

## **GROWTH AND DEVELOPMENT OF THE HYPOPHARYNGEAL GLANDS AND DETECTION OF ESTERASE ISOZYMES ACTIVITY IN ASSOCIATION WITH VARROA INFESTATION IN SOME HONEYBEE RACES**

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### **ABSTRACT**

In the Egyptian and camiolan races and the hybrid bees, the fresh weight in mg per head maintained its high level (record) during spring and summer compared to autumn season. Moreover, the fresh weight revealed higher estimates in the Camiolan and Hybrid bees than in the Egyptian race in all seasons. As for the dry weight, it has also been concluded that both the Hybrid and Camiolan populations, in all seasons exhibited higher estimates in relation to the Egyptian race. The measurements of fresh weight per thorax showed that all the three populations exhibited their highest estimates during summer season.

In addition, both Camiolan and Hybrid bees were significantly superior to the Egyptian race in all seasons. A similar picture was detected in the thorax dry weight. Determination of nitrogen content per thorax showed that, the least significant differences between the three populations did not occur in spring and autumn. However the tested bees differed significantly during summer.

The development of hypopharyngeal glands showed significant differences between the three populations. The Hybrid bees exhibited the highest development of these glands followed by the Carniolan and the Egyptian races.

The esterase isozymes activities were assayed in healthy and infested pupal stages. While some esterases showed gradual decrease in activity followed by complete disappearance in successive pupal stages, others had highly active bands of isozymes in association with infestation of pupae by one or two varroa mites (*Varroa jobsoni*)

### **INTRODUCTION**

Honeybees, like most animals, require protein, carbohydrates, minerals, fats, vitamins and water for normal growth and development. These nutritional needs are satisfied by the collection of nectar, pollen and water.

Haydak (1935) pointed out that the weight and nitrogen content of newly emerging bees are directly influenced by the pollen consumption of the nurse bees and the fluctuation in the pollen income of the colonies.

Maurizio (1950) proved that the low concentration increased the length of life more than the high one. The development of hypopharyngeal gland and fat body was promoted by high pollen concentration. According to DE-Groot (1954), the bees fed excessive concentrations of protein often had decreased longevity, which is attributed to protein poisoning.

JABLONKA (1964) showed that 15-30 days old worker bees kept for 15 days in and fed a 50% sugar solution had a lower development of hypopharyngeal glands, as compared to 1-day old bees fed a normal diet.

Haydak and Dietz (1965) reported that any appropriate protein source is satisfactory for the growth of emerging bees and development of their hypopharyngeal glands. According to Standifer et al. (1970), the addition of 10% concentration of pollen gum weed and sugar to the food promoted more development of the hypopharyngeal glands than 1 and 5% concentrations. However, the low pollen concentration (1 and 5%) prolonged the life span of worker bees rather than the higher one (El-Barbary, 1989).

De Jong et al (1982) indicated that 6-25% weight loss in the worker, depended on the degree of infestations by varroa mite. According to Weinberg and Madel (1985), feeding activity of the mites during the brood stage causes appreciable losses (15-50%) in haemolymph protein content and total haemolymph volume of the emerging bees.

Ivanov and Spasov (1990) reported that the body, head weights and nitrogen content in the worker of *Apis mellifera carnica* were higher in summer than in bees living in winter season. Morsy (1998) reported that the varroa infestation has greatly affected and decreased the dry weight and protein content of the brood and newly emerged adult workers. Moreover, esterase isozymes were detected in the two strains, Carniolan-Egyptian and Italian-Egyptian honeybee. He also revealed the occurrence of five cathodal and four anodal bands representing the maximum numbers of esterase isozyme bands and mentioned that the infestation with one or two varroa mites enhanced esterase activities. He also suggested that variation in esterase isozyme may be mainly due to strain type more than location.

The aim of this study was to investigate some physiological and genetic characteristics of the two races of the honeybees, the Egyptian *Apis mellifera lamarckii*, and the Carniolan *Apis mellifera carnica*, and also the Hybrid between both races.

