Effect of *Varroa* Infestation on the Morphological and Histological Structure of the Hypopharyngeal Glands of *Apis mellifera* Workers

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Abstract: The ectoparasitic mite *Varroa destructor* is the most dangerous of honeybee *Apis mellifera* parasites, as it causes many losses in apiculture worldwide. Several researches have demonstrated distinct levels of virulence of the mite and increased colony mortality rates due to its infestation; however only a few studies report the mite's effects on specific tissues, glands or other organs in bees, the main secretory products of the hypopharyngeal gland (HPGs) are royal jelly components, as well as other substances such as α -glucosidase. The present study aimed to evaluate the parasitic effect of the mite *V. destructor* on hypopharyngeal glands of *A. mellifera*, to identify changes in the morphometrical Measurements of HPGs acini at different ages. The histological and ultra structures of HPGs are also studied due to its important role in the development of the colony. Morphometrical measurments of HPGs of honey bee workers clear that the mean acinal surface area increased gradually from emergence until it reached the maximal area on the 12 day of worker age (0.0628 ± 0.007 and 0.0356± 0.004 for control and infested workers, respectively). A gradual decrease was taken place towards guarding and foraging activities. Data of histological and ultrastructure investigations came in parallel with the morphometry of the HPGs where the highest number and size of gland acini and secretory vacules; the density of secretory granules within the vacules; the quantity of secretion in the main duct of the glands were seen in 12 days old workers, recording the highest secretory cycle followed by gradual decrease up to 18 day where ireversable degeneration stated to take place with foraging activity. Varroa infestation causes reduced acinal surface area, number of secretory vacules, quantity of secretion in the gland duct and an acceleration towards gland degeneration.

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Key words: *Apis mellifera*, exocrine gland, hypopharyngeal, *Varroa destructor*, ultrastructure, histology

1. Introduction

Honey bees, *Apis mellifera L.* are considered the wings of agriculture due to their important role in crop pollination and hive products (honey, propolis, royal jelly, wax, bee venom, pollen, queens and bee packages). However, honey bees are reliable to be attacked by many insect pests, birds, mites and diseases inducing severe damages.

Nowadays the ectoparasitic mite *Varroa destructor* (Anderson and Trueman, 2000), formally named *Varroa jacobsoni* (Wongsiri *et al.,* 1996; Oldroyd and Wongsiri, 2006). *V. destructor* considered as an important and dangerous ectoparasite of honeybees and can feed on the bodily fluids of larvae, pupae, and adult bees. This mite has been attributed to the recent widespread Colony Collapse Disorder (CCD) as a disease vector (Sasagawa *et al.,* 1999; Van Englesdorp *et al.,* 2007; Anderson *et al.,* 2008). *Apis mellifera*, is critical for crop pollination and honey production. The ectoparasitic mite *V. destructor* is currently a worldwide and serious threat to beekeeping (De Jong *et al.,* 1982). *Varroa jacobsoni Oud.* is associated with the appearance of deformed bees in colonies of *Apis mellifera* and colony death normally occurs within 3–5 years of initial infestation (Korpela *et al.,* 1992). The mite acts as a vector for viruses that may cause problems such as bees growing with defective wings and high bee mortality rate (Rosenkranz *et al*., 2010). This parasitic mite causes weight loss, malformation, a shortened life span in honey bees (De Jong *et al*., 1982, Kovac, Crailsheim, 1988). The existence of a correlation between the infestation with the mite and either viral dispersion, physical deficiencies or shortening of life expectancy (Bailey and Ball 1991, Khalil, 1992).

Only a small number of researches have been published on the tissue, glands or other organs morphological changes (Schneider & Drescher 1987). One of the structures that may be directly affected is the hypopharyngeal gland, which is located in the head and produces a protein-based substance that is used to feed larvae, the queen and the drones.

The hypopharyngeal glands in worker bees in special are of great important and interest due to their secretion (royal jelly) that is the main food for raising honey bee brood and queens i.e. these glands are the backbone of the development of honey bee colony (

Khalil, 1983; Ohashi *et al.,* 1999; Silva and Bowen, 2000)

The present study aimed to evaluate the effect of the *Varroa* mite infestation on hypopharyngeal glands of *A. mellifera* workers during immature stages, through morphometrical, histological and ultra structural of HPGs acini at different ages.

2. Materials and Methods

The present investigation was carried out during (the end June to the end of September) to study the possible effect that may take place to the hypopharyngeal gland of honey bee workers at different ages under the infestation with mite *V. destructor* during immature stages. For this purpose morphometrical, histological and ultra structural (SEM) studies were performed.

A- Expermintal honey bee colonies :

This part of study was performed in the apiary of the Fac. of Agric., Zagazig Univ.; Egypt. Four colonies of Carnio Egyptian hybrid honey bee (two healthy colonies and two infested colonies) were used. The test colonies were established as bee nuclei in July, via inserting severe Varroa infested sealed brood comb up to emerge, by division of strong colonies being nearly of equal strength bee population. Stored honey and pollen and headed by sister queen, nearly of the same age and weights.

