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Assessment of Newly Registered *Varroa destructor* Infestation Control Acaricides in The Colonies of Honey Bees *Apis mellifera* L. Under Egyptian Conditions

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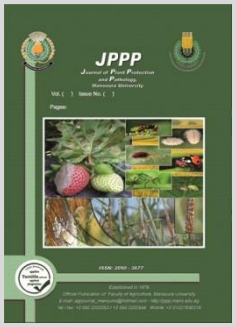

ABSTRACT

Varroa destructor is one of the most deadly pests threatening honey bees and many acaricides have been used worldwide to combat the disease. Thus, this work aimed to evaluate Varoviga® and Bayvarol® acaricides under the Egyptian conditions compared to formic acid. Sixteen honeybee colonies were used and divided into four groups 4 colonies/each group: first group as control, second group treated with Bayvarol acaricide, colonies were treated with Varoviga acaricide as third group, and colonies treated with formic acid as fourth group. Results showed that, formic acid had the major significant effect on varroa mites, followed by Bayvarol then Varoviga acaricides with no significant difference between them. Moreover, the largest increase in honey store areas were found in the colonies treated with formic acid followed by Varoviga and Bayvarol acaricides, with all treatments leading to an increase in brood area without significant variations between them. No chemical residues were present in honey and wax samples that obtained from treated colonies. Regarding the financial coast, Varoviga appears to be an ideal alternative to formic acid, especially when it is not possible to use formic acid. The results of this work indicated that the effective varroa mite acaricides was formic acid, Bayvarol, and Varoviga, respectively.

Keywords: *Formic acid, Bayvarol, Varoviga, Varroa destructor, wax, honey, acaricides residues*

INTRODUCTION

Varroa Mites was first reported in Egypt in 1983 (Maggi *et al*. 2010b). Varroa has become a main problem for apiculturists in the past few years and has found itself extremely economically harmful to the most prosperous people who have been devastated by this mite in the majority of Egyptian Governorates. The varroa (*Varroa destructor*) is regarded as an ectoparasite on *Apis mellifera* L. (Ruffinengo *et al*. 2014 and Alattal *et al*. 2017), which is one of the most destructive plagues in the western honey bee. Nymph and adult mite feeding behavior contributes to deformed, tiny, low-weight and short lifespan of workers' bees (Ramsey *et al*. 2019).

According to seasons of year, the number of mites in the worker cells varied significantly (Barel *et al*. 2011).

The maximum mortality of mites was reported by Rosenkranz *et al*. (2010) in both spring and autumn seasons, while the minimum number was reported in the summer season. The number of mites on bees is only decreased by significant chemical treatments, although they do not affect capped brood Maggi *et al*. (2010a and 2011). Anti varroa products as; Apistan, Apitol, Folbex VA and formic acid were recorded (Kamler *et al*. 2016).

Worldwide beekeepers want an effective Varroa product, that's easy, Simple to treat, cheep to the user and bees and harmless. Thus, the aim of the present study was evaluating the effectiveness of Varroviga ® and Bayvarol ® as new-registered acaricides, compared to conventional formic acid treatment in Egyptian conditions for control of varroa mites. It evaluates its effects under experimental conditions on stored honey and brood areas in the colony of honey bee.

MATERIALS AND METHODS

The present study was conducted in October and November months (autumn season) of 2019 at Giza governorate, Faculty of Agriculture apiary, Cairo University. The average temperature did not exceed 30 °C. Sixteen honeybee colonies of local Carniolian hybrid *Apis mellifera carnica* L, which are equal in strength and exposed to the routine work during the experimental period. Colonies were allocated into four main groups; the first group was control (4 colonies), served as negative control during the whole period of experimentation, the second group was deemed as a monitor with Bayvarol ® (flumethrin 3.4 %) 1 plastic strip / month / colony. The third group has been treated with Varoviga® (a natural product consist of Thymol, Mentol, organic oil and camphor) each colony sprayed with 3 gm dissolved in 12 ml of distilled water weekly as mentioned by the instructor. Four colonies were treated as fourth group with formic acid 65% (standard regular varroa treatment) 10 ml on absorbed pad / colony / week for the assessment of new acaricides. Glycerin-covered plastic sheets were placed at the base of each colony and weekly monitoring and recording of a number of dropped mites. *V. destructor* was measured using the following formula for the efficiency of various acaricides against the mite (Pawar, 2008).

