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Salama Ahmed Salama
Faculty of Science, Jazan University KSA
Faculty of Science, Damanhour University, Egypt.

Correspondence:
Salama Ahmed Salama
Faculty of Science, Jazan University KSA
Faculty of Science, Damanhour University, Egypt.

Effect of some food additives on honey bee hypopharyngeal glands (*Apis mellifera*) in the south of Saudi Arabia

Salama Ahmed Salama

Abstract
This study illustrated the effects of selected Lamiaceae family plant extracts *Lavandula coronopifola, Ocimum basilicum, Ocimum filamentosum, Orthosiphon thymiflorus, Otostegia fruticosa* and Plectranthus asirensis on certain parameters telling the general condition of hypopharyngeal gland of honeybees *Apis mellifera.* In Apis *mellifera* workers the hypopharyngeal glands (HPGs) treated with diets containing food additives was matched to workers from the control honeybee colony treated with sugar syrup only. In the present research maximum length of hypopharyngeal gland acini (129 µm) for workers fed on diet-containing *Lavandula coronopifola*, followed by workers fed on *Ocimum filamentosum* diets (119µm), *Ocimum basilicum* (116µm) and no improvement for workers fed on *Otostegia fruticose* and *Orthosiphon thymiflorus* containing diets but there is negligible decrease in length of acini of workers bees fed on Plectranthus asirensis containing diet (105 µm) when compared with workers bees fed on sugar syrup only. The acini width in the worker colony's 12-day-old workers ranged from 87.2 to 102.9µm, and in bees of the same age in the control colony ranged from 100.8 to 158.2 µm. We observed that honey bee workers fed in these food additives (cell builders) had substantially greater diameter of acinar than workers in the equal age control colonies. The morphological and histological characteristics of HPGs in 12-days-old bee workers have been checked and noticed that food additives stimulated the growth of HPGs in breeding workers compared to workers feeding on sugar solution only. Furthermore, we described the channel diameter of HPGs in a bee worker and compared it to the control colony. The findings are evidence of that. It is clear that hypopharyngeal gland has the highest mean average canal diameter of (32.6 µm) was recorded for workers fed on the diet Lavandula coronopifola, followed by those fed on Ocimum filamentosum diets (31.59µm), Ocimum basilicum (28.53µm). At the other side, the of canal diameter of hypopharyngeal gland has not increased for workers fed at Otostegia fruticose and Orthosiphon thymiflorus containing diets but there is significant reduction in diameter of canal in workers bees fed on Plectranthus asirensis containing diet (10.48 µm) when compared with bees workers fed on sugar syrup (control). In general *Lavandula coronopifola* and *Ocimum filamentosum* substitute diets were superior to Ocimum basilicum and are recommended for addition to the diets of honeybee colonies.

Keywords: Acini, Apis mellifera, honey bee, hypopharyngeal gland

Introduction
Only present in the heads of worker bees, the hypopharyngeal glands (HPG) secrete the components of royal jelly, which is the brood and queen's most important food. The glands are well formed with large actively secreting acini in young workers. In quality queen rearing, the production of HPGs, which produce royal jelly, i.e. food for larvae, is of great importance. HPGs are found in a honeybee worker's and a queen's head, but grow only in workers. Hypopharyngeal glands (HPGs) in honey bees are age-dependent paired glands connected by various secretions to different social behaviors
(Liu *et al.* 2013; Ueno *et al.* 2015). Each gland consists of small oval bodies (acini) that are linked to terminal and axial secretory ducts. Active secretion reaches of hypopharyngeal gland grow into completely developed in new workers bees (6–13 days old bees) by great efficient secretion and HPG were mostly studied in workers (Lass **and Crailsheim 1996; Rahman *et al.* 2014)**. But, these glands de-generate as soon as the bee initiates foraging

behavior outside the colony **(Robinson 1992)**. The activity of the HPGs is mainly dependent on the acini size, which changes with age to express age-polytheism in honey bee's workers **(Deseyn and Billen 2005; Johnson 2010; Robinson 1992)**. Royal jelly is a protein-based food fed to larvae and to produce healthy queens **(Knecht and Kaatz 1990; Ohashi et al. 1999)**. These glands are also vulnerable to vari- ous stresses, such as starvation, heat, and Varroa infestation, which may result in the reduction and degeneration of the glands **(Khalil 1992; Yousef et al. 2014).**

