

## EFFECT OF SOME MATERIALS FOR CONTROLLING VARROA MITE ON THE HONEYBEE DRONES (*APIS MELLIFERA* L.)

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### Abstract

The present investigation was carried out to evaluate the effects of different miticides, fluvalinate, Amitraz, oxalic acid, formic acid and thymol on honeybee drones. Drones were measured for body weight, length, width of fore wing and number of sperms in one seminal vesicle. Drones treated with fluvalinate, Amitraz, Oxalic acid and thymol had significantly less weight and less wing length than the control group. The least values of body weight and wing length observed in colonies treated with fluvalinate recorded 0.186gm and 10.615mm respectively, while the highest values of body weight was 0.205gm and wing length was 10.957mm observed with formic acid. Wing width in drones exposed to fluvalinate, Amitraz and oxalic acid were significantly lower than control. Drones exposed to fluvalinate and Ammitraz had significantly lower sperm count than drones of control recorded  $4.33 \times 10^6$ ,  $3.55 \times 10^6$  and  $5.39 \times 10^6$  respectively. A positive correlation between sperm number and wing size was recorded.

**Key words:** miticides-drones-*Apis mellifera*-wing size-sperm number.

### INTRODUCTION

Mites that parasitize honeybees have become a global problem that threatens the survival of both managed and feral honeybee colonies, as well as agricultural crops that depend on bee pollination. Tracheal and varroa mites are major contributing factors to the loss of honeybee colonies.

*Varroa destructor* feed on larval and pupal honeybees as they develop to adult. This feeding causes infested worker bees to have reduced body weight as adults (De Jong *et. al.*, 1982,), sometimes to have deformed wings and abdomens and to have a reduced life span (Ritter *et. al.*, 1984). Drones are about eight times more likely to be parasitized than workers bees (Fuch, 1990) and the effects of parasitism on drones can be devastating. Rinderer *et. al.*, 1998 found that only 59.7% of drones emerged in *V. destructor* infested colonies.

Varroa infestation also tended to have negative effects on drone weights mucus gland, seminal vesicle weights and the numbers of spermatozoa. Mating of commercial queens depends upon an ample supply of drones, they must be produced in colonies treated to reduce the numbers of *V. destructor*.

The most common method for mite control are the use of synthetic chemical miticides, as well as natural products, such as organic acids and plant derived oils. Despite the use of these chemicals, colony numbers have continued to decline. In addition, other problems have emerged from the use of miticides in colonies, including the accumulation of chemical residues in bees' wax, as well as mite resistance to the chemicals. Several studies have been conducted showing negative effects of several miticides on colonies' health and individuals in the hives (Rinderer *et al.* 1999).

The present study aimed to investigate the effect of different miticides used for the control of honeybee mite on the drones with regard to body size and spermatozoa production.

## **MATERIALS AND METHODS**

The experiment was carried out in the apiary and laboratory of plant protection Research Institute Department (Sharkia branch) to evaluate the effect of some miticides used in controlling *varroa destructor* on drones honeybee.

### **(1) Drone rearing**

A total of 18 colonies were used for the experiment, 3 colonies per treatment. Naturally mated sister queens grafted from the same mother were placed in each hive. As queen in colonies laid eggs in drone cells. Colonies had been treated with the used miticides. Two days before drones emergence, drone eggs were held above queen excluders which allowed the collection of all emerged drones.

When drones emerged, they were held in cages made with queen excluder material. The drone cages placed into the hive allowing workers to enter and feed the drones (Collins 2004). Drones remained in the colonies 14 – 20 days to allow full sexual maturation.

### **(2) Treatments**

- 1- Apistan (10% fluvalinate) was administrated as one Apistan strip/colony. The strips were inserted vertically between brood combs. The strips removed after 21 days.

- 2- Mitac (amitraz 0.2%): was treated by applying 10ml from the solution on cotton pieces placed under the broad combs. The treatment remain for 21 period.
- 3- Formic acid (60%): applied by using varroa form where 120ml formic acid evaporate through carton pieces. The treatment applied for 21 days period.
- 4- Oxalic acid (5%): sprayed by 5ml from the solution on the bees between combs, four times at 4 days intervals.
- 5- Thymol crystals (0.75gm/colony) were kept in a perforated sack to be hung between brood combs. The treatment applied at 10 for 21 days intervals.

