

Original Article

Evaluation of Oxalic Acid Treatments against the Mite *Varroa destructor* and Secondary Effects on Honey Bees *Apis mellifera*

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Abstract

Background: The *Varroa destructor* varroasis is a very serious parasite of honeybee *Apis mellifera*. The objective of this study was to evaluate the effectiveness of *Varroa* treatment using organic acid (oxalic acid) in Algeria identifying its side effects on bee colonies.

Methods: Treatment was conducted in one apiary consisting 30 colonies kept in Langstroth hives kind. Oxalic acid dripped directly on bees 5ml of this solution of oxalic acid per lane occupied by a syringe. Three doses were tested: 4.2, 3.2 and 2.1% oxalic acid is 100, 75 and 50 g of oxalic acid dehydrate in one litter of sugar syrup (1water to 1 surge) concentration.

Results: The percentage of average efficiency obtained for the first dose was 81%, 72.19% for the second dose, and 65% for third one, while the dose of 100 g oxalic acid causes a weakening of honey bee colonies.

Conclusion: The experiments revealed that clear variation in the treatment efficiency among colonies that this might be related to brood presence therefore in order to assure the treatment efficiency oxalic acid should be part of a bigger strategy of *Varroa* treatment.

Keywords: *Varroa destructor*, *Apis mellifera*, Oxalic acid, Effectiveness, Algeria

Introduction

Varroa Disease is a parasitic mite of adult and brood stages of honeybees due to an external parasitic mite, *Varroa destructor* Anderson and Trueman, 2000. *Varroa* is responsible for an outbreak in *Apis mellifera* since its transfer from the Asian honeybee, *A. cerana*, the original host (Colin 1999). It is considered as the major factor responsible for colony losses worldwide (Martin et al. 2012, Nazzi et al. 2012).

Parasitism of the bee by this mite causes deformation and weakness of the young workers. Heavy infestation causes death before the emergence of nymphs and the birth of mutilated bees (Boecking and Genersch 2008). *Varroa* heavily decrease the general weight up to 30% of individuals (Bowen-Walker and

Gunn 2001), the total hemolymph volume and its protein content (Bowen-Walker et al. 1999). It was estimated to be between 10% and 50% reduction in total protein of parasitized nymphs (Dandeu et al. 1991).

The work of Yang and Cox-Foster (2005) clearly show that the *Varroa* weakens the immune system of bees and makes it more susceptible to viral and bacterial infections. *Varroa* by its role as a vector injects the virus it carries directly into the hemolymph of the honeybee. On the other hand, as an activator through the bite of *Varroa* allows the activation of certain viruses present in a latent state in the hemolymph of the honeybee (Tentcheva et al. 2004).

In Algeria, the *Varroa* mite has become a

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major concern of a beekeeper since the discovery of the first cases of infestation with *Varroa* in the country. Many acaricide were used to fight against this disease (Adjlane et al. 2012). Today, chemical control with strips containing pyrethrinoid (Apistan®, Bayvarol®) can be used to a limited extent because these products are no longer optimal efficiency due to mites resistant against these products (Adjlane et al. 2013).

It is becoming crucial to provide beekeepers with new treatments for control *Varroa* mite, oxalic acid is considered as one the natural products that became widely used as alternative treatment. In addition, it was used for this purpose since the early '80s. In early tests, a reduction of 20 to 30% was observed in *Varroa* infestation after spraying it on each side of populated frame with honeybees (Charrière and Imdorf 1995). However, this method of treatment was criticized due to the bees disrupting after the frames moving, the danger oxalic acid vapours on the beekeepers Respiratory system in addition to being a very laborious work and time-consuming Charrière et al. (1998). However, the sugar role in the oxalic acid treatment is not clear, it is proposed that the sugar addition increase the solution oral absorption by honeybees and improves its adhesion to their bodies.

Many experiments worldwide were conducted to test the efficiency of this acid in *Varroa* treatment (Nanetti et al. 1995, Nanetti and Stradi 1997, Imdorf and Charriere 1998, Higes 1999, Gregorc and Planinc 2001, 2002, Moosbeckhofer et al. 2003, Nozal et al. 2003, Hatjina and Haristos 2005, Bacandritsos et al. 2007, Giri gin and Aydin 2010, Giovenazzo and Dubreuil 2011, Mert and Yucel 2011, Gregorc and Planinc 2012). These studies showed that the population density, amount of brood, climatic conditions, honey availability, migratory beekeeping, hive management and the development of *Varroa* population do directly influence the treatment efficiency. Thus, there are no

scientific studies under Algerian conditions.

