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RESEARCH ARTICLE

Efficacies of Formic, Oxalic and Lactic Acids Against *Varroa destructor* in Naturally Infested Honeybee (*Apis mellifera* L.) Colonies in Turkey [1]

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Summary

This study was performed to determine the efficacies of formic, oxalic and lactic acids in the control of the common ecto-parasite *Varroa destructor* on naturally infested honeybee (*Apis mellifera* L.) colonies in the Marmara region of Turkey. Experimental colonies were divided in to five homogeneous groups of eight hives each. Formic, oxalic and lactic acids and coumaphos (Perizin®, a chemical with known efficacy, for comparison) were each applied to one group, with the fifth group serving as the untreated control. Each colony was treated during three seasons, two autumn seasons and one spring. The efficacy of the acids was measured by the Henderson–Tilton formula, and the significance between the acids was determined via Tukey's multiple comparison test. In the first autumn, the highest percentage of the efficacy were detected in oxalic acid (93.7%) followed by coumaphos (92.5%), formic acid (84.7%) and lactic acid (54.3%). In spring, the efficacy rate was 65.6% in coumaphos while these rates were 21.4% in formic acid and 8.3% in oxalic and lactic acid each one. On the other hand, in the second autumn, the efficacy rate was 96.9% in coumaphos, 95.6% in formic acid, 93.3% in oxalic acid and 87.7% in lactic acid, respectively. During the trials, we observed no reduction in the queen bees' egg laying or in adult bee deaths due to the treatments.

Keywords: *Honeybee, Efficacy, Organic acids, Varroa destructor, Turkey*

Türkiye'de *Varroa destructor* ile Doğal Enfeste Bal Arısı (*Apis mellifera* L.) Kolonilerinde Formik, Okzalik Laktik ve Asitlerin Etkinliği

Özet

Bu çalışma, bal arılarının (*Apis mellifera* L.), Türkiye dahil olmak üzere tüm dünyada yaygın paraziti olan *Varroa destructor*'un kontrolünde kullanılan formik asit, okzalik asit ve laktik asidin, Türkiye'nin Marmara Bölgesi koşullarında etkinliğinin belirlenmesi amacıyla yapılmıştır. Deneme kolonileri homojen olarak her biri sekiz kovandan oluşan beş gruba ayrılmıştır. Gruplara formik, okzalik ve laktik asidin yanı sıra etkisi bilinen kimyasal ilaç olarak coumaphos (Perizin®) uygulanmış ve bir grup tedavi edilmeden kontrol olarak bırakılmıştır. Her koloni iki sonbahar - bir ilkbahar olmak üzere üç sezon tedavi edilmiştir. Asitlerin etkileri Henderson - Tilton formülüyle, asitler arası önem ise Tukey'in çoklu karşılaştırma testi ile belirlenmiştir. İlk sonbaharda en yüksek etki %93.7 ile okzalik asitte görülmüş, coumaphos %92.5, formik asit %84.7 ve laktik asit %54.3 etki göstermiştir. İlkbahar sezonunda coumaphos %65.6, formik asit %21.4, okzalik asit ve laktik asit %8.3; ikinci sonbahar sezonunda (üçüncü sezonda) ise coumaphos %96.9, formik asit %95.6, okzalik asit %93.3 ve laktik asit %87.7 etki göstermişlerdir. Denemeler sırasında ana arının yumurtlamasında bir azalma veya ergin arı ölümleri gözlenmemiştir.

Anahtar sözcükler: *Bal arısı, Etkinlik, Organik asitler, Varroa destructor, Türkiye*

INTRODUCTION

Varroa mites (*Varroa destructor)* are the most important parasite worldwide for beekeeping, including in Turkey [1,2]. They parasitize pupae and adult bees and reproduce in the pupal brood cells. Infestation can cause serious damage, such as the loss of up to 25% of adult weight, severe deformations of the wing, reduced

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longevity of worker, drone and queen honeybees and the collapse of untreated colonies within a few years [3]. The use of acaricides should be minimised in beekeeping to avoid both the build-up of residues and their by-products in honey and wax and to reduce the potential of acaricide resistance [4].

Varroa destructor came from the Thrace region to Turkey in 1977, spread across the country in a short time and caused 600.000 colony deaths [5]. It has been described genetically [6] and morphologically [7].

