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EFFECT OF FLUVALINATE ON ACTIVITY OF HONEY BEE INDIVIDUALS

[58]

Ali[1], M.A.M.

ABSTRACT

Twenty concentrations of 98% purity of technical grade fluvalinate (Apistan®) were assayed at 23°C under laboratory and field condition to examine its toxic effect on honey bees (*Apis mellifera* L.). In field trial, dilution of 20 mg fluvalinate/ml acetone was topically applied to evaluate its effect on queen acceptance, mating ratio, superseding and colony vigor. In the experiment, virgin queens were divided into three different groups of queens treated as follows:- (i) acetone only as untreated check, (ii) 20mg/ml of 98% fluvalinate and (iii) queens received treatment only after mating. The data showed that concentrations between 1 -20 mg/ml fluvalinate caused 10 - 100% mortality of workers, meanwhile, 0.05 – 50 mg/ml caused mortality of drones ranging from 11.43-100%. Concentrations between 30 – 100 and 20 -80 mg/ml caused 30 -100 and 20 -100% mortality for queens 24 and 48 hours after treatments, respectively. The $LC50^{n}$ were 3.768, 4.064 and 53.447 for worker, drone and queen honey bees, respectively. In field trial the queen acceptance was 80, 70 and 75% and queen mating was 65, 60 and 75%. Queen superceding was 0, 40 and 6.67% for groups (i), (ii) and (iii), respectively. Brood areas were 2296.77, 2445.96 and 2447.17 cm^2 for groups (i), (ii) and (iii), respectively and numbers of frames field with brood and those covered with bees were 2.38, 2.63 and 2.56 for the former and 5.00, 5.42 and 5.38 for the later aspect for groups i, ii and iii, respectively.

Key words: *Apis mellifera*, Apistan®, Fluvalinate, Honey bee, Varroa destructor

INTRODUCTION

The ectoparasitic, *Varroa destructor* (**Anderson and Trueman, 2000**) has become a formidable problem for beekeepers all over the world. Infestation with Varroa caused a reduction between 10-20% of pupal weight in honey bee workers and drones (**Choi and Woo, 1974**). **Schneider (1986)** found a correlation between the degree of infestation of drone brood and reduction in their weight at emergence, as well as in the size of seminal vesicles and mucus glands. Furthermore, the number of spermatozoa decreased by 50% when a

1- Plant Protection Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Khiema, Cairo, Egypt

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drone pupa was infested with more than 3 mites. Deficiencies in drones could reduce their sperm contribution at mating especially if the queen mates with an insufficient number of drones (**Rinderer *et al* 1998**). Infested newly honey bee queens were not surviving introduction into colonies or were being superseded within the following two to three months. Varroa mites affect queen establishment by interfering with drone production, survival or mating insufficient number of drones may result in an early supersedure of queens (**Camargo and Goncalves, 1971; Webster, 1998 and Williams, 1998**).

The effectiveness of fluvalinate (Apistan™ Queen taps) for controlling mites associated with honey bees is well documented (**Koeniger and Chmiedlewski, 1988; Lubinevski *et al* 1988; Pettis *et al* 1988; and Ruijter and Van Den Eijnde, 1989**). The nontoxic effect of fluvalinate on honey bees has also been studied by (**Stoner *et al* 1984; Taylor *et al* 1987 and Waller *et al* 1988**). However, there have been recent concerns by beekeepers and queen breeders that this miticide might affect the viability of honey bee queens being shipped from queen breeders to beekeepers. (**Williams *et al* 1994**) examined the effect of 1% fluvalinate plastic strips (Apistan™ Queen taps) on honey bee workers and queens and found that it had no significant effect on supersedure, queen acceptance, survivability and brood production, at 25°C. However, at 30°C and 35°C this treatment significantly affected worker and queen mortality.

Honey bees treated with Apistan Queen Taps exhibited greater susceptibility to bifenthrin than untreated bees in laboratory bioassays (**Ellis *et al* 1997**). It was advised that as a precaution beekeepers should avoid treating colonies with Apistan strips at times of year when bees are likely to forage on bifenthrin-treated crops. However, drones that were able to survive to mating age were equally competitive, regardless of varroa infestation or Apistan® treatment. (**Allen *et al* 1999**). Furthermore less fit drones from varroa-infested and Apistan-treated colonies would be more likely to mate with queens.

The aim of the present work is to study the toxicity of different concentrations of fluvalinate (the active ingredient of Apistan®) to honey bee workers, drones and queens. Such a compound is recommended for varroa mite control in honey bee colonies not only in Egypt but also in many other countries.

