

A STUDY OF SOME PHYSIOLOGICAL PARAMETERS IN DIFFERENT RACES OF THE HONEYBEE WORKERS *Apis mellifera* L.

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ABSTRACT

The newly emerged F₁ hybrid workers had the highest mean weights for the head and thorax either fresh or dry. The Carniolan race came next, while the Syrian workers had the lowest weights in spring and summer. The hypopharyngeal glands showed a similar trend in their development after feeding the newly emerged workers for ten days on candy containing 10% of pollen grains. The F₁ hybrid workers showed the highest rate of development, followed by the Carniolan race. The lowest mean was recorded in those of the Syrian race. Some major elements, namely Potassium (K), Calcium (Ca), Sodium (Na) and Magnesium (Mg) in the head of the newly emerged workers of the Syrian and Carniolan races and their F₁ hybrid in spring and summer seasons were determined by Atomic absorption techniques. The statistical analysis proved strongly that there were significant differences between the honeybee workers reared in spring and summer in their content of the (K), (Ca), (Na) and (Mg) in the three tested bees.

Concerning to the thorax fresh weight, dry weight and nitrogen content for each tested bees during the two seasons. Statistical analysis indicated that there were significant differences between the means of the two races and their F₁ hybrid in spring and summer. Only one exception occurred in the case of nitrogen content of the Carniolan race.

INTRODUCTION

In a normal colony, newly emerged bees having plenty of pollen, were able to raise the development of hypopharyngeal glands, weight and nitrogen content of their bodies (Haydak, 1934). He also found in (1970) that pollen consumption is diminished at the age 8-10 days. Herbert and Shimanuki (1979) found that low pollen content (1% and 5%) increased the life span of workers.

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. Minerals are not essential for the development of the hypopharyngeal glands, but brood rearing was increased by the addition of ash to the diet (Haydak and Dietz, 1965). The major elements in each body region were K., Ca., Na and Mg with the greatest concentration in the head (Nation and Robinson, 1971). They also pointed out that although there are differences between bees collected in the late summer and those collected in early spring, more detailed experiments are needed to evaluate the true contribution of the season.

The aim of this investigation is to compare two races of honeybees namely the Syrian race, *Apis mellifera syriaca*, Carniolan race, *Apis mellifera carnica* and their F1 hybrid in relation to growth (Changes in dry weight and nitrogen content) and the development of the hypopharyngeal glands. Determination of the concentration of some major elements during spring and summer seasons was also studied.

MATERIALS AND METHODS

The work and experiments were carried out in the Apiary and laboratory of the Department of Economic Entomology, Faculty of Agriculture, Alexandria University.

Five colonies of the Syrian and Carniolan races and their F1 hybrid were established for the experiments. Syrian bees and newly mated queens were brought from the Syrian Beekeeping Development Project, Damascus, Syria. Carniolan queens were obtained from the Egyptian Ministry of Agriculture and Land Reclamation. F1 hybrid was secured when Syrian virgin queens were naturally reared from mother colonies, then transported into Benton cages to an isolated region for Carniolan colonies in the North of the Delta.

Newly emerged workers aged 0-24 hours for each tested bees (50 workers/ replicate) were obtained during spring (April) and summer (August). To prevent the emerging bees from consuming any pollen or honey in the brood comb, all areas of the comb containing these materials were covered with aluminum foil. Each of the two races and their F1 hybrid was represented by three replicates and 150 workers (50 workers/ replicate). The workers were killed using chloroform, and their heads and thoraces were separated, each weighed and preserved in a freezer. When the number of samples was completed, they were dried at 102°C for 24 hours (Haydak, 1959). Each dried sample was homogenized in a mortar. The heads were used to determine some of the major elements K, Na, Ca and Mg, while thoraces were used to estimate the percentage of nitrogen content.