## MATERIAL AND METHODS

The material used in this study were the two races of *Apis mellifera*, the Egyptian race, *A. mellifera lamarckii* and the Carniolan race, *A. mellifera carnica* as well as the Hybrid. The colonies of each race were headed by newly mated queens of nearly equal age.

For the experimental work three Egyptian colonies each headed by a newly mated queen, were brought from the Bee Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation El-Dokky, Cairo. Three Carniolan colonies each headed by a newly mated queen were also secured. Three Hybrid colonies randomly mated (Carniolan queens X unknown drones) were provided by the Apiary of the Faculty of Agriculture in Alexandria.

### (a) Determination of fresh and dry weights and nitrogen content in the honeybee workers

Worker bees not more than 24 hours old were collected after emerging from combs of sealed brood held in an incubator at  $32 \pm 1^\circ\text{C}$ . To deprive the emerging bees from getting food, all areas of the comb containing honey or

pollen were covered with aluminum foil. Four replicates were applied from each experimental colony and each replicate contained 100 newly emerged workers. The bees were killed with chloroform and then the 400 bees were removed from each experimental cage. The heads and thoraces were separated. Fresh weights were dried at 102 °C for 24 hours to a constant weight (Haydak, 1959). After getting dry, the samples were weighed, homogenized in a mortar and kept in small vials, covered with parafilm paper and stored in desiccators. The nitrogen content was determined for thoraces by coloration method described by the Association of Official Agricultural Chemists (1970) and Evenhuis and De-Waard (1980).

**(b) Development of hypopharyngeal glands :**

The food was given to the colonies in a candy form (2 parts of honey + 5 parts of powdered sugar by weight). The concentration of pollen was 10% in relation to the weight of the candy. The candy patty diets were sheeted with aluminum foil and frozen at 5°C for few days till used.

The food candy was available to the newly emerged workers ad libitum (unlimited amounts) in a feeder of a small plastic cup placed in a cage of plastic material. Several narrow holes were made in the wall of the cage to prevent the accumulation of water vapour. A small section of comb filled with water was fixed to the wall of the cage to provide water. Newly emerged workers of less than 24 hours old from Carniolan, Egyptian and Hybrid bees were collected on emerging from combs of sealed brood held in an incubator at  $32 \pm 1^\circ\text{C}$ . To prevent the emerging bees from consuming any pollen or honey in the brood comb, all areas of the comb containing food were covered with aluminum foil. Groups each of 50 newly emerged workers were raised in four replicates in an experimental cage covered with muslin cloth. The cages were kept in an incubator at  $30 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  Relative humidity.

The newly emerged workers were left on the food provided for 10 days. After that 10 adult bees were taken from each experimental cage, and immediately frozen until the time of dissection. The hypopharyngeal glands were laid bare with a level cut through the head using a razor blade. The worker bee was put in a dissection plate and was flooded with distilled water as a dissection fluid. The glands were taken out from the front region of the head, and lined up in drops of distilled water on a glass slide. Since the right and left hypopharyngeal glands are often slightly different in their degrees of development, both were always evaluated. The development of the glands was investigated under the microscope at x 100 magnification power.

The development of hypopharyngeal glands was evaluated by referring to four stages ranging from 1 which represents no development to 4 which represented complete development. This assessment was adopted after Mourizio (1954).

The F test was applied for the analysis of variance. The L.S.D. was used to determine the significant differences between the means according to Snedecor and Cochran (1971).

**(C) Detection of esterase isozymes:**

Samples of sealed worker brood were collected from each colony of the Egyptian, Carniolan and Hybrid bee workers. Three developmental stages of the pupae were used in the biochemical genetic studies. The individuals of

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the pupal stages were of different ages at the time of sampling, white-eye (13-14 days), pink-eye (15-16 days), and black-eye (17-18 days). The samples of pupal stages were divided into healthy and non healthy pupae infested with one or two varroa mites. (*Varroa jacobsoni*). Each sample was kept in a vial in a deep freezer till using.

