Fayoum Journal of Agricultural Research and Development
ISSN:1110- 7790

EFFECT OF VENOM COLLECTION ON HYGIENIC AND HOARDING BEHAVIOR IN HONEYBEE

Fatma. H. Merzban[2]*, Abd El-Haleem M. Ismail[1] and Atef, A. Abd El-Gayed[1]
[1]Plant Protection Department, Faculty of Agriculture, Fayoum University
[2]Plant Protection Research Institute, Agricultural Research Center, Giza

ABSTRACT
The present experiment was carried out at the apiary of the Bee Research Dept., Plant Protection Res. Inst., Agric. Res. Cent., Ministry Agric., Dokki, Giza, Egypt in August 2018 to study the effect of venom collection on hygienic and hoarding behavior in two honeybee races; *i.e.*, carniolan, *A.m. carnica* and Italian, *A.m. ligustica*.
Results indicated that hygienic behavior significantly increased ($P < 0.05$) after bee venom collection than before collection in both races and there were insignificant differences between the two races in all tested intervals. While hoarding behavior significantly decreased after bee venom collection than before collection in both races and there were insignificant differences between the two races in all tested intervals.

Key words: Honeybee, Venom collection, hygienic and hoarding behavior

INTRODUCTION
Bee venom (BV) is a transparent liquid that dries up easily even at room temperature forming grayish-white crystals that has an ornamental pungent smell. Bee venom is soluble in water and insoluble in alcohol and ammonium sulfate. Dried venom takes on a light yellow color and some commercial preparations are brown, which is thought to be due to oxidation of some proteins in the venom **Owen and Sloley, 1988.**
BV is a valuable product for the pharmaceutical industry, and its production and its place in the market are expected to be of great concern. It's composition is complex, *i.e.* a mixture of active peptides (melittin, adolapin, apamin, mast cell-degranulating peptide (MCD), secapin), enzymes (phospholipase A2 and B, hyaluronidase, phosphatase and α-glucosidase), biogenic amines (histamine, dopamine, noradrenalin), amino acids (aminobutyric and a-amino acids), phospholipids, sugars, volatiles, minerals and other components. These components have variety of pharmaceutical and therapeutical properties against certain diseases, *e.g.* arthritis, chronic pain, multiple sclerosis, and some types of cancer. It is also widely used in cosmetology **Argena et al., 2021**.

* Corresponding outhor: Fatma. H. Merzban.
Received29/9/ 2021
Accepted:10/10/ 2021

BV content varies substantially according to internal factors related to the bee itself, including age, strain and caste, and external factors, such as seasons and methods of BV collection **Abd El-Wahed, 2018.**

Hygienic behavior is a key defense mechanism in honeybees. This behavior is carried out by workers against various brood diseases, such as the bacteria *Paenibacillus larvae* , the chalkbrood fungus *Ascosphaera apis* and the mite *Varroa destructor*. Hygienic behavior involves the detection by worker honeybees of dead or infected brood, followed by the uncapping of the wax cell and the removal of the larva or pupa **Pérez-Sato et al., 2009**.

Worker bees that demonstrate hygienic 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 **Spivak and Gilliam, 1998.**

The first reaction to electric shock that was noticed was that after venom extraction the bees became ravenously hungry. They avidly devoured large quantities of honey **Palmer, 1961.** Greater surface area of comb available to bees, in laboratory cages, resulted in a great rate of hoarding (Rinderer and Baxter, 1980). Hoarding behavior in honey bees, *A.mellifera*, has been explored in laboratory cages **Spivak and Reuter, 2001**.

The present study aims to study the effect of venom collection on hygienic and hoarding behavior of bee colonies.

