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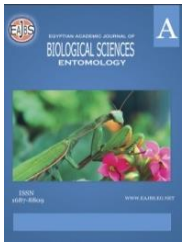

Study of the Efficacy of Oxalic Acid and Thymovar (Thymol) against the Parasitic Mite of the Honey Bee, *Varroa destrutor*

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ABSTRACT

Varroasis is a dangerous pathogen of the honey bee caused by the mite Varroa destructor, the aim of this work is to assess the effectiveness of two natural products (oxalic acid and thymol) in the fight against varroasis. The experiment covered 40 colonies in the central region of Algeria. Two doses of oxalic acid were used: 30 and 45 grams per liter of sugar syrup. For thymol (Thymovar), two treatments were carried out: ½ strip with one and two applications for each group of colonies. The 1/2 strip formulation in two applications at an interval of two weeks apart seems the most effective; the 45 gm solution of oxalic acid has a higher efficacy compared to that of 30 gm, that the effectiveness of oxalic acid is directly linked to the presence of brood at the time of the intervention. these two treatments are of control measure to be integrated by beekeepers as part of an alternative control strategy.

INTRODUCTION

In Algeria, varroa has become one of the major concerns of the beekeeper since the discovery of the first case of Varroa infestation in the east of the country. The fight against Varroa. destructor is therefore an essential component of beekeeping management (Adjlane *et al.*, 2012; Adjlane and Haddad, 2014, Adjlane *et al.*, 2015). In apiaries, users have sometimes observed a reduction in the effectiveness of therapeutics and the problem of miticide resistance has been raised (Adjlane *et al.*, 2013; Adjane and Haddad, 2017). The phenomenon of resistance to several chemical molecules has been reported by several authors (Lodesani *et al*, 1995; Vandame *et al.*, 1995; Elzen *et al.*, 1988; milani and Della Vedova, 2002 and Garcia- Salinas *et al.*, 2006). Varroasis clinical symptoms include brood and bee disorders (Charriere *et al.*, 2012). One of the main signs of pathology is the presence of irregular or lacunar brood with atrophied dead nymphs under the operculum. On adult bees, the symptoms are mainly linked to the presence of workers with deformed wings, dragging, and dead bees. The parasitism of Varroa destructor acts on adult bees and on brood according to three actions: spoiler, mechanical, and vector. Repeated intakes of hemolymph by Varroa lead to a decrease in its haemolymph volume but also in its protein level, which compromises the development of the nymph (Bowen-Walker *et al.*, 1999). The decrease in total protein fluctuates between 10 and 50% in parasitized nymphs (Dandeu *et al.*, 1991). The work of

YANG and COX-Foster (2005) clearly shows that Varroa weakens the bee's immune system and makes it more susceptible to viral and bacterial infestations. Parasitism leads to malformations and weakness of the young worker. A heavy infestation causes the death of nymphs before the emergence and birth of mutilated bees. (Boecking and Genersch, 2008). The role of the mite in the transmission and pathogenesis of certain viruses appears to be twofold. On the one hand, Varroa, through its role as a vector, injects the viruses it carries directly into the bee's hemolymph. On the other hand, an activator role through the mite bite allows the activation of certain viruses, present in a latent state in bee hemolymph (Tentcheva *et al.*, 2004).

This work aims to test series of natural products such as organic acids, and thymol in the fight against varroasis in order to integrate them into a global beekeeper strategy.

MATERIALS AND METHODS

Test Location:

The experiment focused on 40 colonies belonging to a professional beekeeper in the region of Mitidja, more exactly in the region of Blida (36 ° 34 '59" N and 3 ° 0' 0" E) during the period from September to December 2018. La Mitidja is a vast alluvial plain in northern Algeria. It is a depression about 100 km long and 15 to 20 km wide narrowed between the Atlas Blidéen in the south and the Sahel in the north, largely open to the sea with a total area of 1,400 km2 and an agricultural area. from 120,000 to 130,000 hectares. This region has appreciable melliferous and nectar-bearing potentials which allow it to ensure a considerable development of beekeeping production.

Instructions for Use of Oxalic Acid and Thymol Treatments:

The application of oxalic acid is carried out using the drip method: This involves dripping 5 ml of this oxalic acid solution directly onto the bees per occupied lane using a syringe.

Two types of doses are used during our trial:

- 30 gm of oxalic acid dihydrate diluted in 1 liter of sugar syrup 1: 1.
- 45 gm of oxalic acid dihydrate diluted in 1 liter of 1: 1 sugar syrup.