Starch gel electrophoresis was carried out according to El-Metainy *et al.* (1977). The electrode buffer and staining solution were prepared according to Shaw (1965) and Shaw and Keen (1967). The samples were homogenized in separate couples in 0.1 ml of distilled water according to Bitondi and Mestriner (1983). Each homogenate was absorbed on a 2 x 10 mm strip of filter paper, placed on the middle surface of a gel plate and kept at 4°C. for about one hour, and the filter papers were then removed. Electrophoresis was carried out with a constant current of 13-14 v/cm for 2 hours at 4°C. Then the gel plates were stained at room temperature with esterase staining solution for at least 30 minutes. The best plates were selected, photographed and their diagrams were drawn.

## RESULTS AND DISCUSSION

### (a) Growth rate of the honeybee workers

#### (1) Determination of fresh and dry weight of the head in the newly emerged workers:

The mean fresh weight of the head of the newly emerged workers in the two races and the Hybrid during different seasons were calculated and the data are summarized in Table (1). In spring, the mean fresh weight of the head was highest (10.07 mg) in the Carniolan race, followed by Hybrid bees (7.91 mg). The lowest value (7.11 mg) was recorded in those of the Egyptian race. The statistical analysis showed that there were significant differences between the two races and the Hybrid in spring. The same trend was noticed in bees reared in summer. The means also revealed that differences between Carniolan and Hybrid bees were not significant. However, significant differences were detected between the Egyptian race and both the Carniolan and Hybrid bees.

In autumn, a similar picture was found, where differences between the Hybrid bees and the Carniolan race were again insignificant. Moreover, the Egyptian race showed the lowest significance among the three groups.

**Table (1): Means of the head fresh weight between the tested bees in each season .**

Season	Tested bees		
	Egyptian	Carniolan	Hybrid
Spring	7.11 c	10.07 a	7.91 b
Summer	8.68 b	10.58 a	10.38 a
Autumn	6.56 b	8.68 a	9.07 a

L.S.D. (tested bees) = 0.55

Differences between means denoted by common alphabetical letters are not significant at 5% level.

Generally, it could be concluded that in all populations the bees reared in summer and spring seasons showed the highest means, compared with those bees reared in autumn.

The mean dry weight of the head of the newly emerged honeybee workers in the three tested groups during different seasons were also calculated and the data are summarized in Table (2). In spring, the highest mean of the head dry weight (2.37 mg) was recorded in the Carniolan race, followed by the Hybrid bees (2.06 mg). The lowest record 2.02 mg was obtained the Egyptian race. The statistical analysis indicated that there were significant differences between the Carniolan bees and both the Hybrid and Egyptian bees. In summer, the means of the head dry weight were 2.58 mg, 2.52 mg and 2.04 mg. in the Hybrid, Carniolan and Egyptian bees, respectively. The means were significantly higher in both the Hybrid and Carniolan bees than those of the Egyptian race. The mean in both the Hybrid and Carniolan bees did not differ significantly from each other. A similar picture occurred in autumn, as the highest mean dry weight of the head was recorded in Hybrid bees, followed by the Carniolan. The lowest record was obtained in the bees of the Egyptian race. The mean records in were also significantly higher in both the Hybrid and Carniolan bees than those of the Egyptian race in summer and autumn. Generally, it could be mentioned that the mean dry weight of the head of the Carniolan and Hybrid bees were higher than that of the Egyptian race during the three seasons.

**Table (2): Means of the head dry weight between the tested bees in each season :**

Season	Tested bees		
	Egyptian	Carniolan	Hybrid
Spring	2.02 b	2.37 a	2.06 b
Summer	2.04 b	2.52 a	2.58 a
Autumn	1.89 b	2.20 a	2.22 a

L.S.D. (tested bees) = 0.07

Differences between means denoted by common alphabetical letters are not significant at 5% level.

**(2) Determination of thorax fresh and dry weight in the newly emerged workers:**

The means of the fresh weight of thorax in (mg) in the two races and the Hybrid in spring, summer and autumn were calculated and the data are presented in Table (3).

