

HAEMOCYTES OF SOME HOMOPTEROUS INSECTS

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ABSTRACT

The haemocytes of the scale insect, *Aspidiotus nerii* Bouch (Fam.: Diaspididae), were examined and compared with those found in the mealy bugs *Icerya aegyptiaca* (Douglas) (Fam.: Margarodidae), *Orthezia insignis* (Douglas) (Fam.: Ortheziidae) and the citrus aphid *Aphis citricola* (Fam.: Aphididae). Five types of haemocytes were found in the studied scale insects and mealy bugs. The cell types common to all species included: Prohaemocyte (Pr), Plasmotocyte (Pl), Granulocyte (Gr), Non Granulocyte (n-Gr) and Oenocyte (Oe). In addition, Spherule cell (Sph) was found in the citrus aphid, only. The classification of the haemocyte types of the subject insects and some possible characters of them were discussed.

The changes in the different haemocytes counts (DHC) and variations in haemocytes surface area were recorded in different developmental stages of citrus aphid.

Also, the effect of Neem Azal-T5% E.C. was evaluated against the citrus aphid, *Aphis citricola* reared on its citrus host under laboratory conditions. Generally, all the tested concentrations decreased the counts of Pr, Gr and n-Gr. However, the application of Neem extract increased the Oe surface area.

INTRODUCTION

There are different descriptions of the haemocytes in the literature in different groups of insects (Jones, 1962; Wigglesworth, 1979). In recent years the tendency has been to simplify these descriptions and to put the emphasis on limited of more or less well defined types (Wigglesworth, 1979; Brehelin & Zachary, 1986). Insect haemocytes are grouped now into 5 to 7 main classes after the round table discussion developed to these cells at the Invertebrate Immunology Conference in Montepellier (Brehelin & Zachary, 1986).

Homopterous insects are extremely important pests of fruit trees and many ornamental plants. They spread successfully and cause defoliation, dryness of young twigs and excretion of honeydew on which grows the sooty mould fungus causing interference with the vital processes of plants.

Some recent researches have been directed mainly towards the economic impact and control of this pest group while there are few researches about the blood picture of the homopterous insects (Lambdin & Joshi, 2001; Gad & El-Meniawi 2005).

The present study was carried out to study the blood picture and haemocyte surface area in four homopterous insects belong to four different families. Also, the present study investigated the changes in the different haemocyte count and variation in haemocyte surface area in different developmental stages of citrus aphid.

One of the implementation of the integrated pest management is the application of new highly selective pesticides with low side effects on insect behaviour, growth and development (Schmutterer, 1988 & 1990; Koul, 1992; Mordue & Blackwell, 1993). Although a lot of information has been published on the effect of azadirachtin on insects mortality, behaviour, growth and development, no research is available on the effect of neem extracts on the insect

blood picture. The efficacy of neem oil against the sucking insect pests was studied on cotton plants by Ahmed *et al.* (1995) and on egg plant by Tayeb *et al.* (1999). There are numerous records on the effect of neem products on several species of aphids, either in the laboratory or in the field (Dimetry & El-hawary, 1995; Dobelin, 1997; Schulz *et al.* 1997; Zuber, 1997). For all the abovementioned, this work is designed also to study effects of Neem Azal T on different haemocyte count and haemocyte surface area which has not been studied before.

MATERIALS AND METHODS

A-The blood picture of some homopterous insects:

Adult scale insect, *Aspidiotus nerii*, mealy bugs *Icerya aegyptiaca*, *Orthezia insignis* and citrus aphid *Aphis citricola* were collected from the different host plants in the garden of the Faculty of Agriculture, Alex. University. Ten adult insects of each family were crushed between clean glass slides. The haemolymph is quickly smeared to a thin film with the edge of another clean slide. The smears are dried and stained with Wright's blood stain (Conn, 1948). They were examined using emersion oil lens of a light microscope. Different counts of all the haemocyte types in random scan of blood films were carried out by using 50 haemocytes from each film. Haemocytes measurements were carried out described by Arnold & Hinks (1976).

B-Changes of different haemocyte counts during the citrus aphid stages:

To study the changes of different haemocyte types during the development of citrus aphid stages, ten individuals from different developmental stages of citrus aphid were collected to prepare the haemolymph slides. Each sample was replicated five times. The number of haemocyte counts were

recorded. In addition, the surface area of each haemocyte type was measured by micrometric slide in all the developmental stages of the subject insect.

