



## MORPHOMETRIC STUDIES ON VENOM GLAND AND ITS RESERVOIR IN HONEYBEE WORKERS (*Apis mellifera*)

Shimaa A. El-Bahnasy<sup>1\*</sup>; A.M. Elbassiouny<sup>2</sup>; H.M. Mahfouz<sup>1</sup> and M.N. El-Bassiony<sup>1</sup>

1. Dept. Plant Prod., Fac. Environ. Agric. Sci., El-Arish Univ., Egypt.

2. Dept. Plant Protec., Fac. Agric., Ain Shams Univ., Egypt.

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### ABSTRACT

This experiment was done during spring and autumn seasons of 2019 and 2020 to study the effect of age, feeding supply and the active season of honeybee workers on morphometric characters of venom sac and venom acid gland, body weight and the ages from 1<sup>th</sup> to 21<sup>th</sup> day old. Results indicated that in spring season, the highest data were recorded for body weight (122 mg) at day 8<sup>th</sup>, venom sac area (1.554 mm<sup>2</sup>) at day 17<sup>th</sup> and the total length of acidic gland (15.76 mm) at 12<sup>th</sup> day old. While the lowest venom sac area (0.942 mm<sup>2</sup>) was recorded at 1<sup>st</sup> day old. In autumn season with plenty of bee bread, the highest data were recorded for body weights at day 9<sup>th</sup> (116mg), the highest both of venom sac area at day 15<sup>th</sup> (1.5346 mm<sup>2</sup>) and the total length of acidic gland at 13<sup>th</sup> day old (15.3mm). With scarcity of bee bread in autumn season, the body weight at day 1<sup>st</sup> recorded (94 mg) while it recorded at day 21<sup>th</sup> (79mg), the highest venom sac area at day 20<sup>th</sup> (1.1933 mm<sup>2</sup>) and the highest total length of acidic gland at 17<sup>th</sup> day old (15.4 mm). With scarcity of bee honey in autumn season, the highest body weights at day 1<sup>st</sup> (94 mg), while the lowest body weights at day 21<sup>th</sup> (72 mg), the highest venom sac area (mm<sup>2</sup>) at day 19<sup>th</sup> (1.1775 mm<sup>2</sup>) and the highest total length of acidic gland at 12<sup>th</sup> day old (15.5 mm).



## INTRODUCTION

Defending the colony with the sting which is believed to have evolved from the egg laying apparatus of the female is characteristic feature of all insects that belong to the order Hymenoptera including bees. The venom is produced in the abdomen of worker bees from a mixture of acidic and alkaline secretions. It is synthesized by the venom glands associated with the sting apparatus of worker and queens, stored in the venom reservoir and injected through the sting apparatus during the stinging process. Its production increases during the first two weeks of the adult worker's life and reaches a maximum when the worker bee becomes involved in hive defense and foraging (Roat *et al.*, 2006). The anatomy of the sting (venom apparatus)

reveals the presence of two important associated exocrine glands which were venom gland (acidic) and dufors gland (alkaline). The secretions of both glands are apocrine and are released into the lumen to be stored in the venom sac (Devi *et al.*, 2016). Honey bee venom (apitoxin), is a transparent liquid, ornamental pungent smell, a bitter taste, hydrolytic blend of proteins with basic pH between 4.5 to 5.5 (Ali, 2012). The active portion of apitoxin is a complex mixture of proteins, peptides and low molecular components (Bogdanov, 2016). Many factors affecting honey bee venom production and its quality such as; honey bee race, age of bees, colony strength, season of collection, feeding supply, race, its defense behavior and method of collection (Haggag *et al.*, 2015). Bee venom (BV) is a major source of

\* Corresponding author: E-mail address: shimaa\_bah@yahoo.com

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secondary metabolites from honeybees (*Apis mellifera*). It comprises peptides, proteins, enzymes, and volatile metabolites, among other bioactive substances. The compounds contribute to the venom's observed biological functions as per its anti-inflammatory and anticancer effects (El-Seedi *et al.*, 2020). Bee venom is a complicated combination of proteins, peptides and low molecular compounds. Its constituents have now been identified. Proteins and peptides are the most important components. Apitoxin (bee toxin) has a complex content that includes various biochemical and pharmacologically active chemicals like histamine, dopamine, and melittin (Hegazi *et al.*, 2014).

This experiment aims to study the effect of age, feeding supply and rearing season of honey bee workers on morphometrical characters of bodyweight, venom sac and venom acid gland to find out which age of honey bee workers that contain the highest quantity of venom inside the venom sac.

## MATERIALS AND METHODS

These experiments were carried out in the apiary of honey bee research center, Faculty of Environmental Agricultural Sciences, El-Arish University, Egypt. The experiment was conducted during spring and autumn seasons of 2019 and 2020.

