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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

IMPACT OF ACARICIDES ON *VARROA DESTRUCTOR* INFESTATION IN HONEY BEE COLONIES (*Apis mellifera* L.) AND THEIR HISTOLOGICAL EFFECTS ON HYPOPHARYNGEAL GLANDS

Bal Arısı Kolonilerinde (*Apis mellifera* L.) *Varroa Destructor* Bulaşıklığı Üzerine Akarisitlerin Etkisi ve Hipofaringeal Glandlar Üzerindeki Histolojik Etkileri

Rasha A. SALEM[1], Hashish M. EL-SAYIED[1], Abdulraouf M. AMRO[1]* Asmaa E. Abd ALLA[2]

[1]Bee Research Department, Plant Protection Research Institution, Agriculture Research Center, Dokki, Giza, EGYPT, E-mail: Rashaadel83@yahoo.com, ORCID No: 0009-0007-7887-9755, E-mail: Mhas214263@yahoo.co.uk., ORCID No: 0000-00015530-2581, Corresponding author / Yazışma Yazarı E-mail: raoof_amro@yahoo.com, ORCID No: 0000-0001-6837-2298.

[2]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Cairo, Giza, EGYPT, E-mail: Supersemsema@agr.cu.edu.eg; ORCID No: 0000-0001-6140-8446

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ABSTRACT

This study aimed to determine the random role of some acaricides, which are the most commonly used in Egypt for controlling *Varroa destructor* on Hypopharyngeal Glands (HPGs) of honey bees (*Apis mellifera* L.). The acaricides used in this study were Varroakiller®, Menthocaros®, Amitraz®, Varroby®, and formic acid. Results showed that the total number of fallen mites was 53.5, 47, 28.6, 26, and 24.5 for Varroakiller®, Menthocaros®, Amitraz®, Varroby®, and formic acid, respectively, compared to 16 mites in the control group. For sealed worker brood areas (SWBA), the treatments included Varroakiller® (327.98 cm^2), formic acid (228.92 cm^2), Varroby® (222.25 cm^2), Menthocaros® (129.72 cm^2), and Amitraz® (101.73 cm^2), while the SWBA for control colonies was 44.83 cm^2. Histological studies of the HPGs showed that although Varroakiller® effectively controlled *Varroa* mites and had no direct impact on worker bees or egg-laying areas, it considerably impacted the HPGs, which could ultimately affect the bee colony. Moreover, formic acid recorded a lower number of fallen *Varroa* with acceptable outcomes for egg-laying areas and sealed workers. This, coupled with the fact that formic acid was deemed the most significant due to its effectiveness over a short period, resulted in a reduction of 100% after 24 days.

Keywords: Acaricides, Amitraz®, Varroby®, Formic acid, Hypopharyngeal glands

ÖZ

Bu çalışma, bal arılarının (Apis mellifera L.) Hipofaringeal Bezleri (HPG'ler) üzerinde *Varroa destructor'u* kontrol etmek için Mısır'da en yaygın olarak kullanılan bazı akarisitlerin rastgele rolünü belirlemeyi amaçlamıştır. Bu çalışmada kullanılan akarisitler Varroakiller®, Menthocaros®, Amitraz®, Varroby® ve formik asittir. Sonuçlar, düşen toplam akar sayısının kontrol grubundaki 16 akara kıyasla Varroakiller®, Menthocaros®, Amitraz®, Varroby® ve formik asit için sırasıyla 53,5, 47, 28,6, 26 ve 24,5 olduğunu göstermiştir. Kapalı işçi kuluçka alanları (SWBA) için, uygulamalar Varroakiller® (129,125 in2), formik asit (90,125 in2), Varroby® (87,50 in^2), Menthocaros® (51,07 in2) ve Amitraz® (40,05 in2) içerirken, kontrol kolonileri için SWBA 17,65 in^2 olarak belirlenmiştir. HPG'lerin histolojik çalışmaları,

Varroakiller®'in Varroa akarlarını etkili bir şekilde kontrol etmesine ve işçi arılar veya yumurtlama alanları üzerinde doğrudan bir etkisi olmamasına rağmen, sonuçta arı kolonisini etkileyebilecek olan HPG'leri önemli ölçüde etkilediğini göstermiştir. Dahası, formik asit yumurta bırakma alanları ve kapalı işçiler için kabul edilebilir sonuçlarla birlikte daha düşük sayıda düşen Varroa kaydetmiştir. Bu durum, formik asidin kısa süredeki etkinliği nedeniyle en önemli olarak kabul edilmesiyle birleştiğinde, 24 gün sonra %100'lük bir azalma yüzdesi ile sonuçlanmıştır.

Anahtar kelimeler: Akarisitler, Amitraz®, Varroby®, Formik asit, Hipofaringeal bezler

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Bu çalışma, Varroa destructor'u kontrol etmek için Mısır'da yaygın olarak kullanılan bazı akarisitlerin (Varroakiller®, Menthocaros®, Amitraz®, Varroby® ve formik asit) bal arılarının HPG'leri üzerindeki potansiyel etkisini araştırmıştır.