**Table (3): Means of thorax fresh weight between the tested bees in each season:**

Season	Tested bees		
	Egyptian	Carniolan	Hybrid
Spring	25.80 b	30.74 a	30.00 a
Summer	29.12 b	36.28 a	34.10 a
Autumn	25.13 b	34.07 a	33.86 a

L.S.D. (tested bees) = 2.57

Differences between means denoted by common alphabetical letters are not significant at 5% level.

In spring, the Carniolan race showed the highest mean of 30.74 mg, followed by 30.00 mg for the Hybrid bees. The lowest mean (25.80 mg) was recorded in the workers of the Egyptian race. The same trend was noticed in summer and autumn seasons. Significance between the tested bees each season showed that there are no significant differences were occurred between Carniolan and Hybrid bees in the three different seasons. Both the Carniolan and Hybrid bees differed significantly from those of Egyptian bees reared in the three seasons.

As for the mean dry weight of the thorax in (mg) in the Egyptian, Carniolan and Hybrid bees during the three seasons were calculated and the data are presented in Table (4). In spring, the Carniolan race exhibited the highest mean of 8.43 mg followed by 7.56 mg for Hybrid bees. The lowest record of 7.06 mg was obtained in those of Egyptian race. A similar picture occurred in summer and autumn seasons. The statistical analysis showed no significant differences between Carniolan and Hybrid bees in the three seasons. The Carniolan and Hybrid bees were higher significantly than those of Egyptian bees in these seasons. From these results, it is evident that the Carniolan and Hybrid bees showed the highest means in case of the thoraces fresh and dry weights in the three seasons. The lowest means were recorded in those of the Egyptian race.

**Table (4): Means of thorax dry weight between the tested bees in each season:**

Season	Tested bees		
	Egyptian	Carniolan	Hybrid
Spring	7.06 b	8.43 a	7.56 a
Summer	7.26 b	9.07 a	8.99 a
Autumn	7.07 b	8.29 a	8.47 a

L.S.D. (tested bees) = 0.35

Differences between means denoted by common alphabetical letters are not significant at 5% level.

**(3) Determination of nitrogen content in the thorax of the newly emerged honeybee workers.**

The mean nitrogen content in (mg) per thorax in the two races and the Hybrid bees during spring summer and autumn were calculated and the data are presented in Table (5). The data showed that the least differences between the tested bees were not significant during spring and autumn. However the bee populations differed significantly during summer.

Generally, it could be mentioned that all the tested bees reared in autumn showed the lowest means of nitrogen content compared with those reared in spring and summer. It could be also strongly mentioned that Carniolan and Hybrid worker bees exhibited higher weight than the Egyptian race.

Accordingly, the results concerning both fresh and dry weight per head for the three populations of bees in various seasons revealed perfect superiority of the Hybrid and Carniolan populations on one hand and the superiority of bees reared during spring and summer seasons, on the other.

**Table (5): Means of thorax nitrogen content between the tested bees in each season:**

Season	Tested bees		
	Egyptian	Carniolan	Hybrid
Spring	6.21 a	6.88 a	5.80 a
Summer	2.97 c	4.72 b	6.79 a
Autumn	2.72 a	2.30 a	2.33 a

L.S.D. (tested bees) = 1.52

Differences between means denoted by common alphabetical letters are not significant at 5% level.

Regarding the results of fresh weight per thorax for the three populations of bees under investigation over different seasons, a high similarity exists between these results and those of fresh weight per head. The three populations exhibit highest fresh weight per thorax in summer season. In addition, both Carniolan and Hybrid bees were significantly superior to the Egyptian race in all seasons. Such results are identical with those of the dry weight per thorax of the same genotype at corresponding seasons. Concerning the weight of newly emerged workers, it has been considered as an important economic character, since higher weight of workers reflects more tolerance against unsuitable biological conditions. Furthermore, the weight of workers fluctuates in accordance with age and activity. It has been also found that the time of year and abundance of nectar and pollen may also affect the weight (Mitchell, 1970).