An artificial infestation with V. destructor mite, was made to two colonies in August the other group ,on the contrary were treated with formic acid (60%) to control Varroa mites, even if there are no apparent infestation (probably hidden) to form the control (healthy colonies) . The onset of the experiment was started when tha infestation rate in the arterially infested colonies reached around 15-20 %, nearly in mid fall (autumn), the test colonies were fed as usual on 1:1 sucrose solution at 2 weeks intervals.

B- Sampling :

On emergence, about 100 newly emerged workers in each colony of both healthy and infested were marked with colour paint on their thorax . emergence and left in their colony by applying five blown of smokes from bee smoker to let bee to calm down and to reduce, their tendency to kill the newly emerged painted workers.

Ten honey bee workers were picked up from the marked bees at the ages of 0 (newly emerged), 3, 6, 9, 12, 15 and 18 days old from the healthy (control) and infested colonies, to investigate the variation in morphometrical and histological structures of them. In addition, a signal sample of 12 days-old worker (completely developed) of both healthy and infested ones, to investigate the variation in ultra structure (scanning electron microscope) of HPGs.

C- Morphometry of HPGs :

Five honey bee workers of 0,3,6,9, 12,15 and 18 days-old were picked up from both healthy and infested colonies to determine the morphometry of the hypopharyngeal glands. Using a binocular microscope, HPGs at respective ages were dissected out from workers head using modified blades, in insect saline solution (NaCl 8.766 g., CaCl2 0.188 g., KCl 0.746 g., MgCl2 0.407 g., NaHCO3 0.336 g., sucrose 30.807 g., and trehalose 1.892 g., pH 7.6). The HPGs were then of five healthy and infected glands were measured morphometrically under binocular light microscope by using a micrometer eye lens in mm (maximal length and width) of (20) acini for each gland. Acinal surface area was calculated according to Maurizio's formula (1954):

$$\text{Acinal surface} = \pi \times \frac{a \times b}{2}$$

where a = maximal length, b = maximal width and $\pi = 3.14$

The measured values were then modified (corrected) according to the power of ocular and objective lenses.

D- Isolation of glands and preparation of histological sections

Histological studies of hypopharyngeal glands of honey bee workers were maintained in the laboratory of Zoology Department, Faculty of Science, Zagazig University; Egypt.

One sample (five workers) from each cage of healthy "control" and infected honey bee worker were collected at 0,3,6,9, 12,15 and 18 days old post inoculation, to study the variation of histological structure of HPGs in both colonies. Bee heads of each developmental stage were dissected hypopharyngeal glands in the Apiculture and Senculture Laboratory, Plant Protection depart., Fac. Agric., Zagazig Univ.,Egypt and then transferred the sample in insect saline (NaCl 8.766 g., CaCl2 0.188 g., KCl 0.746 g., MgCl2 0.407 g., NaHCO3 0.336 g., sucrose 30.807 g., and trehalose 1.892 g., pH 7.6) and examined by using (CXL Binocular compound light microscope optic) "microscopic examination". In order to obtain sections in wax, the further processes as described by (Hussein *et al*., 1990) were used.

The samples were immediately immersed in the fixative solution. For histological studies the samples were fixed in 10% formalin solution for 24h, followed by staining with Haematoxylin & Eosin. Samples were dehydrated in a standard ethanol series: 70%,80%, 90%, 95%, and 100% 10 min for each sample. The glands were cleared in histoclear

"xylene" for 2 h. All previous processes were carried out by using rotary tissue processor (Leica TP 1020), then were impregnated by placing the samples in 1:1 histoclear "xylene": wax for 1 h in the incubator at 60 °C followed by three changes embedding in pure paraffin wax for 45 min, 1 h and 3 h, respectively at 60°C. The tissue was embedding consol system (Leica EG 1150 H). The blocks were cut into 4 µm. thickness using automatic rotary microtome (Leica RM 2255, Germany). The sections were mounted on chemically clean glass slides without using any adhesive material. For studying general structure of the developmental stages of hypopharyngeal glands of honeybee workers in sections, the haematoxylin and eosin stains were prepared and used as described by (Drury and Wallington 1980) and (Bancroft and Gamble 2002).

E- Ultrastructural analysis

Ultra structural studies of hypopharyngeal glands of honey bee workers were maintained by using high vaccum mode at the Regional Center of Mycology and Biotechnology (RCMB) in Al-Azhar University, Nasr City, Cairo, Egypt.

For scanning electron microscopy (SEM), specimens "glands" were fixed in 2.5% glutaraldehyde (Sigma) for one hour at 4°C then washed with 0.1 M sodium cacodylate buffer solution (pH 7.2) for about 15 minutes.