Thymovar (thymol) is a product in the form of a sponge cloth (5 x 14.5 cm) which serves as a support for the active substance.

The application method consists of placing a Thymovar® plate directly on the edge of the brood frame, two batches were installed during this test:

- Lot n ° 1: 10 colonies treated with Thymovar at the rate of two applications of 1/2 strip spaced two weeks apart.
- Batch n ° 2: 10 colonies treated with Thymovar at the rate of an application of 1/2 strip for four weeks.

After the Thymovar treatments, a control treatment with Apivar is performed. The active ingredient of this product is amitraz, the method of application of the Apivar treatment consists of putting two strips per hive, each strip contains 0.5 mg of amitraz.

Evaluated Criteria:

1. Count of Varroa Collected on The Diapers:

Dead Varroa were collected and counted weekly throughout the test period using the greased langes covering the bottom of the hive.

2- Checking the Effectiveness of Oxalic Acid:

The effectiveness of oxalic acid and Thymovar is calculated as (Imdorf *et al.*, 2003):

$$\text{Efficacy of oxalic acid / Thymovar} = \frac{A}{B} \times 100$$

A: Number of Varroa that died during treatment with oxalic acid / Thymovar
B: Number of Varroa that died during treatment with oxalic acid / Thymovar + Apivar.

RESULTS AND DISCUSSION

Treatment with Oxalic Acid:

The results of the efficacy of oxalic acid are shown in Figures 1 and 2. Treatment with oxalic acid applied by the drip method exerts an acaricid effect which depends on the dose used. Indeed, the solution of 45 g of oxalic acid has a higher efficiency compared to that of 30 g. The average percentage mortality of efficacy obtained for the first dose is 76.35% against a rate of 67.52% for the second dose. Despite the uniform applications of oxalic acid for both groups, we find great variability in efficiency from one colony to another for each group. This difference in efficacy is in all likelihood related to the amount of capped brood present in each colony, i.e. the number of mites that are protected during treatment.