### **(3) Body weight**

Samples of 30 emerged drones from the treated and untreated (control) colonies were weighed individually.

### **(4) Length and width of wings**

After the drones had been weighed, the fore were dissected in buffered solution (0.09% NaCl). The wings were mounted on glass slides using glycerin (Damus and Otis 1997). The length and width of fore wing were measured and recorded with the stereo binocular microscope using a calibrated eye piece micrometer.

### **(5) Number of spermatozoa**

When drones were at least 14 days, they were considered sexually mature. Eight drones collected from each colony, a total 16 seminal vesicles for each treatment were analyzed as two colonies were used for each treatment. The drones were dissected and one seminal vesicle was removed from each drone. Each seminal vesicle was macerated in 10ml 0.5% saline solution. It was punctured and the spermatozoa dispersed by the sucking and expelling action of a pipette. Total spermatozoa from the one seminal vesicle was estimated using a hemocytometer and light microscope (Rinderer *et al*/1985).

Data were tabulated and subjected to a statistical analysis and the treatment means were compared by L.S.D. test using Costat Program 1986.

## RESULTS

### (1) The effect of miticides on drones' weight

The weights of newlyemerged drones treated with miticides are summarized in table (1). The lowest mean drone weight was  $0.186 \pm 0.10$  gm observed in drones exposed to fluvalinate. There was a significant difference between the mean weights of treated and untreated drones  $P < 0.05$ .

Weight of drones exposed to fluvalinate, Amitraz, oxalic acid and thymol were significantly lower than the untreated check. Colonies treated with formic acid had no effect on the body weight.

Table. 1. The mean weight, length and width of fore wing of honeybee (*Apis mellifera*) drones treated with different miticides

Treatments	Mean drone weight (gm)	Fore wing			
		Length (mm)	Reduction %	Width (mm)	Reduction %
Fluvalinate (Apistan)	$0.186 \pm 0.010$ b	$10.615 \pm 0.260$ d	5.36	$3.472 \pm 0.173$ b	4.57
Amitraz	$0.186 \pm 0.010$ b	$10.663 \pm 0.262$ cd	4.93	$3.556 \pm 0.134$ ab	2.27
Oxalic acid	$0.188 \pm 0.012$ b	$10.787 \pm 0.200$ c	3.83	$3.474 \pm 0.168$ b	4.52
Formic acid	$0.205 \pm 0.116$ a	$10.957 \pm 0.155$ b	2.31	$3.647 \pm 0.144$ a	—
Thymol	$0.192 \pm 0.023$ b	$10.809 \pm 0.200$ c	3.63	$3.597 \pm 0.115$ a	1.16
Control (untreated)	$0.211 \pm 0.007$ a	$11.216 \pm 0.301$ a		$3.639 \pm 0.252$ a	
P	0.0000***	0.0000***		0.0016**	
LSD at 0.05	0.0072	0.1471		0.1066	

### (2) The effect of miticides on the length and width of fore wing

Table (1) showed that the lowest wing length and width were observed in drones exposed to fluvalinate recorded  $10.615 \pm 0.260$  mm and  $3.472 \pm 0.173$  mm respectively compared with drones reared in the control conditions  $11.216 \pm 0.301$  mm and  $3.639 \pm 0.22$  mm.

There was a significant difference between the mean length and width of fore drone wings that treated with different miticides ( $P < 0.05$ ).

Wing length of drones exposed to fluvalinate, amitraz, formic acid, oxalic acid and thymol during development were significantly lower than that of control. Wing width in drones exposed to fluvalinate, amitraz and oxalic acid were significantly lower than the untreated check. Rearing drones in colonies treated with formic acid or thymol had no significant effect on wing width drones compared with untreated ones.

### (3) The effect of miticides on the number of spermatozoa

Mean sperm number in one seminal vesicle of  $5.39 \pm 1.37 \times 10^6$  was found for the control drones but the treatments means for fluvalinate, amitraz, oxalic acid, formic acid and thymol were  $4.33 \pm 3.03 \times 10^6$ ,  $3.55 \pm 1.13 \times 10^6$ ,  $4.70 \pm 1.85 \times 10^6$ ,  $5.32 \pm 1.45 \times 10^6$  and  $5.02 \pm 1.32 \times 10^6$  respectively.

There was a significant difference between sperm numbers in control drones and drones exposed to fluvalinate and Amitraz ( $P < 0.05$ ). Drones exposed to fluvalinate and Amitraz have significant lower sperm counts than those of control, thymol, oxalic acid and formic acid treatments.