MATERIALS AND METHODS

1. Effect of venom collection on hygienic behavior

To assess hygienic behavior, eight honey bee colonies of Carniolian and Italian races (four colonies/ race) were compared on August of 2018. Colonies were prepared earlier to be ready for the experiment. An area (2.5 × 2.5 inch) of centered capped worker brood (*ca.* 100 cells/comb/colony) was bordered and killed by piercing a fine wooden pin into each cell. Then such comb was returned to its relevant colony. The percentages (%) of brood removed in each colony were recorded throughout eight hours. The number of removed brood/100 cells was recorded every two hours before and after collection of venom in honey bee colonies. This experiment was carried out during two days. The hygienic behavior percentages for each treated colony were obtained according to the formulation given by **El-Saeady et al. 2016** as the following:

$$\%\ \text{increasing in hygienic behavior} = \frac{No.\ of\ removed\ brood\ before\ application}{No.\ of\ removed\ brood\ after\ application} \times 100$$

To test syrup hoarding behavior, colonies of Carniolan and Italian races (four colonies/ race) were experimented on August of 2018. Such colonies were prepared earlier to be ready for this test.

Colonies were fed water and sucrose solution (50% v: w) in plastic frame feeders. One liter of sucrose solution/colony was added in each feeder. The amount of syrup taken from the feeder was recorded every two hours. The experiment was done in two subsequent days. The consumption was estimated by subtracting the amount consumed of the total sucrose solution volume **El-Saeady et al., 2016.**

RESULTS AND DISCUSSION

1.Effect of venom collection on hygienic behavior

1.1.Effect of venom collection on hygienic behavior of Italian colonies

Before venom collection, there was significant increase in hygienic behavior every two hours interval (after pin-killing) to the tested period (eight hours). Also, there was significant increase between the 1st two hours and each of the 4th, 6th, and 8th hour intervals. Mean values of cleaned dead brood cells increased from 8 cells on the 2nd hour interval to 29 cells on the 8th hour one. After venom collection, there was significant increase in hygienic behavior (after pin-killing) every two hours interval to the 8th hour during the tested period (eight hours). Also, there was significant increase between the1st two hours and each of the 4th, 6th, and 8th hour intervals. Mean values of cleaned dead brood cells increased from 38 cells on the 2nd hour to 49 cells on the 8th hour intervals.

Hygienic behavior after 4h collection was significantly higher than that before 4h collection (increased from 15 to 43 cells, respectively). Hygienic behavior after 6h collection was significantly higher than that before 6h (increased from 20 to 48 cells, respectively).

Hygienic behavior significantly ($P < 0.05$) increased after venom collection than before (increased from 29 cells at the 8th hour before collection to 49 cells at the 8th hour after) (Figure 1)

.

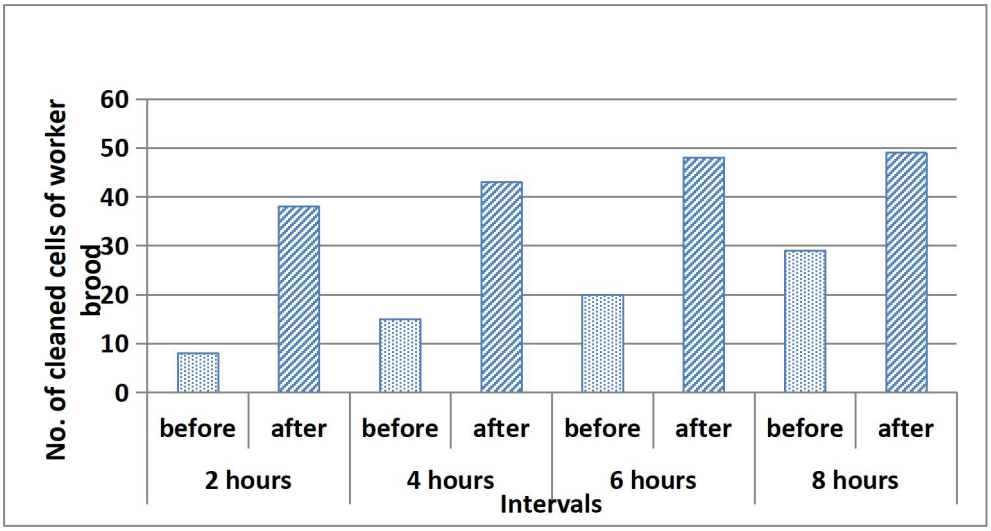

Figure1: Comparison of hygienic behavior before and after venom collection in Italian colonies.

