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Study on Types, Total and Differential Haemocytes counts of Usherhopper, *Poekilocerus bufonius* Klug

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ABSTRACT. This study presented the identification of types, the total and differential haemocytes counts of usherhopper, *Poekilocerus bufonius.* Different histochemical tests were used to distinguish between various haemocyte types. Five types were morphologically characterized; namely, prohaemocytes (PRs), plasmotocytes (PLs), granulocytes (GRs), spherulocytes (SPs) and adipohemocytes (Ads). Total haemocytes counts in nymphal stages and both adult's male and female were 1300/mm^3, 892/mm^3 and 838/mm^3, respectively. The relative percentage of types and differential haemocytes counts of nymph, male and female were calculated. The results were discussed on the light of the existing references with emphases on the possible effect of the food consumed by usherhopper.

Introduction

Several histochemical studies have been carried out on the identification and classification of insect haemocytes using light microscope[1-7]. There are many discrepancies in the categories of haemocyte types ranging from four to seven[6] and between three and nine[1,3,4,7-9]. In addition, there is confusion between various haemocyte types such as prohemocytes and plamatocytes as well as granulocytes and adipohemocytes[2,5]. However, many types of haemocytes were reported in different insect species; namely, prohaemocytes (PRs), plamatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), oenocytoids (Oes), coagulocytes (Cos), adipohemocytes (Ads), reticular and other cells.

Total haemocyte counts (THC) and differential haemocyte counts (DHC) were determined in the haemolymph of different stages of various insects species[10-13].

Usherhopper, *Poekilocerus bufonius* Klug (pyrgomorphidae), a black and yellow spotted locust feed on the milkweed (usher), *Calotropis procera* (Ait.), which is a perennial shrubby plant with broad, evergreen leaves and grows wildly in most arid region of Saudi Arabia. Usher is toxic to most vertebrates and invertebrates[14]. The toxicity is due to the presence of a high content of cardiac glycosides (SGs) in the latex[14, 15]. However, usherhopper ingest, sequesters and concentrates CGs in a bilobed poison gland[15, 16] and ejects them when disturbed as a means of defense against predators[16, 17]. The haemolymph CGs content of both male and female are almost similar to that of poison gland all year round[15]. There has been no study on the types, total haemocytes counts (THC) and differential haemocyte counts (DHC) of *P. bufonius.* So, the present study was undertaken.

Materials and Methods

Various stages of male and female usherhopper, *Poekilocerus bufonius,* were collected from different locations around Jeddah. They were maintained under laboratory conditions (20-25ºC) and were fed on branches of fresh usher.

To prepare blood smears, one leg of an insect (male, female and nymph of both sexes) were amputated and drops were smeared on microslides. The smears were fixed in methanol, washed in distilled water and stained with Giemsa and histochemical stains such as Periodic Acid Sciffs reagent for carbohydrates, Acid hematein for Phospholipid and Alcian blue for mucopolysaccharides.

Total haemocyte counts (THC) and differential haemocyte counts (DHC)

Total and differential haemocyte counts were carried as described by Harzarika and Gupta[18] and Chiang, *et al.*[19]. Helige true count haemocytometer was used to dilute haemolymph, and to avoid coagulation. Three drops of ethylenediaminetetraacetic acid (EDTA) buffer solution (0.1M glucose, 0.10mM EDTA, 0.45M sodium chloride, 30mM sodium citrate and 26mM citric acid) was added to the collected samples. The appropriate volume was well shaken and semiaired on Neubauer chamber slide. Degenerated and mitotic cells were included in the THC[20].