Reviewing the obtained results it could be showed, a significant positive correlation between wing length and sperm numbers of different miticides treatments ( $p = 0.035$ ).

The values of mean sperm content in drones treated with different miticides are shown in table (2).

Table. 2. The mean spermatozoa number in one seminal vesicle of sexually mature of honeybee drones of (*Apis mellifera*) treated with different miticides.

Treatments	Spermatozoa number/seminal vesicle ( $\pm$ SD)	Reduction %
Fluvalinate (Apistan)	$4.33 \pm 3.03 \times 10^6$ ab	19.78
Amitraz	$3.55 \pm 1.13 \times 10^6$ b	34.26
Oxalic acid	$4.70 \pm 1.85 \times 10^6$ a	12.82
Formic acid	$5.32 \pm 1.45 \times 10^6$ a	1.29
Thymol	$5.02 \pm 1.32 \times 10^6$ a	6.86
Control (untreated)	$5.39 \pm 1.37 \times 10^6$ a	
P	0.0151*	
LSD 0.05	$1.1346 \times 10^6$	

## DISCUSSION

Miticides treatments caused several negative effects on drone bee, including decrease in body weight, wing size and spermatozoal production when compared to drones of control colonies. Exposure to fluvalinate, amitraz, oxalic acid, and thymol during development and maturity caused significant reduction in body weight. Drones in formic acid treated colonies showed no significant differences from control, this finding is in agreement with (Rinderer et al 1998) documented a 5% reduction in body weight in drones exposed to Apistan, while De Guzman et al 1999 stated that formic acid treatment did not adversely affected drone weight.

Treatment of drones honeybee with different miticides caused reduction in wing length ranged from 5.36% in drones exposed to fluvalinate, to 2.31% in drones exposed to formic acid compared with control drones. Also, fluvalinate, amitraz and oxalic acid treatments significantly decreased the wing width of drones honeybee by approximately 4.57% ,2.27% and 4.52 respectively compared to the control .

No significant difference was observed in drones wing width that exposed to formic acid and thymol compared to the control drones. Surviving drones in control colonies had the highest means number of spermatozoa with  $5.39 \pm 1.37 \times 10^6$  per seminal vesicle or  $10.8 \times 10^6$  per drone are strongly in agreement with the finding of (Elbassiouny 1992) who recorded  $10.62 \times 10^6$  spermatozoa on carnelian drones.

Drones in fluvalinate and amitraz treated colonies showed significant differences from control with an average of  $4.33 \pm 2.03 \times 10^6$  and  $3.55 \pm 1.13 \times 10^6$  sperm per seminal vesicle respectively. Previous studies have shown that exposure of drones to fluvalinate reduced the amount of sperm found in the seminal vesicles of drones (Fell and Tignor 2001, Rinderer *et al* 1999). However, (Burley 2007) found that fluvalinate treatment did not adversely affect sperm production.

Our study show no significant decrease in sperm production of drones exposed to formic acid, oxalic acid and thymol compared with spermatozoa number reared in control conditions. In contrast, (De Guzman *et al* 1999) observed that formic acid treatment resulted in surviving drones having a higher average number of spermatozoa than control colonies. Also, (Burley 2007) showed a significant decrease in sperm production of drones exposed to thymol.

### **Correlation between wing length and number of spermatozoa**

Wing length used as an indicator for drones size and weight (Berg 1992). The result of our study shows a significant positive correlation of wing length and sperm number in drones treated with fluvalinate, Amitraz, oxalic acid, formic acid and thymol. Schulns *et al* (2003) observed a significant decrease in spermatozoa production in smaller drones reared in worker cell as opposed to drone sized cells, showing that sperm production in drones depend on body size.

The significant positive correlation of drone body size and sperm number also supported by the fact that Africanized honeybees in South America have significantly lower body weights and at the same time lower sperm numbers than European drones (Rinderer *et al*, 1985).

In our study we find that exposure of drones to fluvalinate, amitraz, oxalic acid, thymol and formic acid during development and maturity lower the wing length by 5.36%, 4.93%, 3.83%,3.63% and 2.31 % respectively compared with control drones, which show a positive correlation with sperm number where, the previous treatment produce 19.78%, 34.26%, 12.82% ,6.86 % and 1.29% respectively less spermatozoa than control drones.