To determine some major elements the method of Nation and Robinson (1971) was adopted. Dried head samples were ashed and determined weight from each sample (0.1gm), were put into porcelain crucibles and placed in a muffle furnace at a temperature of 100 °C. Temperature was raised slowly to 500-550 °C and held for 12 hours. When cooled samples were removed, 0.5 ml 2-N HNO₃ was added and slowly dried at 100 °C. The temperature was again raised to 400-500 °C for three hours. 1.0 ml 1-N HCl was added to each crucible. Solutions of ash from 0.1 gm (dry weight) sample were prepared up to a volume of ten ml. by adding distilled water. The final concentration of HCL was 0.1 N. A techtron model AA-100 Atomic absorption spectrophotometer at the central laboratory in Faculty of Science was used. The working maximum values of concentration of elements involved in this study were as follows. K, 10 ppm; Na, 25 ppm; Ca 1 ppm and Mg, 0.5 ppm, suitable dilution for each element according to premier analysis of examined samples was prepared.

The total nitrogen content in the thoraces of newly emerged workers was determined using Kjeldahl method described by Bremner (1965) as follows; a dried sample (0.1 gm) was digested by using 5 ml concentrated sulphuric acid and 1.1 gm of digestion mixture, which constructed from 100 gm K_2SO_4 , 10gm $CuSO_4$ and 1.0 gm selenium, and digested on digestion block unit for overnight. When the digest was clear (turns light green or milk white), it was distilled using 20 ml of NaOH (40%). Twenty ml of 4% boric acid and four drops of bromocresol green- methyl red indicator solution were used to collect the distilled ammonia which was titrated with 0.05- NH_2SO_4 .

The development of hypopharyngeal gland was followed in ten old days workers after providing food in a candy form containing 10% pollen grains. Candy was prepared, sheeted with aluminum foil and kept freezer till used. Experimental cages that were of a plastic material were used. The food was available to the newly emerged workers in a feeder of a small plastic cup with unlimited amount ad-Libitum . A small section of empty comb was fixed to the wall of the cage for providing few water drops. Newly emerged workers aged 0.24 hours were confined in the experimental cages (50 workers/ cage as a replicate) and then covered by muslin cloth. Four replicates were used for each of the three tested workers. The cages population with bees were kept for ten days in an incubator at 30 °C under 65-70% R.H. Ten bees were taken from each cage, preserved in a small vial and frozen until ready for dissection. The hypopharyngeal gland was laid bare with a level cut through the head using a razor blade. The worker bee was put in a dissection plate and flooded with distilled water as a dissection fluid. The gland was taken out from the front region of the head under a binocular and lined up in drops of distilled water on a slide. Both right and left glands were evaluated under the microscope. The degree of development was evaluated by referring to the 4 stages Maurizio (1954). Stage 1 representing no gland development and stage 4 representing complete development.

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).

RESULTS AND DISCUSSION

Determination of fresh weight, dry weight and the hypopharyngeal glands development of the head in the newly emerged workers.

The means of fresh and dry weight of the head in the Carniolan and Syrian races and their F1 hybrid workers were determined during spring and summer seasons. The results are given in Table (1) .

In spring, the data showed that the highest mean of the head fresh weight was 10.65 mg in case of the F1 hybrid, followed in a descending order by the Carniolan race 10.00 mg and lastly 9.51 mg for the Syrian race. Statistical analysis showed that there were significant differences between the three tested workers. The same trend was detected during the summer season, where the means were 10.31mg, 9.91 mg and 9.26 mg in the F1 hybrid, Carniolan and Syrian bees, respectively. Statistical analysis showed that, the differences between the means were significant.

In case of, the mean of the head dry weight, the results proved that in spring season the highest mean was 2.57 mg in case of the F1 hybrid, followed by Carniolan 2.35 mg. The lowest value 2.27 mg was recorded in the Syrian race. Statistical analysis indicated that there were significant differences between the three tested bees.