Beekeepers in Saudi Arabia are spending large amounts of money for importing exotic bee races because the population of indigenous bees is too scarce and honey production is too low to meet the increasing demands of the local market **(Al-Ghamdi et al. 2017).**

The HPGs in the honeybee are a paired organ in front of the brain between the compound eyes, situated in the worker bee's head. The ducts open into the hypopharynx's sub-oral portion. Each of the glands consists of several small oval bodies or acini, short sections connected to the secretory duct axial or terminal.10 to 15 individual cellular bodies decide each acinus and each cell is connected through a thin duct to the axial duct (6). The size of the gland increases and decreases with a worker's age. Glands are inactive in newly-emerged workers and the secretory vesicles mature by the age of 3 days (7). Six-day old bees develop gland secretion and the glands secrete the enzymes at the age of 9 days and collaborate in the manufacture of royal jelly (8). However, deposition activity increases at the age of 15 days and the cells are already beginning to form lysosomes involved in the degeneration processes.

Material and methods

Honeybee source

The honeybee strain, *Apis mellifera carnica*, used in this study we found from the outdoor apiary biology department faculty of science Jazan University. Twenty four typical Langstroth hives, of one chamber containing 9 combs with sufficient brood and stored food, were selected to conduct the ***6*** **experiments**. The hives were headed with young and very active egg-laying queens and categorized to six groups with four hives for each.

Diets preparation

Each group of colonies was provided with a different artificial diet containing food additives as follows: 1) *Lavandula coronopifola* + sucrose + water (1:1:1, v:v:v), 2) *Ocimum filamentosum* + sucrose + water (1:1:1, v:v:v), 3) *Ocimum basilicum* + sucrose + water (1:1:1, v:v:v),4) *Otostegia fruticose* + sucrose + water (1:1:1, v:v:v), 5) *Orthosiphon thymiflorus* + sucrose + water (1:1:1, v:v:v), 6) Plectranthus asirensis + sucrose + water (1:1:1, v:v:v) and 7) sucrose + water (1:1, v:v) was considered as control. The diets were prepared and offered in a solution form.

Food consumption rate

Fortnightly, each hive received half liter of each diet described above. The diet was poured directly in the lateral feeder. Two days after feeding, the remaining amounts of solutions were collected and their volumes were measured. The percent of each diet consumed by honeybees was calculated. The experiment was conducted for one year and the seasonal consumption rates were recorded.

Morphometric studies

Two sealed brood frames with capped honey were selected from the hives and in an incubator reserved at 32-34°C and 60 ± 5% RH and provided with water *ad libitum*. Newly emerged workers (within 24 hours) were individually colour coded with metallic paint marker to define their age precisely. They were defined as being zero day old and reintroduced into the colonies of the aforementioned four groups at the day of coding. Twenty 12 and 18 days old workers were sampled and decapitated to measure head width, length and fresh weight. The heads were dissected according to the method of **Hrassnigg and Crailsheim (1998b)** in NaCl solution (7.5 g/L) under a stereomicroscope. The HPGs were removed and mounted on a glass slide. For each bee, the diameters of approximately 16 adjacent acini with a calibrated ocular micrometer were determined perpendicular to the long axis of the oval acinus. The acini volumes were calculated, captivating an acinus as a circle and its diameter as the shorter axis. The experiment was conducted for all seasons except autumn, when maize and date palm are found and colonies usually do not need pollen substitutes.