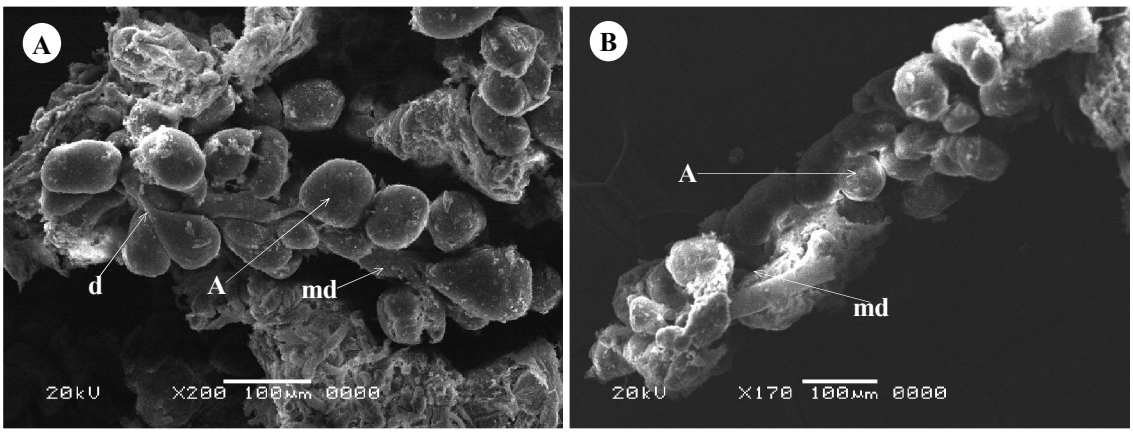
glands were postfixed in 2% osmium tetroxide in cacodylate buffer for one hour at room temperature. They were then washed three times in the same buffer for 10 min each. Dehydration by serial dilution of ethanol (50%, 70%, 80%, 90%, 95%, 100 %) with agitation using automic tissue processor (Lecia EM TP, Lecia Microsystems, Austria), the glands remained in each concentration of ethanol for 5 minutes, then embedded in acetone solution.

The specimens were subjected to critical point, drying by using CO2 critical point drier (Model: Audosamdri- 815, Tousimis, Rockville, Maryland, USA). The specimens were coated with gold in a sputter coating apparatus (SPI-Module, USA), gold coat enhances the electron scattering from the specimen surface, envisaged by electron scanning microscope. The specimens were examined with scanning electron microscopy (Model : JSM-5500 LV, JEOL Ltd – Japan at an accelerating voltage of " 10-15V").

F-Statistical Analysis

Statistical differences between HPGs mean gland (length, width and acinal surface) for two samples, results were reported as the mean ± S. E. Differences between means were determined by using one- way analysis of variance (ANOVA) according to (Steel and Torrie, 1980) except the comparison between the healthy (control) and infected HPGs honey bee workers were reported as the mean ± S.E. Difference between means were determined by using T test according to Samuels (1989). Changes were considered as non- significant, significant and highly significant, when the P-value was $P > 0.05$, $P < 0.05$ and $P < 0.01$, respectively.

3. Results and Discussion



Figure(1): Ultra structure (SEM) of the HPG of *A. mellifera* workers at 12-day old; (A) HPG Control. (B) HPG infested with the mite *Varroa destructor*. A, acinus; md, main duct; d, duct.

The ultra structure (SEM) of the HPG (fig. 1A) showed that it is composed of a paired structure, every side consists of a long, slender main channel with alveolar clusters of glandular secretory cells open. There are approximately 550 of these alveolar units associated with each of the channels. These alveolar clusters, known as acini, consist of 8-12 glandular cells, which are each connected to a duct cell. These duct cells will form a bundle, and connect the secretory cells with the main channel. The

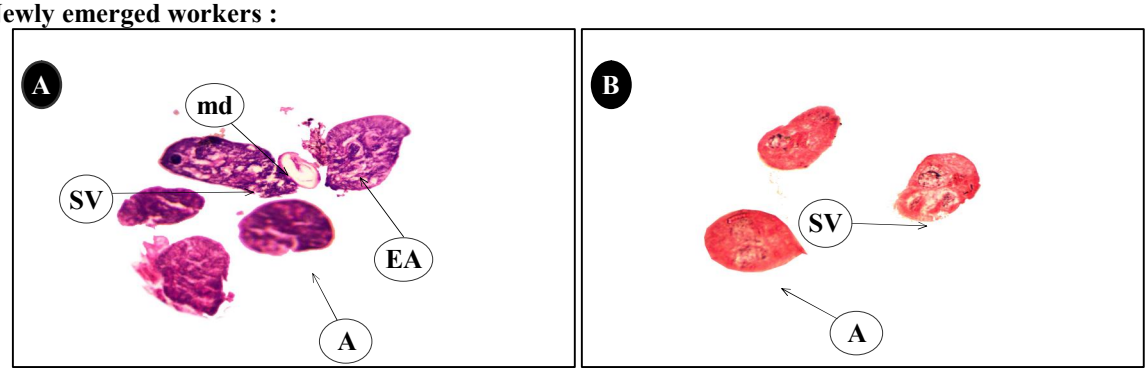
secretion will be transported further by the duct cell into the main channel. (Noirot and Quennedey, 1991; Quennedey, 1998). The two main channels open up into the suboral plate of the hypopharynx, so that the secretion ends up being released trough the mouth of the bee.(Silva de Moraes and Bowen 2000; Deseyn and Billen 2005). While the infested sample (fig.1B) showed a small size of acini and a rupturure of the main duct, due to infestation with mite *(V. destructor*).

