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# The Rational Use of Oxalic Acid Against to "*Varroa Destructor*"; Regional Scale Pilot Scheme

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ABSTRACT

*Varroa destructor* mite poses a serious problem for the future of bee populations around the world. Today, there are many commercial drugs with the same and different active ingredients on the market to chemically control over of *Varroa destructor*. More frequent chemical applications for against *Varroa destructor* increases stress resilience, colony losses, loss of yield and residue problems in bee products. The scope of this project is aimed to determine the appropriate control method of Varroa by investigating the efficiency values of the evaporation and dropping methods of Oxalic acid. Experimental area were chosen three different apiaries. 28 colonies were determined in each apiary and equalization studies (area with brood, number of bees with bees, age of queen bees, honey, pollen, etc.) were carried out in these colonies. The determined colonies were randomly divided into 4 groups as 7 colonies. The first group is the control group, the second group is applying 2 g of oxalic acid by vaporizing, the third group is 4% oxalic acid 5 ml of sugar syrup (1:1) is dropped between the frames, and in the fourth group, the fight against a drug that is determined by the beekeeper in the market without interfering with the beekeeper. In order to evaluate the data, samples were taken for four periods, before and after spraying in spring and autumn. While the varroa measurements in the group of syrup, vapor and spraying were found to be statistically less than the control group, the syrup, vapor and spraying groups were statistically similar in terms of varroa measurements. Oxalic acid syrup application showed higher efficiency in spring and autumn than vapor application. There is no statistically difference between both two-application method reveals that it can be used as an effective and safe alternative to chemical control against varroa.

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## Introduction

Bee diseases and pests have an important place among the reasons that limit the yield the most. Considering the economic losses, *Varroa destructor* parasite is among the most important bee disease and pest factors. This parasite causes a decrease in the development rate of colonies, winter loss in honey bees, infection in the colony, decrease in the flight efficiency, nectar and pollen collection capacity of forager bees, body deformations and body weight loss in adult bees. In advanced varroa infection, the colony is destroyed and serious economic losses occur in the apiary. Today, there is hardly any apiary without varroa. In the world and in our country, a definite and permanent method of combating such a widespread bee pest has not yet been developed. The aim of varroa control is not to kill all mites in the colony, but to keep the mite population below the level that will harm the colony (Mert et al., 2007).

The parasite was introduced to Turkey in 1976 via Bulgaria to the Thrace region and from there to the apiaries of beekeepers in Anatolia who went to the region to produce sunflower honey. The parasite, which was seen in the Aegean Region in 1977-78, then infected the apiaries of beekeepers who came from all regions of the country to the Aegean Region, especially Muğla province, to produce pine honey, and spread to the whole country in a very short period of 4-5 years after they returned to their own regions. The parasite caused great destruction in our country in the first years, causing the extinction of approximately 600 thousand colonies and 7000-7500 tons of product loss (Akyol et al., 2005). The disease is widespread all over the world except the Hawaiian Islands, New Zealand South Island, Australia and the tropical region of Africa

(Anderson and Truman, 2000; Aydın, 2011). It was detected in Asia in the 1950s and in the Americas and other continents in the 1980s and has covered all countries. This parasite is not found in islands such as Australia, New Zealand (South Island) and Hawaii due to quarantine measures (Aydın 2005, 2011).

There are 4 important species of Varroa, *Varroa jacobsoni, Varroa destructor, Varroa underwoodi and Varroa rindereri*, which are seen in adult and juvenile honey bees. These four species differ in host selectivity and geographical distribution (Anderson, 2000; Anderson et al., 2000). Adult and female Varroa constitute 96% of the Varroa population in the hive. Although the mouth structure of the female Varroa is piercing and sucking, the mouth structure of the males is not suitable for feeding and is shaped to carry sperm to the females (Zeybek, 1991; Aydın, 2012). Female Varroa live 2-3 months during the season (Spring-Summer) and 5-8 months during the off-season (Fall-Winter). Their life is completely parallel to the activation of the queen bee. The legs are short and strong, with 3 pairs in larvae and 4 pairs in nymphs and adults. All of the hairs on their bodies are called ketomes and these hairs allow the parasite to stay on the bee. Although the parasite usually lives between the head and thorax of the bee, it can also be found between the thorax and abdomen and between the abdominal rings (Webster and Delaphane, 2001; Aydın, 2005).

