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Effect of Bee Venom Collecting on The Behavior of Honeybee Colonies

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ABSTRACT

The present investigation studied bee venom collecting effect on the behavior of honeybee colonies such as (Hygienic and hoarding honeybees behavior, queen right and queen less). Hygienic behavior, the positive effect of using bee venom collection method on hygienic behavior, that increased this behavior (22%) of honey bee worker cells, *Apis mellifera,*while hoarding behavior in honey bees, *Apis mellifera* L., statistical analysis that there was no significant difference between before and after treatment in four colonies. on the other hand, there was an increase in feed conception after treatment, it that seems to the alarming or stimulation of worker honey bee by electrical impulses from bee venom collector so, it increasing the worker hoarding behavior. The queen less had negative effect on venom quantity, and there was positive relation between the number of combs and venom quantity. The relationship between certain characters of honey bee colonies (i.e. stored pollen, stored honey yield and areas, bee population, brood, and foraging activities) and bee venom produced by electrical impulses and quantity of bee venom which collected from colonies variability at different periods of active season showed significant variations in the venom amounts collected at different periods of active season.

INTRODUCTION

The venom produced in the worker bees abdomen is a mixture of acidic and basic secretions with pH 4.5 to 5.5. Apitoxin, or honey bee venom, is a bitter colorless liquid. local inflammation caused by The active portion of the venom which is a complex mixture of proteins, and acts as an anticoagulant.

Hygienic honeybees, *Apis mellifera* L., have the ability to detect, uncap, and remove diseased brood from their nest before the causative organism reaches the infectious stage (Rothenbubler 1964 b)

Rothenbuhler, (1964 a,b,) states that on a colony level, hygienic behavior of adult bees toward infected larvae is the most important AFB resistance mechanism and also reviewed in (Spivak and Gilliam 1998 a,b). Worker bees that demonstrate this behavior rapidly detect, uncap, and remove infected brood from the nest. The spore or infectious stage of the bacterium appears at approximately 10–11 days after egg-hatching, when the prepupae are developing within the 5th instar cuticle under a wax capped cell. Sporulation is accompanied by death of the prepupae (Hansen and Brodsgaard, 1999). When the bacterium is in the vegetative, non-infectious rod stage; Hygienic bees uncap and remove larvae under capped cells i.e., before the prepupae dies and before the sporulation of bacteria in hemocoel, (Woodrow and Holst, 1942). So, the colony may be infected but the infected brood was removed by the hygienic bees before disease visibility. (Marla and Gary 2001)

Hoarding behavior in honey bees, *Apis mellifera* L., has been explored in laboratory cages (Kulincevic and Rothenbuhler 1973) and honey production measurements from field colonies were significantly correlated with such laboratory tests (Kulincevic et al. 1974). Work at our laboratory (Rinderer, unpublished data) suggested that a greater surface area of comb available to bees in laboratory cages resulted in a greater rate of hoarding. The experiments reported here were designed to further explore this effect of empty comb on bees in laboratory cages and also to explore the effect of empty comb on the behavior of field colonies.

When queens are lost from colonies, worker develops ovaries. However, virtually nothing is known about how nutrients are transformed among colony members and the influence this has on worker ovarian development. Examining the role of predigested food and the social interactions between nurse workers and other colony members with respect to the reproductive physiology of queenless workers should reveal the significance of nurse bees as mediators of worker reproduction in honey bee colonies. (Huarong Lin ,1999)

MATERIALS AND METHODS

The present investigation was carried out at the apiary of the Department of beekeeping research, Plant protection Res. Inst., Agric. Res. Cent., Minist. of Agric., Egypt, under the environmental conditions of Giza region during 2013-2014.

1. Bee venom collector device

The following treatments were collected bee venom from colonies by means bee venom collector devices.