Statistical analyses

The knowledge obtained was tested for normality and homogeneity of variances with Klomogrov-Smirnov and Levene's tests, respectively. The parametric data were tested with One-Way ANOVA for analysis of variance followed by honest Tukey's test for determination of significant differences between groups. Pearson's correlation coefficient test was used for correlation analysis. The non-parametric data were tested with Kruskal-Wallis test followed by Mann-Whitney (*U*) test for determination of significant differences between groups. Kendal's tau-b test was used for correlation analysis. Using SPSS Statistics software 17.0©1993-2007, all data was statistically manipulated.

Histochemical investigations

Five 18 days old workers fed different diets were obtained and their hypopharyngeal gland were dissected, fixed in 10% formaldehyde, dehydrated in ascending ethanol series for 10 min each, embedded in paraffin and sectioned. Sections were microscopically examined and photographed.

Bee sampling

At the time of introduction into the colonies and every five days afterwards, nurse bees at 12 days of age, including nurse bees feeding queen larvae in the nurse colony, were sampled daily. Three employees and the experimental group were sampled at 12 days old.

Glands dissection

Workers were arrested with CO2 and the head in Hyes' solution (NaCl 9.0 g, KCl 0.2 g, CaCl 0.2 g, $NaHCO_3$ 0.1 g, 1 l purified water, pH 8.5) was fixed with two entomological needles on elastic base (Xantopren® L blue and general Activator, Heraeus Kulzer, Germany) in a Dish of Petri. Dissection was performed using stereomicroscope (SterREO Discovery.V12, Zeiss). Behind the compound eyes, the external chitinous exoskeleton of the facial area of the head was extracted and prepared for a further histological procedure.

Acini measurements

Stereomicroscope (SterREO Discovery.V12, Zeiss) and camera (Zeiss) have been used in vivo to conduct morphological measurements of the gland lobes (acini) in nurse workers in winter bees. The images were processed and measured later using the AxioVision Rel software4.6. Diameters of 30 randomly chosen acini perpendicular to the longer axis of the oval acinus were measured for three staff of the same age. The shape of an acinus is oval, but only the shorter axis length has been calculated and used for measurement purposes.

Data analysis

Data were statistically evaluated with SPSS 13.0 (SPSS Inc.; Chicago, IL, USA). The Means and Descriptive basic statistical parameters were determined. Significant variations in the diameter of the acini were measured using one-way ANOVA, with a filter as introduction to the cell bar (1 to3) and colony type (nursing colony, control colony). Comparison of the data of different aged employees. Mean acini diameter of HPGs was compared between workers from nurse colony and the control colony, using One-way ANOVA with age as a factor and further compared with cell bar starter as a factor. Scheffe's test was used for data testing. The Acini diameter in the HPG was measured for pupae and winter bees. The Means and Descriptive basic statistical parameters were determined.

A. Collection of plant material and preparation:

Freshly whole plant samples of Lamiaceae species were collected during April 2018 from the Jazan area, Kingdom of Saudi Arabia; Lavandula coronopifola, Ocimum basilicum, Ocimum filamentosum, Orthosiphon thymiflorus, Otostegia fruticosa and Plectranthus asirensis (Figures 1-6 respectively). They were found by the herbarium of the Department of Biology, Faculty of Science, University of Jazan. They were thoroughly washed 2-3 times with running tap water and then dried once with sterile water, then ground into a fine powder. The powder was used for crude extract extraction.

Fig. 1: *Lavandula coronopifola*

Fig. 2: *Ocimum basilicum*

Fig. 3: *Ocimum filamentosum*

Fig. 4: *Orthosiphon thymiflorus*

Fig. 5: *Otostegia fruticose*

Fig. 6: *Plectranthus asirensis*

B. ***Extraction of the plant material***

20g powder Plant mixed with 200mL of sterilized distilled water in tapering flasks. Respectively Extraction was carried out in ring road shaker for 6 hours at room temperature **(Sultana *et al*., 2009)**, filtered and residue was extracted twice. The combined supernatants were dried in a rotating evaporator and kept in a cool dry place till investigation.