The aim of this work was to study the effectiveness of oxalic acid against *Varroa* mites, and to determine the side effects of the application of this treatment on bee colonies

Materials and Methods

The experiment was conducted in the north-central Algeria in the region of Boumerdes, this province is one of the wettest regions in the country. Annual rainfall varies between 500 mm and 1300 mm per year (36° 46 '00 "N, 3° 29' 00" E) (Fig.1). The relief of Boumerdes is divided into several physical units: the plains and valleys to the north, the hills and plateaus in the middle part and the mountains south. Winter temperatures vary between 8 °C and 15 °C. They climb to 25 °C in May to an average of 28 °C to 32 °C in July and August. The average relative humidity is high enough, it varies between 75–90%.

Thirty *Apis mellifera intermissa* colonies kept in standard Langstroth hives. In each colony, a mobile bottom board was installed with wire screen to count dead mites and to avoid mite removal by honeybees. The natural mite mortality was equal for the thirty honeybee colonies.

The application of oxalic acid is produced by the drip method (Imdorf et al. 1997), dripped directly on bees 5 ml of this solution of oxalic acid per lane occupied by a syringe (Imdorf et al. 1997, Brødsgaard et al. 1999).

Oxalic acid is an organic compound with the formula $H_2C_2O_4$. It shows in the form of colourless crystals of oxalic acid dehydrate (71.4% active ingredient). Oxalic acid dehydrate is partially soluble in water (12.5% by weight at 25 °C) and some organic solvents (ethanol, diethyl ether). It has a molecular weight of 126,07g / mol, a density of 1.653, a melting point at 101.5 °C and sublimed at 150 °C (INRS 2005).

The colonies were treated with three different concentrations: 4.2, 3.2 and 2.1% ox-

alic acid is 100, 75 and 50 g of oxalic acid dehydrate in one litter of sugar water (1.1). For each colony, two applications were made at weekly intervals.

Dead *Varroa* were collected and counted every 2 days throughout the trial period using diapers greased covering the bottom of the hive.

To determine the number of mites remaining after treatment with oxalic acid, a control treatment with Apivar was made two weeks later. The effectiveness of oxalic acid is calculated as follows:

$$\text{Efficacy of oxalic acid} = \frac{\text{Number of } \underline{\textit{Varroa}} \text{ died during treatment with oxalic acid}}{\text{Number of } \underline{\textit{Varroa}} \text{ died during treatment with oxalic acid and Apivar}} \times 100$$

The strength of each treated colony was estimated to analyze the possible impact of oxalic acid on the development of the colony.

The data was analysed using SPSS version 5.0 (Chicago, IL, USA) and analysis of variance (ANOVA).

Results

Efficacy results and mortality of *Varroa* during treatment with oxalic acid and control

(Apivar) for the three experimental groups are shown in Tables 1, 2, 3 and Fig. 1. Treatment with oxalic acid applied by the drop-by-drop method gives an efficiency of the dose used. The solution of 100 g of oxalic acid was a higher compared to the other two doses (70 and 50 g) efficiency.

The most efficient dose was 100 g of oxalic acid is 81% (Table 1), while 70 g of oxalic acid efficiency rate varies between 57 and 87% (Table 2), the dose of 50 g of oxalic acid was 65% (Table 3). Statistical analysis revealed a significant difference between the three treatments (F= 7.87, df= 10, P= 0.002).



