

INFLUENCE OF DIFFERENT DIETS ON HONEYBEE WORKERS (*APIS MELLIFERA* L.) HAEMOCYTES

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Abstract

The influence of different diets on the haemolymph of adult honey bee worker was examined. Four diets were compared for measuring their effect on the different haemocytes types and count of the newly emerged workers bees¹. Four groups of newly emerged worker bees were fed with one of the following diets in patty form with pollen and pollen substitute, soya bean, chick pea, yeast. Differential haemocytes counts were evaluated. The types of the haemocytes (plasmocytes –coagulocyte. granulocyte) were recorded, the most abundant types was plasmocytes cells over 42% followed by coagulocyte 33% and granulocytes 24%. The highest total haemocytes count was 1345 cell for pollen, while the lowest total was 931.66 cells for pollen substitute, the examined pollen substitute caused decreased of total haemocytes count.

INTRODUCTION

Haemocytes are fundamental importance in the preservation of an insect haemocytes especially in regards of cellular defense reaction and management of nutrition elements Glinski and Jarosz, (1995). These cells circulating in haemolymph are easily accessible to evaluate an insect haemocytes and analysis of haemocytes enable the estimation of the influence of different factors external factors such analysis should be particularly useful to assess diet and therapeutic treatment for honey bee as well as chemicals for plant protection recommend as not harmful to bees examination of haemocytes many also be useful to determined the effect of environmental factors on range of immune parameters of cellular system of honey bee haemolymph .Total haemocytes counts (THC) ,differential haemocytes count. And metabolic activity. Metabolic activity was used as an indirect estimation of ability of cells to phagocytosis Glinski and Klimont (1987). Insect's haemolymph plays very important role in transport and storage of nutrients and is crucial for the recognition and defense against micro-organism (Bogart *et al*, 2009) .Honey bees possess an open circulatory system and mummies haemocytes are contained in its haemolymph. Plasmocytes, granular cells, cystocytes, sphaerula, and oenocytes are the cells that comprise the bee haemocytes population (Gupta, 1991). They are variable in morphology and functions. They are responsible for the defense fraction

pathogens penetrate haemocoel (Tepass *et al.*, 1999); Fallers and Gregrio, 1995; Glinsk and Jaroza, 1995, and Inoue, *et al.*, 2001), perform phagocytes encapsulation of foreign bodies in the insect body cavity, coagulation to prevent loss of body, module formation, transport of food material (Patton, 1983). The haemocytes can engulf and destroy small foreign objects such as bacteria, fungal, but larger parasites, bacteria or fungal hyphae are encapsulated by several haemocytes and then removed from circulation (Glinski and Jaroz, 2001). The aim of this experiment was to estimate the influence of different feeding diets (pollen and pollen substitute) on the differential haemocytes count in newly emerged honey bee worker.

MATERIALS AND METHODS

This study was carried out in the year 2016 and the observations were made in early summer. The experimental groups of bees (*Apis mellifera* L.) were fed bee pollen with sugar candy (natural proteinaceous food) and pollen substitute was multi component food. This pollen substitute was made of following raw materials; soya powder (A), chick pea (B) and Yeast (C). The pollen substitute was mixed with powdered sugar, distilled water was added to obtain patty. The diet cakes were placed dried over the center of the brood combs and the top of the cake was covered with wax paper to prevent moisture loss. The experimental colonies were subjected to this different treatment, weekly from early summer 2016 and continued for one year. Investigated bees were collected by placing frames with emerged brood from each colony fed with these feeding treatment in incubator set to 33^o C and the relative humidity of 65% till the worker honey bee emerge. A drop of the haemolymph of the newly emerged worker was taken over a clean microscope slide and smear was made. Smears were stained with Giemsa stain and differential haemocytes count (DHC) were evaluated. One hundred cells were counted per slide and oil immersion and phase contrast using light Leitz microscope. The types of the haemocytes plasmatocytes, granulocytes and coagulocyte were recorded using the key of Gupta (1991). About 30 smears of haemolymph for each diet were examined.