Results

In this research, newly evolved caged bees w,ere supplied with various diets containing six different plant extracts. The heads of honeybees nurse workers of 12- days old in summer nourished with different foods were selected from areas around the opened brood at the same age and dissected. Morphological characteristics of the freshly prepared hypopharyngeal glands in these workers Wide acini with a dense structure and a milky-white color were found. (Fig. 12). Hypopharyngeal glands (HPGs) were investigated and it was observed that there are obvious 4 different developmental stages of nurse workers hypopharyngeal gland. These stages were preliminarily categorized agreeing to the number, size and shape of acini as well as the degree of main channel visibility. The hypopharyngeal glands improvement were evaluated by measuring the maximum length of the hypopharyngeal gland acini and its maximum width for each worker. Data presented in Table (1) show that the significant increase in mean maximum width of acini hypopharyngeal gland (102 µm) was recorded for 12- days old workers fed on the Lavandula coronopifola containing diet, followed by those fed on *Ocimum filamentosum* containing diets(99.59 µm), *Orthosiphon thymiflorus* (99.23 µm). Oppositely, the least significant width (87.4 µm) was recorded for workers bees fed on sugar syrup (control) (fig.7and 12) at the same time there is insignificant decrease in mean maximum width of acini hypopharyngeal gland (75 µm) was recorded for 12-days old workers fed on diet the containing *Plectranthus asirensis* when compared with worker bee fed on sucrose syrup.

In addition, the mean maximum length of hypopharyngeal gland acini in 12 days-old bees confined and fed on the test diets. It is clear that highest significant mean maximum length of hypopharyngeal gland acini (129 µm) was recorded for workers fed on the *Lavandula coronopifola* diet, followed by those fed on *Ocimum filamentosum* diets (119µm), *Ocimum basilicum* (116µm). On the other side, there is no change in the maximum length of acini was recorded for workers fed on *Otostegia fruticose* and *Orthosiphon thymiflorus* containing diets but there is insignificant decrease in length of acini of workers bees fed on *Plectranthus asirensis* containing diet (105 µm) when compared with workers bees fed on sugar syrup (control) (111 µm) as shown in the table blew.

Table 1: Acini number and channel diameter (µm) of the pharyngeal glands of honey bees, fed with the addition of plant extracts with sugar syrup.

Species	hypopharyngeal glands	
	12 days	
	Acini length.(µm)	Acini width(µm)
Control	111±2.73	87.4±1.02
Lavandula coronopifola	129±1.53	102abc±0.34
Ocimum basilicum	116±1.27	92.53abc±0.29
Ocimum filamentosum	119±0.28	99.59c±0.31
Orthosiphon thymiflorus	109±1.65	99.23abc±0.72
Otostegia fruticosa	110±1.12	88c±0.35
Plectranthus asirensis	105±1.32	75a±0.58

Table 2: Post Hoc Test for multiple comparisons (LSD: Least Significant Difference) test on acini length of hypopharyngeal gland of 12days old workers of honeybee, significance level (α) = 0.05.

	Control	***Lavandula coronopifola***	***Ocimum basilicum***	***Ocimum filamentosum***	***Orthosiphon thymiflorus***	***Otostegia fruticosa***	***Plectranthus asirensis***
Control	-	*	*	*	IS	IS	IS
Lavandula coronopifola	-	-	IS	IS	IS	*	*
Ocimum basilicum	-	-	-	IS	IS	*	*
Ocimum filamentosum	-	-	-	-	*	*	*
Orthosiphon thymiflorus	-	-	-	-	-	-	-
Otostegia fruticosa	-	-	-	-	-	-	-
Plectranthus asirensis	-	-	-	-	-	-	-

Table 3: Post Hoc Test for multiple comparisons (LSD: Least Significant Difference) test on acini width of hypopharyngeal gland of 12days old workers of honeybee, significance level (α) = 0.05.