Percent mite mortality =

$$\frac{\text{Mite motality in treatment}}{\text{Mite motality in Formic acid treatment + Mite motality in control}} \times 100$$

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Chemical analysis

Wax and honey samples from all experimental colonies have been taken at the end of experiments and stored under -29°C till analysis to determine the residues of the acaricide (Kamler *et al*. 2016). Samples were analyzed using the device Ab sicex 6500+ at Pesticides Residues and Environment Pollution Department, Central Agricultural Pesticides Lab, Agricultural Research Centre, Giza, Egypt.

Sample preparation

Five grams of homogenized sample were mixed in 10 mL of distilled water and sited in a 50 mL centrifuge tube, then 10 mL of 1% acetic acid mixture in acetonitrile added and shaken in a mechanical disrupter for 3 min. one gram of sodium acetate and 4 g of magnesium sulphate were added after that, and the sample was shaken for 3 min.

The mixture was then centrifuged at 3500 rpm, −12◦C for 20 min, and the mixture of 350 mg of PSA, and 1050 mg of MgSO4 was chosen for the validation. The supernatant was shaken with a mixture of sorbents in a mechanical disrupter for 10 min and again centrifuged (Anastassiades *et al*. 2003 and Kiljanek *et al*. 2016)

Determination of some Biological activities of honey bee colonies infested with varroa mites.

Two measurements (at the start and end of treatment) were made for each area of brood and stored honey and recoded in cm^2.

Financial cost

The cost of controlling Varroa mite for one colony per month was calculated for the three different treatments.

Statistical analysis

Number of fallen mites, percentage of efficacy and percentage of brood and honey areas after treatments, were analyzed with F test (RCBD) followed by LSD test using M-STAT (1989) program.

RESULTS AND DISCUSSION

Results

Data obtained in Table (1) showed that all treatments had a significant effect on Varroa infestation reduction. Formic acid had the most significant effect on Varroa mites as the mean fallen individuals reached 62.38 mite followed by Bayvarol® which recorded 44.38 fallen mites. Whereas, Varoviga® had the lowest effect with 27.88 fallen mites.

Regarding the investigation date the first and second weeks had the highest significant effect as the number of fallen mites reach maximum with 50.50 and 46.75 mites, respectively and the last treatment had the least significant number of fallen mites 20.25.

Efficacy of new acaricides

Newly registered acaricides efficacy was evaluated comparing to Formic acid mortality as it is the common regular acaricide used in Egypt. Comparing efficacy of different acaricides (Fig. 1) revealed that, Formic acid had the highest significant mean efficacy with 91.90%, while Varoviga® had the lowest effect on varroa mites (41.07%).

Table 1. Effect of different acaricides on the mean number of fallen varroa mites (*Varroa destructor*) during the experimental period

Investigation week	Varoviga	Bayvarol	Formic acid	Control	Mean
1	45.00	81.00	73.00	3.00	50.50 A
2	41.00	83.00	61.00	2.00	46.75 A
3	30.00	56.00	67.00	8.00	40.25 AB
4	28.00	39.00	32.00	8.00	26.75 BC
5	30.00	32.00	82.00	8.00	38.00 ABC
6	28.00	34.00	69.00	6.00	34.25 ABC
7	12.00	20.00	58.00	4.00	23.50 BC
8	9.00	10.00	57.00	5.00	20.25 C
Mean	27.88 C	44.38 B	62.38 A	5.50 D	

***Values with different letters in the same row or column are significantly different according to LSD test**

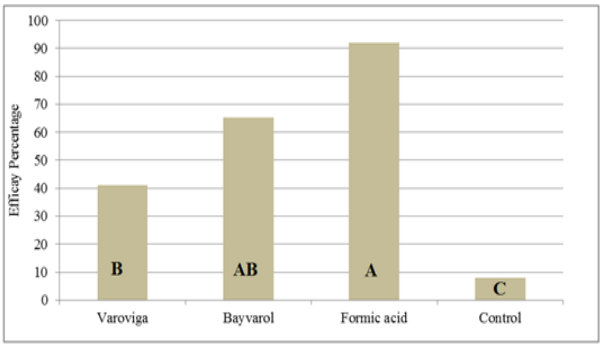

Fig. 1. Comparing different acaricides mean efficacy throughout experiment period

Relation between varroa mites infestation and some biological activities of honey bee colonies.

Varroa mites infestation percentage affected significantly the stored honey and brood areas in tested colonies (Table 2). The superior increase percentage of stored honey was recorded with colonies treated with Formic acid (+ 83.59%) followed by Varoviga® (+ 63.44%) in an intermediate rank. Meanwhile no significant differences were found between stored honey in colonies that did not receive any treatments and those treated with Bayvarol® (+ 34.86 and + 26.17%, respectively). All treatments had a significant increase effect on the brood area with no differences between them, while the control groups had the lowest increase percentage (+ 82.7%).

