

Study of Free Haemocytes of Red Palm Weevil. *Rhynchophorus ferrugineus* (Oliver) (Coleoptera : Curculionidae) of Saudi Arabia

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Abstract. Haemolymph drops were oozed out of the weevil, *Rhynchophorus ferrugineus* (Oliv.) and used for the identification and classification of their free haemocytes. Haemocytes have also been maintained *in vitro* in Wheaton Culture Vessels for short-term cultures to facilitate observations on their live shapes and specific characteristics. Six different types of haemocytes were identified: prohaemocytes, plasmatocytes, granular haemocytes, cystocytes, oenocytoids and adipohaemocytes. The prohaemocytes are juvenile forms; plasmatocytes, granular haemocytes, cystocytes, oenocytoids and adipohaemocytes are metabolites transporting forms, while the plasmatocytes are functionally the immunocytes of the weevil.

Key Words: Blood cells, Haemocytes, Insect Pests, Red Palm Beetle, *Rhynchophorus ferrugineus*

Introduction

Arthropod and other invertebrate haemocytes are comparable to the leucocytes of the vertebrates (Jones 1962, 1975, Ahmed & Khan, 1988. Han and Gupta 1989, Gupta 1979, 1990, 1991, Bardoloi and Hazarika 1992) and constitute an important component of the haemolymph of weevils. Those cells were first discovered and classified into four different categories

by Millara (1747). Due to their phagocytic functions, the plasmatocytes and /or granular haemocytes are in fact the "Immunocytes" of these animals.

Information on the haemocytes of the weevils are meagre. Hence, in the present study an attempt is being made to identify the haemocytes of the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) of Saudi Arabia.

Materials and Methods

A colony of the red palm weevil, *R. ferrugineus* was established in the laboratory to supply the weevils required for the present study. The colony was maintained at 28 ± 2 °C and 65% relative humidity, and the weevils of the colony were fed on sugarcane shoots (Rahlakar *et al.* 1972). One leg of either male, female or larva of the weevil was amputated and drops of haemolymph were smeared onto clean microscope slides. These were air-dried, fixed by absolute methanol and stained with either Leishman's or Giemsa's stain using an acidic buffer, pH 6.5. The slides were then mounted in neutral D.P.X. medium and examined under the microscope.

Phase contrast microscopy was used to study a hanging drop of haemolymph grown in a short-term culture (24-72h) in Eagle's salt medium containing sodium bicarbonate. Short-term cultures of haemocytes in Medium 199 supplemented with bovine calf serum supplied material for studying haemocyte's live shapes and progressive development. The short-term cultures are made in Wheaton culture vessels (Wheaton Instruments, Millville, New Jersey, USA).

Results and Discussion

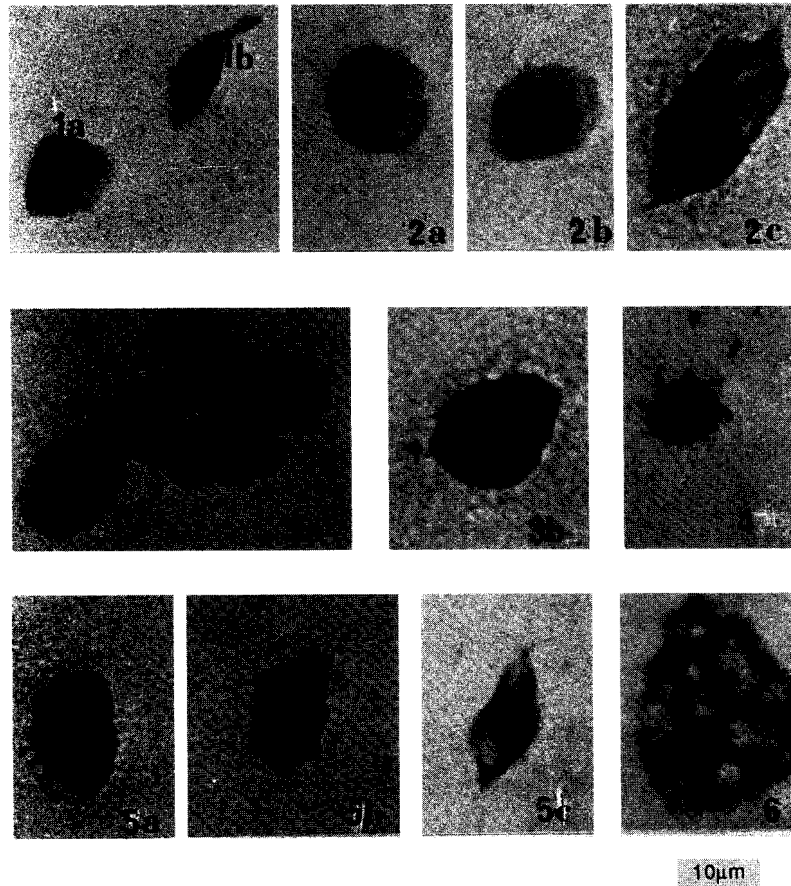
The free haemocytes of *R. ferrugineus* conform to the characteristics of

prohaemocytes, plasmatocytes, granular haemocytes, cystocytes, oenocytoides and adipohaemocytes. The main features of each type are listed below:

Prohaemocytes: These are the smallest of the haemocytes. Their cytoplasm is strongly basophilic and forms a thin layer bound by a uniform, clearly visible cell membrane that uniformly surrounds the comparatively large, spherical, compact and strongly basophilic nucleus which contains scanty nucleoplasm with large, compact chromatin granules. The cytoplasm of these cells varies from 7-10 μm and the nucleus was 4-7, μm , with a cytoplasm nuclear area ratio (AC/AN) of 1.4 ± 0.45 (Figs. 1a & b).