It has been long recognized that the rate of development of bees may be measured through either their thorax dry weight or thorax nitrogen content. In this concern, both criteria have been studied by Haydak (1937a) who found that, in certain cases, the rate of development of young bees can be determined by ascertaining only the thorax dry weight of experimental bees. Moreover, freshly emerged bees, having plenty of pollen for food, gain weight as the nitrogen content of their body increases (Haydak, 1934). It has also been demonstrated that newly emerged honeybees feeding only on sugar solution showed a decrease in body weight and nitrogen content (Haydak, 1937 b).

Determination of dry weight of head and thorax in newly emerged workers and in caged ones was carried out by Yacoub (1998). She found that, in relation to the dry weight of head and thorax at various periods of feeding, the Italian race had the highest significant means followed by the Carniolan and the Egyptian bees. These results are in complete accordance with those found in this study concerning the superiority of the Carniolan race over to the Egyptian bees with respect to these characters.

The nitrogen content in the thorax of the newly emerged honeybee workers indicated that the Egyptian and Carniolan races exhibited significantly lower nitrogen content in summer and autumn seasons rather than in spring, while the Hybrid population showed significant decrease of these parameters only in autumn. This seasonal variation is expected on the basis that the availability of food depends greatly upon season, which would reflect corresponding changes regarding nitrogen content which is in turn related to the rate of development (Haydak, 1937 a).

From another point of view, the differences in nitrogen content between populations among seasons showed no variability between them either in spring or autumn. It was only in summer that the three populations showed significant differences between them, the Hybrid being the highest followed by the Carniolan then the Egyptian population. Such results are expected on the basis of the fact that the great abundance of nectar and pollens during summer season allow various genotypes to express different rates of development and consequently different nitrogen contents compared to spring and autumn seasons.

**(b) Determination of the development of hypopharyngeal glands in honeybee workers :**

After the newly emerged Egyptian, Carniolan and Hybrid worker bees had been fed on their natural food for ten days, the mean of the hypopharyngeal glands development was estimated in the bees and the data are summarized in Table (6). The highest mean of the hypopharyngeal glands (2.93) was recorded in the Hybrid worker bees, followed directly by the Carniolan race (2.53). The lowest mean (2.42) was obtained in those of the Egyptian race. The statistical data in Table (7) prove that there were significant differences between the three groups. According to Maurizio (1954), the development of the hypopharyngeal glands with an average of more than 2.5 is considered as "well developed" while those with a lesser average are considered "poorly developed". Therefore, the Egyptian race may be considered fairly different from either the Carniolan or the Hybrid population. Studying the correlation between head weight and hypopharyngeal glands development in workers reared in colonies with normal and with decreasing amounts of brood, Hrasnigg and Craisheim (1998) found that head fresh weight is correlated with the size of glands and that pollen consumption is positively correlated with gland development. Verification of the results of fresh weight per head together with hypopharyngeal gland development for the three populations in this study greatly supports this view. Similarly, Mohanny (1999) showed that the hypopharyngeal glands reached the maximum development in spring and summer seasons. This result, again supports the presence of a positive correlation between hypopharyngeal glands development and fresh weight per head, since it was found in this investigation that all the three populations revealed significantly higher fresh weight per head in both spring and summer seasons compared with autumn.

**Table (6): Mean of hypopharyngeal glands development in worker bees at age of ten days after their nutrition on honey candy contained pollen.**

Replicates	Tested bees		
	Egyptian	Carniolan	Hybrid
R <sub>1</sub>	2.28	2.65	3.02
R <sub>2</sub>	2.50	2.60	2.78
R <sub>3</sub>	2.15	2.40	2.98
R <sub>4</sub>	2.75	2.45	2.95
Mean	2.42	2.53	2.93

- Each replicate was represented by 10 individuals.



Another analogy of the present results concerning the development of hypopharyngeal glands may also be detected in the work of Yacoub, 2002. She found that both the F<sub>1</sub> Hybrid and the Carniolan bees exhibited higher rate of hypopharyngeal gland development compared to the Syrian race.