### Effect of Different Ages of Honey Bee Workers and Food Supply on Venom Acid Gland Parameters

#### Honey bee Colonies

Honey bee colonies headed with open mated local Carniolian honey bee queens (*Apis mellifera carnica*) were used in the present investigation. Honey bee samples were collected as follows:

- Sixteen sealed brood combs free from bee workers were collected then placed on the top of two strong hives (Incubator hives).
- Every eight combs have on their sides two combs containing honey and pollen grains.

- placing a wire barrier between them to prevent the workers and the queen from moving to the upper floor and covered with thick burlap to keep warm.
- The newly emerged honeybee workers (one day age) collected every day from the sealed brood and weighted using electrical balance then placed in a separate small bee hive with a queen.

To study effect of honeybee worker age on morphometric measurements the small hives were divided into three groups:

**Group 1:** Three honey bee colonies were provided by 2 frames containing bee bread areas.

**Group 2:** Three honey bee colonies were provided by 2 frames containing only bee honey and free from bee bread.

**Group 3:** Three honey bee colonies were provided by 2 frames one of them containing honey and the other was empty.

### Effect of Honey Bee Workers Weights

The experiment was carried out to assessment the relation between honey bee workers weight and length of their venom acid gland and reservoir. Honey bee workers of each age from 1<sup>st</sup> to 21<sup>th</sup> days old were collected every day and weighted at electrical balance.

### Morphometric Measurements

The experiment was carried out to study the effect of age, feeding supply and rearing season of honey bee workers on morphometric characters of bodyweight, venom sac and venom acid gland. The following procedures were executed:

Five adult honey bee workers of each age from 1<sup>st</sup> to 21<sup>th</sup> day old were collected every day. Then put them in the refrigerator until they lose movement. Stings apparatus were isolated using dissection forceps and kept it in (15 ml ethyl alcohol 70% + 30 ml glycerin) before measuring.

The intact sting apparatus of each age picked up carefully, stretched on a slide and examined under binocular, each worker was dissected, the acid gland was removed and transferred to a slide containing alcohol drop. The gland was stretched on the slide with stylets and removed the excess alcohol with filter paper. The length of acidic gland (L.A.G), length of venom sac (L.V.S.) and width of venom sac (W.V.S.) were measured by ocular micrometer and photographed.

The following parameters were measured:

- Length of long branch of venom acidic gland.
- Length of short branch of venom acidic gland.
- The total length of acid gland was calculated by summation the lengths of long and short branches.
- Maximum length (L) and maximum width (W) of venom sac.

The volume of venom sac was calculated according to the formula adopted by **Nentchev and Jordanova (2002)** as follow:

by the following equation:

$$V = \frac{3}{4} \times \pi \times \left( \frac{L+W}{2} \right)^3$$

Where,

V= volume of venom sac.

L= maximum length of venom sac.

W= maximum width of venom sac.

$\pi=3.14$

### Statistical Analysis

Means were compared by LSD test at 0.05 level, using **SAS program (2004)**.

## RESULTS AND DISCUSSION

### Effect of Different Ages of Honey Bee Workers and Food Supply on Venom Acid Gland Parameters

The relations between body weights, the length of acidic gland and venom sac area

in honey bee workers for all ages (from 1<sup>st</sup> to 21<sup>th</sup> age) and the stored areas of bee bread during different seasons were recorded in Table 1. Results showed a difference in body weights (mg) of *Apis mellifera* L. honey bee workers for all ages (from 1<sup>st</sup> to 21<sup>th</sup> day old) during spring season. The honey bee workers showed the highest body weights at day 8<sup>th</sup> (122 mg) followed by day 10<sup>th</sup> (120 mg) with a significant increase up to day 12<sup>th</sup>, then decreased. The lowest body weights at days 1<sup>st</sup> and 19<sup>th</sup> (97 mg). Results in Table 1 show a difference in the parameters of honey bee worker's acid gland (length of both acidic gland, branch 1 length and branch 2 length) and venom sac (length of acidic sac and width of acidic sac). Regarding the effect of honey bee age on venom sac parameters, results indicated that total highest venom sac area (mm<sup>2</sup>) was recorded at day 17<sup>th</sup> (1.554 mm<sup>2</sup>) followed by day 20<sup>th</sup> (1.535 mm<sup>2</sup>) While the lowest venom sac area (mm<sup>2</sup>) was recorded at day 1<sup>st</sup> (0.942 mm<sup>2</sup>) followed by day 2<sup>nd</sup> result also indicated that the highest total length of acidic gland was recorded at 12<sup>th</sup> day old (15.76 mm) followed by 17<sup>th</sup> day old (15.22 mm). While the lowest length of acidic gland was recorded at 1<sup>st</sup> day old (13.36 mm).