The activity of Varroa in the pupal and larval stages of bees is quite high. Adult female Varroa enter 5-5.5 days old bee larval cells before the cells close. Before laying eggs, it feeds on the larva's hemolymph (juvenile hormone in the blood). After receiving enough juvenile hormone and developing ovaries, Varroa lays the first egg 2-3 days after the eyes close. A female Varroa lays 2-6 eggs at 30 hour intervals and it is reported that the first egg is male (n=7 chromosomes) and the following eggs are fertilized (2n=14 chromosomes) female eggs. Females become adults in 8-10 days and males in 6-8 days. While 3 female adults are formed in a worker's eye, 5 female adults can be formed in the edges of the drone's eye (the temperature is lower) due to more food (drone biology is longer) (Zeybek, 1991; Aydın, 2012). While females mate in the closed chamber, male Varroa die following mating. Female Varroa emerge from the brood cells with the development of the fertilized egg and re-enter the honeycomb cell within 3 to 150 days depending on the season and the brood status of the colony. Varroa, which prefer drone cells more, increase 1.8-2.9 times in worker cells and 2.7-3.7 times in drone cells. Varroa is spread through bees by natural swarming, predation, wind and drones entering new hives. Especially the presence of weak colonies and strong colonies in the same apiary is the most common form. The ability of the drone to move between hives has also been reported as an important factor in the rapid spread of the parasite. The adult bee population has started to decrease, the queen's oviposition performance has decreased, dotted holes in the brood cells, symptoms similar to foulbrood rot, and symptoms such as deceased brood remaining in the 'C' shape in the cells are observed (Aydın, 2012).

Today, various methods, including physical, biological and chemical, are used in the control of Varroa. It is not possible to completely eradicate Varroa with the methods applied today. It is necessary to apply continuous treatment for decreasing Varroa density. For this, chemical control is of great importance. As a result of the widespread use of antiparasitic chemical drugs in the control of *Varroa destructor*, beekeeping has faced two important problems. The first one is the resistance of V. destructor to some of these chemicals. Drug-resistant Varroa populations transferred this hereditary trait from generation to generation in a short time. The second major problem is that some toxic components of chemical drugs accumulate in honey, wax and even propolis (Tutkun 2016). The drugs to be used should be safe for human health and the side effects that may occur in bees should be either absent or minimal. Drugs should not leave any residue or odor in the products to be obtained from bees or should not exceed the highest acceptable residue levels. Nowadays, human health and consequently food safety are being studied intensively. At this point, for the control of varroa in relation to bee products, substances that do not have harmful effects on human health and do not carry the risk of residue in honey have started to be sought. Organic acids, especially formic acid, are used in the control of this parasite because they have not yet developed resistance compared to other chemical synthetic compounds, they are cheap, they do not leave residues in honey above the specified limits and they do not harm adult worker bees. However, the use of these compounds is limited by the variability of the application methods used and the fact that the efficacy obtained varies each time and that they sometimes pose a danger to beekeepers due to their application methods. For example, in the hive where formic acid is applied, its concentration in the air varies according to the application method and ambient temperature. Therefore, the exposure time/concentration ratio is different each time. This creates problems in adjusting the concentration of active and toxic substances (Underwood 2003). For this reason, studies are ongoing on various formulations that will control and facilitate the administration routes of these active substances (Rufinengo et al., 2014). Oxalic acid, an organic acid, is naturally present in the structure of honey. If it is present in honey at very high levels (400-900 ppm), it causes a difference in the taste of honey. If oxalic acid application is made in the fall, it does not have any negative effect on the taste of honey and adult bees and brood (Imdorf et al., 1997). Wehling et al. (2003) emphasized that oxalic acid and formic acid applications are effective against varroa and are not harmful to human health as they are naturally present in honey.

In this study, it was aimed to determine the appropriate control method by investigating the effectiveness values of the evaporation and dripping methods of oxalic acid, which is an organic acid with low residue and resistance risk and will not adversely affect human health, in the control of *Varroa destructor* parasite, which poses a serious problem for the future of bee populations worldwide. With the project outputs, it is aimed to raise awareness among beekeepers and to expand its use throughout the country.

## Materials and Methods

### *Material*

*Bee Material*

The bee material consisted of local bees from Bulancak district of Giresun province, which were equalized in terms of brood area, number of bee frames, queen age and food stock. The experiment was conducted in standard Lanstroth type hives.

*Oxalic Acid*

Oxalic acid is a compound in the organic acid class with the formula C2H2O4. Oxalic acid used in the study was obtained from a commercial company.

*Other Tools and Equipment*

In order to determine the level of varroa contamination in adult bees; worker bee samples taken from colonies were taken into glass jars containing 70% ethyl alcohol with the help of a beekeeper's brush. A precision balance with a sensitivity of 0.001 gr was used for the preparation of chemicals for counting. Basic beekeeping materials such as honey pot, partition board, bellows, mask, hand iron were also used for colony maintenance during the study.

