HAEMOGRAM CHANGES IN THE DESERT LOCUST *SCHISTOCERCA GREGARIA* (ORTHOPTERA: ACRIDIDAE) BY DIFFERENT EXTRACTS FROM THE WILD PLANT *FAGONIA BRUGUIERI* (ZYGOPLYLLACEAE).

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Abstract

The total (THC) and differential (DHC) haemolymph counts as well as the morphological and intracellular disorders in haemocytes of nymphs and adults of the desert locust *Schistocerca gregaria* were investigated after treatment of penultimate instar nymphs with *F. bruguieri* extracts. With no exception, THC in haemolymph of the early-aged nymphs increased, irrespective of the extract or concentration level. Reversely, THC in the haemolymph of nymphs at mid- and late-ages was remarkably dropped, regardless to the extract or concentration level. Concerning the adults both the methanolic and petroleum ether extracts exhibited an increasing effect on THC while the n-butanolic extract exerted a serious prohibitory effect.

Three types only of haemocytes were identified in last instar nymphs and newly emerged adults of the present insect species: plasmatocytes, granulocytes and coagulocytes. As a response to the methanolic extract, plasmatocyte counts significantly decreased in the early- and mid-aged nymphs, but remarkably increased in the late-aged ones. Also, plasmatocyte counts in haemolymph of adults were pronouncedly regressed. After treatment with petroleum ether extract, plasmatocyte counts were unexceptionally reduced in nymphs and adults. In addition, a drastic prohibiting action of n-butanolic extract was exerted on plasmatocyte counts in all nymphs, except late-aged ones, and in adults. After treatment with methanolic extract, increasing granulocyte counts were observed in haemolymph of the early- and late-aged nymphs , while mid-aged nymphs had slightly decreased counts or no change. After treatment with petroleum ether extract, considerable increments in the granulocyte counts were observed, irrespective of the stage, age, or concentration level. Reversely, granulocyte counts decreased as a response to the effect of n-butanolic extract in nymphs and adults. Varied effects of the *F. bruguieri* extracts were distinctively recorded in coagulocyte counts, depending on the nymphal age. In addition, remarkably increased counts were determined in haemolymph of adults.

Haemocytes of nymphs and adults of *S. gregaria* were morphologically affected by the methanolic and n-butanolic *F. bruguieri* extracts because some small darkened granulocytes and lysed granulocytes appeared. Also, lysed coagulocytes, with ruptured cell membrane and extruding cytoplasmic contents, were observed. In addition, cytoplasm of some haemocytes appeared with various vacuoles as well as a number of vacuoles appeared in the nuclei of some granulocytes .

KeyWords**:** *Schistocerca gregaria*, *Fagonia bruguieri*, haemolymph, total haemocyte count, differential haemocyte count, cytoplasm, nucleus, plasmatocyte, granulocyte, coagulocyte.

Introduction

Although the classification of insect larvae circulating haemocytes is a subject of controversy and the terminology used to designate each cellular type is often different from one species to another (Ribeiro and Brehelin, 2006), the most common types of haemocytes reported in the literature are: prohaemocytes, granular cells (=granulocytes) and oenocytoids. These haemocyte types have been described from species in diverse orders including Lepidoptera, Diptera, Orthoptera, Blattaria, Coleoptera, Hymenoptera, Hemiptera and Collembola (Ahmad, 1992; Fenoglio *et al*., 1993; Joshi and Lambdin, 1996; Hernandez *et al*., 1999; de Silva *et al*., 2000). However, the differences in the classification of insect haemocytes may arise from several causes such as differences in experimental treatments, observation of living haemocytes as opposed to fixed specimens, morphological changes of haemocytes after withdrawal, differing developmental stages in the test insects and some technical difficulties (George and Ambrose, 2004).

The particular haemocytes reported to be phagocytic varies among insect taxa, and in some cases discrepancies even exist in the literature among studies on the same species (Tojo *et al*., 2000). These differences are likely due in part to difficulties with identifying haemocytes in some insects. Each haemocyte type has a distinct cell morphology, and actin localized in the lamellar extensions of the cell. Azadirachtin or any other naturally originating pesticidal molecule may exert its activity by targeting actins. This finding was reported for some insect species such as *Drosophila melanogaster* and *Spodoptera litura* (Annuradha and Annadurai, 2008).