Gereç ve yöntemler: Bu araştırma, 17 Aralık 2020 ile 25 Şubat 2021 tarihleri arasında Mısır'ın Dokki kentindeki Arı Araştırma Departmanı arılığında bulunan bal arısı kolonileri üzerinde yürütülmüştür. Bu çalışma için, her biri bir yaşında çiftleşmiş bir ana arı tarafından yönetilen eşit güçte 18 yerel hibrit Karniyol bal arısı (A. mellifera) kolonisi seçilmiştir. Tedavi öncesi belirlenen bulaşıklık seviyesi %9 ile %11 arasında değişmekteydi. Seçilen 18 koloni, her biri üç koloniden oluşan altı gruba ayrılmıştır. İlk beş grup akarisitlerle muameleye tahsis edilirken, altıncı grup kontrol muamelesi yapılmadan bırakılmıştır. Seçilen her kovanın tabanına 30 cm² × 45 cm² ölçülerinde vazelin kaplı filtre kağıdı yerleştirilmiştir. Her dört günde bir, sekiz hafta boyunca toplam 11 ölçüm için, kağıtlar farklı uygulamalardan kaynaklanan Varroa akarları saymak üzere laboratuvara götürülmüştür.

Bulgular: Sonuçlara göre düşen toplam akar sayısının kontrol grubundaki 16 akara kıyasla Varroakiller®, Menthocaros®, Amitraz®, Varroby® ve formik asit için sırasıyla 53,5, 47, 28,6, 26 ve 24,5 olduğunu göstermiştir. SWBA için, uygulamalar Varroakiller® (129,125 in2), formik asit (90,125 in2), Varroby® (87,50 in2), Menthocaros® (51,07 in2) ve Amitraz® (40,05 in2) içerirken, kontrol kolonileri için SWBA 17,65 in2 olarak belirlenmiştir. HPG'lerin histolojik çalışmaları, Varroakiller®'in Varroa akarlarını etkili bir şekilde kontrol etmesine ve işçi arılar veya yumurtlama alanları üzerinde doğrudan bir etkisi olmamasına rağmen, sonuçta arı kolonisini etkileyebilecek olan HPG'leri önemli ölçüde etkilediğini göstermiştir. Dahası, formik asit yumurta bırakma alanları ve kapalı işçiler için kabul edilebilir sonuçlarla birlikte daha düşük sayıda düşen Varroa kaydetmiştir. Bu durum, formik asidin kısa süredeki etkinliği nedeniyle en önemli olarak kabul edilmesiyle birleştiğinde, 24 gün sonra %100'lük bir azalma yüzdesi ile sonuçlanmıştır.

Sonuç: Varroa istilası HPG'lerde dejeneratif değişikliklere yol açarak morfolojilerini ve işlevselliklerini etkiler. Bununla birlikte, Varroa'yı kontrol etmek için yaygın olarak kullanılan akarisitlerin HPG'lerin gelişimi üzerindeki etkisi belirsizliğini korumaktadır. Bulgular, Varroakiller®'in Varroa popülasyonlarını önemli ölçüde azalttığını, işçi arılar veya yumurtlama alanları üzerinde doğrudan bir etkisi olmadığını, ancak HPG'ler üzerinde önemli bir etkisi olduğunu göstermiştir. Formik asit kısa bir maruz kalma süresine yol açarak 7. ölçümde (24 gün sonra) %100 azalmaya neden olur ve bu yüzden tedavinin sonuna kadar (19. ölçümde) sabit kalmaktadır. Tedavi edilen koloniler için, arıların tedaviye maruz kaldığı uzun süre nedeniyle tedavi edilen tüm örneklerde histolojik yapı dejeneratif değişiklikler göstermiştir. Ek olarak, Menthocaros® Varroa miktarını önemli ölçüde azaltır ve yumurta ve SWBA gibi alanlar üzerinde mütevazı bir etkiye sahiptir. Bununla birlikte, HPG'ler üzerinde ihmal edilebilir bir etkisi vardır. Akarisitler ve Varroa istilası arasındaki etkileşimi ele alan özel çalışmalar değerli bilgiler sağlayacaktır.

INTRODUCTION

Due to their crucial function in crop pollination and the high quality of their hive products (such as wax, propolis, pollen, royal jelly, bee venom, and honey), honey bees, *A. mellifera*, are known as the “wings of agriculture” (Tiwari *et al*. 2014). Nevertheless, various insect pests, mites, birds, and diseases can harm honey bees (Yousef *et al.* 2014). The parasitic bee mite *V. destructor* is the most destructive pest of honey bees and has a significant negative economic impact on the global beekeeping business (Al-Abbadi and Nazer 2003). Honey bee colonies suffer significant harm from severe infestations of a *Varroa* mite which infest broods, adult bees, and hive waste.