In the past, chemical drugs were usually used for *V. destructor* control, but in recent years organic acids and essential oils have also been used in Turkey because of the possibility of chemical drug resistance and the human health hazard their residues pose in the honey and wax [8,9]. Currently, the selection procedure for resistance against natural acaricides such as organic acids and essential oils is low. Accumulation in wax does not occur, and residues in honey and its by products are sparse and toxicologically unimportant [10].

Temperatures in winter can sometimes fall below freezing, and the region is always quite humid (73% average) due to the Marmara Sea. Average temperature ranges in October, November, December, March and April are 9.9-21.6, 5.7-15.8, 3.5-11.3, 3.5-13.6, 7.2-18.7°C, respectively, in the Bursa province [11].

The primary aim of this study was to determine the efficacies of formic acid, oxalic acid and lactic acid on honeybee colonies that were infested with *V. destructor* in the Marmara region, Bursa province of Turkey. Coumaphos (Perizin), a drug with known efficacy against *V. destructor*, was used as a control to compare these organic acids to. The secondary aim of our work was to establish whether these organic acids are a viable alternative to chemical acaricides (coumaphos in this study) when brood is present and to determine whether they inhibit or decrease the egg laying of queen bees.

MATERIAL and METHODS

Forty *Apis mellifera* honeybee colonies, populated in standard Langstroth hives with six to seven combs, located in the Bursa province, Turkey, were divided into five homogeneous groups. Colonies were treated with coumaphos (Perizin, Bayer) in the previous winter when no brood was present, in proportion to the level of *Varroa* contamination. Formic acid, oxalic acid, lactic acid and coumaphos were applied to each group; one group served as an untreated control. Approximately 100 adult worker bees from outer frames were collected in jars containing cotton with diethyl ether to determine the rate of *Varroa* infestivity before and after treatment[12]. Aliqouted adult bees and mites were counted. Additionally, the bottoms of pollen drawers were cleaned before the trial and were covered with white paper to count dead mites that dropped there after each drug application. Ethical Commission of Uludağ University Veterinary Faculty approval (No: 2/2004) was obtained for all procedures.

Each colony was treated during three seasons: from November 20, 2004 to December 14, 2004; from March 16, 2005 to April 12, 2005; and from October 5, 2005 to November 3, 2005. Average temperatures at the application times were 17.2°C in October, 13.9°C in November, 12.1°C in December, 12.9°C in March and 15.4°C in April.

The application methods of the organic acids and coumaphos were as follows.

→ 80% formic acid was soaked on cardboard in freezer bags and on top of the frames, given 100 ml per hive, at two times in 7-day intervals. Bags were cut by a knife when they put on the frames.

→ 4% oxalic acid was made with sugar syrup (1 L sugar syrup (1:1) + 100 g oxalic acid dihydrate), by dripping between the frames, giving 5 ml for each space, at three times in 7-day intervals.

→ 15% lactic acid was pulverized onto the surface of combs containing bees and brood cells, 5 ml per comb side, three times in 7-day intervals.

→ 2% coumaphos was dripped between the frames, 50 ml per hive, three times in 7-day intervals.

→ One group was not treated and kept as control.

In each season, on the 1st, 3rd, 7th, 14th, 21st and 28th days, the dead mites that had dropped onto the pollen drawers were counted. Approximately 200 adult honeybees were collected from the outer frames of each hive, and the mites on them were counted on the 29th day. Whole brood cell areas were measured during the spring trial to determine if the organic acids had any side-effects on the queen bees' egg laying. The next day, 1:2 sugar syrup (1 L water + 2 kg sugar) was given to the colonies to stimulate ovulation in the queen. One week after the trial, brood areas on the combs were measured again to compare the measurements before the trial with using Kruskal Wallis test [13].