***Method***

*Preparation of Colonies and Treatment Groups in Varroa Control*

The research was carried out in three separate enterprises engaged in fixed beekeeping in Bulancak district of Giresun province where the risk of contamination was minimized. In each enterprise, 28 colonies were determined and equalization studies (brood area, number of frames with bees, queen age, honey, pollen, etc.) were carried out in these colonies. The colonies were randomly divided into four groups of 7 colonies according to the following experimental design.

Group 1 - Control group without any treatment against Varroa parasite

Group 2 - Application of 2 grams of oxalic acid to colonies by evaporation method

Group 3 - Administration of 4% oxalic acid with sugar syrup (1:1) at 5 ml per frame

Group 4 - Fighting with a commercially available drug

No spraying was done in the first group of colonies. Considering the risk of contamination, the colonies were transferred to a different point where there were no bee colonies around and at least 10 km away from the existing apiary. The second and third group colonies were treated with oxalic acid according to the experimental design given above. Considering the risk of contamination in these colonies, they were transferred to a different point in the opposite direction to the first group colonies, where there were no bee colonies around and at least 10 km away from the existing apiary. The fourth group of colonies were left in the beekeeper's own apiary and treated with a commercially available drug that is widely used in Varroa control for positive control. In the spring and fall periods, no treatment against varroa was performed in the colonies divided into groups, and varroa contamination rates were determined before and after treatment.

*Varroa Contamination Rate*

In order to detect Varroa pests, ether was sprayed on the live bee samples brought to the laboratory in sealed containers to kill the bees. At least 100 bees each were placed in a jar filled with 70% ethyl alcohol, the lid was closed, shaken for 30 minutes and left to settle for 10-15 minutes. Varroa were collected on the surface while bees and other residues settled to the bottom. After the process, all the material was passed through a narrow mesh strainer that would not allow the passage of varroa and bees, and the bees and parasites remaining on top were taken on a white blotting paper and the parasites were counted and recorded. Varroa parasites were searched for in the bees on the paper, especially between the wing bases, abdominal segments and feathers under a stereo microscope, and the collected parasites were examined and identified under a stereo microscope. These parasites were collected and placed in small capped bottles and the exact parasite load of the colonies was calculated (Kar et al. 2006).

$$\text{Infestation rate (\%)} = \frac{\text{Varroa count}}{\text{Number of bees}} \times 100$$

*Efficiency value*

The 'Henderson - Tilton Formula' was applied to determine the drug efficacy according to the mite load on the bees sampled before and after the trials (Henderson and Tilton, 1955). According to this formula, the calculation of the efficacy of a drug as a percentage is as follows;

$$\text{AP} = \left[1 - \frac{\text{NMCB} \times \text{NMTA}}{\text{NMCA} \times \text{NMTB}}\right] \times 100$$

AP: Adjusted Percentage
NMCB: Number of mites in the control group before treatment
NMTA: Number of mites in treatment group after treatment
NMCA: Number of mites in the control group after treatment
NMTB: Number of mites in treatment group before treatment

***Statistical Analysis***

Descriptive statistics of the data obtained were calculated and presented as "Mean ± Std. error". Mixed models were used to examine the significance of group, period main effects and group×period interaction term on varroa measurement results. In the model, establishment was included as a random effect and group, period and group×period interaction were included as fixed effects. For multiple comparisons, simple effects analysis was used with Bonferoni correction. The $P<0.05$ criterion was used in the evaluation of all statistical analyses. All analyses were performed using the SPSS (V22.0) package program.

## Results

In the spring period, before the control, all colonies were divided equally into groups and varroa loads were calculated on a group basis. Varroa loads of the first, second, third and fourth groups were 2.97%, 2.99%, 3.26% and 3.26%, respectively. While the varroa loads in the treatment groups decreased after the control treatment, there was an increase of 0.26% in the control group (Table 1).

In the fall period, the varroa loads of the pre-treatment groups were 4.59%, 4.97%, 4.46% and 17.80%, respectively. After the control, varroa load changes were as follows: 3.07% decrease in the first group, 2.56% decrease in the second group and 3.54% decrease in the third group. In the control group, where no control was applied, an increase of 2.85% was observed in varroa load (Table 2). As seen in Tables 1 and 2, an effective control was realized in the treatment groups. In order to determine the effectiveness of oxalic acid applications, applications were made during the periods when the brood area was the lowest (mid-September), while beekeeper applications were made immediately after honey milking (end of August) using long-acting parasitic drugs. In the spring period, the average air temperature was 13.3 centigrade degree in the first treatment and 15 centigrade degree in the second treatment.

In the fall period, the average air temperature was 17.1 centigrade degreein the first application and 17.8 centigrade degree in the second application.