However, during such infestations, pupae fail to mature into adult bees (Anderson and Trueman 2000). A serious physiological disease caused by *V. destructor* feeding on the hemolymph of pupa and adult bees results in a loss of up to 25% of adult weight, decreased worker and drone honey bee longevity, and deformed wings (Kanga and James, 2002). According to Murilhas (2002), colonies with a high infestation rate of *V. destructor* (> 7%) suffer a significant reduction in the bee population, and ultimately, the entire colony collapses unless appropriate measures are taken to treat the mite population.

Honey bees' immune systems are compromised, and the parasitic mite destroys the integument's mechanical protective barriers (Glinski 1991). Synthetic acaricides such as Amitraz®, Coumaphos, fluvalinate, and flumethrin control *V. destructor* (Eguaras *et al*. 2003). According to Tihelka (2018), thymol was used in the screening as a positive control because beekeepers frequently use it as an acaricide. However, thymol may harm bees in various ways, such as being poisonous to bee brood, causing metabolic problems, or altering bee behavior (Charpentier *et al*. 2014, Colin *et al*. 2019). Nevertheless, the consistent use of these pesticides has led to the development of mite resistance in many parts of the world (Pettis 2004), which has significantly reduced the effectiveness of these acaricides for controlling the *Varroa* mite (Lodesani *et al*. 1995).

The HPGs in honey bee workers are part of the digestive system and are in charge of producing royal jelly (Fluri *et al*., 1982), storing glycogen for the flight muscles (Kubo *et al.,* 1996), synthesizing key enzymes for turning nectar into honey (Ueno *et al*., 2009), and maintaining social immunity (da Costa and Cruz-Landim 2000). Optimal gland function, characterized by the highest number and size of gland acini, secretory vacuoles, and density of secretory granules, is observed in 12-day-old workers. With Varroa infestation, these parameters decrease, indicating a decline in gland health (Yousef *et al*. 2014). According to Zhang *et al*. (2023), acaricides exposure may negatively impact colony maintenance, brood development, survival, necrotic and apoptotic cell death. However, the effects of acaricides for controlling *Varroa* on honey bee workers HPGs development remains obscure. Hence, this study investigated the impact of acaricides commonly used by Egyptian beekeepers to control the Varroa destructor mite on honey bee colonies, specifically the histological structure of worker bees' hypopharyngeal glands (HPGs).

MATERIAL AND METHODS

Experimental design

The present investigation was conducted from December 17, 2020, to February 25, 2021, on honey bee colonies located in the apiary of the Bee Research Department at Dokki, Egypt. As documented earlier by Ghoniemy (1998), this period within the study area represents the season with the peak annual density of *Varroa* mites. For this study, we selected 18 colonies of equal strength from local hybrid Carniolan honey bees (*A. mellifera*). Each colony consisted of eight standard frames, seven of them were covered on both sides with bees and was headed by a one-year-old mated queen. The tested colonies remained acaricide-free for more than one year to ensure results that are clear and verifiable. Prior to treatment, we used the powdered sugar roll method (Pietropaoli *et al.,* 2021) to assess varroa infestation, which ranged from 9% to 11%. The 18 selected colonies were divided into six groups, each consisting of three colonies. The first five groups were treated with acaricides, while the sixth group remained untreated as a control. Vaseline-coated filter paper measuring 30 cm² × 45 cm² was placed at the base of each selected hive. To assess the effectiveness of the tested treatments, sheets were collected from the hives every four days over an eight-week period (11 measurements total). These sheets were then taken to the lab for counting the total number of *Varroa* mites that had fallen off the bees.

The tested acaricides

The tested acaricides were Varroakiller®, Menthocaros®, Amitraz®, Varroby®, and formic acid as described in Table 1.

The mean areas of eggs and sealed worker brood

In this experiment, over four mornings, the mean area of eggs was measured in square inches and then converted to square centimeters using a frame divided into one-inch squares, whereas the mean SWBA was measured in square inches every 13 days and then converted to square centimeters. The eggs and brood areas were counted and summed for each colony (Jeffree, 1958). The test colonies were fed with a sugar solution with a 2:1 sugar-to-water ratio (W/W).