	Control	***Lavandula coronopifola***	***Ocimum basilicum***	***Ocimum filamentosum***	***Orthosiphon thymiflorus***	***Otostegia fruticosa***	***Plectranthus asirensis***
Control	-	*	*	*	IS	IS	IS
Lavandula coronopifola	-	-	IS	IS	*	*	*
Ocimum basilicum	-	-	-	*	IS	*	*
Ocimum filamentosum	-	-	-	-	*	*	*
Orthosiphon thymiflorus	-	-	-	-	-	-	-
Otostegia fruticosa	-	-	-	-	-	-	-
Plectranthus asirensis	-	-	-	-	-	-	-

The recorded means of acini number of hypopharyngeal gland in 12 days-old workers bees fed on the test diets lead to significant rise in the number of acini and could be arranged descendingly as follows; *Lavandula coronopifola* diet (684 acini), *Ocimum filamentosum* containing diet (675 acini), *Ocimum basilicum* containing diet workers (526 acini), *Orthosiphon thymiflorus* diet(514 acini), when compared with the untreated worker bees (523acini) on the other side there is insignificant decrease in mean maximum number of hypopharyngeal gland acini was recorded for 12 days-old workers fed on diet the containing *Plectranthus asirensis* (466 acini) and *Otostegia fruticosa*(485 acini) when compared with worker of the equivalent age nourished on sugar syrup soln. (Table 58 and Fig.15). Generally, workers fed on *Lavandula coronopifola* diet showed the highest significant mean acini number, while *Ocimum basilicum* workers showed the lowest significant one was recorded for workers bees nourished on sugar syrup (control) (Table *2* and Fig.10)

Table (4) Acini number and channel diameter (µm) of honey bee pharyngeal glands, supplied with sugar syrup with the addition of plant extracts.

Species	Pharyngeal glands	
	12 days	
	Acini no.	Canal diameter (µm)
Control	523b±1.73	26.4bc±0.23
Lavandula coronopifola	684b±0.57	32.6abc±0.34
Ocimum basilicum	526b±0.57	28.53abc±0.29
Ocimum filamentosum	675c±0.88	31.59c±0.31
Orthosiphon thymiflorus	514b±1.73	23.23abc±0.72
Otostegia fruticosa	485c±1.02	25.40c±0.35
Plectranthus asirensis	466a±1.68	10.48a±0.58

Table 5: Post Hoc Test for multiple comparisons (LSD: Least Significant Difference) test on acini number of hypopharyngeal gland of 12days old workers of honeybee, significance level (α) = 0.05.

	Control	***Lavandula coronopifola***	***Ocimum basilicum***	***Ocimum filamentosum***	***Orthosiphon thymiflorus***	***Otostegia fruticosa***	***Plectranthus asirensis***
Control	-	*	*	*	IS	*	IS
Lavandula coronopifola	-	-	*	IS	*	IS	*
Ocimum basilicum	-	-	-	IS	*	IS	*
Ocimum filamentosum	-	-	-	-	*	*	*
Orthosiphon thymiflorus	-	-	-	-	-	-	-
Otostegia fruticosa	-	-	-	-	-	-	-
Plectranthus asirensis	-	-	-	-	-	-	-

The recorded means of canal diameter of hypopharyngeal gland in 12 days-old workers bees fed on the test diets. It is clear that highest significant mean maximum canal diameter of hypopharyngeal gland (32.6 µm) was recorded for workers fed on the *Lavandula coronopifola* diet, followed by those fed on *Ocimum filamentosum* diets (31.59µm), *Ocimum basilicum* (28.53µm). On the other side, there is no change in the of canal diameter of hypopharyngeal gland was recorded for workers fed on *Otostegia fruticose* and *Orthosiphon thymiflorus* containing diets but there is significant reduction in channel diameter of workers bees fed on *Plectranthus asirensis* containing diet (10.48 µm) when compared with workers bees fed on sugar syrup (control) (26 µm) as shown in the table above (*2*).

Figure (7) The HPGs of workers bees fed on syrup shows, the acini are less, small, ` undeveloped, and with spaces in-between. The acini, side canals and main channel is

visible. In fig 8 the HPGs of workers bees fed on *Otostegia fruticose* there was no significant difference in HPGs of syrup fed bees.

