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BIOCHEMICAL CHANGES IN THE HAEMOLYMPH OF LAYING WORKERS IN HONEYBEE COLONIES.

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ABSTRACT

The haemolymph of normal workers (without ovaries development), workers with ovaries development and recovered workers from development ovaries, were subjected to the following determination; Total protein, GOT, GPT enzymes, SDS Polyacrylamide Gel Electrophoresis.

The results obtained stated that, the total protein, GOT, GPT enzymes, SDS Polyacrylamide Gel Electrophoresis, were clearly higher in laying workers and followed by recovered bees from ovaries development.

INTRODUCTION

The formation of laying workers in honeybee colonies is considered to be one of the problems confronting the beekeepers especially after colonies dequeened either by normal deatn or accidentally (Khodairy, 1990). In spite of the presence of female reproductive system in worker bees, which produces viable eggs, the activation of this system occurs when the colony becomes hopelessly either queenless or broodless (Jay, 1970). The presence of queen in a group of bee workers inhibits the development of the ovaries in the workers and starts in queen absence, and workers brood keeps the worker's ovaries inactive until the new queen emerges to head the colony (seeley, 1985).

The presence of laying workers decreased significantly the number of either accepted new queens introduced into queenless colonies (De Grandi & Martin 1993) or queen larvae (queen cell) in queen rearing colonies (WoyCiechowski & Radwan, 1988). Elbassiouny (2005) found that the appearance of laying workers in honeybee colonies were affected by the age of the queen, where it reached 0.8, 1.3 and 2.2% for colonies headed with queens 10, 20 and 30 month old, respectively.

According to the lack of review concerning the biochemical changes of laying workers, thus the aim of this work is to investigate the biochemical changes in the haemolymph of workers with ovary development as well as workers recovered from ovaries development in honeybee colonies.

MATERIALS AND METHODS

1. Collecting the haemolymph from honeybee samples:-

The tested bees were classified to three groups as follows; the first representing worker bees without ovaries development collected from queen right colonies while the second from queenless colonies bees with development ovaries, whereas the third group of bees representing the bees with ovaries development collected from successfully treated colonies from

laying workers (recovered bees) by introducing mated queens and unsealed as well as sealed brood combs to these colonies.

Not less than 1500 worker bees from each bee groups were subjected to the technique for collection the haemolymph according to the method of Gilliam and Shimanuki (1971).

2. Determination of total protein:-

Not less than 500 worker bees from each bee group were subjected to determine the total protein concentration (gm. / dl) using the colorimetrical method (biuret reaction) described by Henry, (1964).

3. Determination of Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvic Transaminase (GPT):-

Not less than 500 worker bees from each bee group were subjected to determine the Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvic Transaminase (GPT) as (U/L) using colorimetric method according to Retiman and Frankel (1957).

4. SDS Polyacrylamide Gel Electrophoresis:-

The electrophoretic analysis used to identify how long changes occurred in the protein structure in the haemolymph of the normal worker bees, bees with development ovaries, and recovered bees from ovaries development. Not less than 500 worker bees from each bee group were subjected to this study. The technique used for electrophoretic was carried out according to the method of Laemmli, (1970). Analysis of protein fraction and the rate of flow and molecular weight of electrophoretically separated serum protein were made by the computerized Gel. Using Gel- Pro- Analyzer V.3.0 report program (Mass. Comp., Cairo-Egypt).

RESULTS AND DISCUSSION

A- Total protein:-

As shown in table (1) significant differences between normal worker bees without ovaries development, workers with ovaries development and workers recovered from ovaries development, were detected in the total protein (g/dl). The total protein concentration (g/dl) in the haemolymph of laying workers and recovered bees indicated higher levels particularly in the laying workers in comparison with normal and recovered bees.

B- Determination of Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvic Transaminase (GPT):-

As shown in table (1) clear fluctuations were recorded in the levels of GOT, GPT in the haemolymph of the laying workers, recovered bees in comparison with normal one. Concerning GOT; laying workers recorded the highest levels between the tested groups of bees, while the recovered bees recorded the lowest levels. On the other hand the highest GPT levels were recorded in recovered bees followed by laying workers and normal one.

It could be suggested that workers with ovaries development and recovered bees resulted marked increase in the total protein, GOT, and GPT in the haemolymph in comparison with normal one. The changes occurred in

the tested bee groups may be due to the lack of mated queen's pheromones, which affected particularly in the colonies with laying workers. These workers need more protein to activate the ovaries with their eggs and this reflect the high activities of GOT and GPT enzymes.