Haemocytes have been studied mostly in Lepidoptera, Hymenoptera, Coleoptera and Diptera (David and Peter, 1982; Osman *et al*., 1984; Gupta, 1985; Gurwattan *et al*., 1991; Miller and David, 2000; Ayaad *et al*., 2001; Rizk *et al*., 2001; Lavine and Strand, 2002; El-Sheikh, 2002; Zohry, 2006; Ribeiro and Brehelin, 2006; Annuradha and Anuadurai, 2008) as well as Heteroptera (Sanjayan *et al*., 1996) and Hemiptera (Georges and Ambrose, 2004). Little work has been carried out in orthopterans notably the acridids (Barakat *et al*., 2002), hence the present study aimed to characterize the haemocyte types in the desert locust *Schistocerca gregaria* and investigate the effects of some extracts from the wild plant *Fagonia bruguieri* on the total and differential haemocyte counts as well as their effects on the haemocyte morphology.

Materials and Methods

I) Experimental Insect:

The desert locust *Schistocerca gregaria* (Frosk.) (Orthoptera: Acrididae) was used as an experimental insect in the present study. The present culture was originated by a lot of gregarious nymphs obtained from Locust Research Division, Plant Protection Research Institute, Ministry of Agriculture, Doqqi, Giza. An electric bulb (100 watt) was adjusted in the rearing cages to maintain a continuous photoperiod of 12 L: 12 D in each cage as well as in order to maintain an ambient temperature of 32±2°C.

The insects were reared and handled under the crowded conditions outlined by Hunter–Jones (1961). Half hundred adults were placed in each cage for egg laying. The feces, dead locusts and food remains were removed daily before introducing the freshly food. Care was seriously taken to clean these cages at regular intervals and the sand was sterilized in drying oven (at 140°C for 24 hours) to avoid contamination with any pathogenic microorganisms. Fresh clean leaves of berseem *Medicago sativa*, in winter, and the leaves of leguminous plant *Sesbania aegyptiaca*, in summer, were used as a food for insects. On the other hand, the berseem leaves only were offered as food for insects during the experimental work.

II) Plant extracts:

Fagonia bruguieri var. *bruguieri* is a perennial wild herb distributed all deserts in Egypt but profusely spread in Sinai. It is, also, distributed in Arabia, Syria, Jordon, Iraq, Iran, Palestine, Pakistan, Afghanistan and North Africa. It systematically belongs to family Zygophyllaceae. The aerial parts of the plant (leaves, stems and flowers) were collected from the region of Santa Catherin (Sinai) during flowering stage, and were air-dried, powdered and kept in tightly closed amber coloured glass containers for protecting from light, at low temperature.

Dried and pulverized powder of *F. bruguieri* (2 kg) was exhaustively separately extracted with methanol (1.7 Lx3). The combined alcohol extracts were concentrated to 400 ml, diluted with 400 ml of water and the next successively extracted with petroleum ether (5x400 ml) was concentrated to dryness under reduced pressure giving (80 g), while n-butanol (5x400 ml) extracts were concentrated to dryness under reduced pressure giving (60 g).

III) Nymphal Treatments:

The used concentration levels of the methanolic extract were : 3.7, 7.5 % but of the petroleum ether extract and n-butanolic extract were: 15.0, 30.0% . The newly moulted 4^{th} (penultimate) nymphs of *S. gregaria* were fed on fresh leaves of *M.*

sativa after dipping in different concentration levels of each extract. After dipping for three minutes, the treated leaves were allowed to dry before offering to the nymphs. A day after treatment, all nymphs (treated and control) were provided with untreated food plant. Ten replicates (one nymph/replicate) were used for each concentration.

III) Haematological Investigation:

After treatment of the early penultimate instar nymphs with the plant extracts, the successfully moulted last instar nymphs of three ages (early, mid and late) as well as the newly emerged adults were undergone to the haematological investigation.

(1) Haemolymph Collection

For the determination of total or differential haemocyte counts, the haemolymph was collected from the last instar nymphs (of three physiological ages: early, mid and late) as well as from the newly emerged adults, after treatment the early penultimate instar nymphs. The haemolymph was obtained by non-heparinized capillary tube after amputation the hind coxa with fine scissors and gentle pressure on the thorax and abdomen. Three replicates were used and the haemolymph from two individuals was never mixed.