Results in Table 2 indicate that feeding of honey bee workers on pollen and honey combs increase body weights (mg) of honey bee workers venom glands parameters in autumn season. The highest body weights of honey bee workers were observed at day 9<sup>th</sup> (116 mg) followed by day 5<sup>th</sup> (113 mg) with a significant increase up to day 12<sup>th</sup>, then decreased. The lowest body weights at days 2<sup>nd</sup> (87 mg) and 3<sup>rd</sup> (89 mg). Results clearly showed a difference in the parameters of honey bee worker's acid gland (length of acidic gland, branch 1 length and branch 2 length) and venom sac (length of acidic sac and width of acidic sac). The obtained parameters indicated that the highest venom sac area (mm<sup>2</sup>) was recorded at day 15<sup>th</sup>



**Fig.1. Effect of different ages of honey bee workers on venom glands parameters in spring season**

**Table 1. Effect of different ages of honey bee workers on venom glands parameters in spring season**

Age (Day)	Body Weight (mg)	Venom Sac Area (mm <sup>2</sup> )	Acid gland			
			Length (mm)	branch 1 length (mm)	branch 2 length (mm)	Total length (mm)
1	97 <sup>c</sup>	0.942 <sup>e</sup>	8.32 <sup>b</sup>	3.44 <sup>b</sup>	1.6 <sup>d</sup>	13.36
2	101 <sup>c</sup>	0.989 <sup>e</sup>	8.6 <sup>b</sup>	4.1 <sup>a</sup>	1.3 <sup>e</sup>	14
3	105 <sup>b</sup>	1.036 <sup>d</sup>	8.8 <sup>b</sup>	2.8 <sup>c</sup>	1.9 <sup>b</sup>	13.5
4	116 <sup>a</sup>	1.020 <sup>d</sup>	9.14 <sup>b</sup>	2.6 <sup>c</sup>	2 <sup>b</sup>	13.74
5	112 <sup>b</sup>	1.099 <sup>d</sup>	8.84 <sup>b</sup>	2.9 <sup>c</sup>	1.8 <sup>c</sup>	13.54
6	119 <sup>a</sup>	1.130 <sup>d</sup>	9.6 <sup>a</sup>	3.2 <sup>b</sup>	1.8 <sup>c</sup>	14.6
7	118 <sup>a</sup>	1.209 <sup>c</sup>	8.92 <sup>b</sup>	3.1 <sup>b</sup>	2.1 <sup>a</sup>	14.12
8	122 <sup>a</sup>	1.256 <sup>c</sup>	8.82 <sup>b</sup>	3.1 <sup>b</sup>	1.5 <sup>d</sup>	13.42
9	117 <sup>a</sup>	1.264 <sup>c</sup>	9.52 <sup>a</sup>	2.62 <sup>c</sup>	1.4 <sup>e</sup>	13.54
10	120 <sup>a</sup>	1.268 <sup>c</sup>	9.3 <sup>a</sup>	4 <sup>a</sup>	1.8 <sup>c</sup>	15.1
11	115 <sup>a</sup>	1.319 <sup>b</sup>	8.92 <sup>b</sup>	3.6 <sup>b</sup>	1.7 <sup>c</sup>	14.22
12	119 <sup>a</sup>	1.335 <sup>b</sup>	9.46 <sup>a</sup>	4.4 <sup>a</sup>	1.9 <sup>b</sup>	15.76
13	110 <sup>b</sup>	1.374 <sup>b</sup>	9.6 <sup>a</sup>	3.3 <sup>b</sup>	1.7 <sup>c</sup>	14.6
14	111 <sup>b</sup>	1.413 <sup>b</sup>	9.4 <sup>a</sup>	2.92 <sup>c</sup>	1.6 <sup>d</sup>	13.92
15	107 <sup>b</sup>	1.468 <sup>a</sup>	9.8 <sup>a</sup>	2.8 <sup>c</sup>	1.5 <sup>d</sup>	14.1
16	108 <sup>b</sup>	1.444 <sup>a</sup>	10.3 <sup>a</sup>	2.5 <sup>c</sup>	2 <sup>b</sup>	14.8
17	105 <sup>b</sup>	1.554 <sup>a</sup>	9.72 <sup>a</sup>	3.3 <sup>b</sup>	2.2 <sup>a</sup>	15.22
18	98 <sup>c</sup>	1.468 <sup>a</sup>	9.92 <sup>a</sup>	3.1 <sup>b</sup>	2.1 <sup>a</sup>	15.12
19	97 <sup>c</sup>	1.484 <sup>a</sup>	10.1 <sup>a</sup>	2.52 <sup>c</sup>	1.7 <sup>c</sup>	14.32
20	101 <sup>c</sup>	1.535 <sup>a</sup>	9.4 <sup>a</sup>	2.8 <sup>c</sup>	1.9 <sup>b</sup>	14.1
21	102 <sup>c</sup>	1.492 <sup>a</sup>	9.5 <sup>a</sup>	3.4 <sup>b</sup>	1.8 <sup>c</sup>	14.7
	4.93	15.05	1.327			
F (P)	(0.001)	(0.0001)	0.188)	3.811 (0.009)	1.014 (0.455)	
LSD	8.54	0.114	1.041	0.625	0.111	