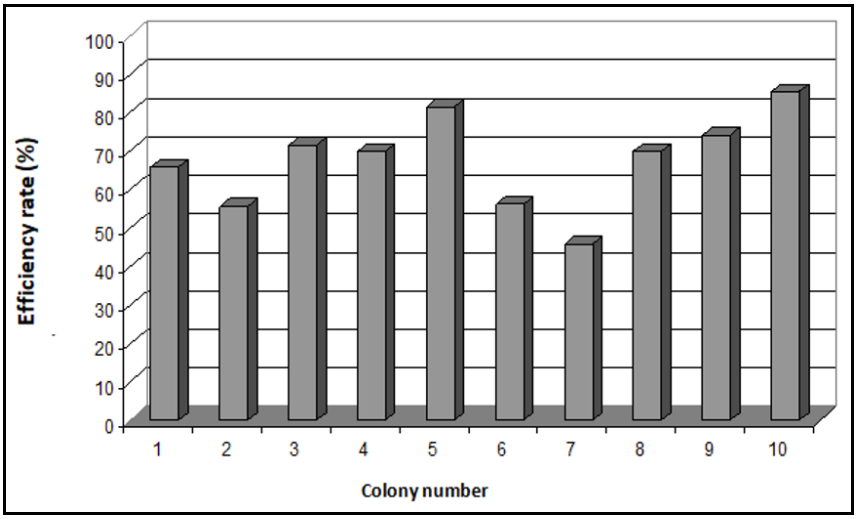

Fig. 1: Efficacy of oxalic acid (30 gm dose) applied by dripping

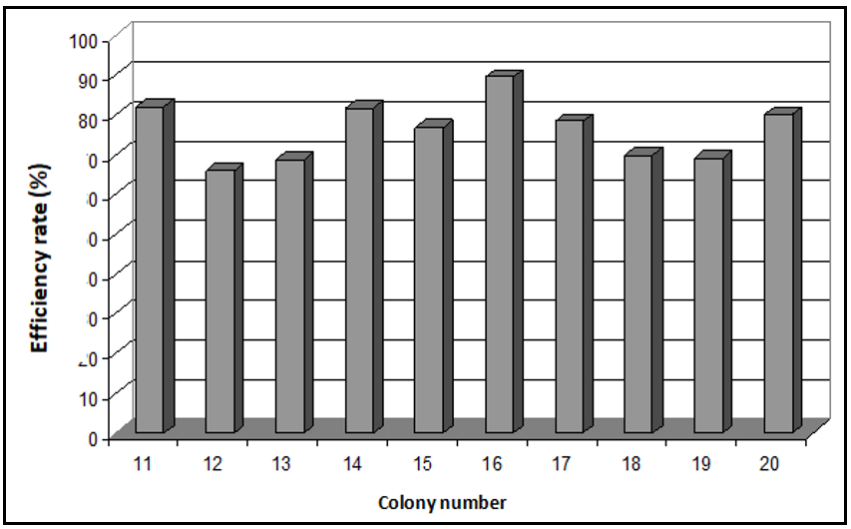

Fig. 2: Efficacy of oxalic acid (Dose of 45 gm) applied by dripping

Indeed, several authors recommend applying this treatment in the absence of brood (Gregorc and Planinc, 2002), since oxalic acid has no effect on Varroa living in the cells of the brood. This is reported by a test carried out by Charriére and Imdorf (1999), where they sprayed 10 colonies which had on average 12 dm2 of capped brood at the time of treatment. The average efficiency was only 61% (against efficiency of 97.4% in the absence of brood). Oxalic acid (OA) is widely used for V. destructor control due to its high efficiency (> 90%) and a low risk of hive contamination (Marinelli et al. 2006; Rademacher and Harz 2006; Bacandritsos *et al.,* 2007).

Blocking reune spawning for a period of 21 days improves the effectiveness of avide against varroa mites (Gregorc *et al.,* 2017). The results of Jack *et al.* (2020) suggest that caging queens to interrupt brood in the fall may have a negative impact on the strength and survival of colonies. We have observed high colony mortality in some treatments with oxalic acid compared to other treatments. Papežíková *et al.* (2017) report after a laboratory study on the effect of oxalic acid a negative effect on workers.

In a study made on products based on oxalic acid, Habbi-Cherifi *et al.* (2018à obtained an efficacy of 89% for Oxuvar and 90% for Apibioxal. Marcangeli and María (2017) del Carmen report efficacy of 86 % for Oxuvar. Moro and Mutineli (2019) also report an efficiency of more than 90% for the new formulation based on oxalic acid.

With regard to the influence of these treatments on the development of the colonies, no anomaly could be highlighted immediately after the oxalic acid treatment except in two colonies (colony 4 and 11) where a decrease in the bee population.AlToufailia *et al.* (2018) determined whether the double application of OA was more harmful than the single application, comparing 12 colonies that had been treated twice with 12 colonies treated individually. There was no difference in colony performance (100% survival in the two groups; 5.5 frames of brood in single-treatment colonies versus 5.3 in double-treatment colonies). Ditto for Aboushaara *et al.* (2017) report that the acid causes side effects on the honey bee. In laboratory work, Sabová *et al.* (2019) indicate that oxalic acid can be harmful to bee brood when it is present during application.

Hatjina and Haristos (2005) showed a detrimental effect on brood development and low efficiency using the OA runoff method in the presence of open brood. Terpin et al (2019) confirm in their results that the use of OA to control mites in the presence of larvae could have a negative effect downstream on the size of the colony population and its wintering capacity.

Treatment with Thymol (Thymovar):

The results of the efficacy of Thymovar during the trials are shown in the following two figures 3 and 4. According to the obtained results, and according to the treatment method applied, the average efficacy for the group of colonies 1 and 2 was 90.61% and 64.31%. So as the results show in our trial, the highest efficacy rate was recorded with the treatment of two 1/2 strip applications two weeks apart. In seven of the ten colonies, the efficiency was more than 90%. the results obtained in Switzerland by Bollhalder (1997) reveal an efficiency rate of 85-97% for the application of two strips of Thymovar at 4-week intervals (treatment duration of 8 weeks) and effectiveness of 66-95 % for the same dosage for a treatment duration of 6 weeks. In Belgium, the average efficiency rate obtained in 46 colonies is 76.5. The correlation analysis between the number of brood frames and the efficiency rate shows that these two data are very little correlated, which explains that the presence of brood does not influence the effectiveness of Thymovar. Indeed, it is a long-term treatment that can affect the varroa staying in the brood as they hatch.

During our tests, apart from a few dead bees found in front of the hive and on the swaddle, we never recorded abnormally high mortality.