Characters of the bee venom device are (Electric shock device, VC-Starter kit)

- Input Voltage:11.5-13.5VDC - Timer ON:0.5 - 2 sec.-
- Timer-OF:3 -5 sec. - Collector Frames:40cm x50 cm
- Operation Mode:semiautomatic - Temperature:-5 C˚to 40 C˚
- Humidity (max): 95% at40 C˚ - Max operating time: 8 hours

2. Effect of bee venom collecting on hygienic behavior.

A- Preparation of Honey bee colonies for hygienic experimental

The assess hygienic behavior, four honey bee colonies from carniolian race were assigned for this study.

Sammataro (1996) recorded The brood removal percentages in each colony after 8 hr in an area (2.5 x 2.5 cm) of centered sealed brood worker (100 cell/one comb/colony) were bordered and killed by piercing a fine wooden pin into each cell and then the comb was replaced to its hive. The number of removing brood per 100 randomized cells were recoded in treated and untreated (control) honey bee colonies and the % of

Increasing in hygienic behavior percentages for each treated colony were recorded according to the formulation as follows:

% increasing in hygienic behavior = 100 - (No. of removed brood before application / No. of removed brood after application x 100).

3. Effect of bee venom collecting on honeybee hoarding behavior.

The honey bee colonies were fed add libitum with water and honey syrup (50%) in plastic feeders; 1200 ml of honey syrup were added to the feeders. The amount of syrup taken from the feeder were recorded at every hour interval for 8 hours before and after treatment with bee venom collector device, The metabolic sugar consumption of the group was estimated by subtracting the amount consumed from the total honey syrup taken from the feeder (Moritz and Hillesheim 1989).

4. Effect of queen right and queen less on bee venom collecting.

Nine colonies Carniolan, *Apis mellifera* L., were divided into three groups of three colonies each as follows:

Group 1. Each colony with four brood combs.
Group 2. Each colony with five brood combs.
Group 3. Each colony with six brood combs.

Each colony had a queen (queen right) were treated by bee venom collector device to collecte the bee venom and recorded the weight in mg, after 15 days the queens of the colonies were removed (queenless) from the colonies, after 1 day the colonies were treated by bee venom collector device to collected the bee venom and was recorded and weight in mg.

Statistical analysis

Data of all treatments were analyzed in a randomized complete block design (ANOVA) by MSTAT-C version 1.41 (Sendecor and Cochran, 1980). And using graph pad prisma version 3.03 for windows, software. All means were compared by Duncan's multiple range test at level 0.05.

RESULTS AND DISCUSSION

1. Effect of collected bee venom on hygienic behavior

Table (1) showed that the positive effect of using bee venom collection method on hygienic behavior, that increased this behavior (22%) of honey bee *Apis mellifera* L. worker cells, The artificially killed brood (pin-killed) from wax cells by adult workers in the experimental colonies were removed outside the hives which is considered as an indicator of hygienic behavior. On other hand the statically analysis clearly showed a significant differences between before ad after treatment of colony. the mean number of cleaned dead brood cell in 100 wax cells before treatments ranged between 45 and 78 with mean 65.3 .These numbers were highly significantly increased after treatment and ranged from 76 to 100 with mean 84.0 / 100 brood cells. The hygienic behavior may be positive reflect to increase the defensive and resistant of honey bee workers against some pests and diseases like American foulbrood, chalk brood and Varroa mites.

Table (1) The mean number of removed dead brood cells in 100 randomized brood cells found in the experimental honey bee colonies before and after treatment with bee venom collecting method

No. hive	Before treatment	After treatment	% Increasing in the hygienic behavior
1	78	100	22.0
2	73	100	27.0
3	45	60	25.0
4	65	76	14.4
Mean	65.25^{b}	84.00^{a}	22.0
$LSD_{0.05}$	9.014		

This means that in honeybee, mechanism the first defense against at least two diseases for example AFB and chalk brood is the hygienic behavior. Hygienic behaviors of workers differentiate and removal of the infected brood before pathogen sporulation. According to (Rothenbuhler, l964 a,b). For responsibility of this behavior two variable genes were suggest; one for cells uncaps and the other gene one for diseased brood removing. Many genetic factors responsible for this complicated behavior were reported and expressed in a phenotype (Mortiz 1988). On the other hand, these bees remove Varroa mites of infected brood and stop reproductive cycle and kill immature stages (Fries *et a1*., 1994). (Gabriela, 2010) found that, Apis meltifera carpatica colonies remove worker brood infested with Varroa destructor mites from the nest (hygienic behavior), and groom the mites off themselves fiom other adult bees (grooming behavior) after using oxalic acid.