1.2.Effect of venom collection on hygienic behavior of Carniolan colonies

Before venom collection, there was significant increase in hygienic behavior every two hour intervals (after pin-killing) to the tested period (eight hours) as the same trend noticed before. Also, there was significant increase between the 1st two hours and each of the 4th, 6th, and 8th hour intervals. Mean values of cleaned dead brood cells increased from 10 in the 2nd hour to 25 cells in the 8th hour intervals, respectively.

After venom collection, there was significant increase in hygienic behavior every two hours to the 8th hour intervals (after pin-killing) to the tested period (eight hours). Also, there was significant increase between the1st two hours interval and each of the 4th, 6th, and 8th hour intervals. Mean values of cleaned dead brood cells increased from 30

in the 2nd hour to 57 cells on the 8th hour intervals.

Hygienic behavior after 2h collection was significantly ($P < 0.05$) higher than that before 2h that increased from 10 before to 30 cells after. Hygienic behavior after 4h collection was significantly ($P < 0.05$) higher than that of 4h before collection which increased from 14 before to 36 cells after collection.

Hygienic behavior after 6h was significantly ($P < 0.05$) higher than that before 6h collection, however it increased from 19 before to 45 cells after collection. Hygienic behavior after 8h collection was significantly ($P < 0.05$) higher than that before 8h collection, since increased from 25 before to 57cells after collection (Figure 2).

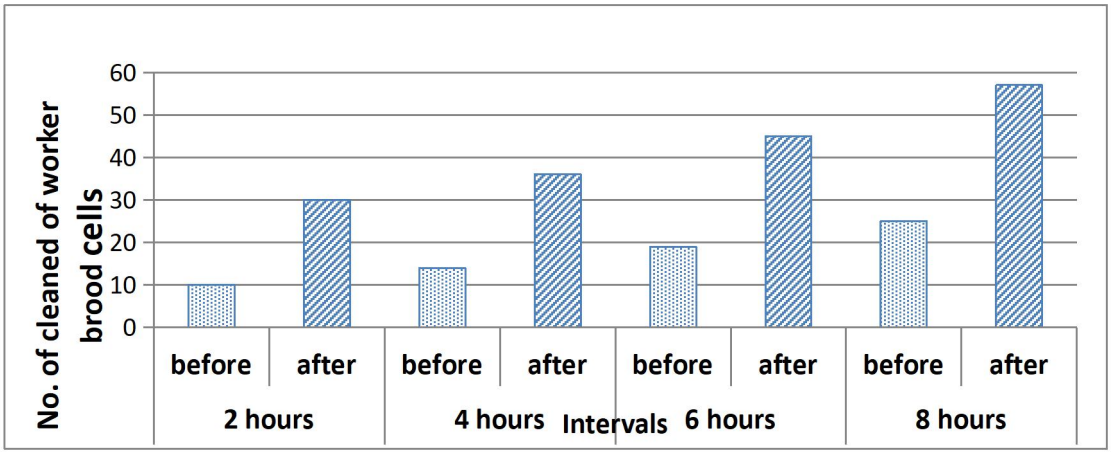

Figure 2: Comparison of hygienic behavior before and after venom collection in Carniolan colonies.

1.3. Comparison of hygienic behavior between Italian and Carniolan colonies

There was insignificant difference ($P > 0.05$) between Italian and Carniolan colonies in all tested intervals (Figure 3).