Fig. 1. The test area on the map of Algeria

Table 1. Determination of the effectiveness of oxalic acid applied by drip for colonies of group I (100g of oxalic acid in 1 liter of syrup) (August-September 2012-Boumerdes area)

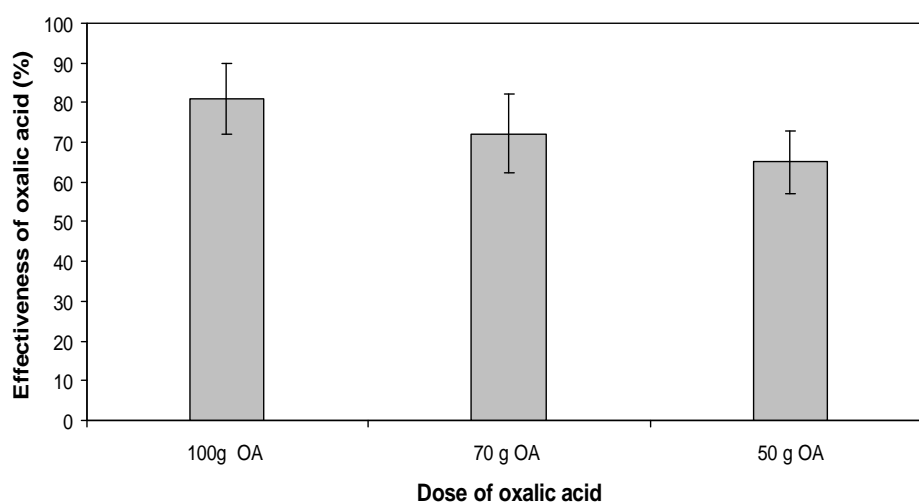
Number of colony	Number of dead <i>Varroa</i> (oxalic acid) (A)	Number of dead <i>Varroa</i> (Apivar®) (B)	Total number of dead <i>Varroa</i> (A+B)	(A) Effectiveness of oxalic acid (B) / (A+B)*100
1	345	271	616	78,50
2	245	251	496	86,34
3	167	15	182	91,65
4	390	114	504	77,31
5	401	175	576	69,54
6	290	65	355	81,65
7	189	61	250	75,45
8	365	178	543	67,20
9	423	54	477	88,65
10	289	19	308	93,76

Table 2. Determination of the effectiveness of oxalic acid applied by drip for colonies of group II (70g of oxalic acid in 1 liter of syrup) (August-September 2012-Boumerdes area)

Number of colony	Number of dead <i>Varroa</i> (oxalic acid) (A)	Number of dead <i>Varroa</i> (Apivar®) (B)	Total number of dead <i>Varroa</i> (A+B)	Effectiveness of oxalic acid (C) / (A+B) *100
11	411	194	605	67,87
12	309	124	433	71,23
13	233	115	348	66,89
14	245	54	299	81,87
15	523	387	910	57,44
16	298	166	464	64,12
17	203	57	260	77,98
18	453	93	545	83,09
19	314	179	493	63,65
20	234	33	266	87,65

Table 3. Determination of the effectiveness of oxalic acid applied by drip for colonies of group III (50g of oxalic acid in 1 liter of syrup) (August-September 2012-Boumerdes area)

Number of colony	Number of dead <i>Varroa</i> (oxalic acid) (A)	Number of dead <i>Varroa</i> (Apivar®) (B)	Total number of dead <i>Varroa</i> (A+B)	(D) Effectiveness of oxalic acid (E) / (A+B) * 100
21	198	102	300	65,90
22	311	247	558	55,65
23	382	150	532	71,78
24	254	160	414	61,34
25	412	275	687	59,89
26	256	101	357	71,65
27	201	113	314	63,87
28	399	290	689	57,88
29	421	93	514	81,90
30	345	229	574	60,1

**Fig. 2.** The average efficiency obtained for each treatment. Different letters indicate a significant difference between the three groups (<0.05, Newman-Keuls test at the 5% level) (August-September 2012-Boumerdes area)

Discussion

Charriere and Imdorf (2001) reported in trials conducted by dripping on colonies without brood in Switzerland showed an average 97% efficiency for the solution of 45 g of oxalic acid, against 96% efficiency for the treatment of 30 g of oxalic acid. In slovenia, the efficacy of oxalic acid treatments between 8 August and 16 September was 41% (Gregorc and Planinc 2012). A lower dose of 10–15 mL/hive (3.5%) showed a good acaricidal effect (98%) when smaller colonies were treated (Moosbeckhofer 2001).

Gregorc and Planinc (2001) treated colonies with brood three times in the summer by applying 4.1–5.2% oxalic acid dehydrate in varying sugar solutions w/w corresponding to approximately 4.5–5.9% oxalic acid dehydrate w/v. An efficacy of 39–52% was achieved.