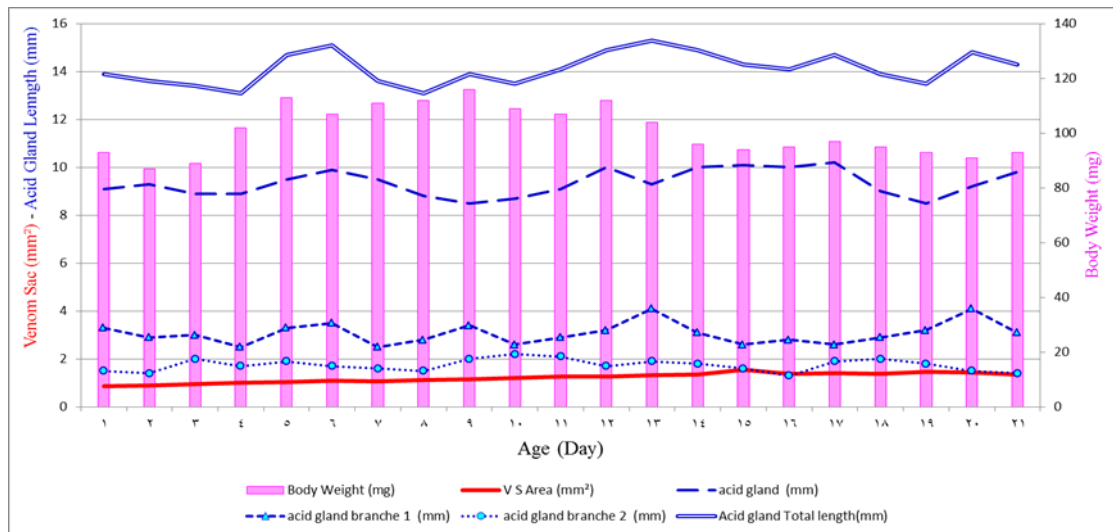
**Table 2. Effect of feeding honey bee workers with pollen and honey combs on venom glands parameters in autumn season**

Age (Day)	Body Weight (mg)	Venom Sac Area (mm <sup>2</sup> )	Acid Gland(mm)			
			Gland	branche1	branche2	Total length
1	93 <sup>j</sup>	0.8478 <sup>q</sup>	9.1 <sup>b</sup>	3.3 <sup>b</sup>	1.5 <sup>b</sup>	13.9
2	87 <sup>m</sup>	0.8949 <sup>p</sup>	9.3 <sup>b</sup>	2.9 <sup>c</sup>	1.4 <sup>b</sup>	13.6
3	89 <sup>l</sup>	0.9420 <sup>o</sup>	8.9 <sup>b</sup>	3.0 <sup>b</sup>	2.0 <sup>a</sup>	13.4
4	102 <sup>g</sup>	0.9891 <sup>n</sup>	8.9 <sup>b</sup>	2.5 <sup>c</sup>	1.7 <sup>b</sup>	13.1
5	113 <sup>b</sup>	1.0362 <sup>m</sup>	9.5 <sup>a</sup>	3.3 <sup>b</sup>	1.9 <sup>a</sup>	14.7
6	107 <sup>e</sup>	1.0990 <sup>k</sup>	9.9 <sup>a</sup>	3.5 <sup>b</sup>	1.7 <sup>b</sup>	15.1
7	111 <sup>c</sup>	1.0715 <sup>l</sup>	9.5 <sup>a</sup>	2.5 <sup>c</sup>	1.6 <sup>b</sup>	13.6
8	112 <sup>b</sup>	1.1304 <sup>jk</sup>	8.8 <sup>b</sup>	2.8 <sup>c</sup>	1.5 <sup>b</sup>	13.1
9	116 <sup>a</sup>	1.1539 <sup>j</sup>	8.5 <sup>b</sup>	3.4 <sup>b</sup>	2 <sup>a</sup>	13.9
10	109 <sup>d</sup>	1.2089 <sup>i</sup>	8.7 <sup>b</sup>	2.6 <sup>c</sup>	2.2 <sup>a</sup>	13.5
11	107 <sup>e</sup>	1.2638 <sup>h</sup>	9.1 <sup>b</sup>	2.9 <sup>c</sup>	2.1 <sup>a</sup>	14.1
12	112 <sup>b</sup>	1.2677 <sup>h</sup>	10.0 <sup>a</sup>	3.2 <sup>b</sup>	1.7 <sup>b</sup>	14.9
13	104 <sup>f</sup>	1.3188 <sup>g</sup>	9.3 <sup>b</sup>	4.1 <sup>a</sup>	1.9 <sup>a</sup>	15.3
14	96 <sup>h</sup>	1.3345 <sup>f</sup>	10.0 <sup>a</sup>	3.1 <sup>b</sup>	1.8 <sup>a</sup>	14.9
15	94 <sup>i</sup>	1.5346 <sup>a</sup>	10.1 <sup>a</sup>	2.6 <sup>c</sup>	1.6 <sup>b</sup>	14.3
16	95 <sup>i</sup>	1.3816 <sup>e</sup>	10.0 <sup>a</sup>	2.8 <sup>c</sup>	1.3 <sup>b</sup>	14.1
17	97 <sup>h</sup>	1.4130 <sup>d</sup>	10.2 <sup>a</sup>	2.6 <sup>c</sup>	1.9 <sup>a</sup>	14.7
18	95 <sup>i</sup>	1.3737 <sup>e</sup>	9.0 <sup>b</sup>	2.9 <sup>c</sup>	2.0 <sup>a</sup>	13.9
19	93 <sup>j</sup>	1.4679 <sup>b</sup>	8.5 <sup>b</sup>	3.2 <sup>b</sup>	1.8 <sup>a</sup>	13.5
20	91 <sup>k</sup>	1.4444 <sup>c</sup>	9.2 <sup>b</sup>	4.1 <sup>a</sup>	1.5 <sup>b</sup>	14.8
21	93 <sup>j</sup>	1.3541 <sup>f</sup>	9.8 <sup>a</sup>	3.1 <sup>b</sup>	1.4 <sup>b</sup>	14.3
			2.566	3.243	1.496	
F (P)	5.944 (0.003)	21.853 (0.0001)	(0.001)	(0.009)	(0.105)	
LSD	1.735	0.021	0.801	0.583	0.482	