Al-Medani (2004) reported that *A.mellifera jemenitica* 85.5% of dead brood was removed during 48 h. diseased brood Sensitivity to odors is increased in bees' exhibit hygienic behavior. Modulatory effect of octopamine, noradrenalin-like neuromodulator enhanced this mechanism. (Palacio *et al*., 1996 & Spivak and Downey, 1998) reported that honeybees removed pierced (pin-killed) brood faster than frozen brood.

Hygienic honeybees, *Apis mellifera* L., have the ability to detect, uncap, and remove diseased brood from their nest before the causative organism reaches the infectious stage (Rothenbuhler, 1964b).

2. Effect of bee venom collecting on honeybee hoarding behavior.

Data in Table (2) showed that the feed intake of sugar syrup before and after treated colonies by bee venom collector, it was obvious from statistical analysis that there was no significant difference between before and after treatment in four colonies. on the other hand, there was an increase in feed conception after treatment, it that seems to the alarming or stimulation of worker honey bee by electrical impulses from bee venom collector so, it increasing the worker hoarding behavior, in addition in collecting treatment the worker honey bee secreted protein (venom) from there body and needed to compensation this by increasing the hoarding behavior.

Table (2) Effect of bee venom collector before and after treatment on hoarding behavior of honey bee workers (ml syrup/hive/1hr).

No.	1 hr.		2 hr.		3 hr.		4 hr.		5hr.		6 hr.	
	before	after	Before	after	before	after	before	after	before	after	before	After
1	33	40	35	30	60	78	55	63	47	54	40	46
%	17	20	18	15	30	39	28	32	24	27	20	23
2	38	25	30	22	40	54	45	40	53	66	60	50
%	19	13	15	11	20	27	23	20	27	38	30	25
3	40	54	45	65	55	67	60	74	53	69	40	63
%	20	27	23	33	28	34	30	37	27	39	20	32
4	38	65	40	72	60	68	45	78	60	78	55	67
%	19	33	20	36	30	34	23	39	30	39	28	34
Mean	37.2	46.0	37.5	47.2	53.7	66.7	51.2	63.7	53.2	71.5	48.7	56.5
Mean %	19	23	19	24	27	33	26	32	27	36	24	28
F	0.989		0.573		3.624		1.800		5.762		1.155	
P	0.358		0.477		0.105		0.228		0.053		0.323	
	Ns		ns		ns		ns		ns		Ns	
$LSD_{0.05}$	21.52		31.49		16.7		22.79		13.76		17.641	

Hoarding behavior is not only aspect of bee behavior that effects the production of a honey, crop, but also include length of life, rat of broad rearing and disease resistance (Kulincevic and Rothenbuhler 1973), Found the number of days required by the bees to remove 20ml sugar syrup ranged from (4.3-13.0 days); shows substantial variation between colonies.

A likely function of hoarding efficiency was clear in context of seasonally varying nectar availability (Crane, 1975 and Rinderer, 1982). there are many research discussed the hoarding behavior (Rinderer, and Baxter, 1980) In laboratory cages, European bees hoarded more sucrose solution, and did so with greater efficiency than Africanized bees, additional empty combs caused both Africanized and European bees to increase both their hoarding intensities and efficiencies, the proportional increase in hoarding intensity was some what less for the africanized bees and the proportional increase in efficiency was similar for Africanized and European bees and differential hoarding rates and differential hoarding efficiencies was occurred between geographical types, European bees hoarded more sucrose solution with greater efficiency. Additional empty combs caused in both Africanized and European bees to increase both their hoarding intensities and efficiencies.