In the model, establishment was included as a random effect and group, period and group×period interaction were included as fixed effects. The random effect of holding was statistically insignificant (P=0.533). This indicates that average varroa measurements were similar across farms. Group, period and group×period interaction terms in the model were statistically significant (P<0.001). In the spring period, the difference between the treatment groups and the control group in terms of pre-treatment varroa loads was not statistically significant. In the autumn period, the difference between the treatment groups and the control group before the control period was statistically significant. In the spring and autumn periods, the difference between the treatment groups was found statistically insignificant, while the difference between the control group and the treatment groups was found statistically significant. When the interaction term in the model was analyzed, it was found that the varroa measurements in the sherbet, steam and own spraying groups were statistically significantly lower than the control group (P<0.05), while the sherbet, steam and own spraying groups were statistically similar in terms of varroa measurements (P>0.05). When the subgroups were analyzed according to the periods within themselves, Sherbet and Steam groups followed a similar trend according to the periods. The lowest varroa was observed after spring, while the highest varroa measurement was observed before fall. When the trend was observed, it was seen that the post-spring and pre-spring periods for these groups were statistically similar in terms of varroa measurements (Table 3).

In the control group, varroa measurements were at the highest point after the fall and statistically significantly higher than the other periods. Post-autumn was followed by pre-autumn, post-spring and pre-spring, and there was no statistically significant difference between pre-spring and post-spring for the control group (P>0.05).

As seen in Table 4, the beekeeper treatments showed the highest efficacy with 92.83% and 82.22% in the spring and fall periods. In the spring period, the efficiency value of oxalic acid slurry treatment was 90.65%, while the efficiency value of steam treatment was 83.58%. In the autumn period, similar results were observed in terms of treatment groups in the spring period. In the autumn period, the efficiency value of oxalic acid syrup application was found to be 71.42%, while the efficiency value of oxalic acid steam application was determined as 58.20%. Oxalic acid slurry application showed higher efficacy than steam application in spring and autumn periods.

Table 1. Varroa contamination rates before and after spring control, %.

| Application | Before the spring combat | | | Mean | After the spring combat | | | Mean | Alteration |
|---|---|---|---|---|---|---|---|---|---|
| | 1.Business | 2.Business | 3.Business | | 1.Business | 2.Business | 3.Business | | |
| Syrup | 3.52 | 2.32 | 3.08 | 2.97 | 0.37 | 0.34 | 0.21 | 0.30 | -2.67 |
| Steam | 3.25 | 2.51 | 3.23 | 2.99 | 0.44 | 0.60 | 0.56 | 0.53 | -2.46 |
| Beekeper App. | 3.85 | 2.77 | 3.46 | 3.36 | 0.34 | 0.29 | 0.17 | 0.26 | -3.10 |
| Control | 3.71 | 2.68 | 3.40 | 3.26 | 3.96 | 2.90 | 3.70 | 3.52 | +0.26 |

Table 2. Varroa infestation rates before and after the fall control period, %.

| Application | Before the spring combat | | | Mean | After the Spring Combat | | | Mean | Alteration |
|---|---|---|---|---|---|---|---|---|---|
| | 1.Business | 2.Business | 3.Business | | 1.Business | 2.Business | 3.Business | | |
| Syrup | 4.99 | 4.37 | 4.42 | 4,59 | 1.39 | 1.99 | 1.19 | 1.52 | -3.07 |
| Steam | 5.19 | 5.05 | 4.68 | 4.97 | 2.40 | 2.60 | 2.25 | 2.41 | -2.56 |
| Beekeper App. | 4.80 | 3.99 | 4.60 | 4.46 | 1.47 | 0.64 | 0.66 | 0.92 | -3.54 |
| Control | 16.76 | 16.00 | 20.65 | 17.80 | 21.34 | 18.25 | 22.38 | 20.65 | +2.85 |

Table 3. Statistical comparison of the periods and control methods of the experimental groups.

| Group | Semestr | | | | P | | | |
|---|---|---|---|---|---|---|---|---|
| | Before Spring | After Spring | Before Fall | After Fall | | | | |
| | Arit.Mean. ± Std. Error | Arit.Mean. ± Std. Error | Arit.Mean. ± Std. Error | Arit.Mean. ± Std. Error | Group | Semestr | Group* Semestr | |
| Syrup | 2.98±0.26 A,ac | 0.31±0.12 B,b | 4.57±0.34 B,a | 1.54±0.25 B,bcd | <0.001 | <0.001 | <0.001 | |
| Steam | 3.02±0.31 A,ac | 0.54±0.14 B,b | 4.96±0.32 B,a | 2.42±0.16 B,bcd | | | | |
| Own Spraying | 3.37±0.25 A,ac | 0.27±0.11 B,b | 4.45±0.23 B,a | 0.9±0.28 B,bd | | | | |
| Control | 3.27±0.29 A,c | 3.53±0.26 A,c | 17.64±1.64 A,b | 20.56±1.58 A,a | | | | |