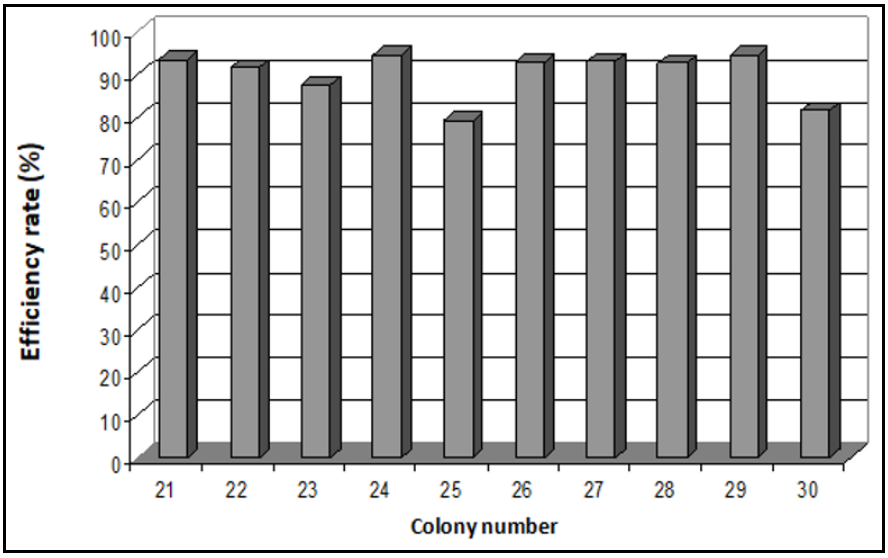

Fig. 3: Effectiveness of Thymovar applied at the rate of two applications of 1/2 strip spaced two weeks apart

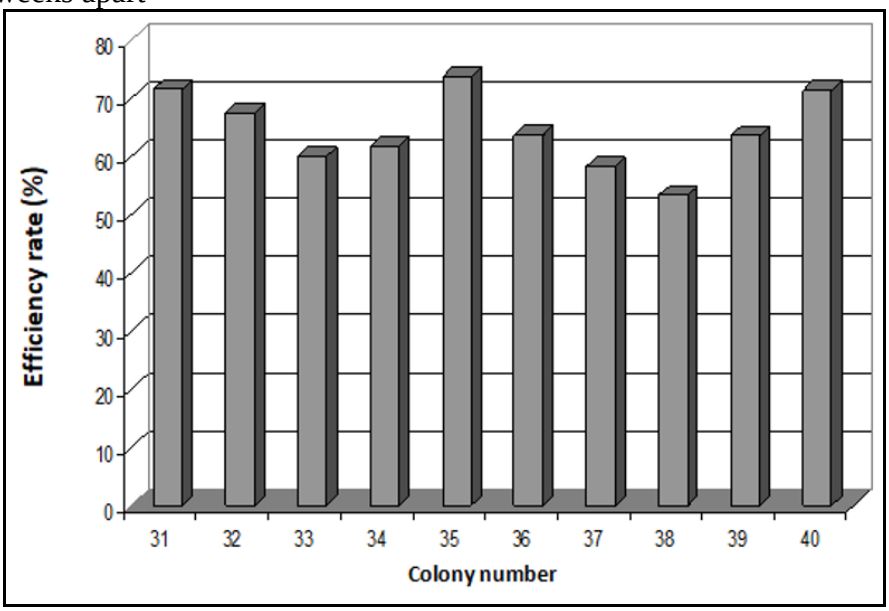

Fig. 4: Efficacy of Thymovar applied with a single application of 1/2 strip for 4 weeks

Akyol and Yeninar (2008) report that the average efficacy of Thymovar ® is 96.91% and 88.66%. According to the same authors, there was no mortality of queen, brood, and adult bee in all the colonies of the group during the research.

According to another work, Thymovar® recorded an average efficiency of 84.7%, was found to be more effective than Apilife Var® (53.8%). The natural mortality of the mites during the test period was approximately 17.8%. An apparent negative impact of treatments on colony development (Coffey and Breen, 2013). In one of the experiments of Naneti and Stradi (1997) in Italy, it was found that Thymovar kills 54 to 66% of varroa mites and in another experiment, this same product reaches an average efficiency percentage of 85%.

It has been established that thymol residues can accumulate in a hive, but they dissipate quickly at a natural hive level of 1.1 mg thymol/kg of honey or less. Thus, the thymol administered to the population of a hive does not modify the taste or the nutritional quality of the honey collected (Bogdanov *et al.*, 1998).

Conclusion

The half-strip formulation in two applications at two-week intervals appears to be the most effective: completely acceptable effectiveness if we take into account the dose used (one strip instead of two) and the treatment period (four weeks), which shows that increasing the presence of strips in colonies can improve the percentage of effectiveness of Thymovar. it appears that the effectiveness of oxalic acid is directly related to the presence of brood at the time of the operation. Consequently, its use requires monitoring of the colonies in order to identify this period, which may be very short. Further trials are needed to clarify this point and to optimize formulation, dosage, and strength.

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