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Influence of some Artificial Diet on Blood Cells of Honeybee Worker Larvae in Three Hybrids

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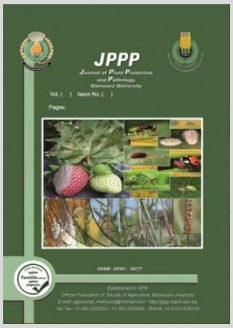

ABSTRACT

The current study investigated the effect of various artificial diets, as pollen substitues, on the blood cells in worker larvae of honeybee. Honeybee colonies were provisioned with four pollen substitutes. Samples of blood were pulled from worker larvae and distributed directly onto glass and examined with 1250x microscope. At most, the prohaemocytes, plasmatocytes and oenocytes recorded the higher numbers in larvae collected from colonies that fed with pollen substitutes than those did not feed (control). But, the coagulescytes with/without granules and spherulescytes recorded lower numbers in larvae collected from colonies that supplied with pollen substitutes than those did not supply (control). The highest amount of prohaemocytes cells in larvae fed with pollen substitutes (diet A, B, D) was accounted for buckfast hybrid followed by the Italian and Craniolan hybrids after one day from feeding. In respect to diet "C", the highest number of prohaemocytes cells was recorded for Craniolan followed by Italian and buckfast after one day from feeding. In all hybrids, the prohaemocytes cells were more in worker larvae fed with pollen substitutes than those did not.

***Keywords*:** Artificial Diet, blood cells, Plasmatocytes, prohaemocytes, Coagulocytes, Spherule, Oenocytes.

INTRODUCTION

The blood is the basis for all the vital processes that take place inside the body of the insect. The most important components of blood are the various blood cells, which form the cornerstone of the insect's immune system (Papadopoulou *et al*., 1993; Abd El-Wahab *et al*., 2016).

There are many factors that affect blood cells. This study is based on testing the effect of various pollen substitutes on the blood cell numbers in larvae of the honeybee workers. The influence of different diets on the haemolymph of honeybee workers was examined (Jędruszuk, 1998a, b) . Jędruszuk (1998a, b) did an experimental study on effect of pollen substitute provided during early summer on haemocytes. In his study, three experimental groups of honeybee fed with one of the following diets: pollen with sugar, pollen substitute alone, or sugar. A sample of haemolymph was pulled from 7–8 day old bee. Then, the haemocyte numbers for each blood cell type (e.g., plasmatocytes, granular haemocytes, and other types of haemocytes pooled together) were recorded and the metabolic activity of haemocytes was noted (Jedruszuk, 1998a, b; Bommarco *et al*., 2013). In absent of protein resource, a significant increase in number of granular haemocytes, a significant decrease in number of other blood cell types and a lower metabolic activity (Jedruszuk, 1998c). Therefore, the present experiments were carried out to study the influence of some artificial diets on blood cells in worker larvae of three honeybee hybrids.

MATERIALS AND METHODS

Forty-eight experimental local honeybee colonies that were equal in strength were provisioned with four different pollen substitutes. The composition of these diets were as follows:

A. 400 g Soybean + 300 g sugar powder + 100 g pressed dates (agwa) + 100 g orange cover + 100 g apple cover.
B. 400 g Chick pea + 300 g sugar powder + 100 g Brower's yeast + 100 g dates (agwa) + 100 g orange cover + 100 g apple cover.
C. 250 g pollen + 100 g Brower's yeast + 300 g sugar powder + 50 g cinnamon powder.
D. 250 g pollen + 300 g sugar powder + 50 g cinnamon powder + 100 g apple cover.

Experimental protocol

1.36 colonies were prepared as follows:

a. 12 Craniolan colonies (brood and worker). Every four colonies had different queens arranged as followed, Craniolan, Italian and bukfast.
b. 12 Italian colonies (brood and worker) each four of it had different queens arranged as followed, Craniolan, Italian and bukfast.
c. 12 bukfast colonies (brood and worker) each four of it had different queens arranged as followed, Craniolan, Italian and bukfast.

2. Blood stains**:** consisted of 25 ml methyl alcohol + 100 ml ethyl alcohol + g powder Wright's stain. This stain then shacked and filtering with filter paper.
3. Buffer solution**:** 3.315 monobasic potassium phosphate + 1.28 dibasic sodium phosphate + 500 ml distilled water.
4. The honeybee colonies were fed with four substitutes (as shown above).
5. Blood samples were collected at two different times. The first at January after 2, 3, 4 and 10 days, the second after

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3, 4, 5 and 11 days after treatment, every day three hive as replicates of the larvae age 2-3-4-5 days then, it pulled the larvae blood samples directly onto glass slides.