Plasmatocytes: These form the bulk of free haemocyte population # ($\geq 70\%$) and are comparatively large, polymorphic cells with small, spherical to oval nuclei. They could be grouped into spherical, oval, and irregular plasmatocytes. The spherical plasmatocytes are 13-19 μm in diameter; their nuclei are generally spherical, 5-8 μm in diameter with an AC/AN ratio of 3.1 ± 1.2 . The cytoplasm of these cells is generally vacuolated (Figs. 2a, b & c).

Granular haemocytes: These are few, mostly spherical cells of moderate size, or oval shaped, 12-16 μm in length and 11-14 μm in width with comparatively small, round eosinophilic nuclei measuring 5-9 μm . Their cytoplasm is mildly basophilic with



few large, round eosinophilic granules and their AC/AN ratio is 2.4 ± 0.9 (Figs. 3a & b).

Cystocytes: These are also few and are similar to the granular haemocytes. They are 8-12 μm in diameter, but have one or more basophilic inclusions. The nucleus is round (5-9 μm) to oval with compactly arranged chromatin granules. The AC/AN ratio is 1.7 ± 0.6 (Fig. 4).

Oenocytoids: These are round or oval in shape with granular and strongly

basophilic cytoplasm that has one or two vacuoles. The nucleus is more basophilic than the cytoplasm, and is round and eccentric in position. These cells are 11-15 μm in diameter, but there are some oval ones which are 12-16 μm in length and 10-15 μm in width. The AC/AN ratio is 2.1 ± 1.4 . (Figs. 5a, b & c).

Adipohaemocytes: These constitute over 5% of the haemocytes in the haemolymph and are moderate in size, 13-

17 μm in length and 11-15 μm in width. They are round with very large cytoplasmic fat inclusions (fat globules), their nuclei are 4-6 μm and the ratio AC/AN is 3.5 ± 1.5 . (Figs. 6).

Similar to the observations of Gupta (1991) in other insects, the prohaemocytes, plasmatocytes and granular haemocytes are the fundamental and most common types of haemocytes in the red palm weevil, *R. ferrugineus*. The prohaemocytes are the germinal blood cells that divide to generate similar as well as other types of haemocytes (Al-Khalifa and Siddiqui, 1985). On the other hand, the plasmatocytes described in the present study are similar to those reported in the haemolymph of scavenger beetle, *Dermestes vulpinus* and *Dermestes maculatus* (Al-Khalifa and Siddiqui, 1985). Together with granular haemocytes, they are also considered to be the immediate derivatives of prohaemocytes (Crossley 1975) an event explicitly augmented by the *in vitro* observations in the present study. Also the inverse relationship of the population of the prohaemocytes and plasmatocytes might predict the interconversion of these two cell types observed by Al-Khalifa and Siddiqui (1985) in other coleopterous insects. However, similar to the observations of Farkas and Zethan (1989) in *Galleria mellonella*, the plasmatocytes and granular haemocytes

were treated in the present study as two separate identities. Furthermore, the plasmatocytes and /or granular haemocytes have also been cumulatively called the "Immunocytes" because they are actively involved in phagocytosis, encapsulation and nodule formation as a defence mechanism (Han and Gupta 1989). The spherulocytes of the other Coleopterous insects were termed here as cystocytes due to their hyaline nature and shape. These are the rarest type of haemocytes and are characterised by having large inclusions in their cytoplasm. Similar observations were also made in insects by (Gupta, 1979). Similar to the observations of (Jones, 1975; and Gupta, 1950) in other insects, the haemocytes with larger vacuoles and eccentric nuclei observed in the red palm weevil studied. The sixth type of haemocytes is the adipohaemocytes which are sometimes called in ticks the nephrocytes (Balashov 1983; Al-Khalifa and Siddiqui, 1993). These cells are known to engulf macromolecules or fat globules from the haemolymph, degrade them and retain them in their cytoplasm (Arnold and Sohi 1974).

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دراسة خلايا الدم الحرة في سوسة النخيل الحمراء من نوع
Rhynchophorus ferrugineus (Oliver) (Coleoptera: Curculionidae)
 في المملكة العربية السعودية

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الملخص : لقد تم جمع قطرات دم كل من الذكور والاناث واليرقات لسوسة النخيل الحمراء ، ومن ثم عملت سحبات علي شرائح نظيفة ، ثبتت بواسطة كحول ميثيلي وبعد ذلك صبغت وفحصت باستعمال المجهر الضوئي للتعرف عليها ولتصنيف خلايا الدم المتواجدة في كل منها إضافة الي ذلك ، تمت زراعة بعض خلايا الدم في مزرعة قصيرة الامد باستعمال اوعية ويتق لزراعة الخلايا (Wheaton Culture Vessels) مما مكنتنا من رؤية اشكال هذه الخلايا الدموية حية ودراسة بعض خصائصها وصفاتها باستعمال مجهر الطور المتباين .

لقد وجد ستة انواع من خلايا الدم متميزة عن بعضها البعض وهي :- خلايا الدم الاولية (Prohaemocytes) (وخلايا الدم البلازمية (Plasmatocmytes) وخلايا الدم المحببة (Granular haemocytes) وخلايا الدم الكروية (Cystocytes) وخلايا الدم الالينوسيتويد (Oenocytoids) وخلايا الدم الدهنية (Adipohaemocytes) نعتبر خلايا الدم الاولية بمثابة الخلايا المنشئة لبقية الانواع ، بينما خلايا الدم البلازمية والمحببة والكروية والالينوسيتويد والدهنية فهي اشكال انتقالية للمواد الغذائية وتميز الخلايا البلازمية ايضاً بكونها تقوم بوظيفة المناعة في السوسة .