A,B: Different letters in the same column indicate statistically significant difference (P<0.05); a,b: Different letters in the same row indicate statistically significant difference (P<0.05)

Table 4. Efficiency Levels of Treatment Groups, % (Henderson, CF and EW Tilton, 1955)

| | Oxalic acid sherbet application | Oxalic acid steam application | Beekeeper application |
|---|---|---|---|
| Spring semester | 90.65 | 83.58 | 92.83 |
| Fall semester | 71.45 | 58.20 | 82.22 |

In varroa control, organic acid is widely used in different doses by dripping method Adjlane et al., (2016); Higes et al., (1999); Del Hoyo et al, (2007); Benfotti and Lucchelli, (1999); Gregorc and Planinc, (2005); Charriere and Indorf, (2002); Gregorc and Planinc, (2001); Gregorc and Planinc, (2004); Marcongeli and Gorcia, (2004); Al Toufaila, (2015), spray method Yücel, (2005); Cengiz, (2012); Girişkin et al, (2010); Çetin, (2010); Al Toufaila, (2015) and evaporation method Radetzki, (2001); Al Toufaila, (2015). Varroa parasite control; Adjlane et al. (2016) achieved 81% efficacy value with 4.2% oxalic acid solution; Al Toufaila, (2015) achieved 93-95% efficacy value with spray, dripping and evaporation methods of oxalic acid; Higes et al. (1999) achieved 94% efficacy value in spring and 73% in autumn with dripping method; Del Hoyo et al, (2007) used 5% oxalic acid solution with dripping method with an efficacy value of 83.8%; Akyol and Yeninar, (2009) dissolved 30 g oxalic dihydrate in 1 liter of sugar water and used 5 ml per frame with 93,40% efficacy value; Benfotti and Lucchelli, (1999) dissolved 100 g oxalic acid in 1 liter of sugar water and used 5 ml per frame with 98-99% efficacy value; Yücel, (2005) 3. 2% oxalic acid solution by spraying 3 ml on each honeycomb surface to 92.1% efficiency value; Cengiz, (2012) 3.2% oxalic acid solution with 5 ml spraying method to 84.90% efficiency value; Girişkin et al, (2010) spraying 4% organic acid solution between the frames with 93.3% efficacy value in fall; Gregorc and Planinc, (2005) using 2.9% oxalic acid sugar syrup solution with 94.42% efficacy value in fall; Nasr et al, (2001) achieved 55% efficacy with 2.8% oxalic acid sugar syrup solution and 90% efficacy with 3.5% oxalic acid sugar syrup solution, 40-50 ml per colony; Charriere and Imdorf, (2002) used 3.4% oxalic acid 47.6% sugar as the first solution; 3.7% oxalic acid/ 26. 1% sugar as the first solution, 3.7% oxalic acid/ 26.9% sugar as the second solution, and 2.9% oxalic acid/ 31.9% sugar as the third solution all had efficacy values above 90%; Gregorc and Planinc, (2004) 2.9% oxalic acid, 31.9% sugar water mixture had an efficacy value of 77.8% in spring and 88.87% in fall; Charriere et al, (1998) reported 98-99% efficacy value of oxalic acid solution in autumn; Çetin (2010) reported 95.56% efficacy value in spring and 92% efficacy value in autumn by using 3% oxalic acid solution as 3-4 ml spray method on each honeycomb surface.

In our study, the efficacy value of the oxalic acid syrup group in the spring period was found to be 90.65%, while the efficacy value of the steam treatment was 83.58%. In the autumn period, the efficiency value of oxalic acid slurry group was 71.45% and the efficiency value of steam treatment was 58.20%. Al Toufaila, (2015); Higes et al., (1999); Del Hoyo et al., (2007); Akyol and Yeninar, (2009): Benfotti and Lucchelli, (1999); Yücel, (2005); Cengiz, (2012); Girişkin et al, (2010); Cornelissen and Blacquiere, (2004); Gregorc and Poklukar, (2003); Gregorc and Planinc, (2001); Gregorc and Planinc, (2004); Marcongeli and Gorcia, (2004); Charriere et al. (1998); Çetin, (2010) reported that the efficacy values were higher than our fall treatment groups and partially similar and partially lower than our spring treatment groups. It is thought that the differences between our study results and the literature data are due to the differences in the type, method and dose of oxalic acid used; the strength of the colonies; in-colony incubation activities; climatic variables, differences in bee races and different treatments applied to the colonies at the beginning of the experiment periodically. Bacandritsos et al. (2007) reported that the varroa efficacy value was 65.3% in colonies with brood activity and 77.3% in colonies without brood activity. In support of the previous literature, Marcongeli and Gorcia (2004) reported that the efficacy value of varroa control was 85.6% in colonies with 3 frames of brood and 75.7% in colonies with 6 frames. Again, Nanetti et al. (2003) reported that the same applications in varroa control gave different results in different locations. In our study, the efficacy value was found to be 90.65% as a result of the application of 4% oxalic acid with sugar syrup (1:1) and 5 ml per frame in the spring period. The efficacy value reported as 90.3% in Italy, where a similar application was made, coincides with the efficacy value we obtained in our study. The efficacy values reported as 94.3% in Germany and 95.6% in Norway were higher than our study values, while the efficacy values reported as 87.8% in Switzerland and 85.0% in Finland were lower than our study efficacy value.