6. Blood samples: The abdomen of larvae was punctured with a fine scissor. The haemolymph drops were received on a glass slide, and then a smear was done (Shapiro, 1968). The smears were left to dry and then slides stained using wright's blood stain by introduced into the jar of Wright's stain for 2-5 minutes and then transferred directly to the jar containing the buffer for 2-5 minutes. The slide is then washed with buffer or distilled water and then dried at a natural air (Arnold and Hinks 1976). The smears were investigated under oil immersion at 1250x and maximum of 100 haemocytes/slide were differentiated based on the classification of Jones (1962) and Akai and Sato (1973).

RESULTS AND DISCUSSION

Data in Fig:1 show the hybrids blood cells number feeding with "A" pollen substitute after one day on the first season.

the highest amount of prohaemocytes cell was (38±5.2) showed at buckfast hybrids followed by the Italian (37±1.7) as the lowest prohaemocytes cell number recorded at Craniolan hybrids (35±0). Whoever all hybrids were recorded prohaemocytes cell more than the control (30).

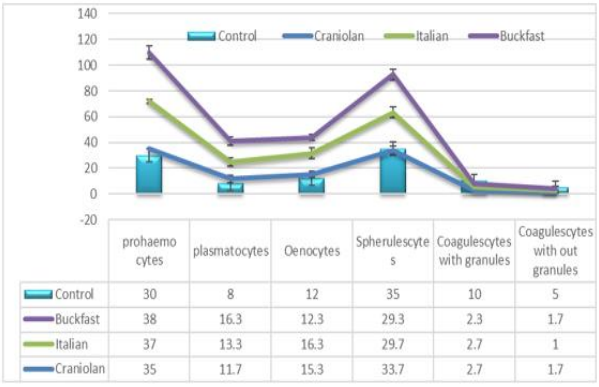
the highest amount of plasmatocytes cell was (16.3±3.2) showed at buckfast hybrids followed by the Italian (13.3±3.2) as the lowest plasmatocytes cell number recorded at Craniolan hybrids (11.7±2.9). Whoever all hybrids were recorded plasmatocytes cell more than the control (8).

the highest amount of Oenocytes cell was (16.3±3.2) showed at Italian hybrids followed by the Craniolan (15.3±2.1) as the lowest Oenocytes cell number recorded at buckfast hybrids (12.3±2.5). Whoever all hybrids were recorded Oenocytes cell more than the control (12).

the highest amount of Spherulescytes cell was (33.7±3.2) showed at Craniolan followed by the Italian hybrids (29.7±4) as the lowest Spherulescytes cell number recorded at buckfast hybrid (29.3±4). Whoever all hybrids were recorded Spherulescytes cell less than the control (35).

the highest amount of Coagulescytes with granules cell was (2.7±2.1) showed at Craniolan and Italian hybrids as the lowest Coagulescytes with granules cell number recorded at buckfast hybrid (2.3±1.1). Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (10).

the highest amount of Coagulescytes without granules cell was (1.7±1.1 and 1.7±1.5) showed at Craniolan followed by the buckfast hybrids respectively. as the lowest Coagulescytes without granules cell number recorded at Italian hybrid (1±0). Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (5).



	prohaemocytes	plasmatocytes	Oenocytes	Spherulescytes	Coagulescytes with granules	Coagulescytes with out granules
Control	30	8	12	35	10	5
Buckfast	38	16.3	12.3	29.3	2.3	1.7
Italian	37	13.3	16.3	29.7	2.7	1
Craniolan	35	11.7	15.3	33.7	2.7	1.7

Fig. 1. Number of hybrid blood cells for worker larvae fed with diet "A" after one days

From the obtained results it could be concluded that, the prohaemocytes, plasmatocytes and Oenocytes recorded blood cells number more than the control. Moreover, the Coagulescytes with/without granules and Spherulescytes recorded blood cells number less than the control. Thus may be due to the pollen substitute.

Data in Fig 2 showed the hybrids blood cells number feeding with "B" substitute after one days. The highest amount of prohaemocytes cell was (47.67±2.52) showed at buckfast followed by the Craniolan hybrids (39.7±9.503) as the lowest prohaemocytes cell number recorded at Italian hybrid (34.67±4.73). Whoever all hybrids were recorded prohaemocytes cell more than the control (30).

