Journal of Animal and Veterinary Advances 10 (9): 1106-1111, 2011
ISSN: 1680-5593

Efficacy Levels of Organic Acids are Used for Controlling Varroa (*Varroa jacobsoni* Qudemans) and Their Effects on Colony Development of Honey Bees (*Apis mellifera* L.)

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Abstract: This study was carried out to determine the effects of using formic and oxalic acids against *Varroa jacobsoni Q.* which is the most hazardous parasite of honey bees (*Apis mellifera* L.) alternately in different seasons on the efficiency in Varroa treatment and colony development of honey bees. About 35 honey bee colonies in same of queen ages (1 year old) from Buckfast race were used in the research. Colonies were equalized for natural varroa levels and adult-brood bee population, prior to the research. Colonies were selected randomly as 2 treatment groups with 14 colonies and one control group with 7 colonies in the autumn and then the treatment goups were divided into 2 for using organic acids alternately so, 5 research groups (O/F, O/O, F/F, F/O and control) were used for the following spring. Varroa levels, treatment effectiveness, adult bee and brood population growth of groups were determined in autumn and spring, before and after the research. In autumn and spring experiments, Varroa infestation levels of the treatment groups were significantly reduced after the oxalic and formic acid applications ($p<0.05$). After, the experiment in autumn, number of adult bee frames in the group treated with formic acid were found significantly lower than before the treatment ($p<0.05$). In this season, brood surface level in the group treated with oxalic acid were increased significantly ($p<0.05$) at the end of the spring experiment. The highest efficiency of organic acid (94.10%) were observed in the 1st group (O/F) but it was not found different from other groups ($p>0.05$).

Key words: Honey bee, varroa organic acids, formic acid, oxalic acid, experiment

INTRODUCTION

Although, beekeeping, colony number and total honey production is getting better increasingly year after year in Turkey which has a huge beekeeping potential by its rich flora and favorable ecology, average honey yield per colony is low. An important reason of this stiuation is a disease called *Varroatosis* caused by Varroa mites (*Varroa jacobsoni* Qudemans). It is also an important problem for the beekeeng of all over the world. Varroa which lives and feeds on larva, pupa and adult of honey bees is a hazardous external mite of honeybee. Varroa causes a decrease in range of growth of colony, flight efficiency and capacity of gathering nectar and pollen of field bees. The mites have several deleterious effects on their host. Parasitized bees lose weight and they may have malformations. It can cause collapsing of honey bee colonies when disease virulence gets increase and economic losses can be occured heavily for beekeeping. Most of studies about chemical, biological, genetic and hormonal fighting methods against the mite have been done in many countries until now. Coumaphos, amitraz, fluvalinate and flumetrin have been the most commonly used treatments in recent years. These products do not have a long future as mites have get resistant and they have not affected the mites which grow in the sealled brood cells (Elzen *et al*., 2000). Lately, the aim of solving these problems, synthetic acaricides for Varroa control have given place to organic acid applications which are natural components of honey (Wehling *et al*., 2003). Organic acids such as formic, oxalic and lactic acids are biopesticides which have been used for Varroa control at the present time. They do not cause loss of quenn bee and do not affect adult bee or brood population negatively when used correct time and dose (Imdorf *et al*., 1996; Milani, 1999; Goodwin *et al*., 2002).

Applications with formic acid that is one of the organic acids used for controlling Varroa have been used since, 1980 in Germany and other European countries and its efficiency varies between 60-92% depends on application methods (Imdorf *et al*., 1997; Calis *et al*., 1998; Goodwin *et al*., 2002). Efficiency of the formic acid

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changes according as weather conditions, application season, evaporation material and distance of this material to the brood area (Charriere *et al*., 1998). Application temperature is very important for obtaining intended efficacy in controlling Varroa by using formic acid. Ambient temperatures are important for the success of application and formic acid dose not show adequate efficacy under the temperature of 10°C (Korpela *et al*., 1992). Furthermore, formic acid evaporates quickly by rapid increase in wheather temperature in sub-trophical climates and it causes honey bee pupas die (Underwood and Currie, 2003). Moreover, application of formic acid is difficult in mediterranean conditions because of its high and changeable wheather temperature (Imdorf *et al*., 1999).