Table 1. Acaricides used against *Varroa destructor in* honey bee colonies

Commercial name	Composition	Form & Concentration	Manner	Add position
Formic acid	CH_2O_2	Liquid 65%	5x10 cm^2 Cardboard slices satisfied by 5 ml in pored sacks	Upper brood combs
Menthocaros®	Thymol (26%) Eycalyptol (22%) Menthol(crystals) (3.8%) Camphor syntgtie (3.8%) Antioxidant (3%) Aromatic oils (11%) Dried coriander (6%) Garic extract around (4.5%) Vaslin (20%)	Sticky substance	5x10 cm^2 Cardboard slices coated one side with 5 gm	On hive bottom opposite the entrance foraging internally
Varroby®	Thymol (30 %) Camphor syntgtie (5%) Carvacrol (5 %) Menthol crystals (5%) Powder sugar (65%)	Yellow powder	topical application (3g. per colony)	On brood combs and adults
Varroakiller®	Molecular formula = C28H22Cl.2FNO3	One strip Contains 3.6 mg Flomethrin	Plastic strip	Strips were inserted vartically between two brood combs
Amitraz® (Mitac)	from amidine compound; N-(2,4-dimethylphenyl)-N-[[(2,4-dimethylphenyl) amino]methyl]]-N-methylmethanimidamide.	Oily liquid EC 200% (w/v)	2 ml mixed with 5 ml food oil in polyethylene packet	On hive bottom under brood combs

Histopathological studies

The histological studies were conducted at the Central Laboratory of the Faculty of Agriculture, Cairo University. Ten adult honey bee workers were used for each treatment, anesthetized by cold exposure (4°C) for 3-5 minutes, and carefully dissected (Dade 1977). Samples from the HPGs of treated bees were collected at the end of the 2-month experiment. They then remained fixed for twenty-four hours in 10% formol saline. After washing with tap water, the subjects were dehydrated using a series of alcohol dilutions (methyl, ethyl, and 100% ethyl). Samples were cleaned in xylene and paraffin-embedded for 24 hours at 56 °C in a hot air oven. A sliding microtome created paraffin beeswax tissue blocks for sectioning at 4-micron thickness. Subsequently, tissues were deparaffinized, mounted on slides, and stained with hematoxylin-eosin for microscopic examination under an electric light microscope, following the protocol described by Bancroft *et al*. (1996).

Statistical analysis

Parametric and non-parametric tests were performed on the data based on their normality, which was tested using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Using MSTAT-C version 1.41, all collected data were entered into tables and statistically evaluated using a randomized complete block design (ANOVA) (Snedecor and Cochran, 1980). Duncan's multiple range test was used to compare all means±SD at a level of 0.05 (Duncan 1955).

RESULTS

Varroa fallen mite

The results obtained in Figure 1 indicate that the total numbers of fallen mites for the different treatments could be arranged in descending order as follows: Varroakiller® (53.5), Menthocaros® (47.0), Amitraz® (28.6), Varroby® (26.0), and formic acid (24.5), respectively, after treating the colony with the different treatments, compared to the control colonies, which had 16 mites. At the seventh measurement, Varroakiller® was observed to be the most effective in eliminating *Varroa*, followed by Menthocaros®, Varroby®, Amitraz®, and formic acid. Regarding the period of exposure, formic acid was deemed the most significant due to its efficiency over a short time, where it resulted in a reduction of 100% at the seventh measurement (after 24 days). For the other treatments, Varroakiller® came in second place, causing a 100% reduction in the 8th measurement, followed by Amitraz® in third place, causing a 100% reduction in the 11th measurement; Menthocaros® in fourth place, causing a 100% reduction in the 12th measurement, and finally Varroby® in the 14th (Fig. 2).

Biological studies
Areas of eggs

According to Table 2, the mean areas of eggs (cm^2/colony/4day) in treated honey bee colonies could be arranged in descending order as follows: Varroakiller® (64.03 cm²), Varroby® (53.54 cm²), formic acid (43.84 cm²), Menthocaros® (35.84 cm²), and Amitraz® (30.86 cm²), while for control colonies it was 18.69 cm². The statistical analyses showed significant differences between the treatments and the control, and among all treatments except for Amitraz® and Menthocaros®.

Areas of sealed worker brood

The data in Table 2 indicate that the mean of SWBA (cm^2/colony/13day) in honey bee colonies treated with different acaricides could be arranged in descending order as follows: Varroakiller® (327.98 cm²), formic acid (228.92 cm²), Varroby® (222.25 cm²), Menthocaros® (129.72 cm²), and Amitraz® (101.73 cm²). By comparison, the SWB of control colonies was 44.83 cm². The statistical analyses indicated significant differences between the control and treatment groups, as well as among the various treatment groups (except between formic acid and Varroby®).