(2) Haemocyte counts :

For measuring the total haemocyte count (THC) The haemolymph was collected into thoma – white blood cell diluting pipette to the mark (0.5). Diluting solution (NaCl – 4.65 gm, KCl – 0.15 gm, $CaCl_2$ – 0.11 gm, Crystal violet – 0.05 gm and acetic acid – 1.25 ml / liter distilled water) was taken up to the mark (l l) on the pipette (dilution is 20 times). The first three drops were discharged to avoid errors. The mixture was dispended to the chamber of counting slide. After three minutes, the total numbers of cells recognized in 64 squares of the four corners were counted. If the cells clumped or uneven distributed, the preparation was discarded. The number of haemocytes per cubic millimeter was calculated according to the formula of Jones (1962) as follows:

$$\frac{\textit{Number of haemocyte counted per champer X dilution X depth factor}}{\textit{Number of 1 mm squares counted}}$$

Where: The depth factor is usually 10.

For estimating the Differential counts (DHCs) of different types of the haemocytes, stained haemolymph preparations were carried out, according to Arnold and Hinks (1979). The haemolymph was smeared on clean glass slides, allowed to dry for 1 – minute, and fixed for 2 – minutes with drops of absolute methyl alcohol. Fixed cells were stained with Giemsa's solution (diluted 1 : 20 in distilled water) for 20 minutes, washed several times with tap water, and dipped in distilled water. The stained smears were air – dried and mounted in DPX with slip cover. The haemocytes were viewed under light microscope at a magnification 10 X 40 = 400 and 100 cells per slide were examined. The cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus were used for the classification of haemocytes using the classification scheme of Barakat *et al*. (2002). The percentages of haemocyte types were calculated by the formula:

$$\frac{\textit{Number of each haemocyte type}}{\textit{Total number of haemocytes examined}} \; \textit{X 100}$$

3) Haemocyte Deformations:

For recording of the haemocyte deformities caused by the plant extracts, electromicrographs were obtained by using a light microscope provided with a camera at a magnification 10 X 40 = 400 .

V) Statistical Analysis Of Data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

Results

Two concentration levels of three extracts from the wild plant *Fagonia bruguieri* (7.5 and 3.7 ppm of methanolic extract and 30.0 and 15.0 ppm of petroleum ether extract or n-butanolic extract) were given to the penultimate instar nymphs of *Schistocerca gregaria* for investigating the effects on total haemocyte count (THC), differential haemocyte counts (DHCs) and the morphological disorders in haemocytes of the last instar nymphs and newly emerged adults.

A) Effects of *F. bruguieri* extracts on the total haemocyte population:

Data of Table (1) demonstrate different responses of THC to the *F. bruguieri* extracts. With no exception, THC in haemolymph of the early-aged nymphs increased, irrespective of the extract or concentration level. Treatment with the

higher concentration level of n-butanolic extract resulted in the most pronouncedly increased THC count (3516.7±202.1 cell/mm^3 compared to 2550.0±180.3 cell/mm^3 of controls). However, the exiguously inducing effect of *F. bruguieri* was detected at the higher concentration level of petroleum ether extract and lower concentration level of n-butanolic extract . Reversely, THC in the haemolymph of nymphs at the mid- and late-ages was remarkably dropped, regardless to the extract or concentration level. The most drastically regressing effect on THC was estimated for n-butanolic extract, at its higher concentration level, and could be expressed in the reduction %: 88.0. Concerning the newly emerged adults, the nymphal treatments with methanolic or petroleum ether extract resulted in a promotion in THC while the n-butanolic extract exhibited a detrimental prohibiting effect on it.

B) Effects of *F. bruguieri* on the differential haemocyte counts :

Three types only of haemocytes were identified in nymphs and adults of the present insect species: plasmatocytes (plas.), granulocytes (gran.) and coagulocytes (coag.). As clearly seen in Fig. (1), plas. count significantly decreased in the haemolymph of early- and mid-aged nymphs (the most sharply dropped count was measured as 21.7±1.5 at the higher concentration level of methanolic extract , compared to 27.0±2.0 of control congeners), but significantly increased ($p<0.005$) in the haemolymph of late-aged ones as a response to the methanolic extract. Also, plas. count in haemolymph of the newly emerged adults was remarkably regressed, especially at the higher concentration level (in 62.2% reduction , $p<0.001$).