Oxalic acid which is one of the organic acids used for controlling Varroa extensively in Europe (Nanetti *et al*., 2003) has a fairly high efficiency in the treatment of broodless colonies (Imdorf *et al*., 1997; Brodsgaard *et al*., 1999). Oxalic acid doesn't affect the mites in sealled brood cells. Therefore, it must be used when the brood production of the colony is minimum. In this wise 90-95% success rate can be obtained in controlling Varroa (Nanetti, 1999; Prandin *et al*., 2000). Oxalic acid is not affected by the ambient temperature in contradistinction to formic acid but it may cause quenn and adult bee population losses in the colony when used high doses and applied >1 (Gregorc and Planinc, 2001).

There are some difficulties in exportation of honey since chemicals and synthetic acaricides used for controlling varroa make residue in honey and bee wax at significant level. By the organic nutritoion is coming into prominence at the present time, synthetic acaricides for Varroa have given place to organic acid applications (Wehling *et al*., 2003). Studies have been done until now have proved that applying organic acids to the coloy expect nectar flow season do not occur residue in honey (Imdorf *et al*., 1996).

The aim of this study is to determine the effects of using formic and oxalic acid applcations which are used against *Varroa jacobsoni* Q. which cause damage for beekeeping alternately in different seasons on the efficiency in Varroa treatment and colony development of honey bees in Aegean region of Turkey where has suitable climates for migration beekeeping.

MATERIALS AND METHODS

Experiment was conducted in autumn and following spring. The autumn experiment date was between 9 October and 12 November in 2005 in village called Yukari Mazi of Bodrum district of Mugla provinence and spring experiment date was between 8 and 29 April in 2006 in village called Parlak of Karaburun district of Izmir provinence. About 35 honey bee colonies from Buckfast race kept in standart Langstroth hives were used. In the autumn experiment for the purpose of determining the Varroa infestation level of adult bees before and after the application 200-250 adult honey bee from each hive which had in same of Queen ages and similar bee populations (4 adult bee frame and 2.34-2.39 dm^2 brood area) were poured into jar filled with detergent/water and were shaken to separate the mites from honeybees (Goodwin and van Eaton, 2001). Adult bees and mites were counted and Varroa infestation level (%) of adult bees in the colony after the application was assigned by dividing varroa number counted in the sample into adult bee number counted in the sample.

About 200 sealed brood cells were unsealed and varroa mites in the cells were counted and then Varro infestation level (%) in the sealed cells of the colonies were calculated. Varroa infestation levels on adult bees and in sealed brood cells were estimated together (De Jong *et al*., 1982). About 35 colonies which were similar with Varroa population level were selected randomly as 3 research groups with 14 colonies in 2 of them and 7 colonies in the other were formed. In late autumn, oxalic acid was applied to the colonies of the 1st group, colonies of second group were treated with formic acid and third group was accepted as a control.

Colonies treated with oxalic acid in the late autumn were divided into two group and half of them were treated with formic acid (1 group O/F) and oxalic acid application was applied to the other of them (2 group O/O). Colonies applied formic acid application in the late autumn were also divided into 2 groups. We arranged that applying oxalic acid to the 1st of them (3 group F/O) and appliying formic acid to the 2nd of them (4 group F/F).

But the 3rd group had only one colony after the overwintering so, this group was eleminated to the experiment. Consequently, groups were formed as 1. group (O/F), 2. group (O/O), 3. group (F/F) and 4. group (K) in early spring. Effects of organic acid applications on colony performance were investigated by evaluating the number of adult bee frame and brood surface area prior and post the experiment in both season.

Colonies in the group applied oxalic acid received a single dose of 5 mL of 3.2% of oxalic acid solution which was prepared by 50% sugar: water solution (44.8 g oxalic acid was added into 1 L water) in late autumn and early spring. The solution were trickled on to honeybees as 5 mL for each space between the frames in the colony by using a syringe. About 1 application was done for each seasons. Colonies in the group apllied formic acid received 20 mL of 65% of formic acid solution which were saturated into absorbent material by a syringe. The material were placed over brood combs in the hive and application was repeated 4 times at 4 day intervals in both seasons. Efficacy of oxalic and formik acid on Varroa was calculated as percentage by Henderson-Tilton's. Wheather, temperature during the experiment was recorded. Datas obtained from experiment were evaluated by using procedure of One-way ANOVA of statistic package programme called SAS (1999) and difference between the groups were determined by Duncan multiple comparision test.