(1.5346 mm<sup>2</sup>) followed by day 19<sup>th</sup> (1.4679 mm<sup>2</sup>) While the lowest venom sac area (mm<sup>2</sup>) at day 1<sup>st</sup> and day 2<sup>nd</sup> (0.8478 and 0.8949 mm<sup>2</sup>), respectively. Morphometric parameters also indicated that the highest total length of acidic gland at 13<sup>th</sup> day old (15.3 mm) followed by 6<sup>th</sup> day old (15.1 mm). While the lowest length of acidic gland was recorded at 4 days old (13.1mm).

Results in Table 3 indicate that feeding of honey bee workers on only bee honey combs decrease body weights (mg) of honey bee workers venom glands parameters in autumn season. There were no significant differences in body weights for all ages of honeybee workers from day 1<sup>st</sup> to day 21<sup>th</sup>. The honey bee workers showed the highest

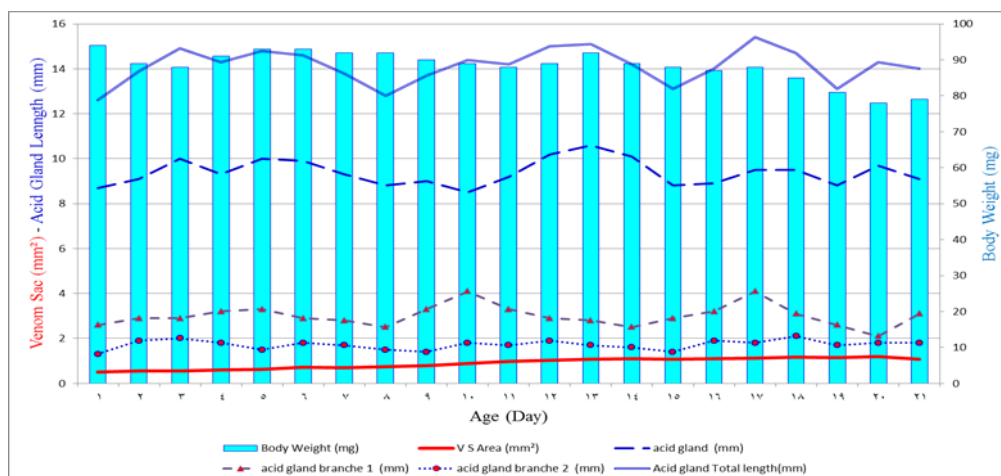
body weights at day 1<sup>st</sup> (94 mg) with a significant gradually decrease to day 21<sup>th</sup>. Results also showed a clearly differences in the parameters of honey bee worker's acid gland (length of acidic gland, branch 1 length and branch 2 length) and venom sac (length of acidic sac and width of acidic sac). Results indicated that the highest venom sac area (mm<sup>2</sup>) was recorded at day 20<sup>th</sup> (1.1933 mg) followed by day 18<sup>th</sup>. While the lowest venom sac area (mm<sup>2</sup>) was recorded at day 1<sup>st</sup> and day 2<sup>nd</sup> (0.4945 mm<sup>2</sup> and 0.5495 mm<sup>2</sup>), respectively. The highest total length of acidic gland was recorded at 17<sup>th</sup> day old (15.4 mm) followed by 13<sup>th</sup> day old (15.1 mm). While the lowest length of acidic gland was recorded at 1<sup>st</sup> day old (12.6 mm).