MATERIAL AND METHODS

This study was conducted in the laboratory of Insect Physiology, Faculty of Agriculture, Ain Shams University and at the apiary of Mr. Mohamed El-Shaer in Fayoum Governorate (Private Sector), between June to September, 2002.

Laboratory bioassays

Preparation of fluvalinate stock solution: Technical grade fluvalinate (98% purity) was provided from Chem. Service, Inc., PA, USA. A stock solution was made by dissolving 0.4891g fluvalinte in 1.1983ml HPLC grade acetone. Twenty concentrations prepared in 200 µ volumes of technical grade fluvalinate were made in acetone and assayed. The concentrations were, 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 10, 5, 1.0, 0.5, 0.1 and 0.05 mg/ml. Solutions were

stored in deep freezer at –5°C in darkness until needed.

Treatment of honey bee: Honey bee workers, drones and queens were collected and the following procedures were carried out:-

(i) Adult workers were placed in queen cages, each contained ten workers and three cages were used for each concentration.

(ii) Adult drones were placed into newly designed cages, measuring 10.5 x 5 cm^2 made from cardboard and surrounded by an eight mesh screen secured by duct tape. Approximately, fifteen drones together with twenty workers were included in each cage and three replicates were used.

(iii) Queen rearing was carried out, according to the procedure of **Ali, (1994)** which was modified from Dolittle technique. Three days after emergence, queens were collected and inserted individually into Benton queen cages. One virgin queen with 10 adult workers was placed in each cage; ten virgin queens (replicates) were used for each concentration.

As an untreated check, an equivalent number of insects were treated with acetone only. All cages were provided with queen candy as a source of food. The cages were immediately transferred to the laboratory and treatments conducted under laboratory conditions of 23°C.

For application, the bees were anesthetized with CO_2 and a volume of 0.5µl of the test solution was applied to the insects abdomen by Hamilton microsyringe. Untreated bees that received acetone only were also included, the accompanying workers with queens and drones were not treated.

The following concentrations were applied for each caste:-

(I) Workers and drones: Twelve doses were tested, 300, 200, 100, 50, 25, 20, 10, 5, 1, 0.50, 0.10, and 0.05 mg/ml.

(II) Queens: Nineteen doses were tested; 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 1, 0.50, 0.10 and 0.05 mg/ml were tested.

The Hamilton microsyring used were thoroughly cleaned three times in three different vials in acetone before and after each use. This process was repeated 10 times between dilutions. Following treatment, the bees were held in dark cardboard boxes at 23°C. Insect mortality was recorded 24 and 48 hours after treatment.

Analysis of data: Data were analyzed and LC_{50}s values with 95% confidence limits were calculated by probit analysis using **POLO methods of LeOra Software(1991).** The LC_{50}, confidence limits and slope was determined using probit analysis as reported by **(Finney, 1970)** and **(Abbot formula, 1925)** was used to correct mortality percentages.

Field evaluation of fluvalinate on queen bees: This evaluation was conducted in private sector apiary located at Fayoum, sixty honey bee nuclei selected from strong colonies were prepared. Each nucleus was consisted of one frame of brood and one frame of honey, the frames covered with adult bees. The nuclei were transferred into two apiaries located at about five kilometers distance between them.

Queen rearing: Carniolan queen honey bees were reared on June 20 and 23, 2002, as mentioned in the laboratory study. The queen cells were cut out and confined under the hemicircle queen

cages on honey frame after 10 days, then transferred into queenless colony until emergence.

Preparation of fluvalinate dilution: 20 mg/ml fluvalinate was selected as this dilution did not cause queen mortality in the laboratory assay. An amount of 0.5 µl was topically applied to queen abdomen.

Preparation of honey bee nuclei: On July 6 and 7, 2002 sixty nuclei of honey bees were prepared from strong colonies. Each nucleus received one frame of brood and one frame of honey, both were covered with adult honey bees.

Treatment of the queens: The experimental virgin queens were marked with a coulored paint dot on their thorax, and then divided into three different groups, each comprising 20 nuclei. Insects of each group were received one of the following treatment: (i) acetone only which served as control, (ii) a concentration of 20 mg/ml of 98%fluvalinate to virgin queens and (iii) queens not receiving treatments except after mating. Queens of groups (i) and (ii) were anesthetized with carbon dioxide for 30 seconds and either 0.5 µl of fluvalinate or acetone was topically applied to its abdomen.