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## تأثير بعض المواد المستخدمة في مكافحة حلم الفاروا على ذكور نحل العسل

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اجريت هذه الدراسة لتقييم تأثير كل من الفوفالينيت (ايبستان) و الميتاك (اميتراز) و حمض الأوكساليك وحمض الفورميك والثيمول والتي يتم استخدامها في مكافحة طفيل الفاروا على ذكور نحل العسل وذلك في معهد بحوث وقاية النباتات فرع الشرقية خلال العام ٢٠١٢ م وقد اشتملت هذه الدراسة على :-

١- تربية ذكور النحل في خلايا يتم معاملتها بالمركبات السابقة.

٢- دراسة تأثير هذه المركبات على ذكور نحل العسل وذلك من حيث:-

- وزن الذكور حديثة الخروج.

- طول وعرض الجناح الأمامي.

- عدد الحيوانات المنوية.

- العلاقة بين طول الجناح وعدد الحيوانات المنوية.

أظهرت الدراسة بعض التأثيرات الناتجة عن استخدام هذه المركبات ويمكن تلخيصها كالآتي:-

١- خفض معنوي في وزن الذكور الناتجة في خلايا تم معاملتها بالفوفالينيت و الاميتراز وحمض اوكساليك و الثيمول حيث بلغ وزن الذكور ٠,١٨٦ ، ٠,١٨٦٣ ، ٠,١٨٨ ، ٠,١٩٢ جم على التوالي وذلك مقارنة بمتوسط وزن الذكور في الخلايا غير المعاملة حيث بلغ الوزن ٠,٢١١ جم.

٢- خفض في طول وعرض الجناح الأمامي حيث سجل اقل قياس لطول وعرض الجناح الأمامي للذكور الناتجة في خلايا يتم معاملتها بالفوفالينيت حيث سجلت ٣,٤٧٢ ، ١٠,٦١٥ مم على التوالي، وكان لباقي المعاملات تأثيراً في خفض طول الجناح حيث سجلت ١٠,٦٦٣ ، ١٠,٧٨٧ ، ١٠,٩٥٧ ، ١٠,٨٠٩ مم للاميتراز وحمض الاكساليك وحمض الفورميك والثيمول على الترتيب وبلغ في المقارنة ١١,٢١٦ مم.

وعلى عرض الجناح حيث سجلت ٣,٥٥٦ ، ٣,٤٧٤ ، ٣,٦٤٧ ، ٣,٥٩٧ مم على الترتيب وفي المقارنة ٣,٦٣٩ مم.

٣- انخفاض معنوي في عدد الحيوانات المنوية في الذكور الناتجة في خلايا يتم معاملتها بكل من الفوفالينيت و الاميتراز حيث بلغ متوسط عدد الحيوانات المنوية ٤,٣٣ × ٦١٠ ، ٣,٥٥

٦١٠× حيوان منوي في الحوصلة المنوية على الترتيب، بينما سجلت باقي المعاملات انخفاضاً غير معنوياً في عدد الحيوانات المنوية فكانت ٥,٠٢ × ٦١٠، ٥,٣٢ × ٦١٠، ٤,٧٠ × ٦١٠ حيوان منوي لكل من الثيمول وحمض الفورميك و حمض الاوكساليك على التوالي مقارنة بالذكور التي نتجت في خلايا غير معاملة ٥,٣٩ × ٦١٠.

٤- اظهرت الدراسة ايضاً وجود علاقة طردية بين طول الجناح الأمامي وعدد الحيوانات المنوية في الخلايا المعاملة بالفلوفانينيت والاميتراز وحمض الأوكساليك والثيمول وحمض الفورميك فكانت نسبة الإختزال في طول الجناح الأمامي ٥,٣٦%، ٤,٩٣%، ٣,٨٣%، ٣,٦٣%، ٢,٣١% على التوالي وكذلك نسبة الأختزال في عدد الحيوانات المنوية/حوصلة منوية ١٩,٧٨%، ٣٤,٢٦%، ١٢,٨٢%، ٦,٨٦%، ١,٢٩% على التوالي.

ويمكن استنتاج ان حمض الفورميك ٦٠% المستخدم في مكافحة حلم الفاروا هو اقل المركبات تأثيراً على ذكور نحل العسل مقارنة بباقي المركبات التي قللت من مقدرة ذكور النحل على عملية تلقيح الملكات العذارى.