**Fig. 2. Effect of feeding honey bee colonies with pollen and honey combs on venom glands parameters in autumn season**

**Table 3. Effect of feeding honey bee workers with bee honey combs on venom glands parameters in autumn season**

Age (Day)	Body Weight (mg)	Venom Sac Area (mm <sup>2</sup> )	Acid Gland (mm)			
			Gland	Branche 1	Branche 2	Total length
1	94 <sup>a</sup>	0.4945 <sup>f</sup>	8.7 <sup>c</sup>	2.6 <sup>c</sup>	1.3 <sup>c</sup>	12.6
2	89 <sup>c</sup>	0.5495 <sup>e</sup>	9.1 <sup>b</sup>	2.9 <sup>b</sup>	1.9 <sup>a</sup>	13.9
3	88 <sup>d</sup>	0.5625 <sup>e</sup>	10 <sup>a</sup>	2.9 <sup>b</sup>	2 <sup>a</sup>	14.9
4	91 <sup>b</sup>	0.5966 <sup>e</sup>	9.3 <sup>b</sup>	3.2 <sup>b</sup>	1.8 <sup>a</sup>	14.3
5	93 <sup>a</sup>	0.628 <sup>e</sup>	10 <sup>a</sup>	3.3 <sup>b</sup>	1.5 <sup>b</sup>	14.8
6	93 <sup>a</sup>	0.7065 <sup>d</sup>	9.9 <sup>a</sup>	2.9 <sup>b</sup>	1.8 <sup>a</sup>	14.6
7	92 <sup>b</sup>	0.6908 <sup>d</sup>	9.3 <sup>b</sup>	2.8 <sup>b</sup>	1.7 <sup>b</sup>	13.8
8	92 <sup>b</sup>	0.7457 <sup>d</sup>	8.8 <sup>c</sup>	2.5 <sup>c</sup>	1.5 <sup>b</sup>	12.8
9	90 <sup>c</sup>	0.785 <sup>d</sup>	9 <sup>b</sup>	3.3 <sup>b</sup>	1.4 <sup>b</sup>	13.7
10	89 <sup>c</sup>	0.8949 <sup>c</sup>	8.5 <sup>c</sup>	4.1 <sup>a</sup>	1.8 <sup>a</sup>	14.4
11	88 <sup>d</sup>	0.9891 <sup>b</sup>	9.2 <sup>b</sup>	3.3 <sup>b</sup>	1.7 <sup>b</sup>	14.2
12	89 <sup>c</sup>	1.0205 <sup>b</sup>	10.2 <sup>a</sup>	2.9 <sup>b</sup>	1.9 <sup>a</sup>	15
13	92 <sup>b</sup>	1.0715 <sup>b</sup>	10.6 <sup>a</sup>	2.8 <sup>b</sup>	1.7 <sup>b</sup>	15.1
14	89 <sup>c</sup>	1.099 <sup>a</sup>	10.1 <sup>a</sup>	2.5 <sup>c</sup>	1.6 <sup>b</sup>	14.2
15	88 <sup>d</sup>	1.0833 <sup>b</sup>	8.8 <sup>c</sup>	2.9 <sup>b</sup>	1.4 <sup>b</sup>	13.1
16	87 <sup>d</sup>	1.099 <sup>a</sup>	8.9 <sup>c</sup>	3.2 <sup>b</sup>	1.9 <sup>a</sup>	14
17	88 <sup>d</sup>	1.1304 <sup>a</sup>	9.5 <sup>b</sup>	4.1 <sup>a</sup>	1.8 <sup>a</sup>	15.4
18	85 <sup>e</sup>	1.1775 <sup>a</sup>	9.5 <sup>b</sup>	3.1 <sup>b</sup>	2.1 <sup>a</sup>	14.7
19	81 <sup>f</sup>	1.1539 <sup>a</sup>	8.8 <sup>c</sup>	2.6 <sup>c</sup>	1.7 <sup>b</sup>	13.1
20	78 <sup>g</sup>	1.1933 <sup>a</sup>	9.7 <sup>b</sup>	2.1 <sup>c</sup>	1.8 <sup>a</sup>	14.3
21	79 <sup>g</sup>	1.0715 <sup>b</sup>	9.1 <sup>b</sup>	3.1 <sup>b</sup>	1.8 <sup>a</sup>	14
F (P)	3.277 (000.8)	34.346 (0.004)	3.32 (0.001)	4.607(0.004)	1.951(0.019)	
LSD	1.148	0.097	0.746	0.523	0.348	



**Fig. 3. Effect of feeding honey bee workers with bee honey combs on venom glands parameters in autumn season**

Results in Table 4 indicate that feeding of honey bee workers on one bee honey comb and another one empty decrease body weights (mg) of honey bee workers venom glands parameters in autumn season. There were no significant differences in body weights for all ages of honeybee workers from day 1<sup>st</sup> to day 21<sup>th</sup>. The honeybee workers showed the highest body weights at day 1<sup>st</sup> (94 mg) with a significant decrease down to day 21<sup>th</sup> (72 mg). Results indicated that the highest venom sac area (mm<sup>2</sup>) was recorded at day 19<sup>th</sup> (1.1775mm<sup>2</sup>) followed by day 18<sup>th</sup> (1.1539 mm<sup>2</sup>) While the lowest venom sac area (mm<sup>2</sup>) was recorded at day 3<sup>rd</sup> (0.4945 mm<sup>2</sup>). The highest total length of acidic gland was recorded at 12<sup>th</sup> day old (15.5mm) while the lowest length of acidic gland was recorded at 14<sup>th</sup> day old (12.9 mm).