All tested results were statistically analysed and compared using "F" test and Least Significant Differences (L.S.D) at probability level 0.01.

C -Effect of Neem extract on the blood picture and haemocyte surface area of citrus aphid adults:

Neem oil was used as Neem Azal - T5%E.C. which is produced by Bayer A.G, Germany. It was used as distilled water emulsion at the concentrations of 0.1, 0.3 and 0.4%. Ten adults were transferred to each treated citrus leaf, two hours after application to allow the liquid to dry. Then after 24 hours five adults were collected to count the different haemocytes, and to measure the haemocyte surface area. Each blood sample for each concentration was replicated five times.

RESULTS AND DISCUSSION

A-The blood pictures of the tested homopterous insects:

Five types of haemocytes were identified in the female adults of the tested scale insect and mealy bugs, while six types were observed in the citrus aphid. Although the studied mealy bugs belong to two different families, there was no difference between them in the haemocytes counts and surface area, therefore, they are considered as one group of insects, (Table 1, 2 and Fig. 1). According to Arnold & Hinks (1976) the identification of the obtained haemocytes description are:

1-Prohaemocytes(pr):

Prohaemocyte cells are characterized by their small size with a round or spherical shape. The nucleus is relatively large, centrally located and nearly fills the cell. Table, 1 illustrates the proportion of the Pr ranged between 9 ± 0.1 cell /mm³ in the scale insects and mealy bugs to 14 ± 0.2 cell /mm³ in citrus aphid. The highest value of Pr surface area was recorded in the mealy bugs ($35.6 \pm 1.3 \mu\text{m}^2$) and the lowest values were $23.7 \pm 1.6 \mu\text{m}^2$ and $25 \pm 1.4 \mu\text{m}^2$ in the scale insect and citrus aphid, respectively, (Table, 2).

2-Plasmatocytes(Pl):

The Pl appears polymorphic (i.e spindle shape or oval). The nucleus is similar to that of prohaemocyte, but is frequently elongated. The cytoplasm may be granular, and sometimes contains small droplets. The plasmatocytes in most orders of insects are reported to play an important role in cellular encapsulation (Akai & Sato, 1979). By comparing the proportion and surface area of this type in the subject insects it was found that Pl constitute 35.6 ± 0.7 cell /mm³ in the citrus

aphid and 14 ± 1.3 cell /mm³ in the scale insect and mealy bugs (Table, 1). The surface area of Pl was 42.2 ± 1.2 and $44 \pm 1.1 \mu\text{m}^2$ in the scale insect and citrus aphid, respectively, while it was $46 \pm 1.1 \mu\text{m}^2$ in the mealy bugs (Table, 2).

3-Granulocytes(Gr.):

The Gr. was usually found to be round with dense granules and it was characterized by numerous cytoplasm projections. The nucleus is usually difficult to be seen due to the numerous granules. The proportions of this type were 18 ± 0.3 , 17.6 ± 0.5 , 32.6 ± 2.1 cell /mm³ for the scale insect, mealy bugs and citrus aphid, respectively (Table, 1). The surface areas of the Gr were $42.2 \pm 1.2 \mu\text{m}^2$ and $36.3 \pm 1.3 \mu\text{m}^2$ in the scale insect and citrus aphid while it was $38.6 \pm 1.4 \mu\text{m}^2$ in mealy bugs (Table, 2).

4-Non- granulocytes (n-Gr):

This type was usually spherical or oval cells, it contains faintly rose coloured granules. The nucleus usually centrally located and stained pale blue. Gr and n-Gr are reported to participate in phagocytosis (Akai & Sato, 1979), encapsulation and secretion of immunological factors (Yeaton, 1983). The proportion of the n-Gr were 14 ± 1.3 , 17 ± 0.6 and 30.3 ± 1.2 cell /mm³ in scale insect, mealy bugs and citrus aphid, respectively (Table, 1). The haemocyte surface areas were 35.6 ± 1.6 , 38.6 ± 1.4 and $36.3 \pm 1.5 \mu\text{m}^2$ in the scale insect, mealy bugs and citrus aphid, respectively (Table, 2).

5-Oenocytes (Oe) :

Oenocytes were the largest haemocyte type, they were round or oval cells. They are characterized by an opaque appearance. The nucleus is usually central. As shown in Table (1) the proportion of Oe were 5 ± 0.1 , 8 ± 0.1 and 14.5 ± 0.1 cell /mm³ in the scale insect, mealy bugs and citrus aphid, respectively.