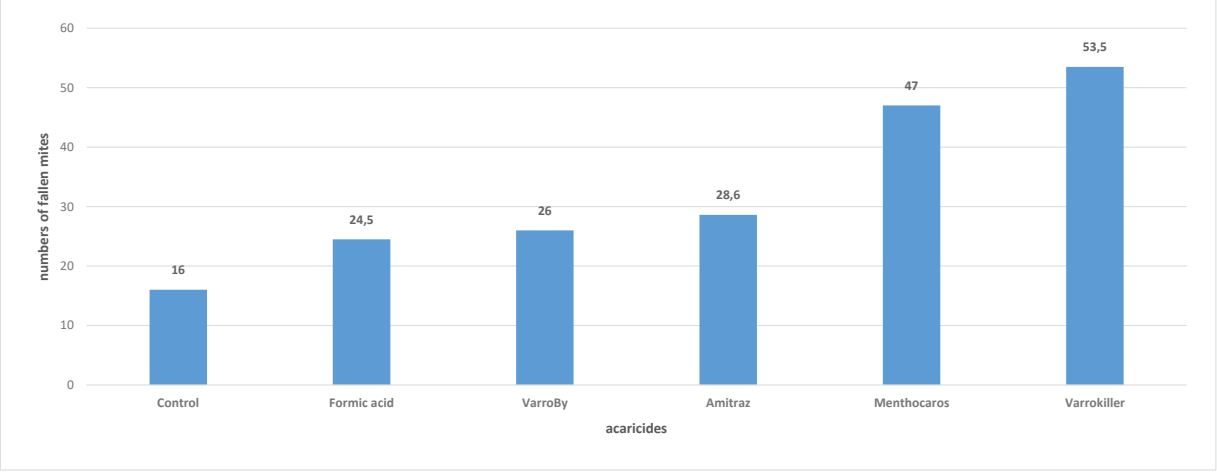


Figure 1. Total fallen mites after treated with different acaricide treatments.

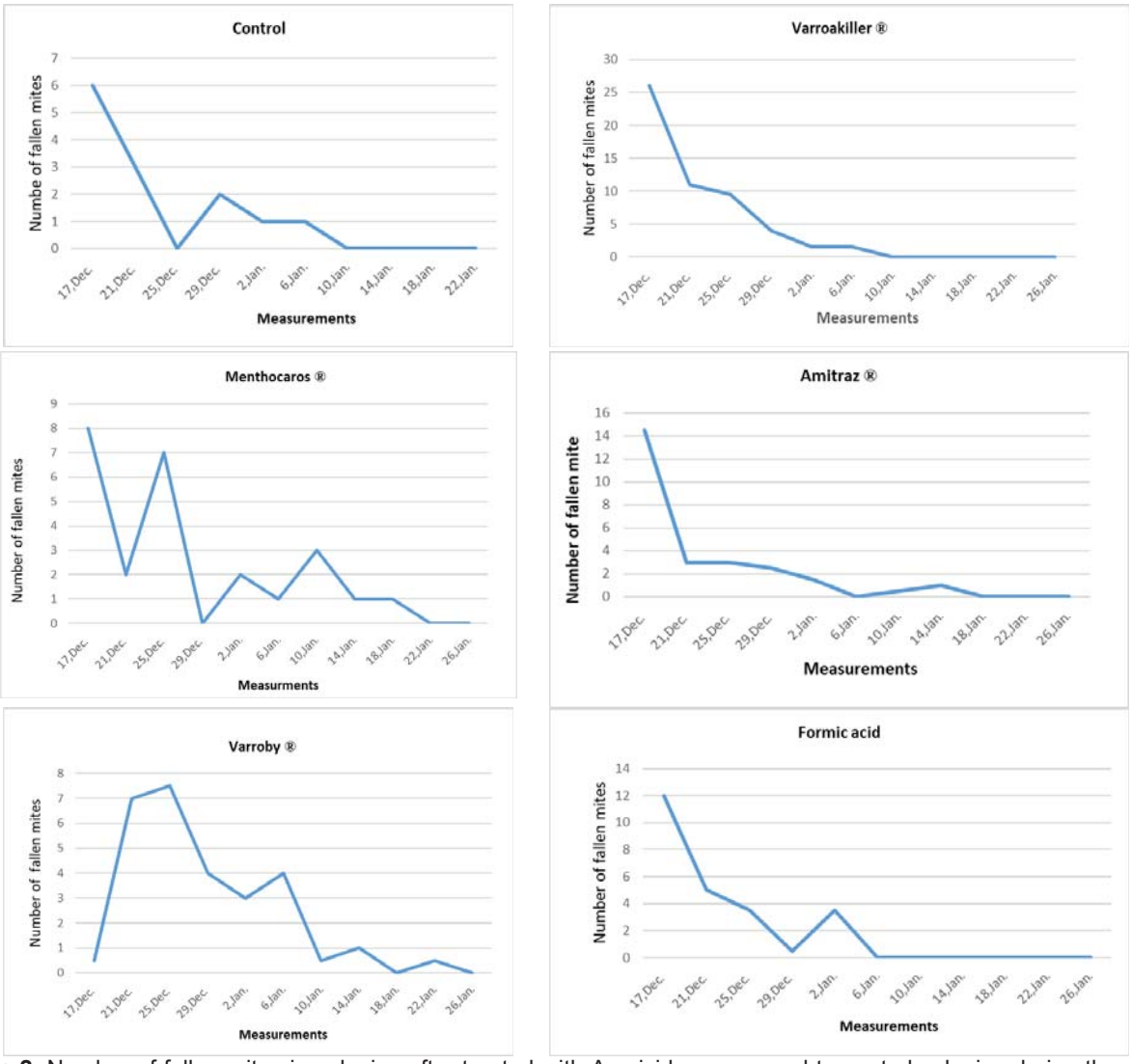


Figure 2. Number of fallen mites in colonies after treated with Acaricides compared to control colonies during the study period.