Whereas the decreasing levels of total protein, GOT and GPT in the recovered bees indicate the effect of produced pheromones from the re-introduced mated queens, unsealed and sealed brood, on the inhibition ovaries of this bees. These physiological changes explain that the recovered bees with inhibition ovaries were not needed to the protein, which reflected the lowest enzymes activity. However, the levels of enzymes and total protein were still higher in recovered bees in comparison with the normal one. It could be suggested that these biochemical changes will take time to decrease these levels as in normal bees.

In general; from the obtained results it could be stated that the biochemical changes in the workers with ovaries development and recovered bees may caused abnormal physiological and morphological changes to the haemolymph of the tested bees.

Table (1): Total protein and Transaminase (GOT, GPT) enzymes of normal, laying workers and recovered worker bees from ovaries development.

Type of Analysis	Normal u/L	Developed u/L	Recovery u/L
G.O.T	180	680	20
G.P.T	22	110	242
Total protein	30.3	44.5	33.2

U/L: unite per liter.

SDS Polyacrylamide Gel Electrophoresis:-

Protein electrophoretic pattern was carried out to determine how long the biochemical changes occurred in the haemolymph protein of the normal worker bees, bees with development ovaries and recovered bees from ovaries development.

As shown in table (2) and fig (1) there were clearly differences in the haemolymph protein pattern between normal and laying workers as well as workers after recovery. Higher numbers of the total protein bands were recorded in the haemolymph of laying workers and after recovery. The number of protein bands were 18, 20 bands in healthy and laying workers, respectively. While in worker bees recovered from ovaries development the number of protein bands were 21 bands.

The haemolymph protein bands of healthy workers (without ovaries development) ranged between 188.814.48 and 33.624.36 KDa, while in laying workers it was ranged between 173.873.15 and 33.959.86 KDa. Whereas in recovery workers from ovary development the molecular weight ranged between 170.985.26 and 33.879.55 KDa.

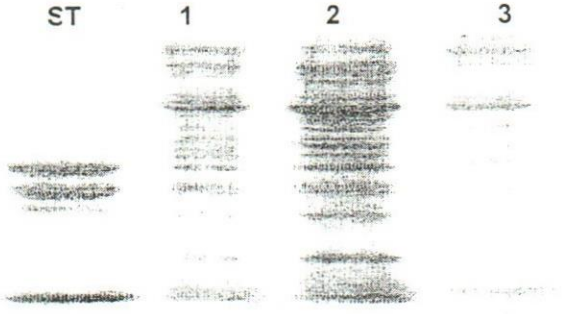
It could be concluded that ovaries development and its recovery of worker bees caused clear changes in the amino acids including their

numbers and molecular weight. Some amino acids were not found and others new were recorded in bees with ovary development and the recovered bees, while others were still detected in the three tested groups of bees.

Table (2): The haemolymph protein bands of normal, laying and recovered workers from ovaries development.

No. of protein bands	Molecular weight of the haemolymph protein bands (KDa.)			
	Protein standard	Normal workers	laying workers	Recovered workers
1		118.814.48		
2		171.572.75	173.873.15	170.985.26
3		157.671.75	156.854.39	155.812.24
4				148.738.37
5			146.627.36	143.311.21
6		134.919.00	137.839.44	132.796.71
7		126.575.15	126.224.80	125.828.84
8	116.000.00	119.418.08	116.240.11	117.685.53
9			110.301.49	111.303.49
10		103.926.95	102.916.82	102.754.70
11		98.789.67	96.567.61	96.642.02
12		95.327.53	93.366.41	92.598.02
13				89.053.65
14		87.439.67	86.627.44	85.963.88
15	97.184.000	81.724.64	79.775.00	79.361.32
16		73.154.76	71.831.94	
17	66.409.00	70.989.75	69.061.60	69.035.62
18	45.000.00	60.859.77	58.239.70	58.293.92
19			53.934.99	54.622.71
20		47.678.68	43.975.65	43.947.85
21		37.917.67	38.141.29	37.665.59
22		35.505.99	35.190.07	35.162.56
23	36.487.00	33.624.36	33.959.86	33.879.55
Total of protein bands		18	20	21

KDa: Unit of kilo Dalton.



10 % SDS poly acrylamide gel

Fig. (1): The Electrophoretic haemolymph protein patterns of normal, laying workers and recovered bees from ovary development.
ST: Molecular weight of the protein Standard
1- Normal workers.
2-Laying workers.
3-Recovered bees.

It could be suggested that the new amino acids in laying workers and recovered bees are related to increasing the levels of Transaminase enzymes of those bees.

