and an additional treatment with coumaphos (Perizin®) was applied to all hives, with the exception of the PZ ones, which received no additional treatment.

Table 2. Doses and Varroa mite fallen in the different treatments.

Group	Treatment	Dose per hive	Ph solutions	Varroa fallen in treatment	Total Varroa fallen	Efficacy (%)	
				Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	V.C.
PR	Propionic acid	1.5 g	2.5	418.7± 375.9	1665.3 ± 1173.5	24.4 ± 9.1	37.1
MA	Malic acid	18 g	1.2	60.9 ± 62.5	1450.1 ± 1299.1	3.7 ± 2.6	70.7
AC	Acetic acid	10 g	2	174.0 ± 108.2	1973.5 ± 920.0	8.1 ± 2.1	26.6
CIT	Citric acid	10 g	1.4	31.4 ± 20.6	1122.3 ± 465.6	2.6 ± 1.3	48.2
OX	Oxalic acid	3 g	<1	1770.2 ± 1074.2	2251.1 ± 1068.8	75.5 ± 12.6	16.7
PZ	Perizin®	SP	-	2468.2 ± 1753.4	2629.5 ± 1854.9	90.5 ± 8.13	9.0
APV	Apivar®	SP	-	112.9 ± 135.1	1217.0 ± 1021.7	92.3 ± 8.2	8.9
TC	Sugar solution	None	6.5	2292.1 ± 556.4	2519.6 ± 566.9	8.9 ± 6.8	76.4
NMC	Negative control	None	-	204.0 ± 173.0	2080.7 ± 1406.1	9.5 ± 2.8	29.5

PR, propionic acid group. MA, malic acid group. AC, acetic acid group. CIT, citric acid group. OA, oxalic acid group. APV, positive control with Apivar®. TC, Trickling control with 50% sugar solution. PZ, positive control trickling with Perizin®. NMC, natural mortality of Varroa control. V.C., Variation coefficient. SP, standard prescription.

2.3. Bee mortality.

Bee mortality was measured using a trap (Pérez *et al.,* 2001) in the hive entrance. Dead bees were collected and counted on every visit to apiary every two or three days. The bee mortality caused by treatments was compared with the bee mortality of the non-treated hives (NMC). This bee mortality value was given the rate of 100% to compare it percentage-wise with the mortality in the treated hives.

2.4. Statistical analysis

To study the acaricide effectiveness of each treatment, a one-way ANOVA model was used. Homogeneity of variances was determined by means of a Levene test. The F test or the Welch and Brown-Forsythe robust test were applied according to the homogeneity of variances. To check post-hoc, Tukey's tests or Games-Howell were used (for a fiducial limit level α=0.05). Every group was compared with the Varroa natural mortality control group using a Dunnett's T test. All statistical studies were performed with the SPSS 11.5 Computer Program.

3. Results.

Table 2 shows the autumn efficacy of the different treatments. In the control groups (PZ, APV and NMC), the number of Varroa fallen indicated as first or second treatment made reference to Varroa mortality in both periods in order to compare the same point with the other treatments.

Significant differences were observed in mite mortality between groups (df=8; F=222.57; P<0.001). Higher effectiveness was obtained in the OX, PZ and APV groups. For PZ and APV, commercial products, it was significantly higher (P<0.05). No significant differences (P>0.05) were observed between MA, CIT, AC and TC or NMC groups, where efficacies of <10% were found in all of them (Fig. 1, Table 2). Nor did we observe any difference between the TC or NMC groups (P>0.05). The efficacy of propionic acid (PR) was different from that of all other groups (P<0.05). PR obtained poorer results than oxalic acid (P<0.05) but had better results than the other organic acids.

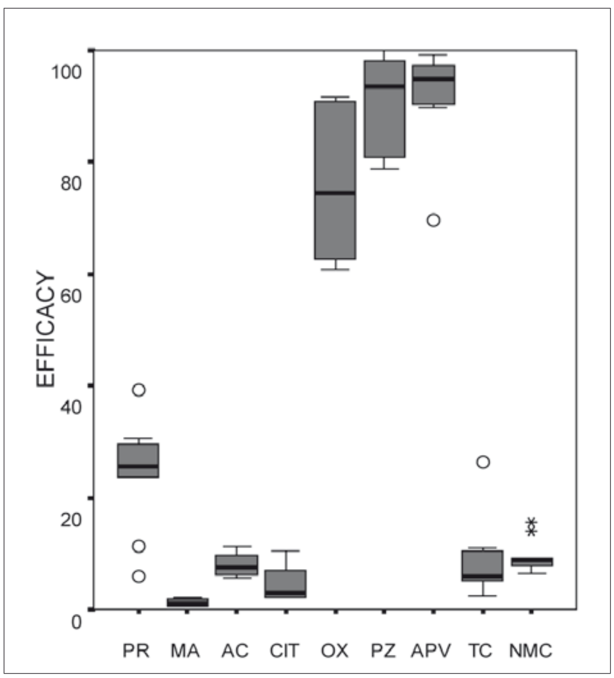

Fig. 1. Efficacy boxplot of different treatments. PR, propionic acid; MA, malic acid; AC, acetic acid; CIT, citric acid; OX, oxalic acid; APV, Apivar®; TC, Trickling control; PZ, Perizin®; NMC, natural mortality of Varroa.