Results

The observation obtained using light microscope, in the present study, showed five types of haemocytes in the haemolymph of all stages and both sexes of *P. bufonius;* namely, prohaemocyte, plasmatocyte, granulocyte, spherulocyte and adipohemocyte. However, there were polymorphs of all haemocyte

types. Prohaemocytes were characterized by their small size, spherical shape and central large round nucleus, occupied most of cell cytoplasm, which form a very thin layer surround the nucleus (Fig. 1,2,3 and 4). Histochemical study (Table 1) indicated that PRs react moderately to PAS stain (Fig. 2), very strong to alcian blue (Fig. 3) and negatively to hematein test (Fig. 4). Plasmatocytes were amongst the most polymorphic and prominent types (Fig. 5). Their shapes ranged from spindle, with very pointed end, to oval and have a large centrally placed nucleus. Plasmatocytes were negatively stained with acid hematein (Fig. 6), however very strongly stained with alcian blue (Fig. 7) and PAS (Fig. 8). Granulocytes varied in shape and size, and were characterized by the presence of a small nucleus and large amount of different size granules (Fig. 9). Very few GRs have small cytoplasmic processes. Histochemical test showed that GRs were stained moderately with the three types of stains used in this study (Fig, 10 A, B. C, D, E). Spherulocytes (SPs) appeared in different shapes (polymorphic) with a small eccentric nucleus, spherules and membrane – bounded vacuoles in the cytoplasm (Fig. 11). These reacted positively with the histochemical stains (Fig. 12 A, B, C) used in this study. Adipohemocytes were very few when compared with other types of haemocytes and ranged in shape from circular to irregular (Fig. 13A, B, C and D). They contained a very large amount of lipid droplets, which occupied most of the cytoplasm. Treatment of adipohemocytes with acid hematein showed very strong reaction (Fig. 13B, C and D). All ADs from adults demonstrated negative reactions to staining with PAS (Fig. 14) and alcian blue (Fig. 15). However, ADs of nymph showed a positive reaction (Fig. 16).

The THC of hemocytes of different sex (nymph and adult) of usherhopper varied considerably (Fig.17A, B and 18A and B). The numbers of cells per mm^3 were more or less similar of both sexes, male (892 cells) and female (838 cells). The nymphs of both sexes contained more THC than the adults (1300 cells/ mm^3). However, the percentage of each cell types varied (Fig. 19A, B and 20A and B). It can be seen that apart from adult males (27%), the GRs accounted for 46%, 62% and 57% of the haemocytes of female, female nymph and male nymph, respectively. In addition, the PRs form between 5-8 % of nymph THC of both sexes, and 15-24% of adult male and female. However, the PLs and GRs are the most prominent blood cell types of all stages (Fig. 17 and 18), and account for around 70% of the THC.

Discussion

The results of the present study, using light microscope and histochemical tests, demonstrated that the haemolymph of the various stages and sexes of usherhopper contained five types of haemocytes; being prohaemocyte (PRs),

