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POTENTIAL EFFICACY OF HONEYBEE, *Apis mellifera* L., VENOM AGAINST THE ECTOPARASITIC MITE, *Varroa destructor* (*Anderson* and *Trueman*)

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ABSTRACT

The present work was carried out at the apiary of honeybee research center, Faculty of Environmental Agricultural Sciences, El-Arish University, Egypt during August - October of 2020 to evaluate the efficacy of honeybee venom at different concentrations (0.1, 0.01 and 0.001g/ 1L sucrose syrup) compared with a commercial acaricide, fluvalinate in controlling *varroa* mites in honeybee colonies. Moreover, the effects of these treatments on brood rearing activity were observed. Obtained results showed that weekly level of fallen *Varroa* on hive bottom board was high (average 92.7 mites/colony) after using fluvalinate strips compared to bee venom averaged 60.3, 46 and 28.3 mites/colony for 0.1,0.01 and 0.001concentrations, respectively. While the untreated colonies (control) averaged 7.3 mites/colony. Level of worker brood rearing was high in colonies treated with 0.1g bee venom, averaged 5441, 5857 and 6215 sealed cells/colony in the 2nd, 3rd and 4th brood cycles (every 12 days) intervals, respectively compared to other venom treatments. However, the least number of sealed worker brood (3607 cells/ colony) was recorded in the 4th interval influvalinate treatment.

INTRODUCTION

The effective defense against disease is the most essential behavior of a bee colony. The individual bee's immune system functions in a similar way to that of vertebrate animals. Effective defense mechanism can lead to self-healing, *e.g*. the social behavior in a bee colony that removing many pathogens or parasites as possible as from the hive.

Many attempts were carried out to improve the quality of honeybee products, but frequently encountered the main obstacle in apiculture, *i.e*. diseases and pests of honeybees. Therefore, it is very important to prevent or control bee maladies **(Wahba *et al*., 2020).**

Honeybee colonies are subject to infestation by certain insects, mites and diseases. The ectoparasitic mite, *Varroa destructor*, is considered as one of the most serious pests of honeybee, causing tremendous damage to colonies and consequent great economic loss to bee keeping industry **(Al-Abbadi and Nazer, 2003)**.

Many chemicals were used for controlling *Varroa* mites in the honeybee colonies **(Nowar *et al*., 2018; Olmstead *et al*., 2019)**. The use of manufactured chemicals, *e.g*. pesticides and antibiotics to control diseases and pests inside bee colonies represents a risk to consumer health and reduces the efficiency of vital honeybee products.

Natural materials secreted by honeybees, *e.g*. bee venom and other products can combat certain diseases and parasites of honeybee colonies. Consequently, pure and biologically- active honey bee products,

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free from any harmful residues are obtained **(Wang *et al*., 2014**).

Few soft chemicals (organic acids) and natural products, especially essential oils, have shown effectiveness against *Varroa* mites, *e.g*. formic and oxalic acids and thymol (**Abu Bakar *et al.*, 2019; Malika *et al.*, 2019; Guang *et al.*, 2020; Sabahi *et al.*, 2020; Jack *et al.*, 2021).**

Many trials were applied to control *Varroa* mite using natural essential oils or their derivatives, *e.g*. **El-Bassiouny *et al.*, (2006)** found that thymol and Santonica mixture caused an obvious reduction of the rate of mite infestation either in sealed brood cells or on adult bees, and increase in number of mites fallen on the floor of treated colonies. Moreover, worker brood rearing pattern was relatively normal.

The current work was carried out to evaluate the effect of bee venom as a natural product compared with an acaricide (fluvalinate) in controlling *Varroa* mites in honeybee colonies. In addition, effect of these treatments on worker brood rearing activity was noticed.

MATERIALS AND METHODS

Location

This experimental period was carried out at the apiary of honeybee research center, Faculty of Environmental Agricultural Sciences, El-Arish University, Egypt during August and October of the year2020 to evaluate the efficacy of bee venom, compared to an acaricide (fluvalinate strip) in controlling *Varroa* mites infesting honeybee colonies. In addition, the effect of these treatments on brood rearing activity was observed.