RESULTS AND DISCUSSION

Results belonged to season of autumn: Daily avarage weather temperature was determined as 17.22±2.91°C in 9 October and 12 November in 2005 in village called Yukari Mazi of Bodrum district of Mugla provinence in autumn. There was no significant difference between the experiment groups about Varroa infestation level and average Varroa infestation level of the groups varied between 13.58 and 14.17% in autumn season. According to the varance analysis results Significant ($p<0.05$) decrease were determined at varroa infestation level of the groups applied oxalic and formic acid in comparison with control group after the applications (Table 1). After the application in autumn, the most reduction at varroa infestation level was detected in the formic acid group and it was found significantly ($p<0.05$) higher than the group of control (Table 1). Accorting to the Henderson-Tilton's Formula, the most high efficiency of organic acid was determined in the oxalic acid group but there was no significant difference between the formic and oxalic acid groups in point of this feature (Table 1). Minimum Varro infestation level (2.29%) were obtained from the formic acid group and maximum Varro infestation level (13.35%) were get from the control group after the experiment in autumn (Table 1). Number of adult bee frame was not change in the control and oxalic acid groups. However, there was significant ($p<0.05$) decrease in number of adult bee frame in the formic acid group. Average brood surface area in the group applied ocalic acid after the experiment were found significantly ($p<0.05$) higher than before, the experiment (Table 2).

Results belonged to season of spring: Daily avarage weather temperature was determined as 17.22±2.91°C in 8 and 29 April in 2006 in village called Parlak of Karaburun district of Izmir provinence during the spring experiment. After the overwintering some of the colonies in the experiment groups perished and 1st group (O/F) had 4 colonies, 2nd 1 (O/O) had 5 colonies, 3rd 1 had 3 colonies and control group had 4 colonies in the begining of the spring.

Varroa infestation level of the 1st group (O/F) after the experiment was found significantly ($p<0.05$) lower than before, the experiment. However, Varroa infestation level of the control group before the experiment reached to 9.04% after the experiment and this increase in Varroa infestation level was determined significantly ($p<0.05$) higher (Table 3).

Varroa infestation level in the control group was of 80% of organic acid efficiency in similar, conditions but disagreed with Imdorf *et al.* (2003) who reported 60% organic acid efficiency. About 88.81% efficiency of organic acid obtained from 2nd group (O/O) is similar, ith the findings that 89.6 and 96.8% of efficiency by found significantly ($p<0.05$) higher than the other groups after the experiment (Table 3). According to the Henderson-Tilton's Formula, the highest organic acid efficiency (94.10%) was obtained from the 1st group (O/F) (Table 3).

No significant difference was found between the groups about number of adult bee frame and brood surface area before and after the organic acid applicataions in the spring experiment (Table 4). Maximum organic acid efficiency (80.95%) was obtained from the

Table 1: Average Varroa infestation level (%) (mean±SE) and organic acid efficiency (%) before and after the experiment in spring

Groups	n	Varroa infestation level before the experiment (%)	Varroa infestation level after the experiment (%)	Organic acid efficiency (%)
I Oxalic acid	14	13.94±2.56^{a}	3.71±1.16^{b}	83.21±6.10
II Formic acid	14	13.58±2.47^{a}	2.29±0.87^{b}	81.64±6.91
III Control	7	14.17±3.39^{a}	13.35±3.20^{a}	-
Mean	-	13.84±1.53	5.27±1.18	82.43±4.40

^{a,b}: Means with no common superscript in the same column for each effect differ significantly ($p<0.05$)

Table 2: Development of adult bee and brood population of the experiment groups in autumn

Madde I. groups	Madde II. Number of adult bee frame before the experiment	Madde III Number of adult bee frame after the experiment	Madde IV. Brood surface area before the experiment (dm^2)	Madde V. Brood surface area after the experiment (dm^2)
I Oxalic acid	4^{ab}	4.08±0.14^{ab}	2.36±0.25^{a}	4.9±0.62^{b}
II Formic acid	4^{ab}	3.7±0.15^{a}	2.39±0.44^{a}	2.66±0.37^{a}
III Control	4^{ab}	4.33±0.21^{b}	2.34±0.90^{a}	4.09±0.89^{a}
Mean	4	4±0.10	2.37±0.26	3.93±0.39

^{a,b}: Means with no common superscript in the same column for each effect differ significantly ($p<0.05$)

Table 3: Average Varroa infestation level (%) (Ortalama±SE) and organic acid efficiency (%) before and after the experiment in spring