Referring to data illustrated in the same figure, the early- and late-aged nymphs had haemolymph with increasing gran. counts (the highest stimulating effect was exhibited in the late-aged nymphs, at the higher concentration level of methanolic extract, in increasing % : of 79.6), while the mid-aged nymphs had slightly decreased gran. count (at the higher concentration level) or no changed gran. count (at the lower concentration level). With regard to the newly emerged adults, the gran. count was tremendously declined by the action of the present methanolic extract (41.7±2.3 and 29.0±1.7 at the higher and lower concentration levels, respectively, in comparison with 51.0±2.0 of control adults).

In respect to the coag. counts, data diagramed in the aforementioned figure exiguously show varied effect of methanolic extract depending on the nymphal age. The coag. counts were distinctively reduced at the two ends of nymphal instar (the

most drastically reducing effect was detected in 39.6% reduction, at the higher concentration level, in the late-aged nymphs), but enhanced at the mid age (43.3±0.6, $p<0.001$, at the higher concentration level , compared to 37.0±1.0 of controls). In addition, elaborately increased coag. counts were determined in the newly emerged adults, regardless to the concentration level.

Results of DHCs after treatment of the penultimate instar nymphs with petroleum ether extract were statistically analyzed and illustrated in Fig. (2). Unexceptionally, plas. counts were detrimentally reduced in nymphs and adults (the highest reduction in plas. counts in adults was calculated in 83.9%, at the higher concentration level) , while gran. counts markedly increased, irrespective of the developmental stage, age, or concentration level (the largest increment was measured as 52.0±2.6 , at the lower concentration level , in the late-aged nymphs , compared to 28.0±1.0% of control nymphs). Dealing with the coag. counts, the previously mentioned figure obviously show considerably decreasing counts in the late-aged nymphs (52.7±1.5 and 43.7±1.5% at 30.0 and 15.0 ppm, vs. 66.7±2.3% of control nymphs), but significantly increasing counts in the nymphs of other ages as well as in the newly emerged adults. The most remarkably increased coag. counts in adults were measured as: 70.4 and 64.2%, at the higher and lower concentration levels, respectively.

As shown in Fig. (3), a serious prohibiting action of n-butanolic extract of *F. bruguieri* on plas. counts in haemolymph of all nymphs , except the late-aged ones, as well as of the newly emerged adults was recorded. The plas. counts in only the late-aged nymphs were induced by n-butanolic extract (increasing %s: 94.3 and 18.9 , at the higher and lower concentration levels, respectively). Similar promoting effect of the present *F. bruguieri* extract was explored on the gran. counts allover the nymphal instar. Otherwise, haemolymph of adults appeared with sharply dropped gran. counts (30.0±1.7 and 43.0±1.0, at the higher and lower concentration levels, respectively , in comparison with 51.0±2.0 of control congeners). Also, the coag. counts were promoted by n-butanolic extract to increase in haemolymph of all nymphs, except the late-aged ones, and adults. Only the late-aged nymphs had decreasing coag. counts (reduction %s: 9.0 and 6.6, at the higher and lower concentration levels, respectively).

Table (1): Change percentages of the total hemocyte count in diffferent developmental stages of *Schistocerca gregaria* after treatment of the early penultimate instar nymphs with *Fagonia bruguieri* extracts

Extract	Conc. ppm (x 10^4)	Last instar nymphs			Newly emerged adults
		Early-aged	Mid-aged	Late-aged	
Methanolic	7.5	+1.3	-35.9	-35.1	+35.4
	3.7	+2.6	-26.3	-3.4	+3.1
Petroleum ether	30	+25.5	-27.1	-35.3	+36.5
	15	+5.9	-1.9	-28.5	+31.8
n-butanolic	30	+37.9	-88.0	-18.6	-34.4
	15	+19.6	-68.6	-20.9	-22.9

Conc.: concentration level.