It could be concluded that the appearance of laying workers and its recovery bee workers caused marked increase in the total protein (g/dl), GOT, GPT and in the numbers of protein bands. The biochemical changes of the haemolymph of the development ovaries and its recovered from laying workers caused abnormal behavior, which affected on their physiological and morphological statues in the tested bees. This behavior may be occurs according to the absent or present the released pheromones from queenless, requeened colonies as well as their brood (unsealed & sealed). The ovaries of worker honeybees usually remain undeveloped, but they may show some development in the presence of a queen at certain times (Jay, 1968) and especially in winter (Maurizio, 1954) in colonies with poor queens, when colony is preparing to swarm (Free, 1987). The brood pheromones that inhibits ovary development of worker larvae several thousand strong and removal of the brood greatly increased ovary development. Whereas removal of the queen from broodless colonies only slightly increased ovary development (Free, 1987). Ovary development of honeybee worker is inhibited by mated adult queens. A mixture of pheromones produced by the mandibular glands of the queen inhibits development of worker ovaries, (DE Groot and Voogd, 1954; Velthuis, 1970b; Free, 1977). The development ovaries in honeybee worker are inhibited by unsealed of workers brood and ovaries will begin to atrophy rapidly by giving so much open brood (Mobus, 1983; Jay, 1970). Beside that, the effect of sealed brood has a role in inhibition of ovary development of worker honeybees (Jay and Jay, 1976; Free, 1977). Meister (1957) found that the balance of amino acids in insect is the result of various biochemical reactions carried out by a group of enzymes amino transferase. Glinski and Jarosz (1985), Weinberg and Madel (1985), Schatton (1985), Schatton and Engels (1988), Achou and Soltani (1997) and Sarag El-Din (1999), they concluded that infested honeybee worker with varroa mites caused different reduction in the haemolymph protein contents. Kantchav *et. al.*, (1997) found significant changes in the total protein content and Isozyme content and also in the protein fraction in the blood infested newly worker honeybees. Zakaria (2002) found changes in GOT, GPT activity were parallel with the change in the protein and amino acids concentrations during the life cycle of the american bollworm. Miller and Ratnieks (2001) mentioned that worker bees eventually begin laying eggs in honeybee colonies that have lost their queens and have failed to rear a replacement. In contrast, workers tend to lack developed ovaries and tend to suppress drone production by worker messmates in colonies with queens. Hofmman (1961) found that when the new queen mates and initiates egg laying normal worker activities are resumed. Colonies are termed "hopelessly queenless" when the workers fail to construct emergency queen cells and no fertilized eggs or larvae less than four days old remain as a potential source of constructing emergency queen cells

Zakaria (2002) stated that a decreased in the total protein concentration (g /dl) in the haemolymph of infested newly emerged workers with Varroa

mites. GOT and GPT activities were increased in the haemolymph of infested workers as increasing number of mites/bee. New protein bands were appeared in haemolymph of infested worker with molecular weight ranged between 51-72 KD., that may result from the beak-down of the polypeptides by the protein hydrolysis enzymes injected into the haemolymph of infested worker, or due to the newly synthesized protein resulted of GOT&GPT activities. Seven protein bands were not changed in the haemolymph of non-infested workers with Varroa mites.

REFERENCES

Acho, M. and Soltani, N. (1997): Impact of *Varroa jacobsoni* Oud. on the morphometry and the biochemical composition of haemolymph in honeybee *Apis mellifera* L. parasitica, 53 (4): 127-134; 25 ref.

De Grandi, H.G. and Martin, J.H. (1993): Behaviour of egg Laying virgin and mated queen honeybees (*Apis mellifera* L.) and the composition of brood in their colonies. J. Apic. Res. 32 (1): 19-26.

DE Groot, A.P. and Voogd, S. (1954): On the ovary development in queenless worker bees (*Apis mellifera* L.). Experientia (Basel), 10: 384-385.

Elbassiouny, A.M. (2005): Occurrence of laying workers at different honeybee colonies status. Arab Univ. J. Agric. Sci., Ain shams Univ., Cairo, 13 (2): 513-519.

Free, J.B. (1977): The social organization of honeybees. The institute of biologs studies in biology no. 81. presented by Britain. pp. 68.

Free, J.B. (1987): Pheromones of social bees. pp. 218, London, Chapman and Hall.

Gilliam, M. and Shimanuki, H. (1971): Blood cells of the worker honeybee. J. Apic. Res., 10 (2): 79-85.

Glinski, Z. and Jarosz, J. (1985): Distribution of protein fraction in the blood of worker brood blood of *Apis mellifera* L. J. Apic. Res., 25 (2): 80-85.