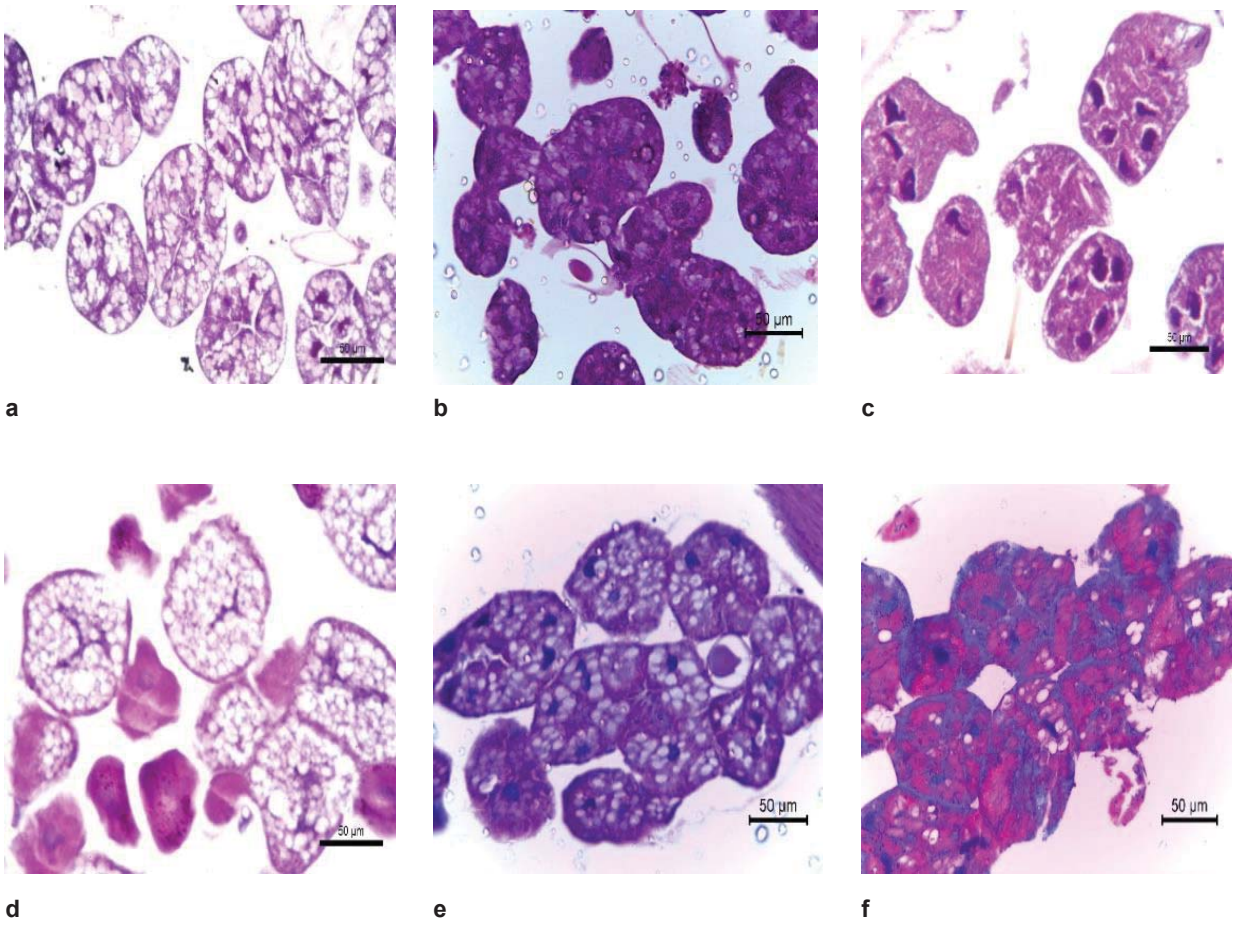
Histopathological studies

The histological changes of the worker bee HPGs were assessed and presented in Figure 3. In the control colonies, a normal histological structure of gland acini and secretory vacuoles was observed (Fig. 3a). Moderate histological changes were noticed in the treatments of formic acid and Menthocaros®, where treated bees with formic acid demonstrated atrophy in some gland acini while others exhibited vacuolization in the cytoplasm without nuclei (Fig. 3b). For Menthocaros®, the HPGs showed loss of intracytoplasmic vacuoles with the disintegration of the nuclei in the gland acini (Fig. 3c). On the other hand, there were significant histological changes in the treatments of Varroakiller®, Amitraz®, and Varroby® (Fig. 3 d,e,f). The HPGs of treated bees with Varroakiller® revealed granular basophilic material in the compact eosinophilic cytoplasm with pyknotic nuclei in gland acini (Fig. 3d). Treated bees with Amitraz® showed fine basophilic granules distributed in the cytoplasm of gland acini (Fig.3 e). In the Varroby® treatment, pyknotic nuclei with deep eosinophilic compact cytoplasm were detected in the atrophied epithelial lining cells of gland acini (Fig. 3f). (Means in the same column followed by the same letter do not differ significantly at the 5% level of probability. $p>0.05$).