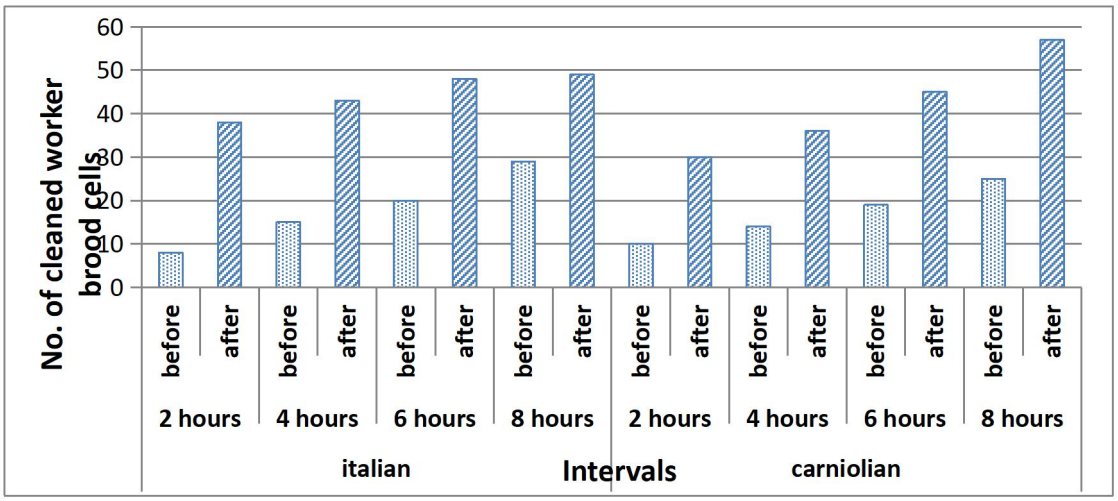

Figure 3: Hygienic behavior in Italian and Carniolan colonies before and after venom collection.

Bee venom collection has positive effect on hygienic behavior of tested honey bee colonies. This behavior increased by 22% in workers. The artificially killed brood (pin-killed) cells in the experimental colonies were removed outside the hives by adult workers. This is considered as an indicator of hygienic behavior. Also, the statistical analysis clearly showed significant difference between before and after treatment of colony **El-Saeady et al., 2016**.
The increase in hygienic behavior positively reflects the defensiveness and resistance of honey bee workers against pests and diseases, *e.g.* American foulbrood, chalkbrood and *Varroa* mites. Workers of hygienic behavior differentiate and remove the infected brood before sporulation of the pathogen (
Khan and Ghramh, 2021.

Many genetic factors are responsible for this complicated behavior which was expressed in a phenotype. On the other hand, these bees removed *Varroa* mites of infected brood, stopped reproductive cycle and killed immature stages **Mortiz, 1988 and Fries et al., 1994**, *e.g. A. m. carpatica* workers removed worker brood infested with *Varroa destructor* mites from the nest, and groomed the mites off themselves from other adult bees (grooming behavior) after using oxalic acid **Chioveanu et al., 2010**.

Also, *A. m. jemenitica* workers removed 85.5% of dead brood during 48 h. This mechanism was enhanced by effect of octopamine, a noradrenalin-like neuromodulator. Also, pierced (pin-killed) brood were removed faster than those frozen ones **Al-Madani, 2004**.

Hygienic behavior was compared in tested honey bee races, *i.e. A.m. jemenitica* and *A.m. carnica* after 24, 48 & 72 of killing the brood. Both races exhibited hygienic behavior, but with different rates. *A.m. jemenitica* removed 100% of dead brood through 24 h, while *A.m. carnica* removed only 55% in the same period. Hygienic behavior is greatly different among and within bee populations and subspecies **Balhareth et al., 2012**.

2. Effect of venom collection on syrup hoarding behavior

2.1.Effect of venom collection on hoarding behavior of Italian colonies

Before venom collection, there was significant decrease between the 4 hours and 8 hours (after supplying the feeding). The mean values of the volume of consumed feeding decreased from 35.71% at the 4th hour to 15.54 % at the 8thhour. Hoarding behavior significantly decreased after bee venom collection than before collection.
After 4h collection was significantly lower than before 4h collection; it decreased from 35.71% before collection to 26.26% after collection. After 6h collection was significantly lower than 6h before collection; it decreased from 21.34% before collection to 25.86% after collection (Table,1).

Table 1: Comparison of hoarding behavior before and after venom collection of Italian colonies

Rep.	2 hours		4 hours		6 hours		8 hours	
	Before	After	Before	After	Before	After	Before	After
Colony1	14.16	47.62	39.17	30.95	28.33	23.81	18.33	21.43
Colony2	42.73	32.76	34.54	27.59	13.18	20.69	9.54	18.97
Colony3	36.11	17.5	30.56	32.5	18.52	35.00	14.82	15.00
Colony4	18.18	20.83	38.18	20.83	24.55	31.25	19.09	27.08
Mean	27.41	28.28	35.71	26.26	21.34	25.86	15.54	19.60

22. The effect of collecting bee venom on hoarding behavior in Carniolan colonies

Before venom collection, there was significant decrease between the 4th hour and the 8th hour (after supplying the feeding). Mean values of the volume of consumed feeding decreased from 45.10% on the 4th hour to 11.76% on the 8th hour.