RESULTS AND DISCUSSION

Haemocytes play an essential role in defending insects against invading parasites and pathogens and is the effective physiological mechanism of phagocytes encapsulation, and other related defense mechanism were primarily duty to availability of circulation immune cells particularly plasmatocytes and granulocyte. Feeding honey bee with different diets may help enhancing the immunity of bees to their positive effect on the types and percentage of haemocytes.

Three haemocytes types were observed in the haemolymph of the newly emerged honey bee worker were fed on different kind of diet are presented in

fig (1) table (1) and (2). They were classified as plasmocytes, coagulocyte and granulocytes or other Shimanuki (1971), following Jedruszik (1997). Plasmocytes were small round cells with compact, round nuclei and thin hyaline neutrophilic. Granular haemocytes were large, oval or ellipsoidal cells with granular nucleic and vacuolated cytoplasm. Coagulocyte are spherical or oval cells, the nucleus is relatively small, rounded or elongate and is centrally located. The predominant type of the haemocytes of bees fed with the four test diet was the plasmocytes cells followed by the coagulocyte and granulocytes. Our results are in agreement with those of previous studies which mentioned that the plasmocytes were the most numerous cells in the haemolymph of young honey bee from free flying colonies Jedruszuk, (1998) .a.b and c

The results also confirm observation made earlier by Wieland's *et al.*, (1987) examination of plasmocytes only may not be sensitive enough, as the next consequence of different diet but also they were a large decrease in proportion of metabolically active cells, which suggests a reduced ability to phagocytes. The plasmocytes recorded the highest occurrence in bee fed different diets 41.2 % while the low percentage was found for both (Co) and (Gr) 32%, 24%, while they revealed respectively. There were significant differences between the effect of different pollen and pollen substitute on honey bee haemocytes. Data in table (2) clear that the total haemocytes count of the worker adult fed different diets was 1345 cells per mm³, the lowest for pollen grain, while total haemocytes count worker adult was 931.66 cells mm³ for pollen substitute. There was significant difference between the pollen and the all other different diets. From the previous results it was noticed that the plasmocytes are the most abundant cells followed by (Co) and (Gr). These results agreed with those of Szymas and Jedruszuk (2003), who mentioned that there was significant higher percent type plasmocytes and a significant decrease of (Gr) in the haemolymph of bees fed with pollen substitute, compared with the haemocytes systems of bees fed with pollen. Phagocytosis and encapsulation are two common types of defense reaction in honey bee against invading pathogenesis. Encapsulation began where granular cells attached to the foreign target and this was followed by attachment of multiple layers of plasmocytes to form the outer layer of the capsule Louis and Michael (1996); Gliński and Jaroza (2001) and Gliński and Buczek, (2003).

The physiological mechanism effect of phagocytosis encapsulation and other related defense mechanisms was due to the availability of circulatory in nuchal cells particularly plasmocytes and granulocytes Sanjayan *et al.*, (1996). Bee haemocytes may directly kill bacteria, fungal spores and other small foraging molecules phagocytes process Gotz (1986).

Researchers found a correlation between diet diversity (poly Loral pollen – pollen substitute) and healthy immune system in honey bees in laboratory experiment, and that bees fed on different types of pollen had higher levels of glucoses oxidase than bees that were fed only on type of pollen even if that on type had higher protein content bees Trinde (1969). As mentioned earlier glucose as oxidase is used by the bees to preserve honey and protect the hive against pathogens (Alaux *et al.*, 2010) the effect of different diets were assessed by measuring haemocytes concentration and haemocytes are necessary for phagocytosis and encapsulation of parasite and fat bodies provide antimicrobial peptide synthesis. (Alaux *et al.*, 2010), while haemocytes activity did not seen to be affected by the pollen source, both bat body and glucose oxidase activity were significantly enhanced. The authors concluded that varied diet yielded more of amino acids necessary to increase antiseptic protection of the hive (Alaux *et al.*, 2010)