Table (1): Means of hypopharyngeal gland, acinal length (mm), width (mm), and surface (mm^2) in different days of life *A.mellifera* honeybee workers both healthy (Control) and infected:

Age (day)	Sample	hypopharyngeal gland acinal sizes			
		length (mm)	width (mm)	surface (mm^2)	P-value
		Mean ± S.E. (Min – Max)	Mean ± S.E. (Min – Max)	Mean ± S.E. (Min – Max)	
0-day old	Con.	0.1085 ± 0.008** (0.0971 - 0.1126)	0.0812 ± 0.007** (0.0723 - 0.0897)	0.0138 ± 0.001** (0.0110 -0.0159)	13.2816
	Inf.	0.0886 ± 0.007** (0.0693 - 0.1031)	0.0691 ± 0.005** (0.0603 - 0.0816)	0.0096 ± 0.001** (0.0066 -0.0132)	
3-days old	Con.	0.1526 ± 0.014** (0.1474 - 0.1599)	0.1211 ± 0.013** (0.1109 - 0.1325)	0.0290 ± 0.002** (0.0257 -0.0333)	26.200
	Inf.	0.1144 ± 0.011** (0.1002 - 0.1357)	0.0884 ± 0.009** (0.0677 - 0.1085)	0.0159 ± 0.001** (0.0107 -0.0231)	
6-days old	Con.	0.1863 ± 0.019** (0.1751 - 0.1932)	0.1435 ± 0.016** (0.1389 - 0.1501)	0.0420 ± 0.005** (0.0382 -0.0455)	15.1092
	Inf.	0.1378 ± 0.014** (0.1166 - 0.1582)	0.1033 ± 0.009** (0.0809 - 0.1206)	0.0223 ± 0.003** (0.0148 -0.0300)	
9-days old	Con.	0.2102 ± 0.025** (0.1994 - 0.2213)	0.1567 ± 0.016** (0.1399 - 0.1700)	0.0517 ± 0.006** (0.0438 -0.0591)	16.4666
	Inf.	0.1492 ± 0.016** (0.1300 - 0.1681)	0.1154 ± 0.012** (0.0975 - 0.1325)	0.0270 ± 0.003** (0.0199 -0.0350)	
12-days old	Con.	0.2296 ± 0.032** (0.2185 - 0.2417)	0.1741 ± 0.021** (0.1595 - 0.1885)	0.0628 ± 0.007** (0.0547 -0.0715)	15.0878
	Inf.	0.1676 ± 0.019** (0.1452 - 0.1895)	0.1354 ± 0.015** (0.1205 - 0.1489)	0.0356 ± 0.004** (0.0275 -0.0443)	
15-days old	Con.	0.1693 ± 0.020*-3 (0.1577 - 0.1780)	0.1309 ± 0.017*-3 (0.1225 - 0.1393)	0.0348 ± 0.004** (0.0303 -0.0389)	16.300
	Inf.	0.1218 ± 0.015 ns-3 (0.1001 - 0.1443)	0.0968 ± 0.011*-6 (0.0712 - 0.1201)	0.0185 ± 0.002*-3 (0.0112 -0.0272)	
18-days old	Con.	0.1211 ± 0.014 ns-0 (0.1117 - 0.1308)	0.0990 ± 0.007** (0.0876 - 0.1052)	0.0188 ± 0.002** (0.0154 -0.0216)	17.401
	Inf.	0.0908 ± 0.010 ns-0 (0.0723 - 0.1113)	0.0712 ± 0.008 ns-3 (0.0606 - 0.0853)	0.0101± 0.001 ns-0 (0.0069 -0.0149)	
L.S.D (0.05 - 0.01)	Con.	(0.013 - 0.017)	(0.009 - 0.012)	(0.003 - 0.004)	calculated value
	Inf.	(0.009 - 0.011)	(0.006 - 0.009)	(0.002 - 0.003)	