Groups	n	Varroa infestation level before the experiment (%)	Varroa infestation level after the experiment (%)	Organic acid efficiency (%)
I O/F	4	7.47±1.14[ab]	1.31±0.54[ax]	94.10±2.46
II O/O	5	6.28±2.15[a]	2.86±1.46[ax]	88.81±4.39
III F/F	3	3.21±1.45[a]	1.17±0.00[ax]	74.45±15.97
IV K	4	3.29±0.55[a]	9.04±0.83[by]	-
Mean	-	5.25±0.85	3.59±1.03	85.79±5.65

[a,b]: Means with no common superscript in the same lines for each effect differ significantly ($p<0.05$) [x,y]: Means with no common superscript in the same column for each effect differ significantly ($p<0.05$)

Table 4: Development of adult bee and brood population of the experiment groups in spring

Groups	No. of adult bee frame before the experiment	No. of adult bee frame after the experiment	Brood surface area before the experiment (dm^2)	Brood surface area after the experiment (dm^2)
I O/F	3.5±0.640	4.33±0.66	19.32±8.50	19.34±4.37
II O/O	4.8±1.010	4.33±1.33	18.86±6.70	13.15±7.98
III F/F	2.66±0.33	3±0.57.00	17.05±8.59	4.94±1.24
IV K	2.75±0.62	3.33±0.88	8.15±2.37	18.19±4.81
Mean	3.56±0.42	3.75±0.42	15.96±3.30	13.90±2.79

oxalic acid goup in autumn and 94.1% of efficiency was detected in the 1st group (O/F) in spring. This efficiency level was in rapport with 95% efficiency reported by Mutinelli *et al.* (1997), Higes *et al.* (1997) and 98% of efficiency by Cornelissen and Blackquiere (2004). All application groups. Achievement above 74% was obtained from all application groups in the experiment.

The highest organic acid efficiency was achieved in the oxalic acid group in autumn when brood production was minimum and in the formic acid group in spring when brood area was maximum. These results agreed with Gregorc and Planinc (2001) who reported above trickling method reported by Nanetti and Stradi. Minimum efficiency of organic acid (74.4%) was obtained from the group treated formic acid in both season. This efficiency is <93.3, 94.48 and 80% efficiency values reported by Eguaras *et al.* (2002), respectively.

This result is parallel with Yucel who reported that formic acid is more sensible to weather temperature than other organic acids. Thus, Goodwin *et al.* (2002) reported that formic acid has comperatively changeable efficiency in regions have different climates and even between the hives in the same apiary. Similarly, Charriere *et al.* (1998) determined that huge variation can occur in the efficiency of formic acid between the colonies. This occasion exhibit that by developing the forms of formic acid (jel or suchlike) which let it evaporate slowly in the hive may obtain more determinative results.

There was no negative effect on growth of adult bee in the groups in both season so, this finding is similar, with the findings that reported by Fries *et al.* (1991). Brood development in the group treated with formic acid in both seasons was found comparatively less than the other groups even if it was not signicant. So, this result supports Ostermann and Currie (2004)'s observation that formic acid can affect negatively brood development in honeybee colonies. This occasion is related to opinion that because of formic acid is effective on the comb surface more time than other the other organic acids it downgrade laying of quennbee and brood development reported by Hansen and Guldborg (1998). Thus, the result obtained from this experiment is similar with the opinion which in consequence of young larvae is more sensitive to formic acid it cause brood population decrease expressed by Bolli *et al.* (1993).

Brood development before the oxalic acid application was significantly increase after the application in the experiment. This finding is agree with the findings reported by Brodsgaard *et al.* (1999), Spinks (2001) and Imdorf *et al.* (2003) that oxalic acid does not affect negatively to brood population and it tolerates succesfully by honeybees.

The highest achievement about organic acid efficiency, adult bee and brood development was obtained from the group (O/F) treated with oxalic acid in autumn and formic acid in spring supports the finding that using organic acids alternately gives the best result for organic fighting against Varroa signed by Imdorf *et al.* (2003).

CONCLUSION

In the present study, all of the organic acid combinations used in the experiment was effective in the organic fighting against varroa but applying formic acid in early spring after the oxalic acid application in previous autumn gave more succesfull results even if it was not important significantly. Adult bee and brood development are not affected negatively by this organic acid combination so, this indicates that it would be use succesfully for controlling Varroa. As a result of icreasing in consciousness of healty life much more, studies should need to be conducted to try the other organic acids and organic acid combinations that causing no residue problem in beehive products and having no negative effects for human health used for organic fighting against Varroa and to find out suitable fighting methods against Varroa for every direction and every neighbourhood.

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