After venom collection, there was significant decrease between the 4th hour and the 8th hour (after supplying the feeding). There was significant decrease between the 6th hour and the 8th hour (after supplying the feeding). Mean values of the volume of consumed feeding decreased from 18.30 % on the 4th hour to 24.84 %on the 8th hour.

Hoarding behavior significantly ($P < 0.05$) decreased after bee venom collection than before collection. Hoarding behavior 4h after collection was significantly ($P < 0.05$) lower than 4h before collection; it decreased from 26.71 % before collection to 11.76 % after collection. After 6h collection was significantly lower than 6h before collection; it decreased from 22.98 % before collection to 18.30 % after collection.

Table 2: Comparison of hoarding behavior before and after venom collection in Carniolan colonies

Rep.	2 hours		4 hours		6 hours		8 hours	
	Before	After	Before	After	Before	After	Before	After
Colony1	15.63	46.05	39.06	13.16	26.58	17.11	18.75	23.68
1Colony2	33.55	45.09	26.71	11.76	22.98	18.30	16.77	24.84
Colony3	43.36	44.16	18.56	5.19	20.62	19.48	15.64	25.97
Colony4	33.54	45.10	26.71	11.76	22.98	18.30	16.77	24.84
Mean	33.35	45.10	26.71	11.76	22.98	18.30	16.77	24.84

Data in Table (2) show the intake of sucrose syrup before and after treating colonies by the bee venom collector. Statistical analysis of data shows a significant difference between before and after treatment in the four colonies. On the other hand, there is a decrease in feed consumption after treatment, these results demonstrate that bee venom collection decreases hoarding behavior, yet regulates the feeding process in even quantities around the day.

Previous studies have demonstrated a short-term effect of colony food stores on behavioral regulation. **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 &Baxter 1980 and El-Saeady et al 2016** stated that hoarding behavior was increased by addition of empty comb to honey bee colonies**.**

Hoarding test indicated that no significant differences existed between before and after the alarming or stimulation of worker honey bee by electrical impulses. Similarly, in field experiments, the mean yield of honey, obtained from colonies in which venom was collected, was not significantly different than controls **El-Saeady et al .,2016. Argena et al., 2021** indicated that bee colonies used for the collection of BV in field studies consumed more syrup after the collection than before, although the differences were not significant. They attributed the increase in feed conception to the stimulation of worker honey bees to collect more food to compensate for the loss of secreted protein (venom).

REFERENCES

Abd El-Wahed, A. A., Khalifa, S. A. M., Sheikh, B. Y., Farag and Fayaz, A. S. 2018: Bee Venom Composition: From Chemistry to Biological Activity. Studies in Natural Products Chemistry, 60: 459-480.

Al-Madani, M.H.E. (2004): Biological study on *Varroa jacobsoni* Oud. and its control in the Republic of Yemen, M.Sc. Thesis. San'aa University, yemen.199 .

Argena, N., Tananaki, C., Thrasyvoulou, A., Goras, G., Kanelis, D. And Liolios, V. 2021: Seasonal variation on bee venom collection. The impact on some biological aspects on Apis mellifera. J. Hell. Vet. Med. Soc., 72(2): 2861-2868.

Balhareth, H. M, Alqarni, Abdulaziz, S. and, Owayss, A. A. 2012: Comparison of Hygienic and Grooming Behaviors of Indigenous and Exotic Honeybee (*Apis mellifera)* Races in Central Saudi Arabia. Int. J. Agric. Biol, 14, 1005-1008.

Chioveanu, G., Dobrescu, C. and Dobre, G. 2010: preliminary evaluation of the grooming behavior to *Apis mellifera* carpatica in relation to the use of bee vital, an alternative preparation for varroa control. Apimondia, First World Conference on Organic Beekeeping,27-290.

El-Saeady, A. Diab, A. Shehata, I., Nafea, E. and Metwaly, A. 2016: Effect of Bee Venom Collecting on The Behavior of Honeybee Colonies, J. Plant Prot. and Path., Mansoura Univ., 7 (6), 347– 351.

Free, J. B. 1955: The division of labor within bumblebee colonies. Insects. Soc., 2: 195–212.