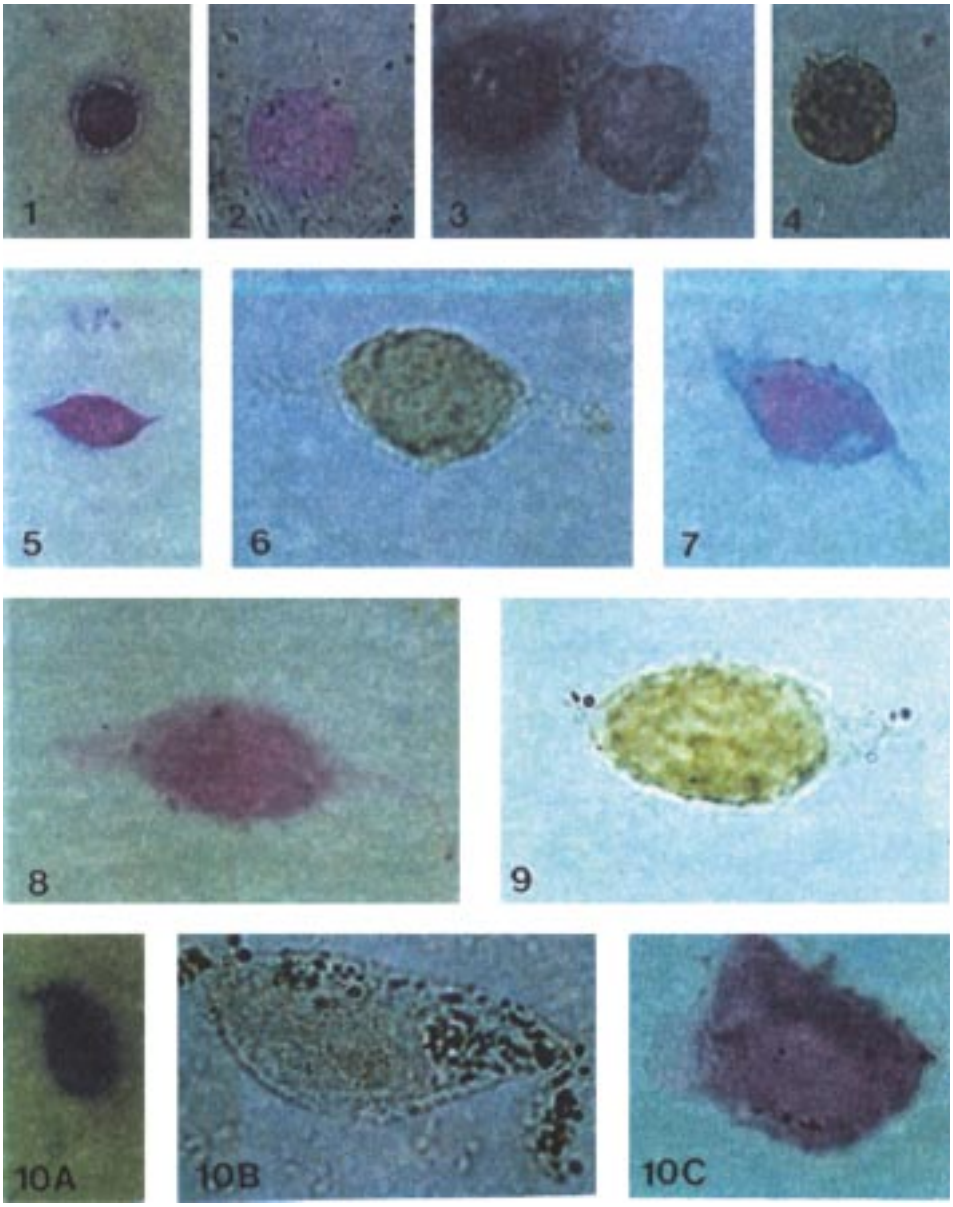

Fig. 1. Prohemocyte stained with Giemsa showing large nucleus occupying most of the cell cytoplasm (× 400).

Fig. 2. Prohemocyte stained with alcain blue indicating the presence of few amounts of mucoproteins (× 1000).

Fig. 3. Prohemocyte showing moderate reaction with PAS (× 1000).

Fig. 4. Prohemocyte showing negative stained with acid hematein (× 1000).

FIG. 5. Plasmatocyte stained with Giemsa and characterized by spindle shape and pointed end (× 400).

FIG. 6. Plasmatocyte showing negative reaction to acid hematein (× 1000).

FIG. 7. Plasmatocyte stained positively with alcain blue (× 1000).

FIG. 8. Plasmatocyte showing positive reaction to PAS (× 1000).

FIG. 9. Plasmatocyte demonstrate trace of positive to acid hematein (×1000).

FIG. 10. Granulocyte stained with Giemsa (A) (× 400), react positively with acid hematein (B) (× 1000) and PAS from male (C) (× 1000) and female (D) (× 1000). Note the amount of granules in the cytoplasm (Fig. 10B and E) (× 1000).