Queen introduction to the nuclei: Treated and untreated virgin queens were transferred to the apiaries and introduced into their nuclei by placing them individually under hemicircular cage between the frames. The virgin queens were left under these cages for three days, they were then released into their colonies by making a little hole against the cage.

Queen acceptance and mating ratio: Fifteen days after releasing the queens, all experimental nuclei were examined to obtain the acceptance and mating ratios. A week later, the now mated queens from the untreated group (iii) were pulled from their colonies and marked by the same colony number, transferred to the laboratory and topically treated with the same concentration of fluvalinate (0.5 µl). Bees were then immediately transferred back to their apiary and introduced into their colonies. The following parameters were studied: queenless colonies, mating ratio, colonies that had superseded their queens, queen right colonies but without eggs or brood, drone layer queens, queen right colonies with a good brood pattern as well as brood area in cm^2, and number of frames filled with brood or covered with adult bees.

Experimental design and analysis: The experimental design was a completely randomized design. Results were analyzed using SAS **(SAS Institute, 1985)**. The general linear modules procedure to test for differences (alpha= 0.05) and applied the least significant differences as a mean separation test were used.

RESULTS

Honey bee mortality

As exhibited in Tables (1 and 2), the concentrations of 98% fluvalinate causing 10 to 100% mortality to workers ranged from one to 20 mg/ml. Meanwhile concentrations less than 1 mg/ml did not affect honey bee workers. Concentrations from 0.05 to 50 mg/ml caused mortality ranging from 31.11 to 100% of drone honey bees (Table, 1), meanwhile mortality in untreated drones was 22.22%. Fluvalinate concentrations up to 20 mg/ml did not cause mortality to queen bees, meanwhile concentrations between 30 to 100 mg/ml caused 30 to 100% mortality.

Probit regression toxicity estimated for fluvalinate topically applied to honey

Table 1. Mean percentages of mortality of honey bee workers, drones and queens after treatment with different concentrations of 98%fluvalinate, incubated at 23° C.

Conc. (mg/ml)	Percent of mortality after 24 hrs for		Conc. (mg/ml)	Percent of mortality of queens** after	
	Workers*	Drones*		24 hrs	48 hrs
Untreated check	0.00	22.22 ± 0.33	Untreated check	0	0
0.05	0.00	31.11 ± 1.20	10.0	0	0
0.10	0.00	40.00 ± 0.58	20.0	0	20 ± 0.13
0.50	0.00	44.44 ± 0.33	30.0	30 ± 0.15	30 ± 0.16
1	10.00 ± 0.58	60.00 ± 1.53	40.0	30 ± 0.15	40 ± 0.15
5	63.33 ± 0.33	65.12 ± 1.85	50.0	50 ± 0.17	50 ± 0.17
10	78.79 ± 1.20	68.89 ± 0.33	60.0	50 ± 0.17	70 ± 0.15
20	100 ± 0.00	84.44 ± 0.67	70.0	60 ± 0.16	80 ± 0.13
25	100 ± 0.00	93.33 ± 0.58	80.0	60 ± 0.16	100 ± 0.00
50	100 ± 0.00	100 ± 0.00	90.0	80 ± 0.13	100 ± 0.00
100	100 ± 0.00	100 ± 0.00	100	100 ± 0.00	100 ± 0.00

* 200 and 300 mg/ml caused 100% mortality for workers and drones 24 hrs after treatment.

** 0.05, 0.1, 0.5, 1.00, and 5.00 mg/ml did not cause queen mortality 24 or 48 hrs after treatment, while 150, 200, 250 and 300 mg/ml caused 100% mortality.

Table 2. Responses of honey bee individuals to topically applied fluvalinate (98% purity)

Aspects	Honey Bee Castes		
	Workers*	Drones**	Queens*
No. of observation	395	583	200
df.	10	10	17
Slope ± S.E	2.638 ± 0.305	1.417 ± 0.384	4.173 ± 0.702
Chi sq.	4.209	15.921	6.513
LC_{10} (mg/ml)	1.231 (0.745 - 1.740)	0.506 (0.001 – 2.338)	26.354 (16.887 – 33.691)
LC_{50} (mg/ml)	3.768 (2.857 – 4.799)	4.064 (0.201 – 8.974)	53.447 (44.411 – 62.619)
LC_{90} (mg/ml)	11.533 (8.814 – 16.444)	32.620 (19.370 – 80.175)	108.396 (88.388-153.794)

*LC values are given in mg per insect followed by the 95% confidence limits (C.L)

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Table 3. Number of accepted, mated, superseded, drone layer queens and colonies with good brood pattern in colonies had queens treated with 20 mg/ml of 98%fluvalinate