## Conclusion

Honey bee populations, which play an important role in the pollination of plants, are decreasing day by day due to internal and external factors, which is a significant threat to the sustainability of foodstuffs. In terms of animal health, Varroa destructor mite poses a serious problem for the future of bee populations in the world. The disadvantages of many drugs with the same or different active ingredients in the market for the chemical control of Varroa destructor are the development of resistance, the residue of some toxic components in honey, beeswax and even propolis, the fact that the main effectiveness occurs only at high doses and the need for frequent dosing. Frequent dosing for an effective control leads to stress development in bees, colony losses, yield loss and residue problems in bee products. For these reasons, the use of oxalic acid, one of the organic acids, has come to the forefront in the control of Varroa destructor, especially in European countries.

In this study, although there were differences between the drug efficacy values of the treatment groups, the change in the varroa load rates of the treatment groups in the spring and fall periods was statistically insignificant. These results revealed that the treatment groups exhibited an effective control against Varroa destructor in the spring and fall periods. It is seen that beekeepers achieved success in the fight against Varroa destructor with their own preferred drugs. The fact that there was no difference between steam and syrup applications of oxalic acid and beekeeper applications reveals that oxalic acid can be used as an effective and safe alternative to chemical control against Varroa destructor. Considering the statistical results of the efficacy values and varroa change rates of the application groups, oxalic acid does not leave any residue on bee products and can be used especially in the late spring period.

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**References**

Adjlane N, Tarek EO, Haddad N. 2016. Evaluation of oxalic acid treatments against the mite *Varroa destructor* and secondary effects on honey bees Apis mellifera. Journal of Arthropod-Borne Diseases, 10(4), 501.

Akyol E, Özkök D. Varroa (*Varroa destructor*) mücadelesinde organik asitlerin kullanımı. Uludağ Arıcılık Dergisi. 2005; 05(4): 167-174.

Akyol E, Yeninar H. 2009. Use of oxalic acid to control *Varroa destructor* in honeybee (*Apis mellifera* L.) colonies. Turk. J. Vet. Anim. Sci. 33(4): 285-288. DOI: https://doi.org/10.3906/vet-0712-16

Al Toufailia H, Scandian L, Ratnieks FL. 2015. Towards integrated control of varroa: 2. comparing application methods and doses of oxalic acid on the mortality of phoretic Varroa destructor mites and their honey bee hosts. Journal of Apicultural Research, 54(2), 108-120. DOI: https://doi.org/10.1080/00218839.2015.1106777

Anderson DL, Trueman JWH. 2000. *Varroa jacobsoni* is more than one species. Exp Appl Acarol. 24, 165-189. DOI: https://doi.org/10.1023/A:1006456720416

Anderson DL. 2000. Variation in the parasitic mite *Varroa jacobsoni*. Apidologie. 31, 281-292. DOI: https://doi.org/10.1051/apido:2000122

Aydın L. 2005a. *Varroa destructor*'un kontrolünde yeni stratejiler. Uludağ Arıcılık Dergisi. 5(2), 59-62.

Aydın L. 2005b. Sonbaharda balarısı hastalık ve zararlılarının kontrolü. Uludağ Arıcılık Dergisi. 5(4), 59-62.

Aydın L. 2011. The current status of Varroosis in Turkey and Its control. The 2nd Beekeeping Conference Israel-Turkey, 6-10th February Kfar Menahem Israel.

Aydın L. 2012. Varroa ilaçları ve kontrol programı. Internatıonal III. Muğla Beekeeping and Pine Honey Congress. 1-4 Kasım Muğla, Türkiye.

Azad IS, Ai-Marzouk A. 2008. Autochthonous aquaculture probiotics-A critical analysis. Research Journal of Biotechnology, 171-177.