**Table (7) : Means of hypopharyngeal glands development**

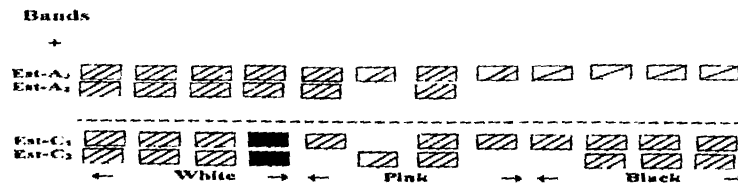
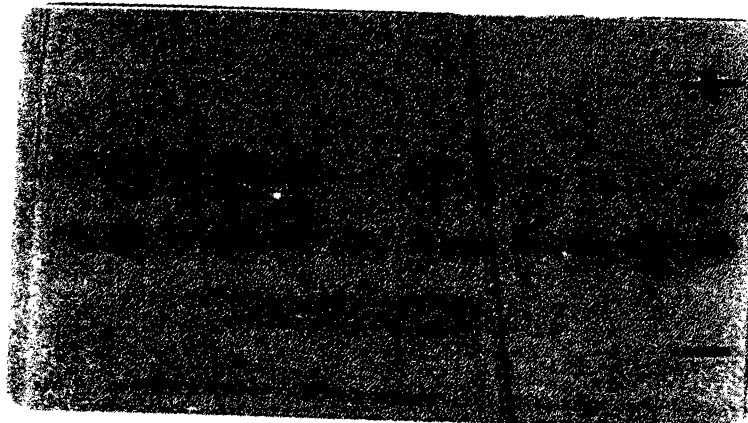
Tested bees	Mean of hypopharyngeal glands development
Egyptian	2.42 c
Carniolan	2.53 b
Hybrid	2.93 a

L.S.D. between tested bees (0.05)

Differences between means denoted by common alphabetical letters are not significantly different at 5% level.

**(C) Detection of esterase isozymes activity in association with varroa infestation in the honeybee workers**

In view of the differences encountered in esterase isozyme pattern as a discriminating tool between the Egyptian, Carniolan and the Hybrid populations, these patterns in healthy and varroa infested pupae at different stages of development were also investigated. Photography and descriptive zymogram of the esterase pattern in different pupal stages of the Egyptian healthy race are shown in Figure(1).



**Fig. (1):** Photograph and descriptive zymogram showing the Esterase isozymes pattern in pupal stages (white, pink and blackeye) of Egyptian healthy.

Key of band densities v. high [solid black] > [diagonal lines] > [white] > [white] v. low

It can be seen that the white-eyed stage exhibits two cathodal, Est-C<sub>1</sub> and Est-C<sub>2</sub>, and two anodal, Est-A<sub>2</sub> and Est-A<sub>3</sub>. In addition, the pink pupal stage shows a rather inconsistency regarding these bands, where absence, of individual bands was observed. e.g., Est-C<sub>1</sub> and Est-A<sub>2</sub> in slot 6 and Est-C<sub>2</sub> and Est-A<sub>2</sub> in slot 8. As for the black eyed pupal stage (Fig. 1), it can be seen that Est-C<sub>1</sub> and Est-C<sub>2</sub> are both present except for slot 9 where Est-C<sub>2</sub> has disappeared. Regarding the anodal pole of this stage, only Est-A<sub>3</sub> band was consistently present.

Considering the esterase pattern exhibited by different Carniolan pupal stages (Figure 2), it can be clearly observed that the number of cathodal and anodal bands has increased.

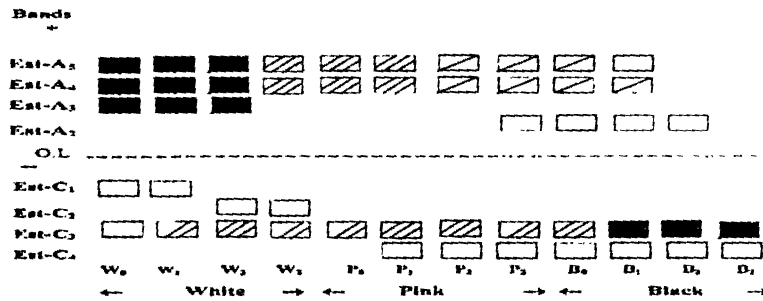


Fig. (2): Photograph and descriptive zymogram showing the Esterase isozymes pattern in pupal stages (white, pink and black eye) of Carniolan race infested by 1 and 2 varroa mites with their controls.