Henry, R. (1964): IN clinical chemistry. Principles and techniques. P 182.

Hofmman, I. (1961): Uber die Arbeitsteilung in weiselrichtigen und weisellosen Kleinvolkern der Honigbiene. Z. Bienenforsch. 5: 267-279.

Jay, S.C. (1968): Factors influencing ovary development of worker honeybees under natural condition. Can. J. Zool. 46: 345-347.

Jay, S.C. (1970): The effect of various combinations of immature queen and worker bees on the ovary development of worker honeybees in colonies with or without queens. Can. J. Zool. 48: 169-173.

Jay, S.C. and Jay, D.H. (1976): The effect of various types of brood comb on the ovary development of worker honeybees. Can. J. Zool. 54(10): 1724-1726.

Kantchav, K.; Getchev, I.; Parvanov, P. and Bonovska, M. (1997): Total haemolymph protein content, protein fraction and lysosem concentration in newly emerged worker bee, *Apis mellifera* L. infested by *Varroa jacobsoni* Oud. veterinary science, 29 (1-2): 475-478 (Apic. Abst. 1141/99).

Khodairy, M.M. (1990): Studied on laying workers of honeybee *Apis mellifera* L. Msc. Thesis. Faculty of Agriculture. Assiut University, Assiut, Egypt.

Laemmli, U.K. (1970): Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature, lond., 227: 680-685.

Maurizio, A. (1954): Pollen nutrition and life processes of honeybee. Landwirtsh Jahrb Schweiz, 68 (6): 115-186.

Meister, A. (1957): Biochemistry of the amino acid. Academic press, New York, 175-196.

Mobus, B. (1983): Management of colonies with laying workers. XXIX th International Congress of Apiculture of Apimondia (Budapest) p. 367-368.

Miller, D.G. and Ratnieks, F.L.W. (2001): The timing of worker reproduction and breakdown of policing behaviour in queenless honeybee (*Apis mellifera* L.) societies. Insects Sociaux. 48: 178-184.

Retiman, S. and Frankel, S. (1957): Coloremetric method for aspartate and alanine Transaminases. Am. J. Clin. Path., 28-56.

Sarag El-Dein, F.S.A. (1999): Studies on the varroatosis (*Varroa jacobsoni* Oud.) Ph.D. thesis, Fac. Agric., Kafr El-Sheikh Univ., Egypt.

Seeley, T.D. (1985): Queens and workers: Reproductive division of labor. In: Seeley, T.D (Ed) Honeybee Ecology pp. 23-25. Princeton University press. New jersey, USA.

Schatton, K. (1985): Changes in the haemolymph spectrum of *Apis mellifera* L. as a result of parasitization by *Varroa jacobsoni* Oud. Diplomarbeit der Fakultat fur Biologie, Universitat Tubmgen, G.F.H., vi + 57pp.

Schatton, K. and Engels, W. (1988): Blood proteins and body weight of newly emerged worker honeybee with different levels of parasitization of brood mites *Varroa jacobsoni* Oud. Entomolgia Generalis, 14 (2): 93-101.

Velthuis, H.H.W. (1970 b): Queen substances from the abdomen of the honeybee queen. Z. Vgl. Physiol. 70: 210-222.

Weinberg, K.P. and Medal, G. (1985): The influence on the protein concentration and the haemolymph volume of the brood of worker bees and drones of the honeybee, *Apis mellifera* L. Apidologie, 16 (4): 421-435.

Woyciechowski, M. and Radwan, J. (1988): Kin recognition in rearing and acceptance of queen honeybee. Pszezel nicze zeszyty Nau Kowe. 32: 35-43.

Zakaria, M.E. (2002): Physiological studies on honeybees *Apis mellifera* L. under varroa parasitism *Varroa jacobsoni* Oud. Ph.D. thesis, Fac. Agric., Cairo Univ. Egypt.

التغيرات الحادثة في هيموليمف الشغالات الواضعة للبيض والشغالات التي تم علاجها من نمو المبايض في طوائف نحل العسل.

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تم الحصول على هيموليمف الشغالات الواضعة للبيض وكذلك الشغالات التي تم علاجها من نمو المبايض لمقارنتها بهيموليمف الشغالات السليمة وذلك لتقدير Total protein, GOT, GPT, SDS Polyacrylamide Gel Electrophoresis

وقد أظهرت النتائج أن نشاط Total protein, GOT, GPT, SDS Polyacrylamide Gel Electrophoresis. في الشغالات الواضعة للبيض كان مرتفعا يليه الشغالات التي تم علاجها من نمو المبايض وذلك بالمقارنة بالشغالات السليمة.