All the groups experienced higher bee mortality than the NMC group (Fig. 2). The increased mortality in the trickling control group was very low, and it was a little higher in the group treated with Apivar® and Perizin®. The highest increase was observed in trickling groups treated with organic acids, especially those treated with acetic and propionic acids. The hives trickled with oxalic, citric and malic acid also showed a certain increased mortality when compared with the group treated with Perizin®.

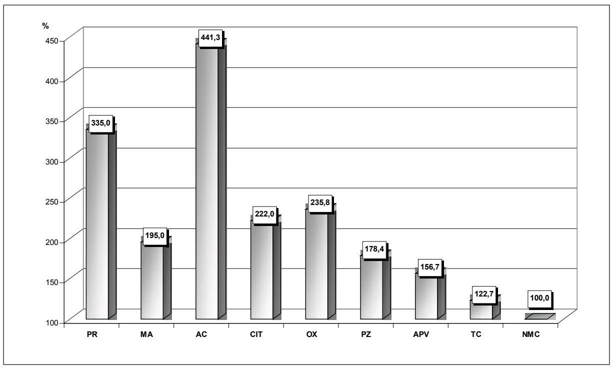

Fig. 2. Increase in honeybee mortality in treated groups over the natural mortality rate considerate as reference value (NMC group, understanding 100%). PR, propionic acid; MA, malic acid; AC, acetic acid; CIT, citric acid; OX, oxalic acid; APV, Apivar®; TC, Trickling control; PZ, Perizin®; NMC, natural mortality of Varroa.

4. Discussion.

Our results show the low acaricide effectiveness of most of the organic acids used in this study to control Varroa infections during the broodless period. The mite mortality observed with malic, citric and acetic acid was not higher than the acari natural mortality rate. In our trials, some Varroa mortality was produced with propionic acid alone, but not enough to be of interest since the results obtained were very far from those obtained with the commercial products.

Very few reported data exist about organic acid effectiveness except oxalic, formic and lactic acids. In our assay, only oxalic acid showed an adequate efficacy. Solutions around 3% oxalic acid have resulted in more than 90% mite mortality in trials similar to ours with respect to timing (Nanetti and Stradi, 1997; Higes *et al.,* 1999; Gregorc and Planic, 2002). In our trials, with two applications of similar concentrations, we obtained around 75% effectiveness, so a single application of the product was not enough to control Varroa in our climate conditions, where the broodless period happens very near to bee cluster. Reported efficacies for formic and lactic acids have been more variable, usually influenced by application methods. This effectiveness ranged between 70-95% for lactic acid (Higes, *et al*., 1997; Brodsgaard *et al.,* 1997) and 61-95% for formic acid (Nanetti *et al.,* 1995; Rademacher *et al.,* 2000), also during the broodless period. Although those reported results were much higher than the ones obtained with the organic acids used in our trials, either lactic or formic acids are not used habitually for the Spanish beekeepers due to bee toxicity, alteration in colony behavior or handling limitations.

The pharmacological action attributed to oxalic acid is based on the low pH of solutions (Nanetti, 1999); however in our assay the pH of the solutions of malic, acetic and citric acids was even lower than in the propionic solution, but the efficacy was higher in the last one. So a low pH does not seem to be quite determinant and any other action mechanism must be contributing to the acaricide effect of the organic acids.

The trickling effect by itself causes some bee mortality. In the TC group the number of dead bees was slightly higher than the natural mortality. This was probably caused by handling, when carrying out the application of the products, and by the negative effect of adding any substance (coumaphos or organic acids). The bee mortality in the AC group was as much as 4-fold higher than in the TC group. The doses of every organic acid selected for field testing were based on laboratory assays made with homogeneous adult honeybees groups, though in hives a mixture of different aged bees is present, and many of them are impregnated by a large amount of the solution during the treatment. This could explain the differences in toxicity levels between field and laboratory assays. Solutions of 10 % citric acid have been reported as non-toxic for Varroa or bees in laboratory assays, and the same concentrations of acetic and propionic acids produce adverse effects on bees but not on Varroa (Koeninger, 1984). Therefore, in a given concentration, citric acid was less active on Varroa than oxalic acid (Milani, 2001).