In this experiment, the efficiency is related to the presence of a quantity of brood in the colonies. In northern Algeria, the brood is present almost throughout the year but with a very small amount in July–August. Indeed, several authors recommend applying this treatment period of absence of brood (Nanetti et al. 1995, Nanetti and Stradi 1997, Imdorf and Charriere 1998, Higes 1999, Gregorc and Planinc 2001, 2002) as oxalic has no effect on *Varroa* staying in sealed brood cells acid.

Investigations from Canada in a climatic region comparing with Central Europe confirmed the results of autumn treatments: 2.8 % oxalic acid dihydrate solution (40–50 mL/hive) killed about 55% of the mites, 3.5% about 90% in colonies with remaining brood (Nasr et al. 2001). Efficiency of up to 100% was reached only in broodless artificial swarms (Büchler 1998).

Experts recommend treatment with oxalic acid using spray application compared to treatment with dripping mode. This treatment guarantees a very good efficacy against *Varroa* coupled with good tolerance to bees. Indeed,

several trials reported efficiency greater than 95% for the spray application of 30 g of oxalic acid in one liter of water (Colin 1997, Imdorf et al. 1997, Charrière et al. 1998).

The mode of action of treatment with oxalic between passes and the amount of frames allows the bees to absorb the acid solution, and therefore the treatment penetrates and flows in their hemolymph. Treatment method using (oxalic acid+sugar) is reported to be efferent due to adding sugar, and it reduces the variation among colonies (Charriere and Imdorf 1999).

Regarding the influence of these treatments on the development of colonies, no abnormalities were detected immediately after treatment with oxalic acid. Studies with a higher concentration of 5% described doubled bee mortality in autumn, bad overwintering (reduced colony strength) of treated colonies and impaired spring development (Charrière 2001). Colonies treated twice with 3% oxalic acid dihydrate solution (7.3 and 6.4 g per comb side, one gram corresponds here approximately to one millilitre) resulted in high bee mortality averaging 170 dead bees after each administration and queen loss in one colony (Nanetti et al. 1995). The effect of oxalic acid on bee larvae was investigated by Gregorc et al. (2004). A solution of 6.5 g oxalic acid dihydrate/ 50 g sugar/ 100 mL water sprayed on honeybee larvae (0.121 mg/ larvae) affected the columnar cells of the midgut, leading to necrosis.

In order to determine the tolerance of bees to these treatments, and avoid a possible weakening of colonies post treatment with oxalic acid, monitoring of treated colonies is performed by repeated checks the strength of the colony during autumn and winter. In general, we observed a significant drop in bee populations during the winter. The colonies treated with oxalic acid solution of 100 g group are weakened by 25%. However, the

colonies of the second group treated with a solution of 70 g of oxalic acid have lost 15% of their bee populations. With the third processing, distribution, there has been a weakening of 10%. It is therefore apparent that the higher the oxalic acid content increases, winter losses more significant.

Although different experimental conditions make direct comparisons difficult, assessments made in Switzerland and Germany tends to a higher loss of bees during winter of 45 g oxalic acid solutions. Lower concentrations have provided better wintering colonies (Nanetti 1999). Higes et al. (1999) showed that oxalic acid has a negative effect on the development of the brood; these authors reported that three queens treated colonies died after applying a solution of 45 g of oxalic acid by spraying. The bad weather and low temperatures prevent persistent bees collect pollen and nectar pollen insufficient reserves generate a slow evolution of colonies (Imdorf et al. 1996). The number of individuals populating the colony is a direct function of floristic conditions, they even dependent on climatic conditions (Vandame 1996). From January, all colonies showed a slight recovery in parallel with the improved weather conditions, and thus flora. However, there were much colonies in group 2 (70 g of oxalic acid) that had resulted in better recovery from the first group.

Conclusion

The experiments revealed clear variation in the treatment efficiency among colonies that this might be related to brood presence therefore in order to assure the treatment efficiency oxalic acid should be part of a bigger strategy of *Varroa* treatment.

The solution of 100 g of oxalic acid appears to be the most efficient comparing to the other two doses but it was poorly tolerated by the honey bees while the (70 and 50 g of oxalic acid) solutions where less effi-

cient in *Varroa* treatment. Therefore, under the similar conditions we recommend the treatment with 70 g of oxalic acid.

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