ns Non significant
*Significant at 0.05
** Highly significant at 0.01

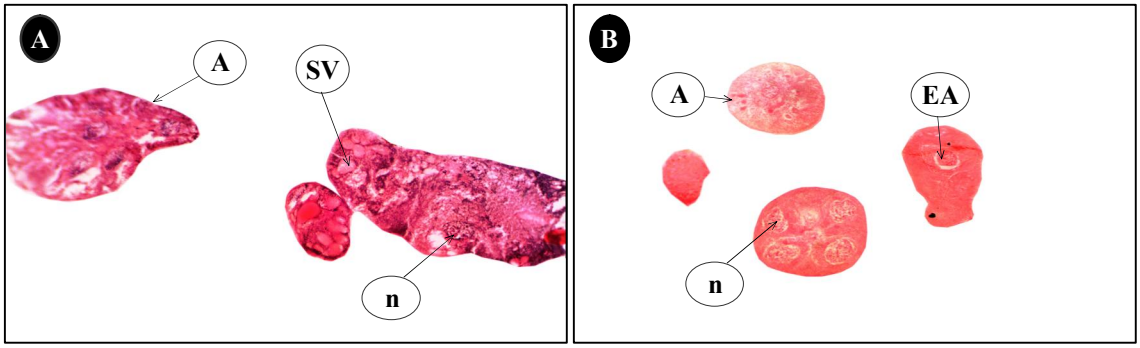
a- Newly emerged workers :



Figure(2): Sections of HPG acini of *A. mellifera* workers at 0-day old; (A) HPG healthy (Con.). (B) HPG infested with the *Varroa destructor*. Image obtained from a light microscope at 100x. A, acinus; md, main duct; EA, end apparatus; SV, secretory vacules; n, nucleus

Data of the morphometrical studies which represented in (Table 1) revealed that the mean HPGs acinal surface (mm2) reached 0.0138 ± 0.001 and 0.0096 ± 0.001 for healthy (control) and *Varroa* infested workers during immature stage, respectively at 0.01 (P value =13.281). Analysis of variance revealed significant difference between the two workers. Histologically HPGs acinus (Fig.2A) are characterized by dense cytoplasm, basophilic neuclei and empty duct in both healthy and infested workers, however the nuclei of infested workers manifested numerous nucleoli, HPGs acinuis (Fig. 2B) showed a decrease in size due to infestation with *V. destructor* mite, so that these results are in agreement with our morphometry. The secretory vacules are absent in both workers indicating that the secretory cycle of the gland was not started yet. Similar data were also reported by (Schneider & Drescher, 1987), found that there is a significant decrease in acini diameter in bees infested with mites, respectively, along with relatively lighter body weight of the emerging infested bees. Similar results regarding weight loss were also observed for bees by (De Jong *et al.*, 1982)

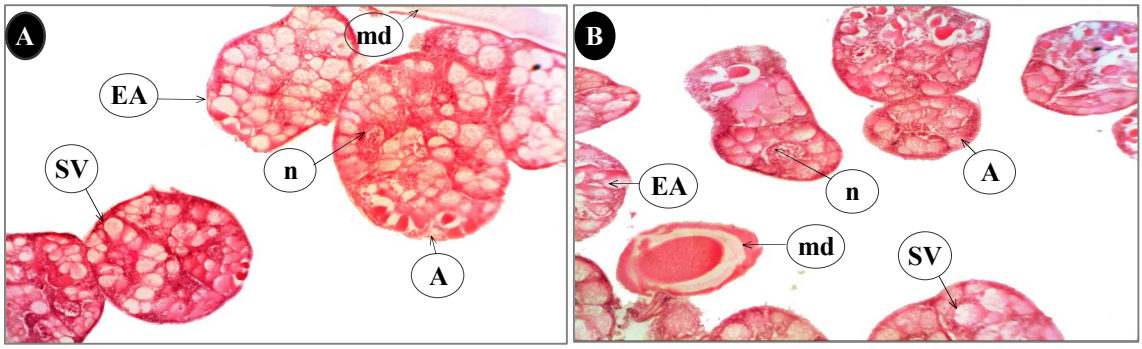
b- Three days old workers :



Figure(3): Sections of hypopharyngeal gland acini of *A. mellifera* workers at 3-days old; (A) HPG healthy. (B) HPG infested with the mite *Varroa destructor*. Image at 100x.

Data in table (1) clear that the mean surface area of HPGs acinus recorded highly significant decrease in the infested worker 0.0290 ± 0.002 and 0.0159 ± 0.001 mm2 for healthy (control) and Varroa- infested workers, respectively at 0.01 (P value = 26.200). Analysis of data clear that healthy workers possessed highly significant increased in the acinal surface area. Histological pictures revealed larger gland acinus with clear neuclei with distinct dispersed chromatin and nucleoli. Moreover, the secretory cells of healthy workers contained large vacules full of secretory granules, but in small number. The cytoplasm is still dense granulated (Fig. 3A). On the other hand the gland in infested workers is less developed as the secretory vacules are still absent, the infested HPGs acinuis showed a decrease in size due to infestation with mite (Fig. 3B), so that these results are in agreement with our morphometry.