Bacandritsos N, Papanastasiou I, Saitanis C, Nanetti A, Roinioti E. 2007. Efficacy of repeated trickle applications of oxalic acid in syrup for varroosis control in Apis mellifera: Influence of meteorological conditions and presence of brood. Veterinary Parasitology, 148(2), 174-178. DOI: https://doi.org/10.1016/j.vetpar.2007.06.001

Bahreini R, Tahmasebi GH, Nowzari J, Talebi M. 2004. A study of the efficacy of formic acid in controlling Varroa destructor and its correlation with temperature in Iran. Journal of Apicultural Research, 43(4), 158-161. DOI:https://doi.org/10.1080/00218839.2004.11101129

Bailey L, Ball BV. 1991. Honey bee pathology (2nd Ed.). Academic Press; London, UK. pp. 31-34.

Büchler R. 2000. Das Aufträufeln von Oxalsäurelösung auf die Wintertraube beeinträchtigt die Auswinterungsstärke. Allg Dtsch Imkerztg, 34(11).

Bonfonti E, Lucchelli E. 1998. New Experiments with Oxalic Acid. Apicultural Abstracts. 50(1):62.

Charriere JD, Imdorf A, Fluri P. 1998. The 'FAM Liebefeld' formic acid dispenser for *Varroa* Control. Swiss Bee Research Centre, 1-4.

Charriére JD, Imdorf A. 2002. Oxalic acid treatment by trickling against Varroa destructor: recommendations for use in central Europe and under temperate climate conditions. Bee world, 83(2), 51-60. DOI: https://doi.org/10.1080/0005772X.2002.11099541

Cornelissen B, Blacquiere T. 2004. Effectiveness of autumn and winter treatments for *Varroa* control. First European Conference of Apidology, 10-23 September, 109-110 p, Udine, Italy.

Çetin M. 2010. Bal Arısı (*Apis mellifera* L.) Kolonilerinde Varroa destructor'un kontrolünde bitkisel, kimyasal ve biyoteknik uygulama yöntemlerinin karşılaştırılması, Ç.Ü. Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, 74 Sayfa, Adana.

Del Hoyo M, Mariani F, Vidondo P, Basualdo M, Bedascarrasbure YE. 2001. The therapeutic efficacy of a new formulation (OxavarR) for the treatment of Varroasis in honeybees. Proc. 37th Apic. Congr. 28 Oct-1 Nov 2001, Durban - S. Africa.

Imdorf A, Charriere JD, Bachofen B. 1997. Efficiency checking of the *Varroa jacobsoni*control methods by means ofoxalic acid. Apiacta. 32 (3): 89–91.

Imdorf A, Charriere JD. Kilchenmann V. Tschan A. Bachofen B. 1997. The integrated control of varroatosis without using the persistent varroacide substances. The XXXIV th International Apicultural Congress of Apimondia. 1-6 September. Poster No: 369/176, Antwerp, Belgium.

Gregorc A, Planinc I. 2001. Acaricidal effect of oxalic acid in honey bee (*Apis mellifera* L.) colonies. Apidologie. 32:333-340.

Gregorc A, Planinc I. 2004. Dynamics of falling varroa mites in honeybee (*Apis mellifera*) colonies following oxalic acid treatments. Acta. Vet. Brno., 73 (3), 385-391.

Gregorc A, Planinc I. 2004. The control of *Varroa destructor* in honey bee colonies using the thymol-based acarcide-Apiguard. Amer. Bee J., 145 (8), 672-675.

Gregorc A, Poklukar J. 2003. Rotenone and oxalic acid as alternative acaricidal treatments for *Varroa destructor* in honeybee colonies**.** Vet. Parasit., 111 (4), 351-360. DOI: https://doi.org/10.1016/S0304-4017(02)00408-9

Girişgin AO, Aydın L. 2010. Efficacies of formic, oxalic and lactic acids against *Varroa destructor* in naturally infested honeybee (*Apis mellifera* L.) colonies in Turkey. Kafkas Univ Vet Fak Dergisi. 16 (6): 941-945. DOI: https://doi.org/10.9775/kvfd.2010.1965

Henderson CF, Tilton EW. 1955. Tests with acaricides against the brown wheat mite. Journal of economic entomology, 48(2), 157-161.

Higes M, Meana A, Suarez M, Lorente J. 1999. Negative long-term effects on bee colonies treated with oxalic acid against Varroa jacobsoni Oud. Apidologie. 30 (4); 289-292. DOI: 10.1051/apido:19990404

Kar S, Kaya M, Güven E, Karaer Z. 2006. Yeni geliştirilen tespit kabı ile ergin arılarda Varroa enfestasyonunun belirlenmesi. Uludağ Arıcılık Dergisi, 6(2), 68-73.