Fries, L.S.; Camazine, J. and Sneyd, 1994: Population dynamics of Varro ajacobsoni: a model and a review. Bee World, 75: 5–28.

Khan, K. A. and Ghramh, H. A. 2021: An investigation of the efficacy of hygienic behavior of various honey bee (Apis mellifera) races toward Varroa destructor (Acari: Varroidae) mite infestation. J. King Saud Uni – Sci, 33(3).

Mortiz, R.F.A., 1988: A reevaluation of the two-locus model for hygienic behavior in honeybees (*Apis mellifera*). J. Hered., 79: 257–262.

Owen, D. M. and Sloley B. D. **1988:** 5-hydroxy tryptamine in the venom of the honeybee (*Apis mellifera L.*): variation with season and with insect age. Toxicon, 26 (6): 577-581.

Palmer, D. J. 1961: Extraction of bee venom for research. Bee World, 42(9): 225-226.

Pérez-Sato, J. A., Chline, N., Martin, S. J., Hughes, W. O.H. and Ratnieks, F. L.W. (2009): Multi-level selection for hygienic behaviour in honeybees. Heredity, 102(6): 609-615.

Rinderer, T.E. and Baxter, J.R. 1980: Hoarding behavior of the honey bee: Effect of empty comp, comb color, and genotype, Bee breeding and stock center Lab., Agric. Res. SEA, USDA, Baton Rouge, LA. 70808.

Palmer, D. J. (1961): Extraction of bee venom for research. Bee World, 42(9): 225-226.

Pérez-Sato, J. A., Chline, N., Martin, S. J., Hughes, W. O.H. and Ratnieks, F. L.W. 2009: Multi-level selection for hygienic behaviour in honeybees. Heredity, 102(6): 609-615.

Rinderer, T.E. and Baxter, J.R. 1980: Hoarding behavior of the honey bee: Effect of empty comp, comb color, and genotype, Bee breeding and stock center Lab., Agric. Res. SEA, USDA, Baton Rouge, LA. 70808.

Spivak, M. and Gilliam, M. 1998: Honeybee hygienic behavior, Am. Bee J., 38: 283-296.

Spivak, **M. S. and Reuter**, **G. S. 2001:** Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior. Apidologie, 32: 555–565.

تأثير جمع السم علي السلوك الصحي ونشاط اكتناز المحلول السكري لطوائف نحل العسل

فاطمه حمدي عبد الله مرزبان[2]وعبد الحليم مشرف اسماعيل[1]وعاطف أحمد عبد الجيد[1]

1-قسم وقاية النبات - كلية الزراعة - جامعة الفيوم
2-**معهد بحوث وقاية النباتات - مركز البحوث الزراعية –الدقي-الجيزة**

اجريت الدراسة الحالية في معهد بحوث وقاية النبات ،قسم بحوث النحل بوزارة الزراعة بالجيزة ،مصر ،خلال اغسطس 2018 لدراسة تأثير **جمع السم علي السلوك الصحي لطوائف النحل وعلي نشاط اكتناز المحلول السكري** في سلالتي الكرنيولي و الايطالي.

هناك زيادة معنوية مضطردة في نشاط السلوك الصحي لطوائف النحل خلال ثمان ساعات بفاصل ساعتين بين كل قرائتين و كان نشاط السلوك الصحي بعد فترة جمع السم أعلي منه قبل المعاملة. بدءا من ساعتين (بعد ثقب العيون السداسية (حتي ثمانية ساعات، و بين الساعتين و كلا من الأربع و الست و الثمانية ساعات، وبعد جمع السم عن قبل الجمع. كما كانت هناك فروق معنوية بين طوائف سلالتي النحل الايطالي والكرنيولي في كل الفترات الزمنية المختبرة.
كان نشاط اكتناز المحلول السكري بعد جمع السم أقل معنويا عنه قبل جمع السم بشكل عام.

وكانت الفروق بين سلالتي النحل الايطالي و الكرنيولي في كل الفترات الزمنية المختبرة (ثمان ساعات بفاصل ساعتين) غير معنوية.

الكلمات الدالة: نحل العسل – جمع السم– السلوك الصحي – اكتناز المحلول السكري.