In general, we can conclude that venom secretion from acid gland is stored in venom sac. The newly emerged bees have very little venom amount, but venom quantity gradually increased and accumulated at 16<sup>th</sup> day-old workers. After the age of guard bees reach to 18<sup>th</sup> days old, no venom is secreted. **Nenchev (2003)** recorded that the venom acid gland and

volume of its sac were depended on the age of honey bee worker. The maximum length of venom acid gland was observed at 17<sup>th</sup> day-old and the highest volume of venom sac was observed in honey bee worker at 17.2 day-old. The production of venom is associated with the length of the gland and branching does not influence the quantity of venom. There was no significant difference between the size of the branched and simple glands or in the quantity of venom produced (**Brizola et al., 2006**). **Arruda et al. (2005)** found that the length of this gland in *A. mellifera* workers varied from 6.22 to 21.98 mm with an average of 12.86 mm. This length was dependent on the age of the workers and rearing season. **Zakaria and Mohammed (2004)** indicated that feeding honey bee workers at different ages (7, 14 and 21 days old) on the tested protein foods particularly bee bread and pollen grains were considered as important factors which affected the quantitative, concentration and venom contents particularly guard bees. There was a clear relationship between the number and the molecular weight of the protein bands detected in bee venom at different tested bees ages depending on the type of the supplemental protein diets.

**Table 4. Effect of feeding honey bee workers with bee honey comb and another empty comb on venom glands parameters in autumn season**

Age	Body Weight (mg)	Venom Sac Area (mm <sup>2</sup> )	Acid Gland (mm)			
			Gland	Branche 1	Branche 2	Total length
1	94 <sup>a</sup>	0.5181 <sup>f</sup>	9.6 <sup>a</sup>	2.5 <sup>d</sup>	1.6 <sup>b</sup>	13.7
2	86 <sup>b</sup>	0.522 <sup>f</sup>	9.8 <sup>a</sup>	2.8 <sup>c</sup>	1.7 <sup>a</sup>	14.3
3	89 <sup>a</sup>	0.4945 <sup>f</sup>	10.3 <sup>a</sup>	3 <sup>c</sup>	1.4 <sup>b</sup>	14.7
4	89 <sup>a</sup>	0.5181 <sup>f</sup>	9.7 <sup>a</sup>	2.8 <sup>c</sup>	1.6 <sup>b</sup>	14.1
5	79 <sup>c</sup>	0.5495 <sup>f</sup>	9.9 <sup>a</sup>	2.6 <sup>d</sup>	1.5 <sup>b</sup>	14
6	81 <sup>b</sup>	0.6594 <sup>e</sup>	9.6 <sup>a</sup>	2.9 <sup>c</sup>	1.5 <sup>b</sup>	14
7	83 <sup>b</sup>	0.6358 <sup>e</sup>	8.9 <sup>b</sup>	3.2 <sup>b</sup>	1.5 <sup>b</sup>	13.6
8	77 <sup>c</sup>	0.7418 <sup>d</sup>	8.8 <sup>b</sup>	2.5 <sup>d</sup>	2.1 <sup>a</sup>	13.4
9	67 <sup>d</sup>	0.7771 <sup>d</sup>	9.5 <sup>b</sup>	2.6 <sup>d</sup>	2.2 <sup>a</sup>	14.3
10	79 <sup>c</sup>	0.8478 <sup>c</sup>	10.1 <sup>a</sup>	2.8 <sup>c</sup>	1.7 <sup>a</sup>	14.6
11	84 <sup>b</sup>	0.8635 <sup>c</sup>	9.4 <sup>b</sup>	3.6 <sup>b</sup>	1.7 <sup>a</sup>	14.7
12	89 <sup>a</sup>	0.9027 <sup>b</sup>	9.5 <sup>b</sup>	4.1 <sup>a</sup>	1.9 <sup>a</sup>	15.5
13	94 <sup>a</sup>	1.0205 <sup>b</sup>	8.3 <sup>c</sup>	3.1 <sup>c</sup>	2 <sup>a</sup>	13.4
14	87 <sup>b</sup>	0.9891 <sup>b</sup>	8.6 <sup>c</sup>	2.5 <sup>d</sup>	1.8 <sup>a</sup>	12.9
15	83 <sup>b</sup>	1.099 <sup>a</sup>	8.8 <sup>b</sup>	2.8 <sup>c</sup>	1.6 <sup>b</sup>	13.2
16	81 <sup>b</sup>	1.0715 <sup>b</sup>	9.1 <sup>b</sup>	3.4 <sup>b</sup>	1.7 <sup>a</sup>	14.2
17	77 <sup>c</sup>	1.1304 <sup>a</sup>	8.9 <sup>b</sup>	3.1 <sup>c</sup>	1.4 <sup>b</sup>	13.4
18	75 <sup>c</sup>	1.1539 <sup>a</sup>	9.1 <sup>b</sup>	2.5 <sup>d</sup>	1.8 <sup>a</sup>	13.4
19	76 <sup>c</sup>	1.1775 <sup>a</sup>	8.9 <sup>b</sup>	2.5 <sup>d</sup>	1.7 <sup>a</sup>	13.1
20	74 <sup>c</sup>	1.0715 <sup>b</sup>	9.6 <sup>a</sup>	3.3 <sup>b</sup>	1.9 <sup>a</sup>	14.8
21	72 <sup>d</sup>	1.1304 <sup>a</sup>	8.9 <sup>b</sup>	4.1 <sup>a</sup>	1.7 <sup>a</sup>	14.7
F (P)	7.14 (0.0006)	38.9 (0.004)	2.611 (0.001)	6.506 (0.005)	1.007 (0.004)	
LSD	6.225	0.092	0.762	0.451	0.504	