Treatments

Twenty-seven naturally infested of hybrid Carniolan, *A. m. carnica*, honeybee colonies approximately similar in their strength were used. These colonies were divided into three groups (each of nine colonies). The 1st two groups were treated, while the 3rd one was control. Treatments were as the following:

A) First group: were divided into three subgroups (three colonies/subgroup). Each subgroup was fed weekly with bee venom (one concentration/subgroup) dissolved in1L sucrose syrup, *i.e*.0.1, 0.01 and 0.001 g/L bee venom, respectively.

B) Second group: were treated with an acaricide, fluvalinate strips (recommended apistan strips) replaced every 20 days.

C) Third group: were left untreated (control).

All experimented colonies received sugar syrup (50%).

Efficacy Evaluation of Tested Materials

Percentages of effectiveness of the tested materials against *Varroa* mite were determined according to the formula adopted by **Matinez (1989)** as follow:

$$\text{Effectiveness (\%)} = \frac{Ft - Fc}{Ft} \times 100$$

Where:

Ft: Final count of the fallen mites in the treatment.

Fc: Final count of the fallen mites in control.

Effect of Treatment on Brood Rearing Activity

The number of sealed worker brood were recorded every 12-day intervals in all colonies by using a graduated grid divided into square inches after the honeybees had been shaken from combs **(Jeffree, 1958)**.

Statistical Analysis

Obtained data were recorded and mean values of different treatments were compared by LSD test at 0.05 significance, using **SAS program (2004)**.

RESULTS AND DISCUSSION

Effect of Treatments on *Varroa* Mites

Results on adult bee before treatment are reported in Table 1 show no significant differences between the five experimental groups. The average trends (mean ± SD) of fallen mites counted weekly at the bottom of the hives are reported in Table 1. During the first week after treatment, the number of fallen mites was significantly higher in the treated hives than in the control ones where, the highest number of fallen varroa mites was (92.7 mites) after using fluvalinate strips followed by using bee venom (0.1g) in feeding on sugar solution(60.3mites), bee venom (0.01g) in feeding on sugar solution (46mites) while bee venom (0.001g) in feeding on sugar solution (28,3 mites) and the least number of fallen varroa was recorded in untreated colony (7,3 mites).

Results presented in Table 1 clear that the mean calculated percentage of extermination of the tested materials attained 75.01, 65.55, 49.32 and 80.45% in Carniolan hybrid colonies treated with Bee Venom (.1), Bee Venom (.01), Bee Venom (.001) and Fluvalenate strips, respectively. The results showed that the bee venom is a promising product for controlling *Varroa* mites. it has many advantages easy to use, safe for beekeepers without any side effects, it also causes no honeybee toxicity, no loss of queen either brood or adults, therefore they can be used safely.

Effect of Treatments on Brood and Adult Worker Bees

From the results obtained in Table 2, during the first week after treatment, the highest area of honeybee worker sealed brood was recorded when using fluvalinate strips (5817 cells), followed by(4830cells) when using bee venom (0.1g). However, for the 2nd, 3rd and 4th week after treatment, the highest area of workers sealed brood recorded when using bee venom (0,1 mg) in feeding on sugar solution (5441 cells), (5857 cells) and (6215 cells) respectively. The least area of workers sealed brood was found when using fluvalinate strips in 4th week (3607 cells).

In general, high concentrations of bee venom affected on the number of *Varroa* mites compared to low concentrations. **Mohanny (2010)** evaluated the effect of some honey bee products for controlling *Varroa* mite on adult worker bees and in sealed worker brood. The products were honey solution, bee venom solution, royal jelly solution and honey bee larval extract. It was found that the reduction percentages in *Varroa* mite on adult bees were: 79.38, 74.15, 73.75and 66.54 while the reduction percentages in *Varroa* mite in sealed worker brood were: 90.07, 89.83, 84.61 and 74.08% for the previous materials, resp.