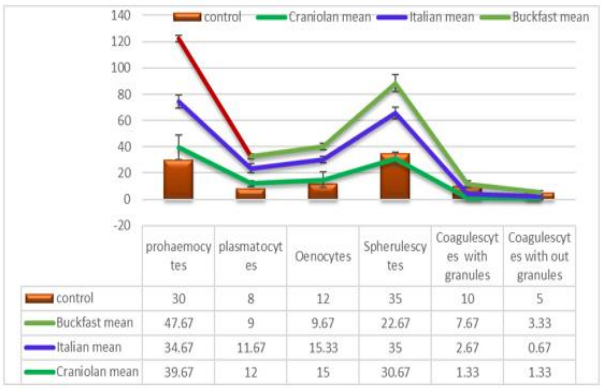
The highest amount of plasmatocytes cell was (12±2) showed at Craniolan followed by the Italian hybrids (11.67±3.51) as the lowest plasmatocytes cell number recorded at buckfast hybrid (9±1.73). Whoever all hybrids were recorded plasmatocytes cell more than the control (8).

The highest amount of Oenocytes cell was (15.33±2.51) showed at Italian followed by the Craniolan hybrids (15±6.08) as the lowest Oenocytes cell number recorded at buckfast hybrid (9.67±2.51). Whoever Italian and Craniolan hybrids were recorded Oenocytes cell more than the control (12) while the buckfast hybrid recorded Oenocytes cell less than the control.

The highest amount of Spherulescytes cell was (35±4.36) showed at Italian followed by the Craniolan hybrids (30.67±5.13) as the lowest Spherulescytes cell number recorded at buckfast hybrid (22.67±6.43). Whoever Italian and buckfast hybrids were recorded Spherulescytes cell less than the control as the Italian hybrid was recorded Spherulescytes cell seem equal the control (35).

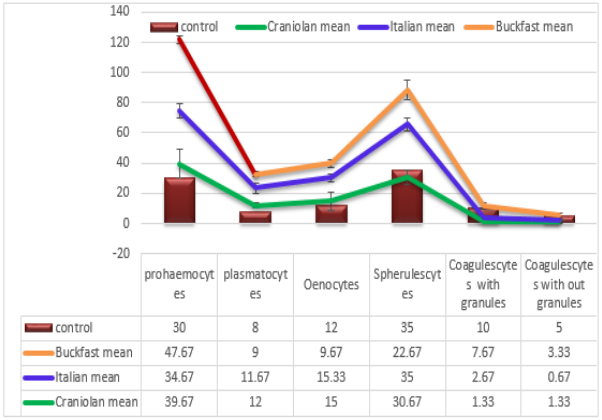
The highest amount of Coagulescytes with granules cell was (7.67±2.52) showed at buckfast hybrid. As the lowest Coagulescytes with granules cell number recorded at Craniolan (1.33±0.58) and Italian (2.67±1.155) hybrids. Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (10).

The highest amount of Coagulescytes without granules cell was (3.33±1.53) showed at the buckfast followed by Craniolan hybrids (1.33±1.53) respectively. As the lowest Coagulescytes without granules cell number recorded at Italian hybrid (0.67±0.58). Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (5).



	prohaemocytes	plasmatocytes	Oenocytes	Spherulescytes	Coagulescytes with granules	Coagulescytes with out granules
control	30	8	12	35	10	5
Buckfast mean	47.67	9	9.67	22.67	7.67	3.33
Italian mean	34.67	11.67	15.33	35	2.67	0.67
Craniolan mean	39.67	12	15	30.67	1.33	1.33

Fig. 2. Number of hybrid blood cells for worker larvae fed with diet "B" after one days



	prohaemocytes	plasmatocytes	Oenocytes	Spherulescytes	Coagulescytes with granules	Coagulescytes with out granules
control	30	8	12	35	10	5
Buckfast mean	47.67	9	9.67	22.67	7.67	3.33
Italian mean	34.67	11.67	15.33	35	2.67	0.67
Craniolan mean	39.67	12	15	30.67	1.33	1.33

Fig. 3. Number of hybrid blood cells for worker larvae fed with diet "C" after one days

From the obtained results it could be concluded that, the prohaemocytes, plasmatocytes and Oenocytes recorded blood cells number more than the control unless at the buckfast hybrid. Moreover, the Coagulescytes with/without granules and Spherulescytes recorded blood cells number less than the control unless at the buckfast hybrid's Spherulescytes cell.