Marcangeli J, Garcia, MDC. 2004. Effect of *Apis mellifera* (Apidae) honeybee brood amount on Oxavar® acaricide efficacy against the mite *Varroa destructor* (Varroidae). Rev. Soc. Entomol. Argent., 63 (3-4), 35-38.

Marinelli E, Pulcini P, Morgia C, De Pace FM, Allegrini F, Persano Oddo L. 2004. Oxalic acid by Varrox® to varroa control in central Italy. Apiacta, 39, 39-43.

MAF 2001. A review of treatment options for control of Varroa mite in New Zealand. Report to the Ministry of Agriculture and Forestry. The Horticulture &Food Research Institute of New Zealand Ltd. Palmerston.

Mert G, Yücel B, Kösoğlu M. 2007. Bal arısı hastalık ve zararlıları ile organik mücadele yöntemleri. Hasad Hayvancılık Dergisi. 261-263.

Mutinelli F, Baggio A, Capolongo F, Piro R, Prandin L, Biasion L. 1997. A scientific note on oxalic acid by topical application for the control of varroosis. Apidologie, 28(6), 461-462.

Muz MN. 2008. Bal arılarında ani koloni sönmesi. Türkiye Parazitoloji Dergisi, 32 (3): 271-275.

Nanetti A, Büchler R, Charrière J, Friesd I, Helland S, Imdorf A. 2003. Oxalic acid treatments for varroa control (a review). Apiacta, 38(1), 81-7.

Nanetti A. 1999. Oxalic acid for mite control-results and rewiev, in: Fries (Ed), Coordination in Europe of Research on Integrated Control of Varroa Mites in Honey Bee Colonies. Commission of the European Commission, Concerted Action 3686, Merelbeke November 13-14, p.7-14.

Nanetti A, Stradi G. 1997. Varroasi: trattamento chimico con acido ossalico in sciroppo zucherino. Ape Nostra Amica, 19, 6–14.

Nasr ME, Servos D, Bannister R, Wilson G. 2001. Efficacy of three miticides (Oxalic acid, Formic acid, Apilife Var) on Varroa destructor and Acarapis woodi in honey bee colonies in Canada. European Group for Integrated Varroa Control, York.

Radetzki T. 2001. Varroa control with oxalic acid evaporation. Assessment of the impact for the bees, Meeting of the European Group for Integrated Varroa Control, York, unpublished data (available on request to the author at Swiss Bee Research Centre, FAM Liebefeld, Bern, Switzerland, http://www.apis.admin.ch).

Sergio RR, Maggi MD, Fuselli S, Fiorella G, Negri P, Brasesco C, Satta A, Florı I, Eguaras MJ. 2014. Bioactivity of microencapsulated essentials oils and perspectives of their use in the control of Varroa destructor. Bulletin of Insectology, 67(1), 81-86.

Tunca Rİ, Çimrin T. 2012. Kırşehir ilinde bal arısı yetiştiricilik aktiviteleri üzerine anket çalışması. Iğdır Üniversitesi Fen Bilimleri Ensitüsü Dergisi, 2(2): 99-108.

Tutkun E. 2016. Arı akarı (Varroa Destructor) mücadelesinde timolün kullanılması. Arıcılık Araştırma Dergisi. 8(1):1-5.

Underwood RM, Currİe RW. 2003. The effects of temperature and dose of formic acid on treatment efficacy against Varroa destructor (Acari:Varroidae), a parasite of *Apis mellifera* (Hymenoptera: Apidae). Experimental and Applied Acarology 29:303–313. DOI: https://doi.org/10.1023/A:1025892906393

Ütük AE, Pişkin FÇ, Deniz A, Balkaya İ. 2011. Varroosis ve nosemosis üzerine retrospektif bir çalışma. Etlik Veteriner Mikrobiyoloji Dergisi, 22: 11-15.

Wallner K, Fries I. 2003. Control of the mite *Varroa destructor* in honeybee colonies. Pesticide Outlook, 14, 80-84. DOI: https://doi.org/10.1039/B301510F

Webster TC, Delaplane KS. 2001. Mites of Honey Bee. Dadant and Sons Publish. p. 280.

Wehling M, Ohe W, Ohe K. 2003. Natural content of formic and oxalic acids in honeys. Apiacta, 38, 257.

Yücel B. 2005. Bal arısı (*Apis mellifera* L.) kolonilerinde varroa (*Varroa jacobsoni* Q.) ile mücadelede farklı organik asitlerin kullanılmasının koloni performansı üzerine etkileri. Hayvansal Üretim, 46(2).

Zeybek H. 1991. Arı hastalıkları ve zararlıları. Tarım Köyişleri Bak. Etlik Hay Hast Araş Enst Müd Yayınları, Ankara Geliş tarihi, 7, 2004.