The surface area of Oe ranged between 62 ± 2.1 and $96.3 \pm 1.5 \mu\text{m}^2$ in the scale insect and citrus aphid to $120.7 \pm 1.3 \mu\text{m}^2$ in mealy bugs (Table, 2).

6-Spherule cells (Sph):

This type was found only in the citrus aphid. Sph cells are identified by their oblate shape, when stained with Wright's stain, contains some or numerous pale red spherules of different sizes which may be due to the maturity of the cell. The cytoplasm is dark blue. Possible functions of Sph were suggested including secretion of some haemolymph proteins (Akai & Sato, 1979). The number of spherule cells was 4 ± 0.2 cell /mm³ and the surface area of this type was $43.5 \pm 1.6 \mu\text{m}^2$ (Tables 1, 2).

The statistical analysis of the obtained data showed that no significant differences were recorded in the Pr count, while there were significant

differences in the cases of Gr, n-Gr, Pl, Oe and Sph in all tested insects. On the other hand, There were significant differences in the surface area of Pr, Gr, n-Gr, Pl, Oe and Sph in all tested insects.

The present work agree with Lambdin & Joshi, (2001) who studied the haemocytes of three

mealy bug species, *Phenacoccus gossypi* Townsend and Cockerell, *Pseudococcus logispinus* (Targioni Tozzetti) and *Dactylopius confuses* (Cockerella) (Hemiptera: Coccoidea) and found four basic cell types in the two pseudococcids and in the cochineal scale.

Table (1): Mean values of the differential haemocyte count in some Homopterous insects.

Some of Homopterous insects	Pr	Gr	nGr	Pl	Oen	Sph
<i>Aspidiotus nerii</i>	9±0.1a	18±0.3b	14±1.3b	14±1.3b	5±0.1b	————
<i>Icerya aegyptiaca</i>	9±0.1a	17±0.5b	17±0.6b	16±0.1b	8±0.1b	————
<i>Orthezia insignis</i>	10±0.2a	16±0.2b	13±0.1b	19.6±0.3b	7.2±0.1b	————
<i>Aphis citricola</i>	14±0.2a	32.6±2.1a	30.3±1.2a	35.6±0.7a	14.5 ±0.1a	4±0.2

Pr = Prohaemocytes

Gr = Granulocytes

N Gr = Non-Granulocytes

Pl = Plasmotocytes

Oe = Oenocytes

Sph = Spherule cells

Each value represents the mean ± S.E.

Means in same column followed by the same letter (s) are not significantly different according to L.S.D at 0.01 level of probability.

Table (2): The haemocyte surface area in some Homopterous insects.

Some of Homopterous insects	Pr.	Gr.	nGr	Pl.	Oen.	Sph.
<i>Aspidiotus nerii</i>	23.7±1.6b	42.2±1.2a	35.6±1.6a	42.2±1.1a	62±2.1c	————
<i>Icerya aegyptiaca</i>	35.6±1.3a	46.3±1.5a	38.6±1.4a	46±1.1a	96.3±1.5 ab	————
<i>rthezia insignis</i>	34.3±1.2a	44.7±1.2a	34±1.6a	45.3±1.3a	83.4±2.1b	————
<i>Aphis citricola</i>	25±1.4b	42.9±0.2a	36.3±1.5a	44±1.1a	120.7±1.3a	43.5 ±1.6

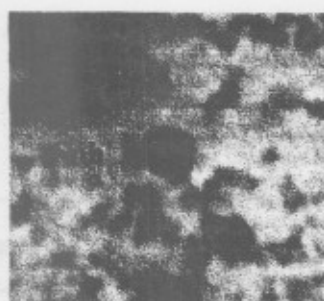
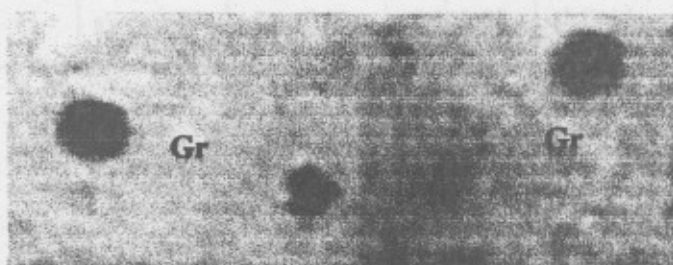
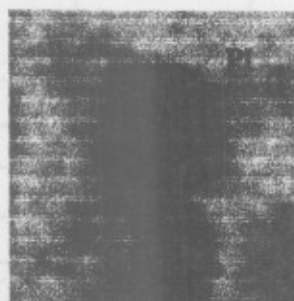
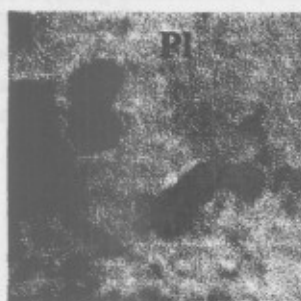