Table 2. Mean areas of eggs, sealed workers brood (cm^2/colony) of honey bee colonies and fallen numbers of mites as a result of acaricides treated. SD=standered division.

Treatments	Area of eggs (mean ± SD) (cm^2/colony/4day)	Area of sealed worker brood (mean ± SD) (cm^2/colony/13 day)	Numbers of fallen mites / day (mean ± SD)
Varroakiller®	64.03^{A} ± 4.53	327.98^{A} ± 17.8	5.35^{A} ± 2.63
Menthocaros®	35.84^{D} ± 0.28	129.72^{C} ± 8.23	4.70^{A} ± 1.14
Amitraz®	30.86^{D} ± 0.75	101.73^{D} ± 10.05	2.60^{BC} ± 1.38
Varroby®	53.54^{B} ± 0.52	222.25^{B} ± 14.27	2.80^{B} ± 0.88
Formic acid	43.84^{C} ± 0.20	228.90^{B} ± 15.21	2.45^{BC} ± 1.22
Control	18.69^{E} ± 2.71	44.83^{E} ± 4.67	1.60^{C} ± 0.53

Means in the same column followed by the same letter do not differ significantly at the 5% level of probability. p>0.05



a b c

d e f

Figure 3. Light micrographs of the HPGs of honey bee workers, after two months of treatment with acaricides. (a) the control bees show the normal histological structure of the acini. (b) treated bees with Formic acid, showing atrophy in some acini while other acini showed vacuolization in the cytoplasm. (c) treated bees with Menthocaros®, showing loss of the intracytoplasmic vacuoles. (d) treated bees with Varroakiller®, showing granular basophilic material in the compact eosinophilic cytoplasm with pyknotic nuclei. (e) treated bees with Amitraz®, showing fine basophilic granules in the cytoplasm of the acini. (f) treated bees with Varroby®, a nuclear pyknosis with deep eosinophilic compact cytoplasm in the atrophied lining epithelial cells of the acini. Scale bar 100 µm.

DISCUSSION

Varroa mite

Understanding the morphological changes and functional implications is crucial for effective beekeeping practices and colony management. The parasitic bee mite *V. destructor* is the most destructive pest of honey bees and has a significant negative economic impact on the global beekeeping business (Al-Abbadi and Nazer, 2003). Acaricides are currently widely used by beekeepers in Egypt to control *Varroa* mites in honey bee colonies. The negative effects of acaricides can be so cryptic that they may be challenging to recognize by beekeepers and may only cause acute poisoning when combined with other stressors (Gregorc *et al.* 2012). Therefore, this study was conducted to determine the effect of acaricides on the *Varroa* mite and their adverse effect on bee colony activities**.** This study represents the first attempt in Egypt to evaluate the effect of some acaricides on the HPGs using histological methods. Our results showed that the total fallen mite counts were 53.5, 47.0, 28.6, 26.0, and 24.5 for Varroakiller®, Menthocaros®, Amitraz®, Varroby®, and formic acid, respectively. This trend of results is consistent with the findings of Abu-Zeid and Ghoniemy (1992), who reported that Bayvarol and Apistan reduced the infestation percentages of *Varroa* mites from 36.00% and 33.00% to 2.7% and 4.7%, respectively.

There are very few acaricides available to manage the parasite, and so the evolution of the mite's resistance to acaricides poses a serious threat to controlling the mite. In our study, although Amitraz effectively has fallen out of an acceptable number of *Varroa* mites, global concerns and warnings necessitate caution regarding its use. Hernández-Rodríguez *et al.* (2021) documented that there is a significant variation in the expected efficacy of coumaphos and pyrethroids across the different region of Spain, indicating the presence of a different ratio of resistant individuals to these acaricides in each population. On the other hand, the expected efficacy of Amitraz was more consistent, though slightly below the expected efficacy according to the label.