The same trend was noticed during the summer season, when the means were 2.38 mg, 2.33 mg, and 2.23 mg in the F1 hybrid, Carniolan and Syrian bees successively. Statistical analysis showed that, the differences between the means were significant. Concerning, the development of the hypopharyngeal glands in the three tested honeybee workers, the data in Table (2) show that the highest mean 3.13 was recorded in the F1 hybrid bees, followed in a descending order by the Carniolan race 2.92. The lowest mean 2.58 was recorded in the Syrian race. Statistical analysis showed that there were significant differences between Syrian bees and both the Carniolan and F1 hybrid worker bees. No significant differences were found between the Carniolan race and the F1 hybrid. Generally, it could be concluded that the hypopharyngeal glands in the two races and their F1 hybrid reached their maximum development. These results are confirmed by the findings of Maurizio (1954), that the hypopharyngeal glands developed with an average of more than 2.5 as well developed, whereas a lesser average was considered as poorly developed.

Maurizio (1950), Standifer *et al* (1970), El-Barbary (1980) and Yakoub (1998) also found that the development of the hypopharyngeal gland was promoted by high pollen concentration (10%). The development of the hypopharyngeal gland is greatly affected by feeding. The relatively more developed hypopharyngeal glands in the F1 hybrid and Carniolan workers in the present study may be attributed to genetic factors inducing higher adaptation and greater protein content in the haemolymph (Abd – Alla, 1997). It is also obvious that the fresh and dry weights of heads can serve as an indicator of development of the hypopharyngeal glands and growth (Hrassnigg and Crailsheim, 1988).

Table(1):Means of fresh and dry weight (mg) of head for the three tested bees in each of spring and summer.

Race	Spring		Summer	
	Fresh weight	Dry weight	Fresh weight	Dry weight
F1 hybrid	10.65 ^a	2.57 ^a	10.31 ^a	2.38 ^a
Carniolan	10.00 ^b	2.35 ^b	9.91 ^b	2.33 ^b
Syrian	9.51 ^c	2.27 ^c	9.26 ^c	2.23 ^c
L.S.D.	0.31	0.05	0.31	0.05

Means followed by the same letter are not significantly different at 0.05 probability level.

Table(2):Mean of hypopharyngeal glands development in the three tested bees

Race	Mean hypopharyngeal glands development
F1 hybrid	3.13 ^a
Carniolan	2.92 ^a
Syrian	2.58 ^b
L.S.D	0.31

Means followed by the same letter are not significantly different at 0.05 probability level.

(a) Determination of some major elements in the head of newly emerged workers during spring and summer seasons.

The means (ppm) of four elements, Potassium (K), Calcium (Ca), Sodium (Na) and Magnesium (Mg) in the three tested honeybee workers in spring and summer were calculated and the data are presented in Table (3).

Table(3): Mean elements (ppm) in the head for the three tested honeybee workers in each of spring and summer seasons.

Race	Spring				Summer			
	K	Ca	Na	Mg	K	Ca	Na	Mg
F1 hybrid	90.00 ^a	36.63 ^b	37.00 ^b	46.30 ^a	64.67 ^a	21.92 ^a	131.33 ^b	101.20 ^a
Carniolan	86.67 ^a	50.23 ^a	50.67 ^a	35.70 ^b	60.00 ^a	26.19 ^a	170.67 ^a	75.07 ^b
Syrian	70.00 ^b	28.67 ^b	30.27 ^b	32.03 ^b	33.33 ^b	20.48 ^a	118.67 ^c	58.30 ^c
L.S.D.	14.43	9.40	11.82	9.13	14.43	9.40	11.82	9.13

Means followed by the same letter are not significantly different at 0.05 probability level.

Concerning the mean of (K) content in the workers head in spring and summer, statistical analysis in Table (3), indicated that there were significant differences between the Syrian race and Carniolan race as well as between the Syrian and F1 hybrid bees. No significant differences between the Carniolan and F1 hybrid bees were found.

In case of (Ca) content in workers reared in spring, the data cleared that there were significant differences between the Carniolan and Syrian races, as well as between the Carniolan and F₁ hybrid bees, but no significant differences occurred between the Syrian race and F1 hybrid bees. However, no significant differences were found between the two races and their F1 hybrid during summer season.