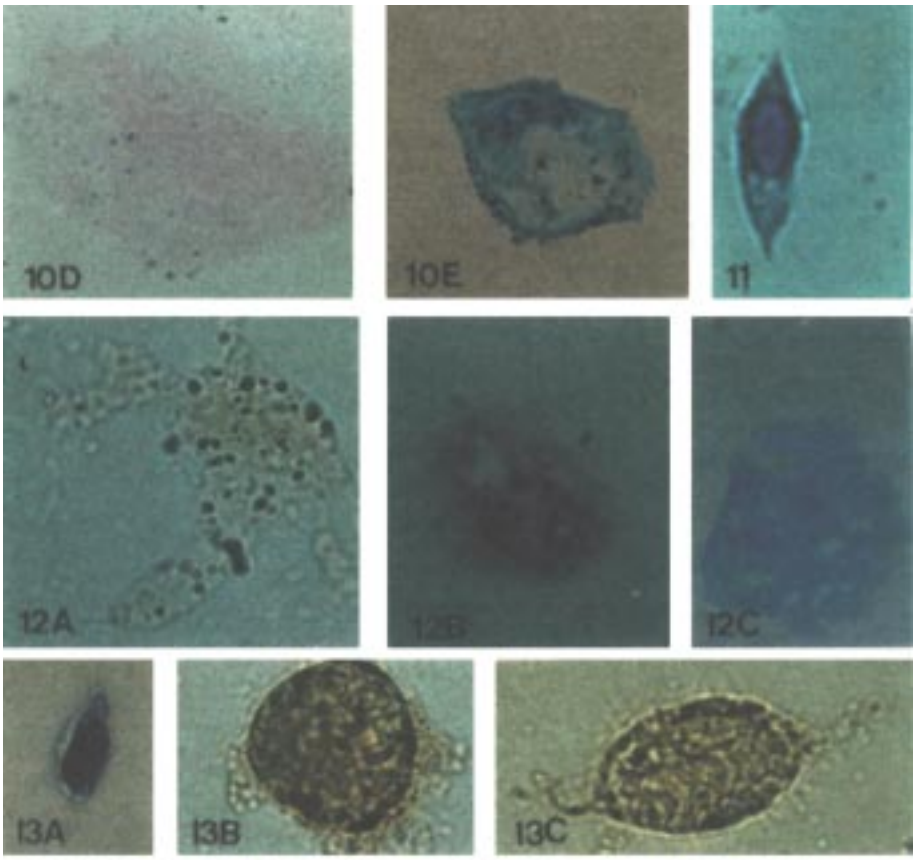

FIG. 11. Spherulocyte stained with Giemsa (× 400).

FIG. 12. Showing various spherulocytes reacts positively with acid hematein (A) (× 1000) PAS (B) (× 1000) and alcian blue (C) (× 1000).

FIG. 13. Demonstrate adipohemocytes stained with Giemsa (A) (× 400) and reacts positively to acid hematein whether it from nymph (B) (× 1000), adult male (C) (× 1000) and adult female (D) (× 1000).