c- Six days old workers :



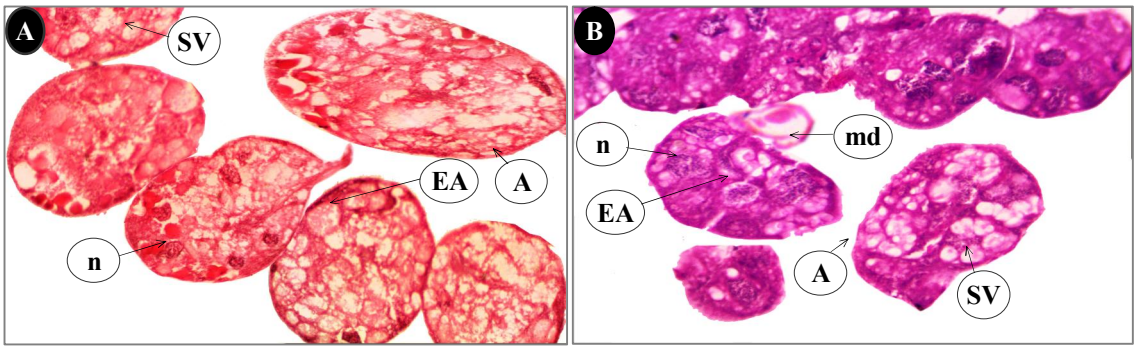
Figure(4): Sections of hypopharyngeal gland acini of *A. mellifera* honeybee workers at 6-days old; (A) HPG healthy (Control). (B) HPG infested with the mite *Varroa destructor*. Image at 100x.

Task regulation in worker honeybees is based mainly on age, with young individuals performing activities inside the nest such as brood care and wax production, while older bees become foragers with extranidal activities. Age-dependent changes in exocrine glands can be expected, because of changing functions (Michener, 1974).

The reduction in the size of HPG has a potential negative effect on the production and quality of substances that are useful to bees.

Obtained results (Table 1) clear that the mean surface area of HPGs acinus increased sharply, reaching 0.0420 ± 0.005 and 0.0223 ± 0.003 mm^2 , respectively for healthy (control) and infested workers at 0.01 (P value = 15.1092). Histological study revealed higher secretory activity of the gland of both workers, more pronounced in healthy (Fig. 4A) one where the secretory cells are filled with secretory vacules that arranged around the neucleus forming rose-like shape. Secretory vacules appear in the picture containing huge number of secretory granules. Generally acinal size, number of vacules and the density of secretory granules are relatively higher in the glands of healthy workers as compared to that of infested one (Fig. 4B).

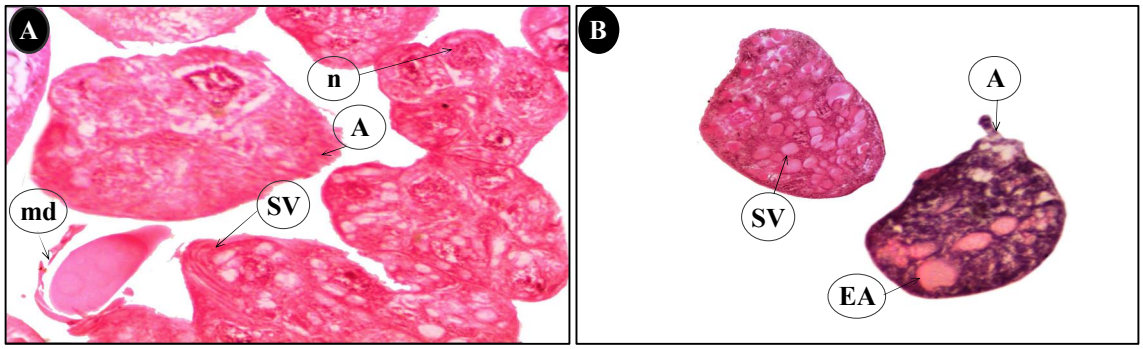
d- Nine days old workers :



Figure(5): Sections of hypopharyngeal gland acini of *A. mellifera* honeybee workers at 9-days old; (A) HPG healthy (Control). (B) HPG infested with the mite *Varroa destructor*. Image obtained from a light microscope at 100x.