As for the mean of (Na) content in the head of workers reared in spring, statistical analysis showed that there were significant differences between the Carniolan and both Syrian race and F1 hybrid bees, but no such differences were found between the Syrian race and F1 hybrid bees. Meanwhile in summer season there were significant differences between the three tested workers. With regard to the mean of (Mg) content in the heads of the workers collected in spring, statistical analysis indicated that there were significant differences between the F1 hybrid bees and the Carniolan and Syrian races, but no significant differences occurred between Carniolan and Syrian races. In summer season, there were significant differences between the three tested honeybee workers.

Concerning, the effects of seasonal variations on the head contents of (K), (Ca), (Na) and (Mg), the statistical analysis in Table (4), proved strongly that, there were significant differences between the honeybee workers of both races and their F1 hybrid reared in spring and summer. These results are supported by the findings of Nation and Robinson (1971), that there were significant differences between bees collected in the late summer and those collected in early spring in their contents of the mentioned elements. However the interpretations of these authors are likewise true

contributions to the effect of seasonal variations on the mineral contents in the head of the adult honeybee workers. With regard to the importance of the tested minerals in bee nutrition, development and activity, the obtained results provide a clear evidence that differences in these biological aspects of the honeybee workers are highly attributed to seasonal variations.

Table(4):Seasonal variations on the mean elements (ppm) in the head for each of the tested workers in spring and summer seasons

Race Season	F1 hybrid				Carniolan				Syrian			
	K	Ca	Na	Mg	K	Ca	Na	Mg	K	Ca	Na	Mg
Spring	80.00 ^a	36.63 ^d	37.00 ^c	46.30 ^d	86.67 ^a	50.23 ^d	50.67 ^c	35.70 ^c	70.00 ^a	28.67 ^d	30.27 ^c	32.03 ^c
Summer	64.67 ^b	21.92 ^e	131.33 ^a	101.20 ^a	60.00 ^b	28.19 ^e	170.67 ^a	75.07 ^a	33.33 ^c	20.48 ^e	118.67 ^a	58.30 ^a
L.S.D.	11.78	7.68	9.65	7.45	11.78	7.68	9.65	7.45	11.78	7.68	9.65	7.45

Means followed by the same letter are not significantly different at 0.05 probability level.

(b)Influence of seasonal variations on the growth of the newly emerged honeybee workers

The effects of seasonal variations on the means of fresh and dry weight and the percentage of nitrogen content of the thorax in newly emerged workers Carniolan and Syrian races and their F1 hybrid in spring and summer were calculated and the data are presented in Table (5).

Table (5) : Seasonal changes on the mean of fresh weight, dry weight (mg) and nitrogen content (%) of the thorax for each tested workers in spring and summer

Race Character Season	Syrian			Carniolan			F1 hybrid		
	Fresh weight	Dry weight	N. content	Fresh weight	Dry weight	N. content	Fresh weight	Dry weight	N. content
Spring	31.94 ^a	8.15 ^a	13.80 ^a	33.06 ^a	8.48 ^a	20.49 ^a	34.53 ^a	9.04 ^a	23.88 ^a
Summer	31.11 ^b	7.95 ^b	11.57 ^b	31.50 ^b	8.12 ^b	18.21 ^a	31.87 ^b	8.32 ^b	16.93 ^b
L.S.D.	0.69	0.07	3.78	0.69	0.07	3.78	0.69	0.07	3.78

Means followed by the same letter are not significantly different at 0.05 probability level.

In F1 hybrid, the mean fresh weight of thorax was higher 34.53 mg in bees reared in spring than those bees reared in summer 31.87 mg. The same trend was noticed with the dry weight, where the mean values were 9.04 mg and 8.32mg in bees reared in spring and summer, respectively. In relation to the percentage of nitrogen content, the highest mean was 23.88% in bees reared in spring, followed by the bees reared in summer 16.93%.