Figure (1) Fresh haemolymph samples taken from the tested homopterous insects. Smears stained with Wright's stain. Prohaemocyte (Pr), Plasmotocyte (Pl), Granulocyte(Gr), Non-Granulocyte (n-Gr), Oenocyte (Oe), Spherule cell (Sph).

B-Changes of different haemocyte counts during the citrus aphid stages:

The examinations and observations of fixed and stained smears from different developmental stages (the four nymphal instars and the newly emerged adults) of *Aphis citricola* revealed six types of haemocytes Pr, Pl, Gr, n-Gr, Oe, and Sph.

As illustrated in Table(3) ,Pr were gradually increased throughout the development from the first nymph to the adult stage. The maximum increase was observed in the adult stage(14 ± 0.3 cell /mm³). A significant difference was recorded in Pr count between adult and nymphs but there were no significant different among second ,third and fourth nymphal instars. The Gr showed a remarkable increase to be 40.7 ± 0.2 cell /mm³ in the third nymphal instar and declined thereafter to be (30.3 ± 0.6 cell /mm³) in the adult stage, while the n- Gr slightly increased to be 18 ± 0.4 cell /mm³ in adult stage. The maximum increase of Pl was recorded for the adult stage(35.7 ± 0.5 cell /mm³). On the other hand, Oe increased gradually to be 14.6 ± 0.3 cell /mm³ in the adult stage. The count of Sph had the same trend of that in Gr, the maximum increase was observed at the fourth nymphal instar to be 5.6 ± 0.5 cell / mm³ and then decreased in adult stage to be 4.3 ± 0.3 cell / mm³ . The present result in this concern agreed to the results of Gad & El- Meniawi ,

(2005) who observed that the immunocytes (Pl and Gr) represented the majority of total haemocytes (about 60%) during the whitefly *Bemisia tabaci* development. In addition, there were no significant differences in Sph counts.

The surface area of Pr increased gradually to be 30.2 ± 0.3 μ m² in the fourth nymphal instar. There are no significant difference in the surface area of Pr during all developmental stages of citrus aphid. The same trend was observed in the Pl surface area (60.1 ± 1.2 μ m²) in the fourth nymphal instar. Also, there were no significant differences in Pl surface area among all developmental stages of citrus aphid. The surface area of n-Gr slightly decreased to be 40.6 ± 1.2 μ m² in the third nymphal instar and then increased to be 60 ± 1.3 μ m² in the adult stage. The same trend was observed in Gr and Oe surface area. The maximum surface areas were observed at the adult stage (50.7 ± 1.7 and 80.8 ± 2.3 μ m² in Gr and Oe, respectively). while the minimum were observed at third nymphal instar (40.6 ± 1.2 and 70 ± 1.7 μ m²) in Gr and Oe, respectively (Fig. 2).

Concerning the surface area of Sph ,the results showed that it increased significantly from the first nymphal instar to adult stage.

The maximum area was observed at adult stage to be 70.8 ± 2.1 μ m².

Table (3):Differential haemocyte counts in different developmental stages of *Aphis citricola*.