Figure (12) The HPGs of workers bees fed on *Lavandula coronopifola* containing diets, the acini increase in number and size and become rounded containing numerous vesicles accumulating secretion but still transparent. The main channel is still totally obvious but most of side canals are not clear as they are concealed by the filled acini with small spaces between them (fig.7,12) The HPGs of bees nourished on *Ocimum filamentosum* diets, is characterized by, the gland lobes develop to the maximum size and become highly functional. The acini are readily grown and become less transparent as well as the main channel and side canals are completely concealed.

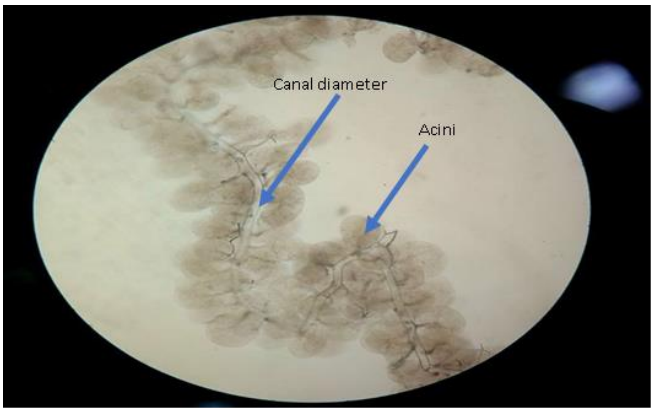

Fig. 7: Honey bee worker's hypopharyngeal gland fed on sugar-containing diets

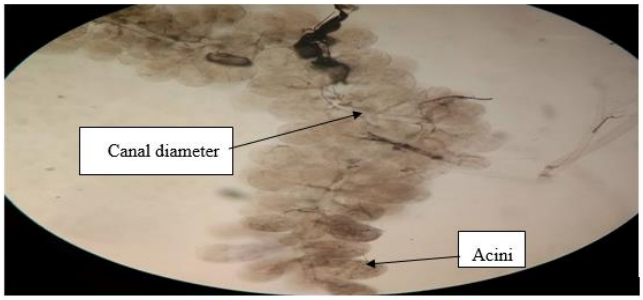

Fig. 8: Honey bee worker's hypopharyngeal gland fed on *Plectranthus asirensis* containing diets

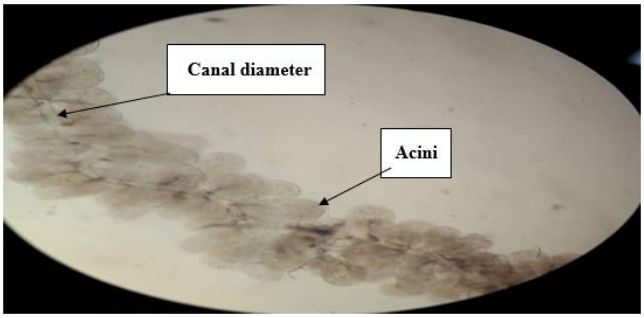

Fig. 9: Honey bee worker's hypopharyngeal gland fed Otostegia fruticosa containing diets

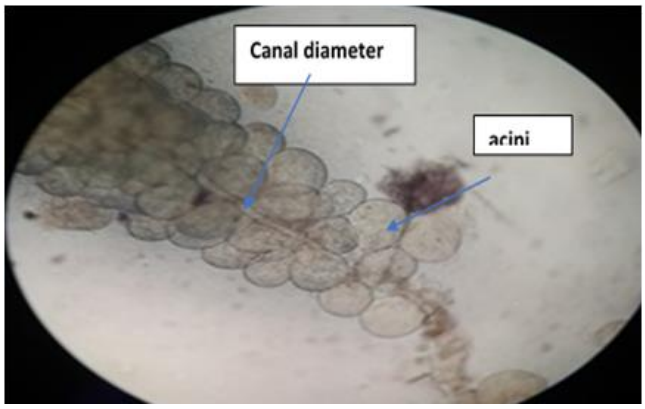

Fig. 10: Honey bee worker's hypopharyngeal gland fed on *Orthosiphon thymiflorus* containing diets