The observed morphometricall studies (Table 1) proved that the mean surface area of HPGs acinus reached 0.0517 ± 0.006 and 0.0270 ± 0.003 mm^2, respectively for healthy (control) and infested workers at 0.01 (P value = 16.4666). Analysis of variance revealed highly significant increase in the HPGs acinal surface (mm^2) in the healthy workers than infested workers. Histologically the gland acinus and secretory cells showed higher secretory activity.The ductules come in form the secretory cells with the secretion towards the end apparatus are cleary appeared specially in the gland of healthy workers (Fig. 5A)e.

e- Twelve days old workers :



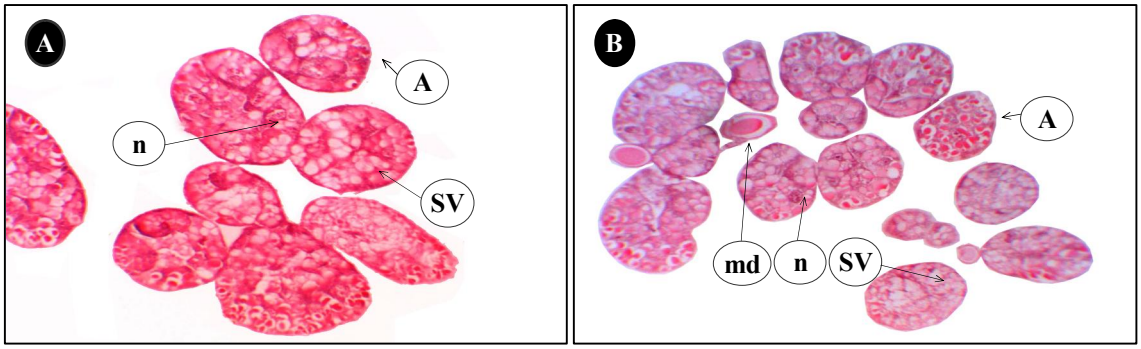
Figure(6): Sections of hypopharyngeal gland acini of *A. mellifera* honeybee workers at 12-days old; (A) HPG healthy (Control). (B) HPG infested with the mite *Varroa destructor*. Image at 100x.

On the other hand the gland of infested workers showed empty secretory vacules, indicating an abnormal absorption of hemolymph material and small nuclei due to mite infestation (Fig. 5B), and the

cytoplasm become dense again refering to the end of the first secretory cycle of the gland, in which it secrets the proteinious part of royal jelly. , but HPGs of infested workers presented cell boundaries with less vacuoles

The morphometry of HPGs acinus revealed the maximum mean surface area at this age in table (1) as it recorded 0.0628 ± 0.007 and 0.0356 ± 0.004 (mm^2), respectively for healthy and infested workers at 0.01 (P value = 15.0878). The difference between the two workers is highly significant in favor of the healthy workers. Histological pictures clear that the main duct of the gland is full of granular secretion, while the gland cells are lack of vacules except very few empty ones in healthy workers (Fig. 6A). However, the vacules were more pronounced in number in *Varroa* infested workers. large numbers of nuclei were clearly observed, the cytoplasm is crowded with more vacuoles, which appear at the neighborhood of the collecting duct due to accumulated secretions in the extracellular region. However, when parasitized with the mite and possibly by their associated viruses, the number of the secretory vacules decreased and this is affect on the secretion production (fig. 6B) Also, we found that some cells still have vesicular RER, while others already have reticular RER in the acini of HPG at 12 days old and this was in agreement with (Cruz-Landim *et al.*, 1987) who proved that organelles modify with age, such as the RER. The endoplasmic reticulum is almost vesicular and becomes more reticular. This situation is also seen in a Melipona species. Probably these different cells do not have the same function at this time. Also, we found that the glandular cells increased more than other worker ages and become larger,because of more dense secretion of accumulated protein (royal jelly) is found around the end apparatus, when workers are known to feed the larvae with royal jelly. This is agreement with morphometrical results (Hrassnigg and Crailsheim, 1998). While the infested HPGs acinuis showed a decrease in size and ruputure of the acinal epicuticle due to infestation with mite, so that these results are in agreement with our morphometry. The secretion produced by the HPGs plays an important role in the development of the brood and queen nutrition. Furthermore, colonies with high levels of infestation may suffer from damages due to parasitism effects such as change in structures, and decrease in the bee's life expectancy.This is agreement with (Wegener *et al.*, 2009) who observed that, the decrease in the size of HPGs in parasitized bees during their development stage may reduce their ability to produce royal jelly and cause abnormal development of the broods, considering that royal jelly is crucial to feeding larvae and queens.

f- Fifteen days old workers :

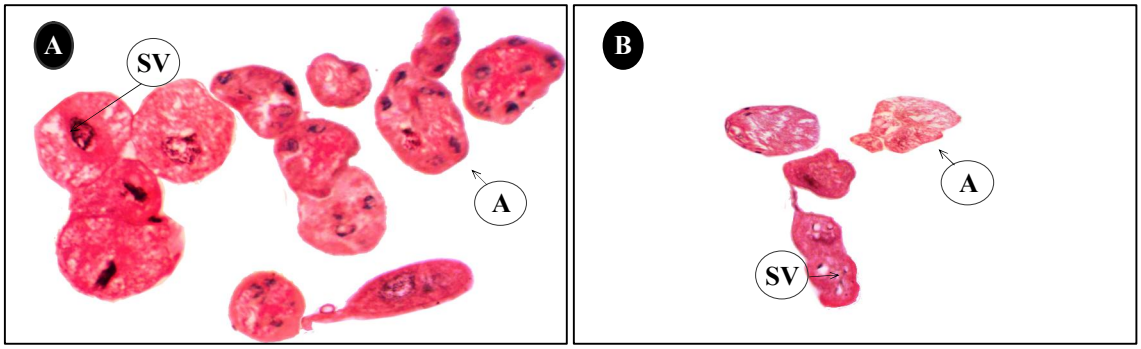


Figure(7): Sections of hypopharyngeal gland acini of *A. mellifera* honeybee workers at 15-days old; (A) HPG healthy (Control). (B) HPG infested with the mite *Varroa destructor*. Image at 100x.