Aspects	Treatments		
	Group I	Group II	Group III
No. of observation	20	20	20
No. of accepted queens	16 (80)	14 (70)	15 (75)
No. of mated queens	13 (65)	12 (60)	15 (75)
No. of superseded queens	0 (0)	0 (0)	6 (40)
No. of drone layer queens	0 (0)	0 (0)	1 (6.67)
No. of colonies with good brood pattern	10 (76.92)	8 (66.67)	8 (53.33)

* Values between brackets represent percentages

Table 4. Number of brood area cm^2, frames of brood and frames covered with adult bees in colonies had queens treated with 20 mg/ml of 98%fluvalinate

Aspects	n	Brood area (cm^2)	Frames of brood	Frames of bees
Group I	13	2296.77 ± 143.67	2.38 ± 0.13	5.00 ± 0.28
Group II	12	2445.96 ± 149.84	2.63 ± 0.18	5.42 ± 0.26
Group III	8	2447.17 ± 249.29	2.56 ± 0.18	5.38 ± 0.50
F. value		0.27	0.69	0.55

bee individuals show that all estimates were significant (t-ratio > 1.96) (Table 2). The LC10 were 1.231, 0.506 and 26.354 mg/ml, while LC50 were 3.768, 4.064 and 53.447 mg/ml and LC90 were 11.533, 32.62 and 108. 396 mg/ml for workers, drones and queens, respectively (Table 2). The queens were the most tolerant to fluvalinate (LC50= 53.447), while the workers were the least tolerant (LC50= 3.768). At the LC10, LC50 and LC90 the drones were 1.08 time more tolerant than workers, while the queens were 13.15 and 14.18 times more tolerant than drones and workers, respectively.

Activities of honey bee colonies

Table, (3) indicates that percentage of queen acceptance was 80% in the control treated with acetone (group i), while this was slightly reduced to 70 and 75% in groups (ii) and (iii), respectively. Colonies that had queens treated after copulation (group iii) had the highest percent of mating i.e. 75%, meanwhile it was 65 and 60 in groups(i) and (ii), respectively. The data also indicate that percent of queen superseding was zero in both group (i) and (ii), meanwhile, it reached 40% in group (iii). However, this latter group (i.e. group iii) exhibited a higher percent of queen superseding as compared with groups (i) and (ii). Group (iii) also exhibited 6.67% drone layer queens, while none were observed in group (i) or group (ii). The percentage of colonies with a good brood pattern was high (76.92%) in group (i) as compared with the two other groups (ii) or (iii) (66.67 and 53.33%), respectively.

However, as exhibited in (Table, 4) for the queen surviving the treatments in the three groups, (I), (ii), (iii), no significant differences were noticed in brood areas which were 2296.77, 2445.96 and 2447.17 cm^2, respectively (F=0.27, df= 32, *P*=0.7622). Also, the number of frames filled with brood and those covered with adult bees at end of the experiment were not significant affected, i.e. 2.38, 2.63 and 2.56 (F= 0.69, df= 32, *P*= 0.51) and 5, 5.42 and 5.38 (F= 0.55, df= 32, *P*= 0.584), respectively.

DISCUSSION

In the present work, fluvalinate has a toxic effect to the different honey bee individuals, although **Stoner *et al* (1984)** and **Taylor *et al* (1987)** reported that fluvalinate was non toxic to honey bees. Toxicity of fluvalinate differed between the castes the queens were the most tolerant to fluvalinate, while the workers were the least tolerant. Also the toxicity of fluvalinate varied with the concentration, at LC10, LC50 and LC90 the drones were 1.08 times more tolerant than workers, while the queens were 13.15 and 14.18 times more tolerant than drones and workers, respectively. Virgin queens topically treated with a dilution of 20mg/ml of fluvalinate then introduced into their colonies did affect the queen acceptance or mating and superseding as compared with untreated queens or queens treated with the same dilution of fluvalinate after mating. Although, **(Williams *et al* 1994)** had also found no effect on bee survivability, queen acceptance. superseding or brood production when treated with 1% fluvalinate at 25°C, meanwhile treatment of queens at 30°C and 35°C significantly affected mortality. Also, **(Webster, 1998) and Wiliams, 1998)** reported that new queens were not surviving introduction into colonies or

were being superseded within the following two or three months of treatment with fluvalinate. The data of the present work also concluded that treated mated queens with dilution of 20mg/ml of fluvalinate affected positively queen superseding (40%) and drone layer (6.67%) as compared with untreated or treated virgin queens.