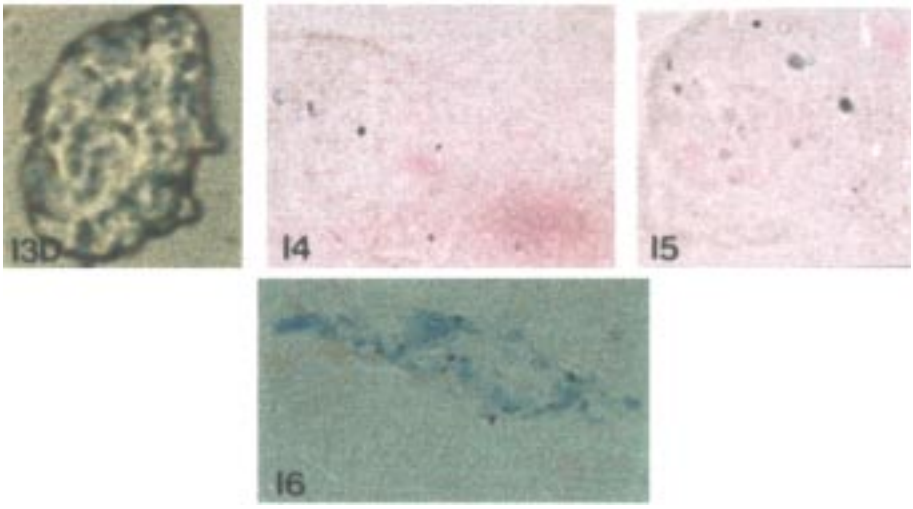

FIG. 14. Adipohemocyte react negatively to PAS (× 1000).

FIG. 15. Adipohemocyte react negatively to alcian blue (× 1000).

FIG. 16. Adipohemocyte of nymph shows positive reaction to alcian blue (×1000).

plasmatocyte (PLs), granulocyte (GRs), spherulocyte (SPs) and adipohemocyte (Ads). Similar results have been reported for various insect[6]. The conflict in terminology, used to describe the same type of haemocytes[2,5,21], between different reports may be due to the various characteristics used for classification of haemocytes. These features included cell shapes[4], morphology, ultrastructure[22-26] and function[6,21,27-29].

Histochemical study (Alcian blue, Acid hematein and PAS) showed that *P. bufonius* contained five types of haemocytes. However, there are different degrees of response to these stains. Similar results have been reported in haemocytes of *Garausius morosus*[6] in which three types of GRs were found. It is possible to suggest that the differences in shape and responses to various stains was due to differences in growth turnover between haemocytes, such as PRs to PLs and others. It has been suggested that the PRs are the stem cells for the other types of haemocytes[29]. This might have occurred, at least, in embryonic stages and not in post embryonic stages[6, 7], which indicated that PRs are the origin of PLs. Some authors used its presence in all stages of insect life as strong evidence to support their role in producing other haemocytes[29, 30]. It has been proposed that many, if not all, cell types are merely stages in the development of a single type and the others are arbitrary selections from a continuous range of variation[31]. The present study indicated that some haemocytes fulfill the function of food storage (GRs, SPs and Ads) since they