The efficacies of drugs were measured with the Henderson-Tilton formula, and significance between the acids was determined via Tukey's multiple comparison tests and defined at the level of 0.05 [14-16].

$$\text{Corrected \%} = \left(1 - \frac{n \text{ in Co before treatment} \times n \text{ in T after treatment}}{n \text{ in Co after treatment} \times n \text{ in T before treatment}}\right) \times 100$$

Where n = mite population, T = treated, Co = control

RESULTS

Applying the seasonal data of *Varroa destructor* population to the Henderson-Tilton formula (see *Materials and Methods*); we obtained the following calculations for the drug efficacies:

1st season (November 2004):

Formic acid efficacy: 1 - [(21 × 34) / (46 × 102)] × 100 = 84.7%
Oxalic acid efficacy: 1 - [(21 × 7) / (46 × 51)] × 100 = 93.7%
Lactic acid efficacy: 1 - [(21 × 30) / (46 × 30)] × 100 = 54.3%
Coumaphos efficacy: 1 - [(21 × 7) / (46 × 43)] × 100 = 92.5%

2nd season (March 2005):

Formic acid efficacy: 1 - [(11 × 4) / (8 × 7)] × 100 = 21.4%
Oxalic acid efficacy: 1 - [(11 × 4) / (8 × 6)] × 100 = 8.3%
Lactic acid efficacy: 1 - [(11 × 2) / (8 × 3)] × 100 = 8.3%
Coumaphos efficacy: 1 - [(11 × 2) / (8 × 8)] × 100 = 65.6%

3rd season (September 2005):

Formic acid efficacy: 1 - [(46 × 4) / (82 × 51)] × 100 = 95.6%
Oxalic acid efficacy: 1 - [(46 × 5) / (82 × 42)] × 100 = 93.3%
Lactic acid efficacy: 1 - [(46 × 7) / (82 × 32)] × 100 = 87.7%
Coumaphos efficacy: 1 - [(46 × 2) / (82 × 37)] × 100 = 96.9%

These efficacy results are summarized in *Table 1*. The efficacy of coumaphos in the second (spring) season was markedly higher than those of the organic acids. Lactic acid demonstrated a moderate effect in both autumn seasons, compared with those of the other two organic acids. Coumaphos reached 96.9% efficacy during the third season (autumn).

Table 1. *Efficacy of organic acids and coumaphos by the Henderson-Tilton formula*

Tablo 1. *Henderson-Tilton formülüne göre organik asitlerin ve kamofosun etkinliği*

Season	Efficacies %			
	Formic Acid	Oxalic Acid	Lactic Acid	Coumaphos
1st	84.7	93.7	54.3	92.5
2nd	21.4	8.3	8.3	65.6
3rd	95.6	93.3	87.7	96.9

The total number of mites dropped in each treatment in each season, as shown in *Table 2*. The number of dropping mites in all treated colonies was the lowest in the second (spring) season compared to the two autumn seasons. Coumaphos treatment caused the highest total number of mites to drop throughout the three seasons, and formic acid has more mites than the other two organic acids.

Table 2. *Mean number of dead mites on pollen drawers on the 1st, 3rd, 7th, 14th, 21st and 28th days (mean ± SEM)*

Tablo 2. *1, 3, 7, 14, 21 ve 28. günlerde polen çekmecelerine düşen ölü akarların ortalama sayıları (ortalama ± SEM)*

Drugs	1st Season	2nd Season	3rd Season	Total
Formic acid	46.25±4.54	7.14±0.95	24.68±3.96	78.07±3.15
Oxalic acid	39.54±5.57	6.47±1.01	15.33±2.91	61.34±3.16
Lactic acid	19.91±3.66	2.77±0.31	18.93±4.96	41.61±2.97
Coumaphos	34.68±5.17	4.33±0.37	53.27±7.28	92.28±4.27
Control	11.77±3.43	1.37±0.23	1.14±0.19	14.28±1.28

During the experiment, dropping mites on drawers were counted for 28 days. The differences between the control and the formic acid, oxalic acid, lactic acid and coumaphos treatments were significant in the first and second season, but not for lactic acid in the third season.

The area of the brood cells was measured before and after the acid treatments during the spring trial. The mean percentage increases of these areas are shown in *Table 3*.

Table 3. *Mean percentage increase of total brood cell area in each acid and control treatment*

Tablo 3. *Her bir asit ve kontrol tedavisinde toplam yavrulu alan artışlarının ortalama yüzdesi*

Drug	n (hive)	Mean Ranks (%)
Formic acid	8	12.57
Oxalic acid	8	17.29
Lactic acid	8	16.57
Control	8	11.57

The Kruskal-Wallis test was used to detect any possible side-effects of the organic acids on each queen bee's egg laying. We measured this in terms of the percentage increase in the total area of brood cells in each colony. The difference value was 0.47, and the chi-square value was 2.51. There was no significant difference between the acid and control groups at the level of 0.05, indicating that none of the acids had a negative effect on the egg laying of the queen bees. Finally, there were no observable side-effects or abnormal bee deaths during any of the trials in the treated or control colonies.