Key of band densities v. high ■ ▨ ▨ ▨ ▨ v. low

This feature for Carniolan was previously observed in newly emerging adults belonging to this race. Four cathodal bands namely Est-C<sub>1</sub>, Est-C<sub>2</sub>, Est-C<sub>3</sub> and Est-C<sub>4</sub> and four anodal bands namely Est-A<sub>2</sub>, Est-A<sub>3</sub>, Est-A<sub>4</sub> and Est-A<sub>5</sub> are also detected. Examining the white-eyed pupal stage, Est-C<sub>1</sub>, Est-C<sub>2</sub> and Est-C<sub>3</sub> were present in different samples, the latter being present in all samples. In the pink pupal stage, Est-C<sub>3</sub> was still existing in addition to Est-C<sub>1</sub> but Est-C<sub>1</sub> and Est-C<sub>2</sub> have disappeared. With regard to the black pupal stage (Fig. 2), both bands Est-C<sub>1</sub> and Est-C<sub>2</sub> may be detected. The anodal bands, Est-A<sub>3</sub>, Est-A<sub>4</sub> and Est-A<sub>5</sub> in white pupal stage, turned to be Est-A<sub>2</sub>, Est-A<sub>1</sub> and Est-A<sub>5</sub> in the pink pupal stage. Then they remained unchanged in the black pupal stage.

As the Hybrid population is considered, different pupal stages presented fairly distinctive patterns of esterase isozymes. Regarding cathodal bands (Figure 3), all the white, pink and black-eyed pupal stages expressed a relatively highly active Est-C<sub>2</sub> band. It is interesting to note the appearance of a new highly active Est-C<sub>1</sub> band in the sample, representing black-eyed pupae infested with two varroa mites. This finding may shed light on the effect of varroa infestation on the gene action regulation. Regarding anodal bands exhibited by the Hybrid pattern (Figure 3), it can be seen that the Est-A<sub>4</sub> was the only band appearing in common in all pupal stages. Mean while, Est-A<sub>3</sub> is consistently present in the white stage. Two additional extra bands, Est-A<sub>2</sub> and Est-A<sub>3</sub> appeared in the pupae infested with one or two varroa. Another interesting observation is the appearance of a new band, Est-A<sub>2</sub> in the black pupal stage infested with two varroa.

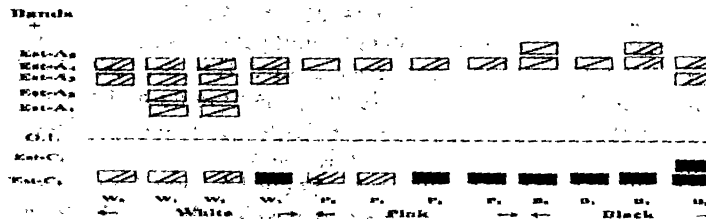
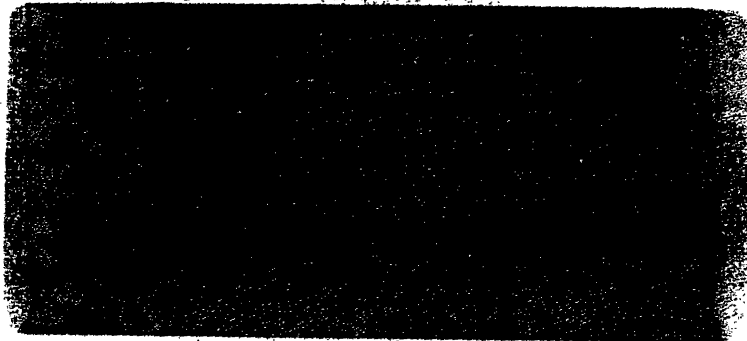


Fig. (3): Photograph and descriptive symogram showing the Esterase isozymes pattern in pupal stages (white, pink and black eye) of Hybrid infested by 1 and 2 varroa mites with their controls.