**Fig. 4. Effect of feeding honey bee workers with one bee honey comb and another one empty on venom glands parameters in autumn season**



**Salman (2009)** recoded that feeding the honeybee colonies on different types of proteinaceous diets caused an obvious increase in gland length and venom sac volume as compared with the control group. The longest gland and volume of venom sac were obtained in spring season followed by summer, autumn and winter seasons, respectively at any tested age of workers.

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## المخلص العربي

دراسات مورفومترية على غدة ومخزن السم في شغالة نحل العسل *Apis mellifera* L.شيماء أحمد البهنسي<sup>1</sup>، عادل محمد البسيوني<sup>2</sup>، حاتم محمد محفوظ<sup>1</sup>، محمد نجيب البسيوني<sup>1</sup>

1. قسم الإنتاج النباتي، كلية العلوم الزراعية البيئية، جامعة العريش، مصر

2. قسم وقاية النبات، كلية الزراعة، جامعة عين شمس، مصر

أجريت هذه التجربة خلال موسمي الربيع والخريف لعامي 2019 و2020 لدراسة تأثير العمر والتغذية وفصول السنة على الخصائص الشكلية لوزن الجسم وكيس السم والغدة الحامضية لشغالات نحل العسل اللاتي تتراوح أعمارهن (من عمر يوم إلى واحد وعشرين يوماً). أشارت النتائج إلى أنه في موسم الربيع، كانت أعلى أوزان للجسم في اليوم الثامن (122 مجم)، وأعلى مساحة لكيس السم (مم<sup>2</sup>) في اليوم السابع عشر (1.554 مم<sup>2</sup>) بينما أدنى مساحة لكيس السم (مم<sup>2</sup>) كانت في اليوم الأول (0.942 مم<sup>2</sup>) وأعلى طول للغدة الحامضية كانت بعمر 12 يوم (15.76 ملم). بينما في موسم الخريف مع توفر خبز النحل فإن أعلى وزن للجسم تم تسجيله في اليوم التاسع (116 مجم)، أعلى مساحة لكيس السم (مم<sup>2</sup>) في اليوم الخامس عشر (1.5346 مم<sup>2</sup>) وأعلى طول للغدة الحامضية تم تسجيله عند عمر الثالث عشر (15.3 ملم) وعند ندرة خبز النحل في فصل الخريف، فإن أعلى وزن للجسم تم تسجيله في اليوم الأول (94 مجم)، بينما أقل وزن للجسم تم تسجيله في اليوم الحادي والعشرين (79 مجم)، أعلى مساحة لكيس السم (مم<sup>2</sup>) تم تسجيله في اليوم العشرين (1.1933 مجم). وأعلى طول للغدة الحامضية تم تسجيله عند عمر 17 يوم (15.4 ملم). وعند ندرة عسل النحل في فصل الخريف، وجد أن أعلى أوزان للجسم تم تسجيله في اليوم الأول (94 مجم)، بينما أدنى وزن للجسم تم تسجيله في اليوم الحادي والعشرين (72 مجم)، أعلى مساحة لكيس السم (مم<sup>2</sup>) تم تسجيله في اليوم التاسع عشر (1.1775 مم<sup>2</sup>) وأعلى طول للغدة الحامضية تم تسجيله في اليوم الثاني عشر (15.5 ملم).

الكلمات الإسترشادية: نحل العسل، كيس السم، غدة السم الحامضية.

## REVIEWERS:

Dr. Asmaa Anwer

Pesticides Residues and Environ. Pollution, Central Agric. Pesticides Lab., Agric. Res. Center, Dokki, Egypt.

Dr. Tarek Abdel-Rahman

Plant Protec. Res. Inst., Giemeza, Agric. Res. Center, Dokki, Giza, Egypt.

| asmaa.anwer111@gmail.com

| tarekessa2006@yahoo.com