Developmental stages	Pr	Gr	nGr	Pl	Oe	Sph
1 st Nymph	$5.7\pm0.6c$	$29.7\pm0.6b$	$13.3\pm0.2c$	$30.8\pm0.3ab$	$6\pm 0.5 c$	$4.4\pm 0.4 a$
2 nd Nymph	$8.3\pm0.8 bc$	$23.7\pm0.3c$	$9\pm 0.5c$	$25.6\pm0.3b$	$5.3\pm 0.5 c$	$3.6\pm 0.5 a$
3 rd Nymph	$10.3\pm0.8b$	$40.7\pm0.2a$	$10.3\pm0.2d$	$28.8\pm0.6ab$	$6.2\pm 0.4 c$	$5.6\pm 0.6 a$
4 th Nymph	$8.6\pm0.6bc$	$34.0\pm 0.5b$	$16\pm0.3 b$	$27.3\pm 0.8b$	$11.6\pm 0.3b$	$5.2\pm0.2 a$
Adult ♀	$14\pm0.3a$	$30.3\pm0.6b$	$18\pm 0.5 a$	$35.7\pm0.5a$	$14.6\pm 0.3a$	$4.3\pm 0.3 a$

*Statistical analysis between stages.

*There are no significant differences among means with same letter.

*Each value is a mean \pm S.E.

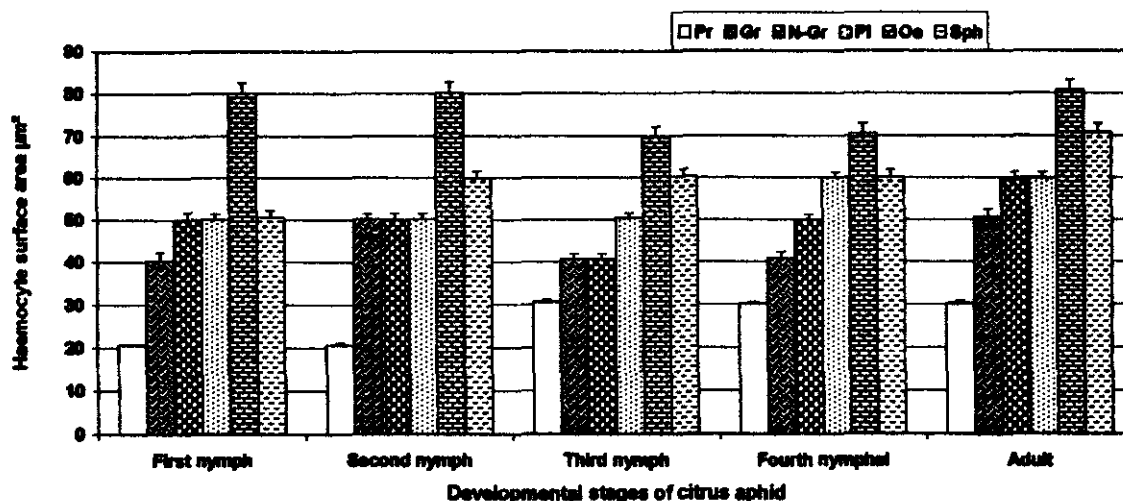


Figure (2) : Haemocytes surface area in different developmental stages of *Aphis citricola*

C-Effect of Neem extract on the adult stage of the citrus aphid :

The present results showed that, all the tested concentrations of Neem-Azal.T(0.1, 0.3,0.4%) showed obvious effects on the blood picture of the citrus aphid.

Table(4):summarizes the variation in DHC of the citrus aphid adult reared on citrus leaves treated with different concentrations of neem extract,it was found that all the tested concentrations significantly decreased the haemocyte counts of Pr, Gr and n-Gr .However, the application of neem extract caused a supreme effect on Oe and Pl counts.

The Pr count significant decreased to be 3 ± 0.1 cell/mm³ in the concentration 0.4%.The same result was observed in Gr ,n-Gr and Pl.The number of Gr decreased to be 5.1 ± 0.1 cell / mm³ in the 0.4% concentration .Also, the treatment decreased the haemocyte of n-Gr to be 3.3 ± 0.5 cell/mm³ in the concentration 0.4%. Although most of haemocyte types decreased but the number of Oe and Pl increased to be 10.7 ± 0.2 cell/mm³ and 16.2 ± 0.1 cell/mm³, respectively at the concentration of 0.4% and also, in all the tested concentrations.

Table (5) shows variations in the surface area of each haemocyte type of citrus aphid adult reared on different concentration of neem extract. The data revealed that no significant difference in the surface

area of Pr ,Gr, n-Gr and Sph among all the tested concentrations .The highest value of Pl surface area was recorded after the treatment with 0.4% neem extract(80 ± 0.4 µm²) followed by that of the concentration of 0.3% neem extract(70.8 ± 0.2 µm²). The same trend was observed in the Oe surface area .The maximum value of the Oe was obtained in the aphids treated with 0.4%neem extract (111.9 ± 0.6 µm²) followed by that of 0.3% neem extract(100 ± 0.7 µm²).