Table 1. percentage of different haemocytes content of worker honey bee feeding with different diets .

group	Pollen (control)	%	Diet with Pollen substitute					
			Soya bean	%	Cheak pea	%	yeast	%
Total haemocytes	1345		1186.66		1055.66		931.66	
plasmocytes		42.52		41.71		41.77		40.71
coagulates		32.88		33.13		33.08		33.08
granulocytes		24.52		25.14		24.9		26.19

Table 2. Influence of feeding bee colonies with different diets on haemocytes number cell per mm³ (R=replicate)

Different diets	R1	R2	R3	Mean	F	p	LSD0.05
Soybean	1225	1165	1170	1186.66	130.60	0.000	50.59
Cheek bean	108.3	1056	1020	1055			
Yeast	957	930	608	931.66			
pollen	1362	1346	1327	1345			

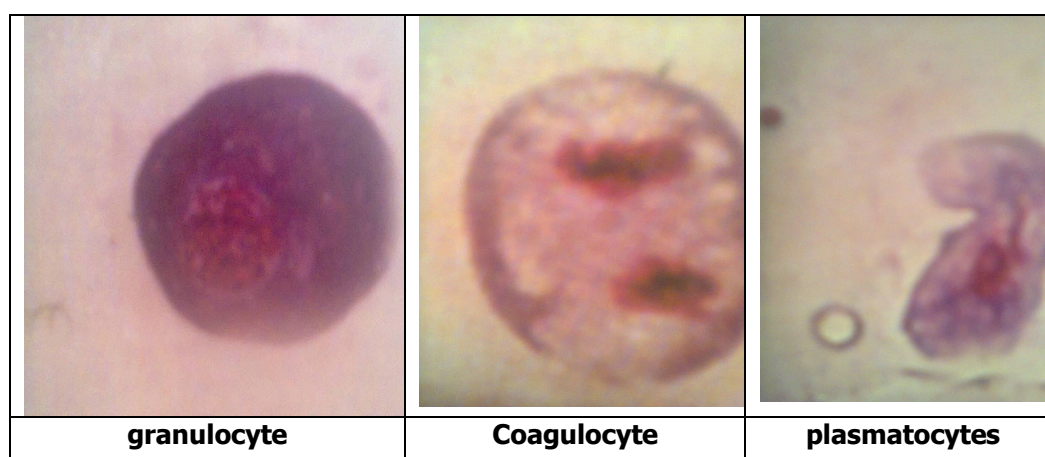


Fig. 1. Kinds of honey bee worker haemocytes

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تأثير التغذية المختلفه على خلايا دم شغالات نحل العسل

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دراسه تأثير التغذية المختلفه على خلايا هيمولف شغالات نحل العسل حديث الخروج 4
انواع من التغذية المختلفه استخدمت فى تأثيرها على بعض انواع خلايا هيمولف شغالات نحل
العسل وهى عباره عن ثلاث مركبات غذائيه كبديل لحبوب اللقاح واستخدمت حبوب اللقاح كمقارنه
لهذه البدائل وهذه البدائل مطحون الفول الصويا - مطحون حمص حلو - وخليط من العجوه
والخميره الطبيه الجافه وقد اظهرت النتائج انه تم تعريف ثلاثه انواع من خلايا الدم
وهى Plasmocysset, coagulocyte, granulocyte طبقا للنتائج التى تم الحصول عليها ظهرت خلايا
البلازموسيت باعلى نسبة فى هيمولف الشغلات وقدرت نسبة وجودها ب42% بينما وجود النوع
الثانى وهو الكلووجلوسيت بنسبه 33% تليه نسبة الجرانيوسيت بنسبه 24% ووضحت النتائج ايضا
ان الطوائف التى غذيت على حبوب القاح اعطت اكبر عدد من خلايا الدم (1345 خلايا) بينما اقل
قيمه سجلت على خلايا الدم فى الطوائف التى غذيت على بدائل حبوب اللقاح (931 خليه).