DISCUSSION

Chemical compounds (e.g., Coumaphos, Amitraz, Tau-fluvalinate) are used widely in Turkey for the control of *Varroa destructor* on honeybees. Consequently, there is the potential for chemical resistance to develop among the mites and for harmful residues of these chemicals to remain in bee products, especially honey and wax [17].

We conducted this study to examine the efficacy of three organic acids as alternative control methods for *V. destructor* in the Bursa province of the Marmara region in Turkey.

Calderone and Nasr [18] have found 56% efficacy and Charriere et al.[19] 95% efficacy for formic acid dispensed via imbibed pads in autumn against *V. destructor*. Greatti et al.[20] have found 47.08% efficacy of formic acid in spring treatment dispensed via imbibed cardboard. Charriere and Imdorf [21] have observed 98% efficacy and Gregorc and Planinc [10] 99.4% efficacy with three oxalic acid trickle treatments in autumn. Kraus and Berg [22] have found 94.2-99.8% efficacy against *V. destructor* with two lactic acid spray treatments in autumn.

There are many recent reports about coumaphos resistance in *Varroa* mites from several countries but not in Turkey [17]. Efficacies of coumaphos between 83% and 100% have been observed by some researchers [23,24].

Counting the mites that drop from a colony onto a bottom board is a reliable diagnostic method to evaluate the efficacy of acaricide treatment [4]. In addition to that method, we used the Henderson-Tilton formula, which can be used to calculate the corrected efficacy percentage in pesticide trials [14].

The highest efficacy was observed in the third season of this trial (autumn) with coumaphos at 96.9%. Formic acid showed the highest single-season efficacy (95.6%, third season) of all the organic acids in all three seasons of the trial. Oxalic acid (93.7%) and lactic acid (87.7%) reached their maximum efficacies in the first and third seasons, respectively.

There was a substantial increase in the efficacy of lactic acid in consecutive seasons. We speculate that the increase in lactic acid efficacy in the third season resulted from regular usage and accommodation of the bees to lactic acid.

If all treatments are evaluated, it is clear that all of the drugs had lower efficacies in the spring because of the presence of brood areas in the combs and also due to a decreased mite number after the fall treatment [10,20,25]. The efficacy of coumaphos was higher than all three organic acids in the spring, when the broods were present.

A natural drug that is currently used or will be used in the future must have been applied during at least two seasons. Because there can be an increasing or decreasing efficacy to *V. destructor* and side-effects on the honeybees, we used three applications of these organic acids and ultimately saw an increase in the efficacy of each acid and in that of the coumaphos as well.

Organic acids must be applied in the early spring and late fall because of the lack of brood cells at those times, so most of the mites are on the bees, making them easy targets for miticide drugs [20,21,26]. Lower doses of these acids are not effective against *Varroa* mites, and higher doses have negative effects such as bee deaths and/or decreased egg laying by queen bees [18,19,21]. These acids are good alternatives for the *Varroa* control, and they do not present the disadvantages of chemical products used for the control of this mite (for example, residues in bee products and resistance among the mites) [9]. In our trials, no cases of honeybee toxicity, loss of queens, loss of brood and/or adult honeybee mortality were recorded. However, organic acid-induced queen losses and interruptions to egg laying have been reported [19,26]. Using natural products like organic acids and essential oils with miticide effects can be an appropriate method to solve the *Varroa* problem because such products are believed to have a low environmental impact and low or null toxicity to bees and humans. Therefore, organic acids appear to be a suitable alternative for *Varroa* mite control, even in Turkey. Effective results were obtained with formic and oxalic acids, with a moderate effect observed for lactic acid, in our experiment.

There are only a few studies on organic acids, mostly formic acid, in *Varroa* control in Turkey [27,28]. The use of formic and oxalic acids is suggested for beekeepers as an alternative to chemical drugs. Lactic acid is not suggested according to our results because of the difficulty associated with its application and its moderate effect on Varroas.

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