In case of Carniolan race, the means of fresh weight of the thorax were 33.06 mg and 31.50 mg in the honeybee workers reared in spring and summer, respectively. The thorax dry weight exhibited a similar trend where the highest mean of 8.48 mg was recorded in bees reared in spring while a lesser mean 8.12 mg was obtained in those bees reared in summer. Concerning the percentage of N-content, the mean values were 20.49% and 18.21% in bees reared in spring and summer seasons, successively. As for the Syrian race, the highest mean of thorax fresh weight 31.11 mg was recorded in bees collected in spring, followed by bees collected in summer 31.11 mg.

A similar picture occurred with the dry weight and nitrogen content, where the mean values were 8.15 mg and 7.95 mg, respectively. In case of N-content, the means were 18.80% and 11.57% in bees collected in spring and summer, respectively. Statistical analysis in Table (5) indicates that there were significant differences between spring and summer bees in the two races and their F1 hybrid. One exception appeared in the case of nitrogen content for the Carniolan race. It seems that there is a positive correlation between thorax weight and nitrogen content. Haydak (1937) reported that in certain cases the rate of development of young bees can be determined by ascertaining only the dry weight of thoraces. It could be mentioned that, in newly emerged bees the deviation in weight, mineral and protein content are dependent on season and food availability outside the hive (Kunert and Crailsheim, 1988) and (Ivanov and Spasov, 1990).

From the obtained mentioned results, generally the F1 hybrid and Carniolan honeybee workers had the highest means in all determinations than those workers of the Syrian race in the two seasons. In conclusion, it could be concluded that hybridization between the Syrian race and the Carniolan race has greatly improved many of the characteristics under investigation. Some of these characteristics were improved and surpassed their original parents which may be due to heterosis or hybrid vigor.

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دراسة بعض القياسات الفسيولوجية في سلالات مختلفة لشغالات نحل العسل وفاء يعقوب - نبيل البربري - اسامة الانصاري - محمد عباس عبد اللطيف

- قدم البحث دراسة مقارنة بين سلالتين من نحل العسل هما سلالة النحل الكرنيولي وسلالة النحل السوري والهجين الناشيء بينهما والنتائج من تلقيح ملكات سوري عزراء بذكور نحل كرنيولي في منطقة معزولة في شمال الدلتا من حيث تقدير معدل نمو وتطور شغالات نحل العسل وكذلك عندها تحت البلعومية وتقدير بعض العناصر الكبرى واوضحت النتائج مايلي :
- ان متوسطات الوزن الرطب والجاف لرؤوس وصدور الشغالات الهجين حديثة الفقس كانت اعلى لتقيم تايبا شغالات سلالة النحل الكرنيولي ثم سلالة النحل السوري على الترتيب وذلك في فصلي الربيع والصيف .
 - ان معدل تطور الغدد تحت البلعومية في الشغالات عمر ١٠ ايام بعد التغذية على كاندى محتوى على نسبة ١٠% من حيوب اللقاح كان عاليا في شغالات الهجين الاول يليها شغالات النحل الكرنيولي ومن ثم الشغالات السوري .
 - لوحظ عند تقدير بعض العناصر الكبرى مثل البوتاسيوم والكالسيوم والصوديوم والماغسيوم فى رؤوس شغالات سلالتى النحل الكرنيولي والسوري وكذلك الهجين خلال فصلي الربيع والصيف ان هناك فروقا معنوية بين الشغالات المرباه فى فصل الربيع عن تلك المرباه فى فصل الصيف .
 - لوحظ عند تقدير متوسطات الوزن الرطب والجاف والمحتوى النيروجينى فى صدور الشغالات فى كل من سلالة النحل الكرنيولي والسوري وكذلك الهجين ان هناك فروقا معنوية بين النحل المربى فى فصل الربيع عن تلك المربى فى الصيف فيما عدا حالة واحدة بالنسبة للمحتوى النتروجينى لصدور شغالات النحل الكرنيولي .
- وعموما يمكن القول انه فى جميع القياسات الفسيولوجية سابقة الذكر ان شغالات النحل الهجين سجلت اعلى قيم للمتوسطات يليه سلالة النحل الكرنيولي . بينما سجلت اقل القيم فى سلالة النحل السوري .