Table (2): Change percentages in the differential hemocyte counts of different evelopmental stages of *S. gregaria* after treatment of the early penultimate instar nymphs with methanolic extract from *F. bruguieri*.

Developmental age		Conc. ppm (x 10^4)	% of defferential hemocyte count		
			Plasmatocytes	Granulocytes	Coagulocytes
Last instar nymphs	Early	7.5	-12.5	+76.0	-34.5
		3.7	-9.0	+21.5	-6.0
	Mid	7.5	-19.6	-2.8	+17.0
		3.7	-3.7	0.0	+2.7
	Late	7.5	+64.2	+79.6	-39.6
		3.7	+13.2	+73.9	-32.1
Newly emerged adult		7.5	-62.2	-18.2	+91.2
		3.7	-10.0	-43.1	+93.5

Conc.: see footnote of Table (1).

Table (3): Change percentages in the differential hemocyte counts of different developmental stages of *S. gregaria* after treatment of the early penultimate instar nymphs with petrolium extract from *F. bruguieri*.

Developmental age	Conc. ppm (x 10^4)	% of defferential hemocyte count		
		Plasmatocytes	Granulocytes	Coagulocytes

Last instar nymphs	Early	30	-62.9	+71.7	+15.9
		15	-76.9	+84.5	+21.7
	Mid	30	-76.7	-1.9	+57.6
		15	-61.9	+11.1	+34.3
	Late	30	-62.3	+61.8	-21.0
		15	-18.9	+85.7	-34.5
Newly emerged adult		30	-83.9	+2.0	+70.4
		15	-78.3	+2.5	+64.2

Conc.: see footnote of Table (1).

Table (4): Change percentages in the differential hemocyte counts of different developmental stages of *S. gregaria* after treatment of the early penultimate instar nymphs with n-butanolic extract from *F. bruguieri*.

Developmental age		Conc. ppm (x 10^4)	% of defferential hemocyte count		
			Plasmatocytes	Granulocytes	Coagulocytes
Last instar nymphs	Early	30	-83.3	+48.9	+48.6
		15	-88.6	+79.0	+36.0
	Mid	30	-90.0	+49.2	+18.1
		15	-82.6	+21.4	+39.7
	Late	30	+94.3	+3.6	-9.0
		15	+18.9	+9.6	-6.6
Newly emerged adult		30	-87.0	-41.2	+157.7
		15	-90.0	-33.3	+148.8

Conc.: see footnote of Table (1).