Key of band densities: High - [Solid Black] - [Diagonal Lines] - [White] - [White] v. low

From the results of esterase patterns in pupal stages shown in (Figures 1,2 and 3 for the Egyptian, Carniolan and Hybrid respectively, it may be concluded that the two races and Hybrid bees exhibited fairly different patterns during their development and differentiation. The appearance of newly high density bands was detected in many cases in association with infestation of pupae by varroa mites. Furthermore the cathodal bands, while expressing highest activities in the adult stage, higher activities in the pupae, specially in earlier stages were attributed mainly to anodal bands.

From the results obtained in this study, it has been concluded that the three populations exhibited fairly different esterase patterns during pupal development and differentiation. Whereas some esterases showed gradual decrease in activity followed by complete disappearance in successive pupal stages, other esterase isozymes formed new and high active bands in association with infestation of pupae by one or two varroa mites. These results may be related to the occurrence of the natural defense mechanisms against varroa infestation. It may be also a useful as indicator for the tolerance behaviour of bee colonies against varroa mites (Abdel-Wahab, 2001).

In accordance with the results obtained in this study, Gadelhak (1999) reported that infestation of the last larval instar and pupal stages with varroa mites has changed dramatically band protein concentration. Similarly, Morsy (1998) found that the infestation of honeybee workers in different pupal stages with one or two varroa mites enhanced esterase activity.

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نمو وتطور الغدد تحت البلعوية وتقدير النشاط للمشابه الأنزيمي الأستيرييز تحت ظروف الإصابة بطفيل الفاروا في بعض سلالات نحل العسل  
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- استهدفت هذه الدراسة نمو شغالات نحل العسل من حيث الوزن الجاف والمحتوى النتروجين وتطور غددها تحت البلعومية وتأثير الإصابة بالفاروا على نشاط المشابه الأنزيمي الأستيرييز لثلاث عشائر مختلفة من نحل العسل وهما سلالة النحل الكرنولي وسلالة النحل المصري والهجين. وأوضحت نتائج الدراسة ما يلي :-
- 1- أظهرت نتائج الوزن الرطب للرأس أن العشائر الثلاث أعطت أعلى تقديرات خلال فصلي الربيع والصيف. مقارنة بفصل الخريف. كذلك أعلى تقديرات لكل من الكرنولي، والهجين مقارنة بالنحل المصري على مستوى جميع الفصول.
  - 2- أوضحت نتائج الوزن الجاف للرأس توافقا تاما مع نتائج الوزن الرطب من حيث الزيادة في قيم فصلي الربيع والصيف مقارنة بالخريف وكذلك التفوق لكل من الكرنولي والهجين مقارنة بالمصري في كافة الفصول.
  - 3- أظهرت تقديرات الوزن الرطب للصدر أن العشائر الثلاث تحت الدراسة بينت أعلى تقديرات خلال الصيف، كذلك فإن قياسات الكرنولي والهجين تفوقت على السلالة المصري على مستوى الفصول الثلاث.
  - 4- أعطت نتائج الوزن الجاف للصدر تقديرات متوافقة تماما مع تقديرات الوزن الرطب في العشائر والمواسم المناظرة.
  - 5- أدى تقدير النمو للغدة تحت البلعومية في العشائر الثلاث الى ظهور اختلاف معنوي فيما بينها حيث أظهر الهجين أعلى معدل نمو للغدة يليه الكرنولي ثم المصري.
  - 6- درست مشابهاة أنزيم الأستيرييز في أعمار مختلفة من نمو العذراء تحت الظروف العادية وظروف الإصابة بالفاروا بكثافات مختلفة وقد تبين ان بعض مشابهاة الأستيرييز يقل نشاطها تدريجيا ثم يختفي تماما خلال تتابع مراحل تطور العذراء. في حين أن مشابهاة أخرى قد أستحدثت فيها حزم جديدة مصاحبة للإصابة بواحدة أو اثنتين من الفاروا.