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(٢) ، ٨١٧ - ٨٢٦ ، ٢٠٠٣

تأثير مركب الفلوفالينات على أفراد طائفة نحل العسل

[٥٨]

محمود عبد السميع محمد على[١]

١- قسم وقاية النبات - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القاهرة - مصر

تم اختيار عشرين تخفيفا من المادة الفعالة لمركب الفلوفالينات ٩٨% نقاوة مذابة فى الأسيتون معمليا (وهو المادة الفعالة لمستحضر الابيستان® المستخدم في مكافحة طفيل الفاروا على طوائف نحل العسل) لدراسة تأثيراته السامة الجانبية على أفراد طائفة نحل العسل، مع حساب التركيز القاتل لنسبة ١٠ ،٥٠ ، ٩٠% من الشغالات والذكور والملكات. أما في الدراسة الحقلية فقد تم تجهيز ستين نواة نحل بكل منها قرص حضنة وقرص عسل وكلاهما مغطى بالنحل .وقد اختير تركيز ٢٠ ملليجرام/مل الذي لم يعط أي نسب موت للملكات العذارى المختبرة تحت الظروف المعملية

لدراسة تأثير الفلوفالينات على قبول الملكات وتلقيحها وإحلال الملكات ومساحة وعدد أقراص الحضنة وعدد الأقراص المغطاة بالنحل. حيث قسمت الملكات العذارى في التجارب الحقلية إلى ثلاث مجموعات الأولى (٢٠ ملكة عذراء) عوملت بالأسيتون فقط (٥و٠ ميكرولتر/ملكة) إدخلت كل منها إلى نواتها (المقارنة)، والمجموعة الثانية (٢٠ ملكة عذراء) عوملت بالفلوفالينات بتركيز ٢٠ ملليجرام/مل (٥و٠ ميكرولتر/ملكة) ثم تم إدخالها إلى نواتهم أما المجموعة الثالثة (٢٠ ملكة عذراء) فقد تم إدخالها إلى نواتها حتى تم تلقيحها وبعد بداية وضع للبُيض تم نقلها للمعمل ومعاملتها بمركب الفلوفالينات بتركيز ٢٠ ملليجرام/مل (٥و٠ ميكرولتر/ملكة) ثم أعيد إدخالها إلى نواتها مرة أخرى .

وقد أوضحت النتائج أن تركيز ١-٢٠ ملليجرام /مل من مادة الفلوفالينات قد أعطت نسب موت تراوحت من ١٠-١٠٠ للشغالات بعد ٢٤ ساعة من المعاملة ، بينما أعطى التركيز الذي تراوح بين٥٠و٠ - ٥٠ ملليجرام /مل نسب موت من ٤٣و١١ - ١٠٠ في حالة الذكور بعد ٢٤ ساعة من المعاملة، بينما أعطى تركيز ٣٠ - ١٠٠ ملليجرام /مل و تركيز ٢٠ - ٨٠ ملليجرام /مل نسب موت تراوحت بين ٣٠-١٠٠ و ٢٠-١٠٠ في حالة الملكات بعد ٢٤و ٤٨ ساعة من المعاملة، على التوالي. كما ظهر أن اقل تركيز سبب موت ٥٠%من الأفراد المختبرة كان ٧٦٨و٣، ٠٦٤و٤، ٤٤٧و٥٣ لكل من الشغالات والذكور والملكات على التوالي. وكانت الذكور اكثر تحملا عن الشغالات بمقدار ٠٨و١ مرة ، بينما كانت الملكات اكثر تحملا عن الشغالات والذكور بمقدار ١٥و١٣، ١٨و١٤على التوالي. كما أوضحت التجارب الحقلية أن نسبة قبول الملكات كانت ٨٠ ، ٧٠ ، ٧٥%، بينما كانت نسبة تلقيح الملكات ٦٥، ٦٠، ٧٥%، وكانت نسبة إحلال الملكات صفر، ٤٠، ٦٧و٦% للمجاميع الأولى والثانية والثالثة على التوالي. وكانت مساحة الحضنة ٧٧و٢٢٩٦، ٩٦و٢٤٤٥، ١٧و٢٤٤٧سم^2، بينما كان عدد أقراص الحضنة ٣٨و٢، ٦٣و٢، ٥٦و٢، و عدد الأقراص المغطاة بالنحل ٠٠و٥ ،٤٢و٥، ٣٨و٥ للمجاميع الأولى والثانية والثالثة على التوالي .

تحكيم: أ.د أحمد على جمعه
أ.د محمد عطية عويس