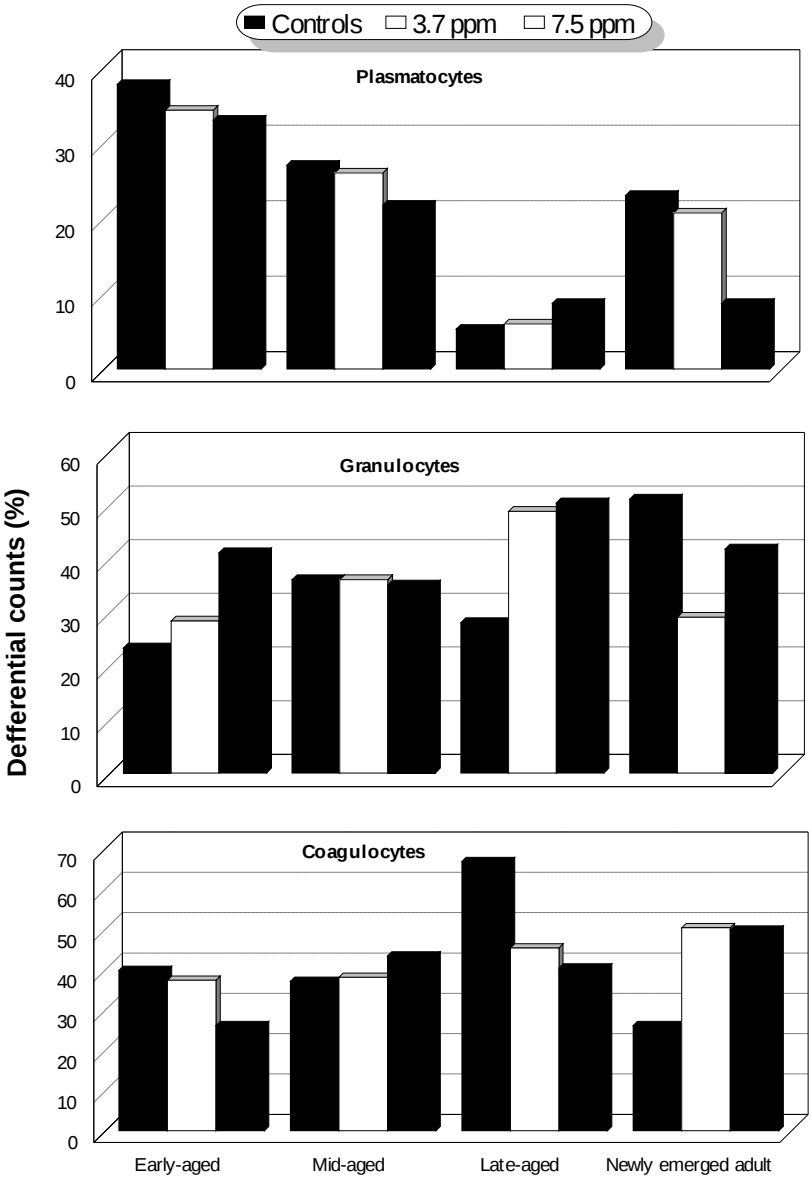


Fig. (1): Effect of the methanolic extract from *Fagonia bruguieri* on the differential haemocyte counts in different developmental stages of *Schistocerca gregaria*.

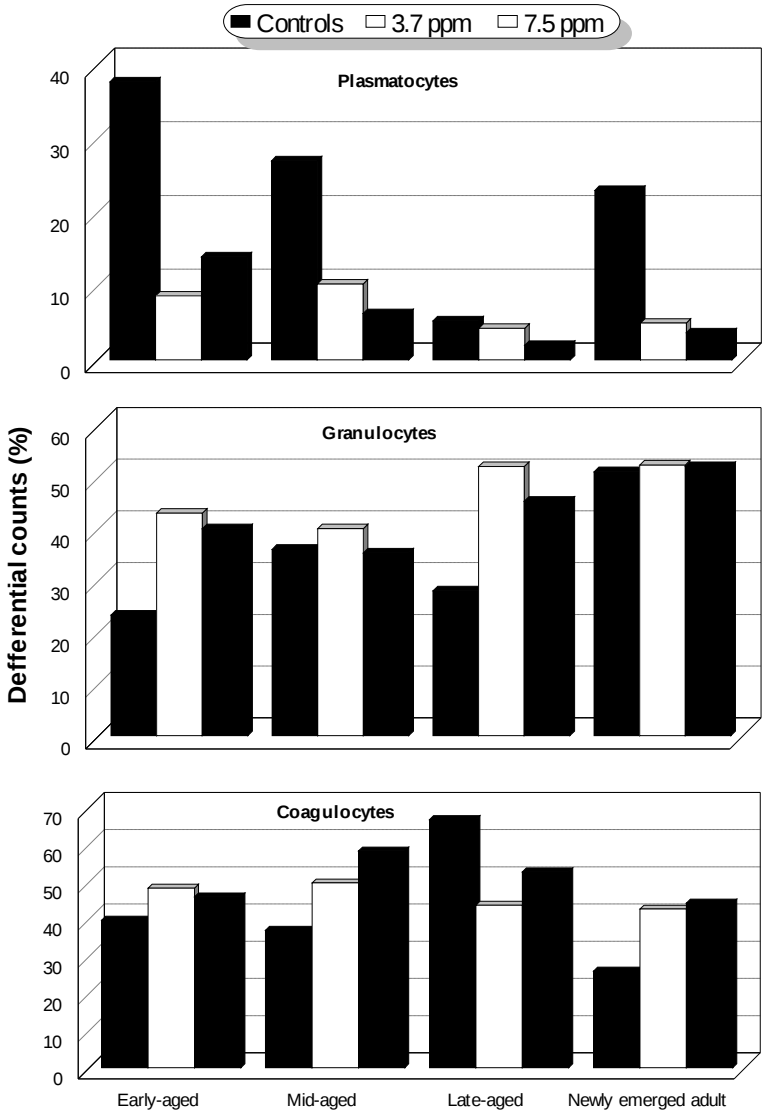


Fig. (2): Effect of the petroleum ether extract from *Fagonia bruguieri* on the differential haemocyte counts in different developmental stages of *Schistocerca gregaria* .