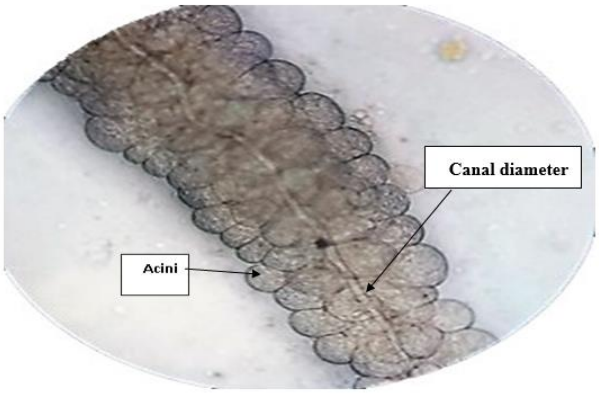

Fig. 11: Honey bee worker's hypopharyngeal gland fed on Ocimum filamentosum Containing diets.

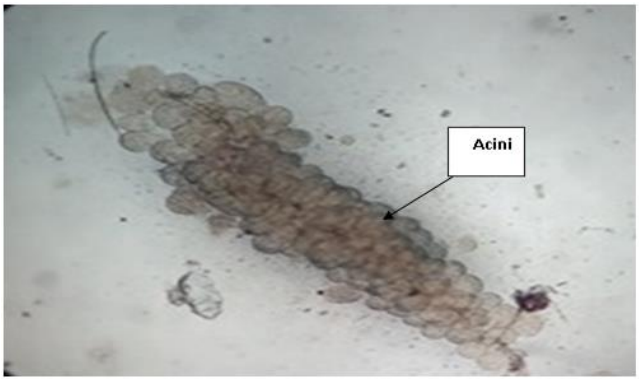

Fig. 12: Honey bee worker's hypopharyngeal gland fed on Ocimum basilicum containing diets.

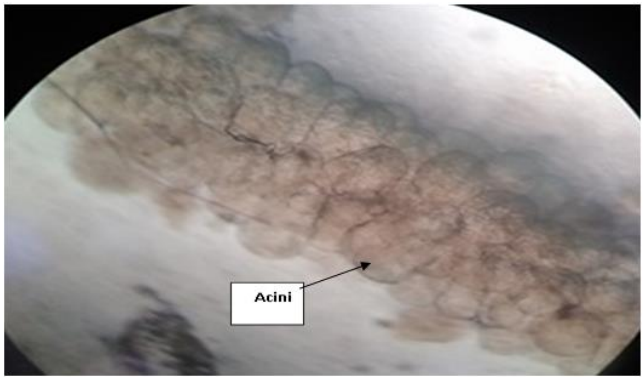

Fig. 13: Honey bee worker's hypopharyngeal gland fed on Lavandula coronopifola containing diets

Discussion

HPGs start to develop in worker pupae about a week before emergence and continue after bees emerge from the brood comb, changing their structure until they die. In summer bees, hypopharyngeal glands are flexible and can reactivate the HPG activity when needed. In this research, we test the ability of glands to elongate their activity and increase their size and number which depend on the age of the quality of food. The HG secretion is the main component of royal jelly, a material rich in protein and other nutrients that feeds young larvae, contributes to caste differentiation because of its morphogenetic properties, and is also the queen's exclusive source of nutrients. **(Michener 2007; Kamakura 2011)**. In The present study results demonstrated that there is significant increase in maximum width of hypopharyngeal gland acini for 12 days-old workers fed on the Lavandula coronopifola containing diet, followed by those fed on *Ocimum filamentosum* containing diets and *Orthosiphon thymiflorus* containing diets. That agree with The quality of food collected by honeybees has an important relationship to the overall hive development, and special attention must be given to the role that food plays on the improvement of the hypopharyngeal glands (HG) **(Wcislo and Cane 1996)**. On the other side, the least width was recorded for workers bees fed on sugar syrup (control) which in the same line with HG of workers bees of *Apis mellifera L*. (Hymenoptera:Apidae) have been morphologically and physiologically studied due to their position on the manufacture of royal jelly **(Cruz-Landim 2009; Seehuus et al. 2007; Gatehouse et al. 2004**). at the same time there is insignificant decrease in mean maximum width of acini hypopharyngeal gland was recorded for 12 days-old workers fed on diet the containing Plectranthus asirensis when compared with worker bee fed on sucrose syrup that agree with The results obtained from this study demonstrated that the type of diet does affect the improvement of the workers' glands agree with **DeGrandiHoffman et al. (2010)** who said that the finest results were detected for the group fed on treatment T3 (honey + pollen). Protein availability affected the size of acini, as groups of honeybees fed with protein enhancements offered acini with greater areas as matched with groups that were fed wholly with pollen and/or honey