Empty combs in honeybee (*Apis mellifera*, L.) nests strongly influence the nectar harvesting of bees (Rinderer, 1981& Rinderer and Abxter 1978, 1980). (Free *et al.*, 1972; Klincevic and Rothenbuhler, 1973) showed increased hoarding intensities (rates) in laboratory cages and increased intensities of foraging activities of bees in field conditions caused by increased amounts of empty combs.

Honey yield measured throughout the entire season was compared to the foraging and hoarding efficiency (sugar syrup/candy) in Buckfast (Bcf) and in Norwegian Black Bee queens x Caucasian drone hybrids (Nor x Cau) using both field and laboratory cage tests. Artificially made nucleus colonies were tested during the field tests under flying cages. Nor x Cau does not seem to be overly sensitive to adverse weather conditions. Bcf bees were efficient both in good and in worse weather and they represent more efficient foraging/hoarding behaviour. Nor x Cau, which may be encountered in the Lublin region did not prove to be a good combination. Results of the cage tests correspond to the honey yield measured in the full size colonies. The amount of syrup foraged in the test under the flying cages corresponded to the amount of the stored syrup supplies. Therefore combining these two tests results in the proper assessment of the hoarding behaviour. Krzysztofolszewski (2005).

The principal of these results is demonstration of colony shortage of carbohydrate stores can affect temporal polyethism in honey bee colonies, but not because worker sense the shortage directly, via worker-nest interactions.

Previous studies have demonstrated a short-term effect of colony food stores on behavioral regulation, as did the present study. Free (1955) showed that the addition of food to storage pots of bumble bees reduced the number of foraging trips made by committed foragers. On Converse, Rinderer and Baxter (1978) stated that hoarding behavior was increased by addition of empty comb to honey bee colonies, and rate of pollen collection increased by depletion of pollen stores in honey bee colonies by competent foragers (Fewell and Winston 1992; Camazine 1993).

Also, significant variations in the collected amounts of venom at different periods of active season were determined by Khodairy and Omar, 2003. they also found that the venom amounts were high in June compared with amounts collected in May and July, they finally proved that the correlations between venom production and each of bee brood, population, stored pollen and honey and foraging activities were positive.

3. Effect of queen right and queenless on bee venom collecting.

Table (3) showed that effect of bee venom collector treatment on queen rate colonies and queenless colonies in different strength (number of brood comb) it is clearly showed that queen less had negative effect on venom quantity, and there was positive relation between the number of combs and venom quantity. It's obvious that queen lees made up normal condition in hives that reflects on worker behavior, especially defense behavior.

Table (3) Effect of bee venom collector on queen right and queen lees colonies.

	No. of combs	Queen	Queen less	F	P	LSD 0.05	Sig.
		١٠٠	٢٢.٥				
		١١٠	١٨.٠				
١	٤	٩٠	٢٨.٠	142.81	٠.٠٠٠٣	١٧.٩٢	***
		100 a B	22.8a C				
		١٨٠	٩٣				
		٢١٠	١١٠				
٢	٥	١٦٥	٧٩	٣٢.٤٣	٠.٠٠٤٧	٤٤.٣٦	**
		185 a A	94 b B				
		٢٢٠	١٥٤				
		٢١٠	١٤٠				
٣	٦	١٨٠	١٦٠	١٥.٠٥٩	٠.٠١٧٨	٣٧.٢٠	*
		203.3 aA	151.bA				
F		٢٥.٨٥	١٠٠.٤١				
P		٠.٠١١	٠.٠٠٠				
LSD 0.05		٣٧.٥٢	٢٢.٢٣٠				
Sig.		**	***				

Workers could receive nutrients during ritualized trophallaxis (Sakagarmi, 1954; Korst and Velthuis, l982) or possibly from other nestmates in a colony to develop their ovaries and become egg-layers (Velthuis, 1985). After dominant workers become egg-layen, they maintain their dominance hierarchy through pheromones (Sakagami,, 1958; Ayasse *et al.,* 1998). When a queen is lost from a colony, a dominance hierarchy is reestablished among the workers of the queenless group by means of intensive agonistic behavior (Korst and Velthuis. 1982; Liebig and Hodlldobler, 1997). Aggressive behavior *is* associated with trophallatic react ions in most hymenoptera species, with the aggressive dominant workers which often forcing submissive worker to offer regurgitated food.