Naglaa *et al.* (2020) reported that the bee venom solution gave the highest values of fallen varroa mite followed by (Formic acid and negative control), respectively in sealed brood and adult. The worker brood rearing activity after the treating with bee venom solution had a major peak of rearing in next summer and spring (653.7 inch^2/ col.) while the spring season (544.2 inch^2/ col.). The general adult population mean of workers recorded for the treated colonies were (25700 worker/colony). The re-use of Apistan® strips would be justified **(Pechhacker, 1991).** However, the assessment that the fluvalinate content of the strips does not decrease over a few weeks is not enough to justify the reuse of Apistan® strips: in particular, one should check that the release of the active ingredient does not change over time. Application period (strips permanence in hive) must be restricted to the minimum necessary (4 weeks) to reduce excessive toxicological and biological risks from residual accumulation **(Moosbeckhofer, 1991). Gregorc *et al.* (2018)** evaluated the acaricides like coumaphos, tau-fluvalinate,

Table 1. Weekly fallen *Varroa* mites due to different treatments in honeybee colonies

Inspection week	Treatment				
	Bee venom concentration (g/L)			Fluvalinate	Control
	0.1	0.01	0.001		
Before treatments					
R1	4	12	11	3	7
R2	9	8	14	12	6
R3	7	5	6	7	11
mean ± SD	6.7± 2.5	8.3±3.5	10.3±4.1	7.3±4.5	8±2.6
F (P)			0.445(0.774) NS		
1st week after treatments					
R1	71	37	15	96	6
R2	63	58	50	103	5
R3	47	43	20	79	11
mean ± SD	60.3^{b}± 12.2	46^{b}±10.8	28.3^{c}±18.9	92.7^{a}±12.3	7.3^{d}±3.2
F (P)			27.226 (0.0001)		
LSD			16.306		
2nd week					
R1	49	50	20	67	3
R2	42	40	11	79	9
R3	33	15	25	71	8
mean ± SD	41.3±14.1	35±18.1	18.7±7.1	72.3±6.1	6.7±3.2
F (P)			18.248 (0.0004)		
LSD			15.402		
3rd week					
R1	23	22	16	59	7
R2	31	20	17	47	8
R3	38	15	12	55	6
mean ± SD	30.7±9.7	19±3.6	15±2.6	53.7±6.1	7±1
F (P)			34.636 (0.004)		
LSD			8.099		
4th week					
R1	21	15	5	14	12
R2	28	14	11	9	1
R3	30	11	20	21	4
mean ± SD	26.3±9.9	13.3±2.1	12±7.5	14.7±6.2	5.7±5.6
F (P)			5.542 (0.02)		
LSD			8.386		
5th week					
R1	15	12	6	8	2
R2	18	10	5	4	8
R3	22	9	11	6	6
mean ± SD	18.3±5.7	10.3±1.5	7.3±3.2	6±2	5.3±3.1
F (P)			10.775 (0.003)		
LSD			4.26		
6th week					
R1	9	11	6	5	4
R2	10	9	5	4	7
R3	11	7	4	2	2
mean ± SD	10±2.9	9±2	5±1	3.7±1.5	4.3±2.5
F (P)			10.821 (003)		
LSD			2.311		
7th week					
R1	4	5	5	2	6
R2	5	5	4	3	5
R3	2	3	5	2	6
mean ± SD	3.7±1.4	4.3±1.1	4.7±0.7	2.3±0.5	5.7±0.6
F (P)			5.111 (0.02)		
LSD			1.44		
8th week					
R1	3	5	4	1	7
R2	2	3	4	2	8
R3	3	2	5	2	4
mean ± SD	2.7±0.7	3.3±1.5	4.3±0.6	1.7±0.5	6.3±2.1
F (P)			6 (0.015)		
LSD			1.93		
Extermination%	75.01	65.55	49.32	80.45	

R = replicate.

Table 2. Effect of controlling of *Varroa* mites with different treatments on sealed worker brood

Inspection week	Treatment				
	Bee venom concentration (g/L)			Fluvalinate	Control
	.1	.01	.001		
	Before Treatments.				
R1	3806	2783	3996	3724	4028
R2	4788	3986	3788	4589	3103
R3	4246	3323	4246	3147	4782
mean ± SD	4280±492	3364±602	4010±229	3820±726	3971±841
F(P)			F0.805(0.555)		
LSD			LSD329		
	1st week after treatments				
R1	4940	3884	3624	5612	3992
R2	5322	4187	4289	6496	4496
R3	4228	3422	3448	5343	3545
mean ± SD	4830±555	3831±385	3787±443	5817±603	4011±475
F(P)			F110.474(0.0001)		
LSD			LSD 218		
	2nd week				
R1	6130	5022	4224	5040	4294
R2	5361	3740	4489	5216	4580
R3	4832	4282	3647	3832	3774
mean ± SD	5441±652	4348±643	4120±430	4696±753	4216±408
F(P)			F4.491(0.033)		
LSD			LSD 666		
	3rd week				
R1	6440	5642	4624	3983	4762
R2	5851	5158	4389	4985	4458
R3	5280	4764	3245	3320	3314
mean ± SD	5857±580	5188±439	4086±737	4096±838	4178±763
F(P)			F 14.868(0.0009)		
LSD			LSD 549		
	4th week				
R1	6604	6184	3924	3083	3673
R2	6216	5582	4289	4186	4155
R3	5825	4986	3646	3552	3614
mean ± SD	6215±389	5584±599	3953±322	3607±553	3814±296
F(P)			F 27.846(0.0001)		
LSD			LSD589		