In the same geographical and climatic scope for our plots, the results of Ghoniemy and Abu-Zeid (1993) highlighted the efficacy of formic acid in suppressing *Varroa* mites. They observed the number of fallen *Varroa* mites per hive over four days at two locations, Qalyobia and Fayoum. Their findings indicated that the number of fallen *Varroa* mites at Qalyobia was 661.25, 302.75, 86.75, and 6.00 per hive for the respective four-day periods, while at Fayoum, the numbers were 319.75, 88.74, 79.50, and 47.50 for the corresponding periods. Untreated colonies in Qalyobia exhibited an average of 29.25, 21.50, 23.00, and 12.50 collected mites per colony. In contrast, those in Fayoum had averages of 12.25, 13.00, 17.00, and 14.25 mites, respectively. Additionally, the percentage of infestation decreased from 51.6% to 7.45% and from 41.82% to 10.90% in the Qalyobia and Fayoum apiaries, respectively. In a related study, Mahmoud *et al*. (2019) reported a mean of 118.00, 55.30, 55.00, 25.00, and 20.00 fallen *Varroa* mites per week after treating honey bee colonies with one Varroakiller® strip in Giza. In contrast, Sharaf El-Din and Elenany (2020) found that formic acid had the most significant impact on *Varroa* mites, with an average of 62.38 mites falling, followed by Bayvarol® at 44.38 fallen mites. Varoviga® showed a minor effect, with only 27.88 fallen mites. However, Aljedani (2021) discovered that garlic, cinnamon, Amitraz®, and thyme oil were the most effective treatments for falling *Varroa* mites, with minimal differences. The resulting values were 4.26 ± 1.57, 4.13 ± 1.84, and 2.73 ± 0.72 for garlic oil, cinnamon oil, and Amitraz®, respectively.

The areas of eggs and sealed worker brood

The SWB results showed that the treatments were arranged in the following order: Varroakiller® (327.98 cm^2), formic acid (228.92 cm^2), Varroby® (222.25 cm^2), Menthocaros® (129.72 cm^2), and Amitraz® (101.73 cm^2). For the control colonies, the SWB was 44.83 cm^2. The statistical analyses demonstrated significant differences between the control and treatment groups and among the various treatment groups (except between formic acid and Varroby®). According to Ghoniemy (1998), in Fayoum, the SWBA in colonies treated with formic acid (applied as cardboard plates placed on top of frames) were 1.158 cm^2. According to Sharaf El-Din and Elenany (2020), treatments with Varoviga, Bayvarol, and formic acid significantly increased the brood area compared to the control group.

Histological studies of the hypopharyngeal glands

The worker honey bee produces the protein fraction of worker and royal jelly, which is fed to developing larvae and queens. The glands responsible for this production are located in the head of the bee and are highly sensitive to the quantity and quality of pollen

and pollen substitutes that the worker bee consumes (Kubo *et al.,* 1996; Ueno *et al*., 2009). Our findings indicated a normal histological structure of gland acini, as well as secretory vacuoles, in control colonies. Treated bees with formic acid showed some gland acini with atrophy, vacuolization in the cytoplasm, and the absence of nuclei. In contrast, treated bees with Menthocaros® exhibited loss of intracytoplasmic vacuoles and nuclear disintegration in gland acini. Treatment with Varroakiller®, Amitraz®, and Varroby® resulted in significant histological changes. Bees treated with Varroakiller® showed granular basophilic in the compact eosinophilic cytoplasm with pyknotic nuclei in gland acini.

In contrast, those treated with Amitraz® demonstrated fine basophilic granules distributed in the cytoplasm of gland acini. Bees treated with Varroby® showed pyknotic nuclei with deep eosinophilic compact cytoplasm in the atrophied lining epithelial cells of gland acini. Our results are consistent with those of de Moraes and Bowen (2000), Gashout *et al*. (2020), and Zhang *et al*. (2023), who reported that acaricide-exposed HPGs of *A. mellifera* exhibited vacuoles, heterogeneous content of secretory vesicles, pyknosis, and degeneration of this gland in workers, with an impact on the amount of royal jelly produced and its proteomic profile. To obtain a comprehensive understanding of the overlapping effects of acaricides and Varroa parasites on HPGs, further research is necessary.

Conclusion

This research determined the effect of the five most popular mite acaricides on honey bee colonies in Egypt. Specific effects vary based on acaricide type, dosage, application method, and the overall health of the bee colony. Varroakiller® significantly reduces *Varroa* populations, has no direct impact on worker bees or egg-laying areas, but has a considerable impact on the HPGs. Formic acid results in a short exposure period, causing a 100% reduction by the 7th measurement (after 24 days), and this percentage remains constant until the end of the treatment (at the 19th measurement). For treated colonies, the histological structure showed degenerative changes in all treated samples due to the long period the bees were exposed to the treatment. Additionally, Menthocaros® significantly decreases *Varroa* quantity and has a modest impact on areas such as eggs and SWBA. However, it has a negligible impact on the HPGs. Therefore, this study confirms that it is essential to consider the impact of these treatments on the bee colony and the quantity used to achieve this result when determining the acaricide's performance rather than relying solely on its ability to reduce *Varroa* populations. The interaction between Varroa infestation and acaricide exposure requires detailed and context-specific investigation and beekeepers should know how these drugs affect bees.

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