Because nurses are the food processor and suppliers, they could play an important role in mediating worker fertility *via* food trophallaxis in these situations, possibly by differentiating worker dominance status since workers are able to recognize development of nestmates ovaries (Visscher and Dukas. 1995). My results indicate thatyoung workers with higher dominance status in a queenless colony would receive .rkersroyal jelly from other wo. (Huarong, 1999).

Test colonies were compared for stinging behavior by counting the number of stings on red suede-leather patches that were dragged lightly across the tops of the exposed frames. A long rod with a clip at one end was used to hold the patches, each of which was 5 cm square. At time 0 the hive lid was removed without using smoke. At 10 s an unused patch was dragged front to back over the frames, and then back to front, then twice side-to-side. The time for each 'pass' was 5 s and the 20-s sequence was repeated three times to give the patch a 60-s exposure to the bees. This test was used only for trials 3, 4 and 5. For each trial the test was done at least twice during the last week of the experiment. The total count of stings for each colony was used in the analysis of defence behaviour. Keith *et al*., (1987)

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تأثير جمع سم النحل على سلوك طوائف نحل العسل

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تم إجراء هذه التجارب في منحل قسم بحوث النحل – معهد بحوث وقاية النباتات بالدقي مركز البحوث الزراعية بالجيزة خلال ٢٠١٣-٢٠١٤ وكان الهدف من الدراسة هو دراسة تاثير جمع سم النحل من طوائف نحل العسل وتاثير ذلك على بعض سلوكيات نحل العسل مثل (تاثيرجمع السم على نشاط الطائفه في تنظيف الحضنه الميته ومعدلات سحب التغذيه وتخزينها بالاضافة الي تاثير وجود ملكة النحل على كمية السم المنتجه تم إستخدام جهاز(Electric shock device, VC-Starter kit) لتجميع كميات السم من الطوائف المختارة اثناء مواسم النشاط. بالنسبة لدراسة تاثيرجمع السم على نشاط الطائفه في تنظيف الحضنه الميته تم تحديد ٤ خلايا متساوية القوة (٤ أقراص حضنة) واختيار قرص حضنة مغلقة من كل خلية وباستخدام دبوس تم قتل ١٠٠ عين سداسية بكل قرص حضنة (حيث تم تحديدها باربع دبابيس) وبعد مرور ٨ ساعات تم قياس النتائج وكررت التجربة في اليوم التالي بعد جمع السم منها وأثبتت النتائج المتحصل عليها ان هناك تحسن ملحوظ في سلوك التنظيف بعد جمع السم من الطائفة. دراسة تاثيرجمع السم على معدلات سحب التغذيه وتخزينها بإستخدام ٤ خلايا متساوية القوة (٤ أقراص حضنة) غذيت ب ١٢٠٠ ملليتر محلول سكري حيث يتم قياس كمية التغذية السكرية المسحوبة بواسطة نحل العسل كل ساعة لمدة ٦ ساعات وذلك قبل جمع السم من هذه الطوائف وتم تكرار نفس التجربة في اليوم التالي بعد جمع سم النحل منها وأثبتت النتائج المتحصل عليها أن جمع السم من الطوائف يزيد معدل سحب التغذية السكرية وتخزينها بدرجة ملحوظة. دراسة تاثير وجود ملكة النحل على كمية السم المنتجه حيث حددت مجموعة مكونة من 3 طوائف نحل العسل وتم جمع السم منها في وجود الملكة وبعد 15 يوم تم جمع السم منها بعد تيتمها بيوم وأثبتت النتائج المتحصل عليها أن أكبر كمية من السم المجموع يمكن الحصول عليها من الطوائف التي تحتوي علي ملكات وبفارق معنوي عن الطوائف اليتيمة عديمة الملكات.