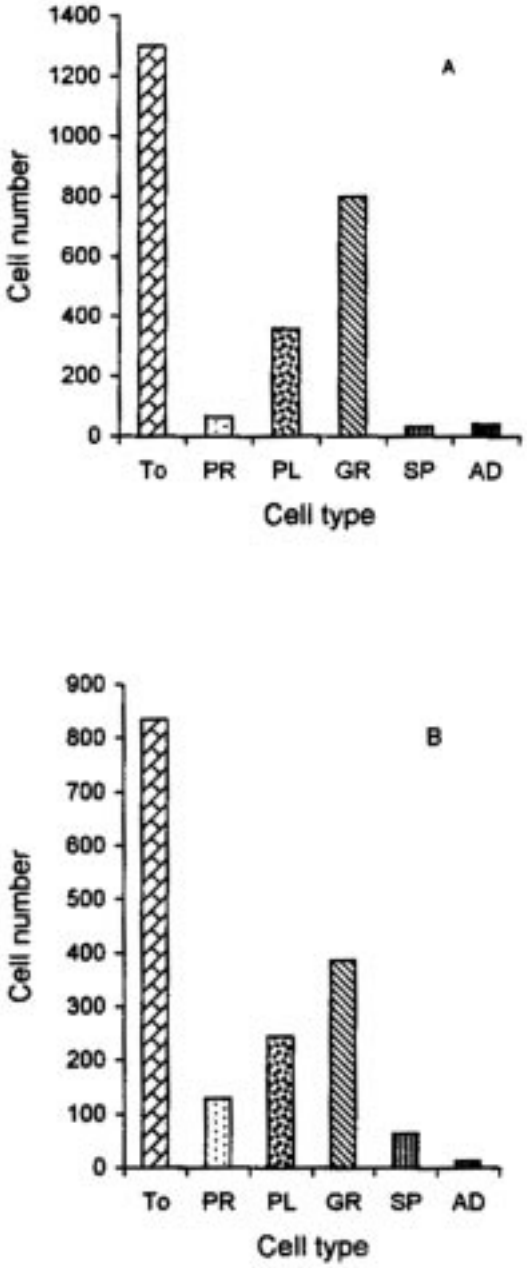

FIG. 17. Total and differential haemocytes counts of nymph (A) and adult (B) female *Poekilocerus bufonius.*

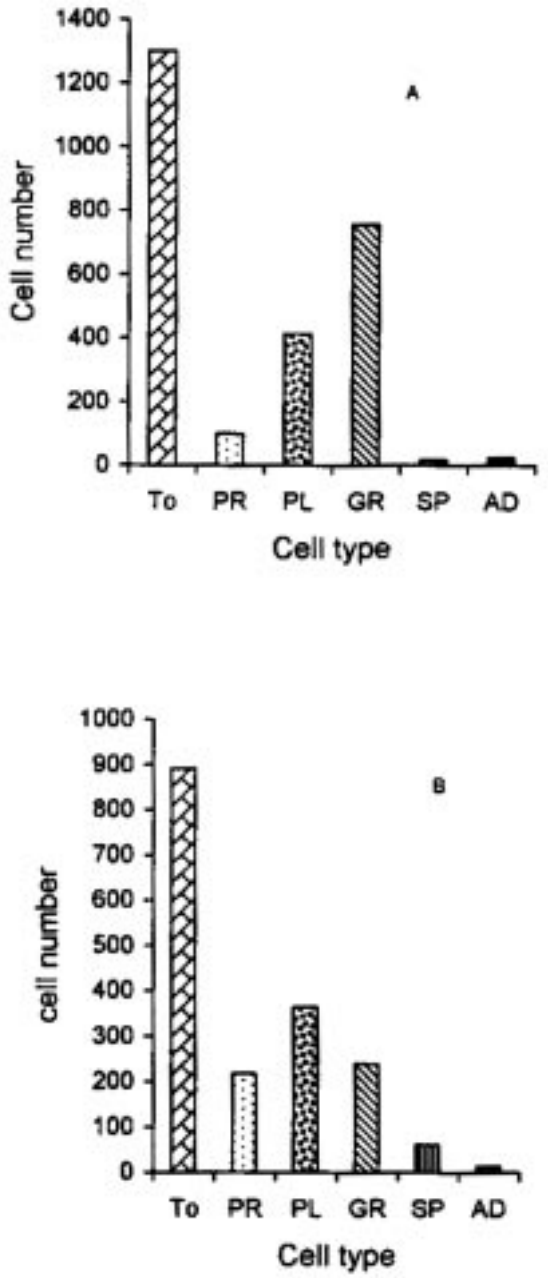

FIG. 18. Total and differential haemocytes counts of nymph (A) and adult (B) male *Poekilocerus bufonius*.

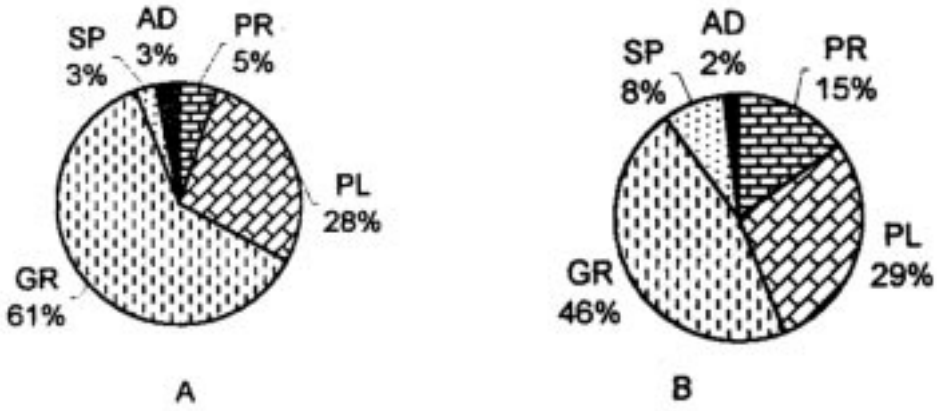

FIG. 19. Percentage of haemocytes of nymph (A) adult female (B) *Poekilocerus bufonius.*