Data in Fig 3 showed the hybrids blood cells number feeding with "C" substitute after one days. The highest amount of prohaemocytes cell was (38.33±2.89) showed at Craniolan (38.33±2.89) followed by the Italian (32±2) hybrids respectively. As the lowest prohaemocytes cell number recorded at buckfast hybrid (30±2.89). Whoever all hybrids were recorded prohaemocytes cell more than the control (30) unless at the buckfast hybrids.

The highest amount of plasmatocytes cell was (14.33±6.03) showed at Craniolan followed by the Italian hybrids (8±3) as the lowest plasmatocytes cell number recorded at buckfast hybrid (7±4.04). Whoever the Craniolan hybrids recorded plasmatocytes cell more than the control (8) while the Italian hybrids has the seam equal control and the at buckfast hybrids recorded plasmatocytes cell less than the control.

The highest amount of Oenocytes cell was (18±2) showed at Italian followed by the buckfast hybrids (15±5.77). As the lowest Oenocytes cell number recorded at Craniolan hybrid (10.67±1.16). Whoever Italian and buckfast hybrids were recorded Oenocytes cell more than the control (12) while the Craniolan hybrids recorded Oenocytes cell less than the control.

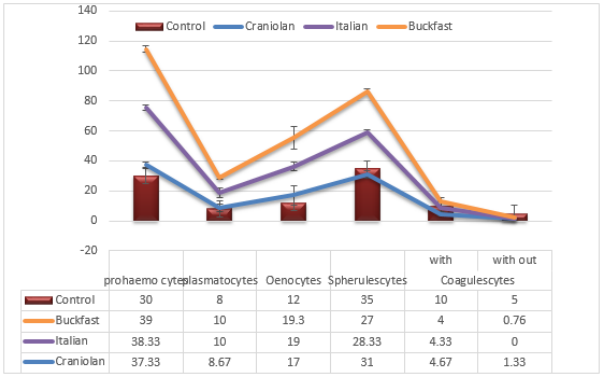
The highest amount of Spherulescytes cell was (40±7.64) showed at buckfast followed by the Italian hybrids (33.33±7.64). As the lowest Spherulescytes cell number recorded at Craniolan hybrid (27±4.36). Whoever the buckfast hybrid recorded Spherulescytes cell more than the control. while the Italian and Craniolan hybrids were recorded Spherulescytes cell less than the control (35)

The highest amount of Coagulescytes with granules cell was (7.67±6.43) showed at Craniolan followed by the Italian hybrids (6.67±4.16). As the lowest Coagulescytes with granules cell number recorded at buckfast hybrid (5±1). Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (10).

The highest amount of Coagulescytes without granules cell was (3±1) showed at the buckfast. As the lowest Coagulescytes without granules cell number recorded at Craniolan (2±2.65) and Italian (2±2) hybrids respectively. Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (5).

From the gotten results it could be concluded that, the prohaemocytes, plasmatocytes and Oenocytes recorded blood cells number more than the control unless at the buckfast and Italian hybrids at prohaemocytes/plasmatocytes and plasmatocytes respectively. Moreover, the Coagulescytes with/without granules and Spherulescytes recorded blood cells number less than the control.

Data in Fig 4 showed the hybrids blood cells number feeding with "D" substitute after one days. The highest amount of prohaemocytes cell was (39±2.64) showed at buckfast followed by the Italian hybrids (38.33±3.78). As the lowest prohaemocytes cell number recorded at Craniolan hybrid (37.33±2.51). Whoever all hybrids were recorded prohaemocytes cell more than the control (30) unless at the buckfast hybrid.



	prohaemo cyte	plasmatocytes	Oenocytes	Spherulescytes	with Coagulescytes	with out Coagulescytes
Control	30	8	12	35	10	5
Buckfast	39	10	19.3	27	4	0.76
Italian	38.33	10	19	28.33	4.33	0
Craniolan	37.33	8.67	17	31	4.67	1.33

Fig. 4. Number of hybrid blood cells for worker larvae fed with diet "D" after one days

The highest amount of plasmatocytes cell was (10±2) showed at buckfast and the Italian hybrids. As the lowest plasmatocytes cell number recorded at Craniolan hybrid (8.67±1.73). Whoever all hybrids recorded plasmatocytes cell more than the control (8).

The highest amount of Oenocytes cell was (19±3.46 and 19.3±1.15) showed at Italian and buckfast hybrids respectively. As the lowest Oenocytes cell number recorded at Craniolan hybrid (17±2.51). Whoever all hybrids were recorded Oenocytes cell more than the control (12).