Although acetic acid is expressly recommended in European ecological beekeeping regulation (EU Council, 1999), we did not get good results for Varroa control during the broodless period, the time when the treatment with organic acids is usually recommended. The bee mortality observed with propionic and acetic acids does not recommend increasing the dosage in order to enhance the efficacy.

In our trials, oxalic acid was selected as reference due to the absence of reported data of the tested acids and in order to compare them. It is the most widely employed organic acid for Varroa control and seems to be the only organic acid useful in Varroa control programs, recently included in Annex II of Regulation (CE) 2377/90 (EMEA, 2004).

5. References.

Bolli, H.K.; Bogdanov, S.; Imdorf, A. and Fluri., P. 1993. Zur Wirkungsweise von Ameisensäsure bei *Varroa jacobsoni* Oud. Und der Honigbiene (*Apis* mellifera L.). *Apidologie,* 24, 51-57.

Brodsgaard, C.; Hansen, H. and Hansen, C. 1997. Efectos del ácido láctico utilizado como único método de lucha contra el ácaro Varroa durante cuatro años sucesivos en colonias de abejas melíferas sin pollo. *Apiacta XXXII,* 81-88.

Colin, M.E.; Ducos, J.; Larribal, E. and Bove, T. 1989. Activité des huiles essentielles de labiés sur *Ascosphaera apis* et traitement d'un rucher. *Apidologie,* 20: 221-228.

(EMEA) European Agency for the Evaluation of Medicinal Products Veterinary Medicines and Inspections. 2004. Consejo de Regulación (EEC) Nº 2377/90. EMEA/MRL/891/03-FINAL.

(EU) Official Journal of the European Communities. 1999. Council Regulation No. 1804 on organic farming, Chapter Beekeeping and Beekeeping Products: of 19 July 1999. L-222.

Fries, I. 1991. Treatment of sealed honey bee brood with formic acid for control of *Varroa jacobsoni*. *Am Bee J*, 131, 313-314.

Greatti, M.; Lob, M.; Barbattini, M. and D'Agro, M. 1992. Efficacia di trattamenti primaverali con acido lattico e acido formico contro *Varroa jacobsoni* Oud. *Apicttlura Moderna*, 83, 49-58.

Gregorc, A. and Planic, I. 2002. The control of *Varroa destructor* using oxalic acid. *Vet J,* 163, 306-310.

Higes, M.; Meana, A.; Suárez, M. and Llorente, J. 1999. Negative long-term effects on bee colonies treated with oxalic acid against *Varroa jacobsoni* Oud. *Apidologie,* 30, 289-292.

Higes, M.; Suarez, M. and Llorente, J.. 1997. Método integral para el control de la varroosis de la abeja melífera (*Apis mellifera*); cría dirigida de zánganos y ácido láctico. *Medicina Veterinaria,* 14, 415-419.

Koeninger, N. 1984. Utilización de ácidos y otras sustancias naturales para atajar la varroasis. *Apiacta* 19, 33-39.

Kunz, S.E. and D.H. Kemp. 1994 Insecticides and acaricides: resistance and environmental impact. *Review Scientific and Technical of Office International des Epizooties,* 13, 1249-1286.

Milani, N. 2001. Activity of oxalic and citric acids on the mite *Varroa* destructor in laboratory assays. *Apidologie,* 32, 127-138.

Mutinelli, F.; Baggio, A.; Capolongo, F.; Piro, R.; Pradin L. and Biasion, L. 1997. A scientific note on oxalic acid by topical application for the control of varroosis. *Apidologie*, 28, 461-462.

Nanetti, A. 1999. Oxalic acid for mite control – results and reviews. Coordination in Europe of research on integrated control of Varroa mites in honey bee colonies. Merelbeke, Belgium, Commission of the European Communities, 6-11.

Nanetti, A. and Stradi, G. 1997. Varroasi: trattamento chimico con acido ossalico in sciroppo zuccherino. *L'APE Nostra Amica,* 5, 6-14.

Nanetti, A.; Massi, S.; Mutinelli, F.; Cremasco, S.; Arcuelo, P. and Artese, F. 1995. Trattamento della varroasi con acido formico: esperienze con diversi metodi di somministrazione. *L'Ape Nostra Amica*, 2, 5-13.

(OEPP) European and Mediterranean Plant Protection Organization. 1992 Guideline of test methods for evaluating the side effects of plant protection products on honeybees. Draft nº 170. Bulletin OEPP/EPPO, 22, 203-215.

Pérez, J.L.; Higes, M.; Suárez, M.; Llorente, J. and Meana, A. 2001. Easy ways to determine honey bee mortality using dead-bee traps. *J Apic Res,* 40, 25-28.

Rademacher, E.; Bruckner, D.; Otten, C.H. and Radtke, J. 2000. El ácido fórmico en el tratamiento de la varroosis. *Vida Apícola,* 101, 41-47.