R = replicate.

amitraz, thymol, and natural plant compounds (hop acids) to control the *Varroa* mite, *Varroa destructor*, of honey bees, in two different settings. All of the tested acaricides significantly increased the overall *Varroa* mortality in the laboratory experiment. a higher *Varroa* mortality was recorded in all of the treatments, compared with the natural *Varroa* mortality during the pretreatment period. tau-Fluvalinate is a pyrethroid acaricide (Apistan®) and is an agonist of the voltage-gatedsodium channel in *varroa* mites. tau-Fluvalinate will bind to the sodium channel and cause adelay in sodium channel closing, and thus result in prolonged sodium inactivation **(Yu, 2008).** tau-Fluvalinate was initially successful at controlling the *Varroa* mite, but its efficacy decreased due to widespread resistance in *Varroa* mite populations worldwide (**Elzen *et al.*, 1999**). Tau-Fluvalinate resistance was established through mutations in the voltage-gated sodium channel, leading to a reduction in the binding ability of the acaricide (**Wang *et al.*, 2003)**. Tau-Fluvalinate is the active ingredient (a.i., 10.0%) in the acaricide product Apistan®, the first product in the U.S. registered in 1990 for use in honey bee colonies to manage *Varroa* mites **(Bogdanov *et al.*, 1998).** Apistan® plastic strips are suspended between frames for 6 to 8 weeks in the hive. The bees come in contact with the strip and acaricide, which is distributed throughout the colony. *Varroa* mites on the bees are then exposed to the acaricide, leading to paralysis **(Johnson *et al.*, 2010).**

These results strongly support bee keepers for using honey bee products like bee venom as a labor-efficient and non – toxic treatment for the control of *Varro*mite.

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الملخص العربي

تقييم دور سم نحل العسل *Apismellifera* L. في مكافحة طفيل الفاروا *(Anderson & Trueman)*

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تم تنفيذ هذا العمل في منحل مركز بحوث نحل العسل بكلية العلوم الزراعية البيئية، جامعة العريش، مصر خلال شهر أغسطس وحتى أكتوبر من عام 2020 لتقييم فاعلية سم النحل (كأحد منتجات النحل) بتركيزات مختلفة (0.01،0.1، 0.001 جم)، مقارنة بالمبيد الكيميائي (شريط الفلوفالينات) في مكافحة طفيل الفاروا. بالإضافة إلى تأثير هذه المعاملات على نشاط تربية الحضنة المختومة. تظهر النتائجبأن عدد أكاروس الفاروا المتساقط أسبوعياً على أرضية خلايا النحل كان 92.7 طفيلاً بعد استخدام شرائط الفلوفالينات. من ناحية أخرى، فإن استخدام سم النحل في المحلول السكري، بتركيزات 0.1 و0.01 و0.001 جم/1 لتر من محلول السكر تساقط 60.3 و46 و28.3 طفيلاً على التوالي. الطوائف الغير معالجة سجلت نسبة تساقط 7.3 طفيلاً. تم تسجيل أكبر عدد من حضنه الشغالات عند استخدام تركيز 0.1 جم من السم والتي كانت 5441 و5857 و6215 خلية للأسبوع الثاني والثالث والرابع (كل 12 يومًا) بعد العلاج على التوالي. تم تسجيل أقل عدد من حضنه الشغالات المختومة عند استخدام شرائح الفلوفالينات في الأسبوع الرابع (3607 خلية).

الكلمات الاسترشادية: نحل العسل، سم النحل، طفيل الفاروا، الكنترول.

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