The highest amount of Spherulescytes cell was (31±6.43) showed at Craniolan followed by the Italian

hybrids (28.33±2.88) as the lowest Spherulescytes cell number recorded at buckfast hybrid (27±7.55). Whoever all the hybrid recorded Spherulescytes cell less than the control (35).

The highest amount of Coagulescytes with granules cell was (4.67±2.51) showed at Craniolan hybrid followed by the Italian hybrids (4.33±1.15). As the lowest Coagulescytes with granules cell number recorded at buckfast hybrid (4±1.73). Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (10).

The highest amount of Coagulescytes without granules cell was (0.76±0.57) showed at the buckfast. As the lowest Coagulescytes without granules cell number recorded at Craniolan (1.33±1.52). The Italian hybrid recorded no Coagulescytes without granules cell. Whoever all hybrids were recorded Coagulescytes with granules cell less than the control (5).

From the obtained results it could be concluded that, the number of prohaemocytes, plasmatocytes and Oenocytes were higher than the control. Moreover, the Coagulescytes with/without granules and Spherulescytes recorded blood cells number less than the control.

As far as we know, no much more reports had discussed the influence of artificial diet on blood cells in honeybee worker's larvae. Gliński and Jarosz (1995) stated that haemocytes are of fundamental importance in the preservation of an insect homeostasis, especially in regards of cellular defense reactions and management of nutritional elements. The cell circulating in haemolymph is easily accessible to evaluate an insect homeostasis and counts of haemocytes that enables studying the effect of different factors on insect homeostasis.

The plasmatocytes are the most numerous cells in haemolymph of young honeybee from free-flying colonies (Jędruszuk, 1998a, b). Haemocytes were classified as plasmatocytes, granular haemocytes, or other (all other types of haemocytes pooled together;). Plasmatocytes were small round cells with compact, round nuclei and thin, hyaline neutrophilic or pale-basophilic cytoplasm (Jędruszuk, 1998c).

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تأثير بعض بدائل حبوب اللقاح على خلايا الدم ليرقات نحل العسل العاملة على خلايا دم يرقات شغالات نحل العسل

أشرف شريف فتحي[1]، عبد البديع عبد الحميد غانم[2]، سمير صالح عوض الله[2]، مروة بسيوني جمعه[1] و محمد عبد الفتاح ابوعبدالله[1]

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الدراسة الحالية فحصت تأثير البيئات الصناعية كبدائل حبوب اللقاح علي عدد خلاليا الدم في يرقات شغالات نحل العسل، حيث تم تغذية طوائف نحل العسل باربع بدائل مختلفة لحبوب اللقاح، ثم تم سحب عينات من الدم من يرقات الشغالات في الطوائف التي غذيت علي بدائل حبوب اللقاح، ثم تم توزيعها مباشرة علي شرائح زجاجية ثم فحصها بواسطة العدسة الزيتية للميكرسكوب بقوة x1250 . تقريبا خلايا الدم من نوع ال prohaemocytes, plasmatocytes and oenocytes سجلت اعلي اعداد في اليرقات المجموعة من الطوائف التي غذيت علي بدائل حبوب اللقاح مقارنة باليرقات المجموعة من طوائف لم تحصل علي بدائل حبوب اللقاح (معاملة الكنترول). ولكن خلايا التجلط المحببة وغير المحببة وخلايا الـ spherulescytes سجلت اقل اعداد في اليرقات التي جمعت من الطوائف التي مدت ببدائل حبوب اللقاح مقارنة بتلك التي لم تمد (معاملة الكنترول). أوضحت النتائج أن اعلي عدد من خلايا الدم prohaemocytes في اليرقات المغذاه علي بدائل حبوب اللقاح (بيئة A, B, D) تم عدها في هجين النحل ال buckfast يعقبها هجين النحل الايطالي ثم الكرنيولي بعد يوم واحد من التغذية. بالنسبة للبيئة C، كما أوضحت النتائج أن اعلي عدد لخلايا الدم من النوع prohaemocytes تم تسجيلها في هجين نحل العسل الكرنيولي يعقبه الايطالي ثم ال buckfast بعد يوم واحد التغذية. عموما في كل هجن النحل، فان خلايا الدم من النوع prohaemocytes كانت اعلي في اليرقات المغذاه ببدائل حبوب اللقاح مقارنة بتلك التي لم تغذي.