Table 2. Effect of different acaricides on the stored honey and brood mean areas in infested honey bee colonies

Treatments	Honey				Brood			
	Varoviga	Bayvarol	Formic acid	Control	Varoviga	Bayvarol	Formic acid	Control
Increase percentage	+ 63.44 AB	+ 26.17 B	+ 83.59 A	+ 34.86 B	+ 245.4 a	+ 245.0 a	+ 252.9 a	+ 82.7 b

***Values with different letters in the same row or column are significantly different according to LSD test**

Financial coast

In the case of Formic acid, 1 liter of formic acid 85% costs 96 Egyptian pound (EGP), this means that 10 ml of 65% formic acid costs 0.73 EGP and the treatment for 2 months costs 5.87 EGP. Regarding Bayvarol the treatment for 2 months coast 60 EGP, and 4.32 EGP for Varoviga.

Chemical analysis

The findings suggest that there are no acaricide residues in the samples of all treatments based on the biochemical analyzes carried out on samples of honey and bee wax taken from all colonies under the experiment.

Discussion

The key issue of beekeeping worldwide is possible hazards arising from the use of acaricides such as residues in bee products or mite resistance. However, the use of acaricides that are applied in various types, such as strip, liquid or powder is widely regulated in Varroa mites (Bolli *et al*. 1993; Satta *et al*. 2005). All treatments have been shown to be effective and reduced varroa infestation with formic acid superiority, followed by Bayvarol ® as a new

varroa infestation control treatment, and then the Varoviga ® with no significant differences between the two new acaricides, That agrees with Gracia *et al*. (2017), who found that no major variations in the degree of decreasing infestation between synthetic and natural acaricides.

Regarding biological activities of honey bee colonies, colonies treated with Formic acid had the highest significant stored honey, which was mentioned by Pietropaoli and Formato (2019), followed by colonies treated with Varoviga® then Bayvarol®, also with no significant differences. However, there were no significant differences between all treatment in increasing brood area. Financial cost is the main barrier facing any new techniques in developing countries due to low individual income. Varoviga® would be more suitable to use as alternative acaricide to Formic acid (especially during summer season) than Bayvarol® because it is much cheaper

CONCLUSION

Varroa destructor is a parasitic mite that considered the most dangerous problems facing the beekeepers around the world including Egypt. The present study evaluates the effectiveness of newly acaricides (Bayvarol and Varoviga) and their residues in honey and bee wax from the treated colonies. The results indicated that the effective varroa mite acaricides was formic acid, Bayvarol, and Varoviga, respectively.

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تقدير فعالية بعض المبيدات الاكاروسية المسجلة حديثا لمكافحة الفاروا في طوائف نحل العسل تحت الظروف المصرية

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يعتبر طفيل الفاروا واحد من أكثر الآفات المميتة التي تهدد طوائف نحل العسل وقد تم استخدام العديد من المبيدات الحشرية في جميع أنحاء العالم لمكافحته. وبالتالي ، يهدف هذا العمل إلى تقييم تأثير كلا من ®Varoviga و®Bayvarol تحت الظروف المصرية مقارنة بحمض الفورميك. تم استخدام ستة عشر طائفة من نحل العسل وقسمت إلى أربع مجموعات 4 طوائف / كل مجموعة: المجموعة الأولى كمجموعة كنترول، المجموعة الثانية تمت معالجتها بمبيد البايفارول ، تمت معاملة المستعمرات بمستحضر الفاروفيغا كمجموعة ثالثة ، وتم معاملة الطوائف بحمض الفورميك كمجموعة رابعة. أظهرت النتائج أن حمض الفورميك كان له فعالية معنوية كبيرة على الفاروا، يليه البيفارول و الفاروفيجا مع عدم وجود فرق معنوي بين الأثنين في الفعالية. علاوة على ذلك، فقد كانت أكبر زيادة في مساحة العسل المخزن في الطوائف المعالجة بحمض الفورميك تليها مبيدات فاروفيغا وبايفارول في نفس المرتبة معا، حيث أدت جميع المعالجات إلى زيادة مساحة الحضنة دون اختلافات معنوية بينها. كما لم يتم العثور على بقايا كيميائية في عينات العسل والشمع التي تم الحصول عليها من الطوائف المعالجة. فيما يتعلق بالناحية المالية، يبدو أن الفاروفيجا بديل مثالي لحمض الفورميك، خاصة عندما لا يكون من الممكن استخدام حمض الفورميك عند ارتفاع درجات الحرارة. أشارت نتائج هذا العمل إلى أن المبيدات الفعالة للفاروا كانت حمض الفورميك وبايفارول وفاروفيجا على التوالي.