The HPGs acini at this age, started to decrease morphometrically, as the recorded surface area reached 0.0348 ± 0.004 and 0.0185± 0.002 mm^2, respectively for healthy (control) and infested workers at 0.01 (P value = 16.300). The histological data showed an inverse situation, where the gland acinus is full of clear vacules, the acini of guards become irregular, elongated and cylindrical in shape and decreased in size than nurse bees or foragers, the numbers of secretory cells decreased and secretion still gathers around the end apparatus, the amount of secretion in the secretory cells is also positively correlated with size of the acini. taken irregeular arrangement and the main duct is also full of this clear secretion (enzyme). The development of the gland is more pronounced in healthy workers (Fig. 7A) than in Varroa infested one (Fig. 7B). This is in agreement with our morphometrical studies and with (Ohashi *et al.*,2000) who found that hypopharyngeal glandular size is known to be positively correlated with gland activity and is influenced by larval feeding. These glands gradually decrease in size

when honeybees become guards, cease feeding, and begin defending the colony. The hypopharyngeal gland secretes a proteinaceous substance which is fed to larvae, queens and drones (Ohashi et al. 1999) Morphologically, acini size of the HG radically changes with age. acini size begins to decrease after 15 days. The volume of the acini, as well as the number of secretory vesicles, decreases, and no vesicles are visible after 3 weeks of age. Gland size is positively correlated with gland activity. It has also been reported that the HPGs size is positively correlated with gland activity. The amount of secretion in the secretory cells is also positively correlated with acini size (Deseyn and Billien, 2005)

g- Eighteen days old workers :



Figure(8): Sections of hypopharyngeal gland acini of *A. mellifera* honeybee workers at 18-days old; (A) HPG healthy (Control). (B) HPG infested with the mite *Varroa destructor*. Image at 100x.

The mean HPGs acinal surface area recorded 0.0188± 0.002 and 0.0101± 0.001 mm^2 for healthy (control) and infested workers, respectively at 0.01 (P value = 17.401). The difference between the two workers is highly significant increase in control than in the infested workers. Histologically HPGs suffered severe irreversable degeneration in both types of workers, where the acinal area reduced, cell membranes disappeared, forming cynsetiume and a lot of neuclei became basophile(fig. 8A).

A little amount of protein (royal jelly), however a highly amount of electron dense (enzymes) secretion, which follows the production of the royal jelly accumulates around and in the end apparatus, this is in agreement with (Sasagawa *et al.*, 1989). The size of the glands in foragers corresponds with that of the undeveloped gland of newly emerged bees. This was also observed by (Simpson *et al.*, 1968). Therefore, size of the gland is positively correlated with gland activity. Older workers no longer feed the brood, so that the gland size decreased and thus gland atrophy is expected (Costa and Cruz-landim, 2005). Meanwhile, when bees become foragers, the hypopharyngeal glands are the site of conversion of nectar to simple sugars by enzymes also, (Huang and Otis,1989) investigated the effect of worker age on the hypopharyngeal glands development. At normal condition they are well developed when bees are nursed and they degenerate when bees become foragers. It depends on age of workers, the colony conditions and the time of the year. The amount of secretion in the secretory cells is also positively correlated with size of the acini. When the worker becomes a forager, the gland no longer has a food-producing function. This partially explains the decrease of size as illustrated by our morphometric results, but also the cytoplasmic structures start to disintegrate.

Also, we observed that HPGs acinus of the infected worker were smaller in size than control due to *V. destructor* infestation (fig 8B).In addition, the mite *V. destructor* is also a vector for several viruses, increasing the virulence rate of these pathogens within the hive. Some viruses that attack *A. mellifera* have tropism to specific regions of the bee's body such as organs or glands, affecting the development of these structures. For example, the DWV which is commonly found in the hives of A. mellifera (Teixeira *et al.*, 2008), causes deformity to the bee's wings and has a tropism to the hypopharyngeal, mandibular and salivary glands, and is also found in smaller concentrations in the gut and other organs. Another virus that may affect the development of hypopharyngeal glands is the ABPV (Lanzi *et al.*, 2006). When the viruses transmitted by the mite may affect the size and morphology of the hypopharyngeal glands (Denholm 1999).

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