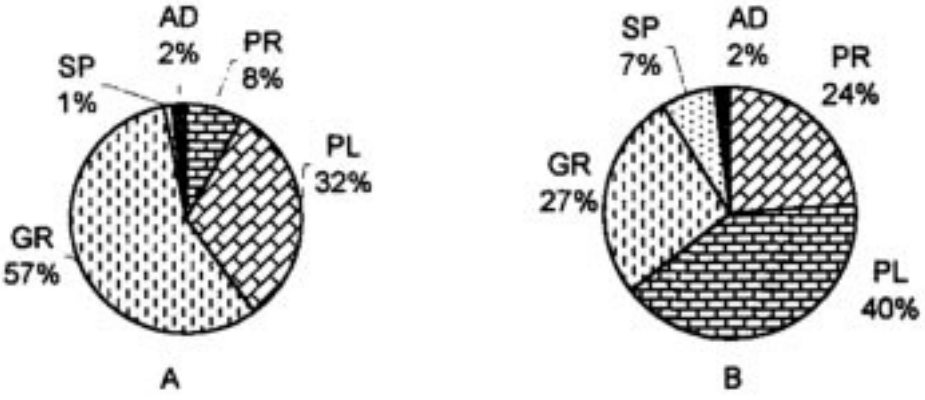

FIG. 20. Percentage of haemocytes of nymph (A) and adult male (B) *Poekilocerus bufonius.*

contained inclusions of carbohydrate and lipid, which decreased on starvation of the insect[32, 33]. The very low THC of usherhopper haemolymph, when compared with that of locusts might have been due to the type of food-consumed[15] and losing the ability to fly[13, 34]. As pointed out in the introduction usherhopper consumed toxic plants, such *Calotropis procera,* which contained cardiac glycosides. The later would have reduced the need for high number of haemocytes such that in locusts[6].

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الأنـواع والعـدد الكلي وأعـداد الأنـواع المختلفـة لخـلايا الدم في نطاط العشر *Poekilocerus bufonius* Klug

علي بن أحمد الرباعي و أحمد إبراهيم السقاف و ندى عثمان إدريس
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المستخلص . تهتم هذه الدراسة بالتعرف على أنواع وأعداد خلايا الدم في نطاط العشر *Poekilocerus bufonius* . استخدمت الصبغات الهستوكيميائية للتمييز المظهري بين الأنواع المختلفة . تم التعرف على خمسة أنواع من خلايا الدم تشمل : الأولية Prohaemocytes ، البلازمية Plasmatocyte ، الحبيبية Granulocytes ، الكروية Spherulocytes والدهنية Adipohemocytes . قدر العدد الكلي في الأطوار الحورية والذكور والإناث البالغة بـ ١٣٠٠/ مم^{٣} ، ٨٩٢/ مم^{٣} و ٨٣٨/ مم^{٣} ، على التوالي . تم حساب النسبة المئوية لكل نوع في الحوريات والحشرات البالغة ورسمت بيانيًا . نوقشت النتائج على ضوء الدراسات السابقة مع التأكيد على تأثير نوع الغذاء الذي يستهلكه نطاط العشر . قلة عدد خلايا الدم في نطاط العشر استبدلت (تم تعويضه) بقدرتها على استهلاك النباتات السامة المحتوية على الجليكوسيدات القلبية المستخدمة كوسيلة دفاع .