The observed increase of Oe number and surface area after the treatment could be attributed to the indirect hormonal effect of neem extract which may affect the blood picture .

Several authors demonstrated the influence of the azadirachtin on the hormonal control of moulting .

Schmultter (1990) reported that it interferes with some transmitters involved in the regulation of ecdysone biosynthesis or release leading to growth regulatory effect . Also, Karam, (1999) reported that the effect of Neem Azal .T on the morphogentic effects of the citrus mealy bugs was similar to the effect of jevenoides . Essawy & Idriss , (1990) demonstrated that the JH affects the haemocyte poietic organ increasing the release of Oenocytes; Gad, (1996) observed a positive correlation between the counts of Oe, Sph and corpora allata activity during the last larval instar of *Philosamia ricini*.

Table (4) : Effect of Neem extract on the haemocytes counts of *Aphis citricola*

The concentration of Neem- Azal-T%	Pr	Gr	nGr	Pl	Oe	Sph
Control	9.1±0.4a	11.1±0.2a	9.5±0.3a	12 ± 0.2b	6 ± 0.1b	5 ± 0.2a
0.1%	4.2±0.3b	7.6±0.5ab	5.4±0.1b	13.4±0.1ab	8.1 ± 0.1ab	4.3±0.2a
0.3%	3.2±0.2b	8 ± 0.2a	4.2±0.1b	15 ± 0.3a	9.4±0.1a	2.2±0.1b
0.4%	3 ± 0.1b	5±0.1b	3.3±0.5b	16.2±0.1a	10.7±0.2a	1.2±0.2b

Table (5): Effect of Neem extract on the haemocyte surface area of *Aphis citricola*.

The concentration of Neem- Azal-T%	Pr	Gr	nGr	Pl	Oe	Sph
Control	35.7±0.1a	51.1±0.2a	39.5±0.3a	56 ± 0.2b	80 ± 0.1b	79± 0.2a
0.1%	32.7±0.3a	52±0.1a	35.4±0.1a	70.4±0.1b	88.1± 0.1ab	84.3±0.2a
0.3%	31.2±0.2a	56 ± 0.2a	34.2±0.1a	70.8± 0.2b	100±0.7a	82.2±0.1a
0.4%	31 ± 0.2a	50.1±0.1a	33.3±0.5a	80±0.4a	111.9±0.6a	75.2±0.2a

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الملخص العربي

خلايا الدم في بعض حشرات رتبة متشابهة الأجنحة

عبد المجيد جاد

قسم الحشرات الاقتصادية - كلية الزراعة - جامعة الإسكندرية

تم في هذا البحث دراسة بعض أنواع خلايا الدم في أربعة عائلات مختلفة من رتبة متشابهة الأجنحة Homoptera Or.: هذه

العائلات هي:

Fam.: Diaspididae ممثلة بالحشرة القشرية المدرعة *Aspidiotus nerii*, Fam.: Margarodidae ممثلة بالبق الدقيقي المصري *Icerya aegyptiaca* (Douglas) و Fam.: Orthezidae ممثلة بالبق الدقيقي *Orthezia insignis* (Douglas) و Aphididae ممثلة بمن الموالح *Aphis citricola* وقد أوضحت النتائج وجود خمسة أنواع من خلايا الدم في جميع الحشرات موضع الدراسة هي: خلايا الدم الأولية Prohaemocytes وخلايا الدم البلازمية Plasmotocytes وخلايا الدم المحببة Granulocytes وخلايا الدم غير المحببة non - Granulocytes وخلايا الدم الليبينية Oenocytes وبالإضافة إلى هذه الأنواع فقد وجد نوع سادس في من الموالح فقط وهي خلايا الدم الكروية Spherule cells وقد قورن بين هذه الأنواع من الخلايا من حيث العدد ومساحة السطح في كل من الحشرات موضع الدراسة.

كذلك تم أيضاً دراسة التغيرات في أنواع خلايا الدم المختلفة وكذلك مساحة سطح الخلايا للأطوار المختلفة لمن الموالح كما تم دراسة تأثير مادة اللدغ - ت (5% زدراختين) بتركيزات 0.1، 0.2، 0.4، 0.6% على أعداد خلايا الدم المختلفة ومساحة سطح الخلايا وذلك في الطور الكامل لمن الموالح .