From the our findings data in this study the workers bees that were fed solely with a sucrose syrup presented the smallest acini length values, This outcome is in agreement to what was presented by **Pernal and Currie (2000)**, who did not note differences, for the same age days, in the amount of protein secretion from glands of honeybees fed on foundations of inferior nutritional values. In comparison, in this analysis, the bees that fed with Lavandula coronopifola diet, which presented acini length significantly bigger than those obtained from the other dietetic treatments.

It is clear that in this study highest maximum length of hypopharyngeal gland acini was recorded for workers fed on the *Lavandula coronopifola* diet, *Ocimum filamentosum* diets and *Ocimum basilicum* this outcomes in agreement with result obtained by **Brodschneider and Crailsheim (2010)** who said that The best results for the acini region were obtained from the honeybee community fed with their natural source of protein (pollen), even though the bees did not completely developed HG in this procedure. This outcome may be valuable in the development of supplemental diets for hives in times of natural shortage of food. In order to reduce the costs, diets that combine pollen with other protein sources should be tested in future studies to evaluate the HG's growth and other structures such as the mandibular glands. Providing the hives with an adequate diet might also act as a means to prevent the occurrence of pathogens and epizootics such as CCD, which has been the cause of millions in losses to the apicultural business worldwide. At the same time there is no change in the maximum length of acini was recorded for workers fed on *Otostegia fruticose* and *Orthosiphon thymiflorus* containing diets but there is insignificant decrease in length of acini of workers bees fed on Plectranthus asirensis containing diet when compared with workers bees fed on sucrose only (control) This findings not in the same line with the result obtained by **Al-Ghamdi *et al.* (2011)** When evaluating food protein concentration and development of HG in Carniolan bees, verified a better development although other groups of the same category of bees were fed with rations with higher protein content and provided with underdeveloped glands, the structure of those fed with apicultural pollen also found that the consumption of diets based on soybean extract, milk powder and brewer's yeast was 56.63 percent lower than the consumption of diets based on apicultural pollen. Therefore the development of diets including fractions of apicultural pollen in their composition may be a good solution to minimize dietary aversion and increase the nutrient absorption, resulting in better overall hive development and improved royal jelly production.

As it clear from the previous data In this study we found that all tested diets with additive food lead to increase in the acini number of hypopharyngeal gland of workers of honey bee when compared with control this in agree with result data obtained by **Zahra and Talal (2008)** observed that the effect of supplemental feeding in hives of A. mellifera an increase in mean acinus size and HG ducts length was promoted with high values obtained in hive supplemented with vitamin C, overcoming soybean-based supplements extracts.

Conclusion

When the hypopharyngeal gland acini are not developed, they are less productive than the glands with the middle-sized acini. The structure of the HPGs changes with the age of individual worker bees and the tasks in the hive. To produce high quantity and quality royal jelly we have to consider the breeding technology, feeding technology and diet additives. HPGs are a flexible organ in young worker bees and are able to actively respond to the needs of the colony, In general *Lavandula coronopifola* and *Ocimum filamentosum* substitute diets were superior to Ocimum basilicum and are recommended for addition to the diets of honeybee colonies.

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