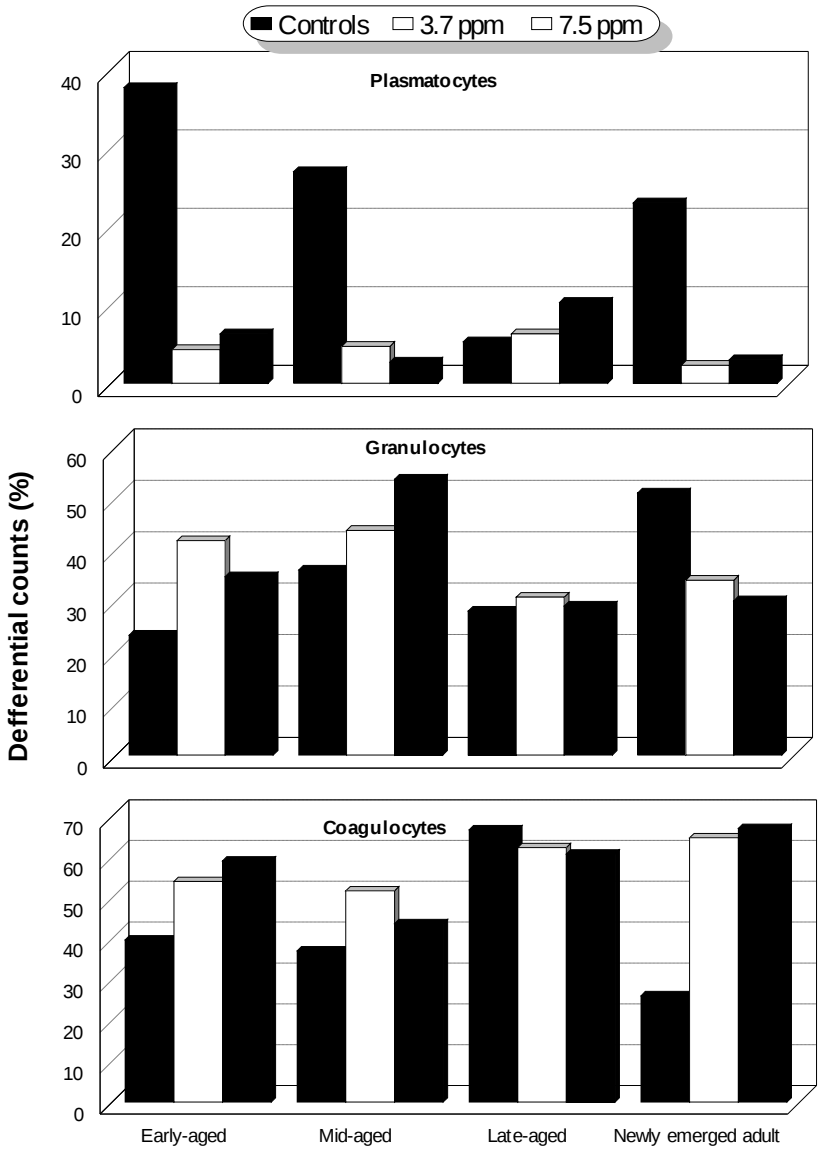


Fig. (3): Effect of the n-butanol extract from *Fagonia bruguieri* on the differential haemocyte counts in different developmental stages of *Schistocerca gregaria* .

C) Characterization of normal haemocytes :

As previously mentioned, only three haemocyte types were recognized in the nymphs and adults of *S. gregaria* in the present study: plasmatocytes, granulocytes (granular cells) and coagulocytes. As clearly shown in Plate (1), the normal (control) plasmatocytes can be described as oval- or spherical-shaped cells containing basophilic cytoplasm (faintly stained). The large rounded nucleus is centric or ecentric and occupied 40-55% of the cell volume, as well as contained scattered chromatin masses. Cytoplasm enclosed a moderate amount of rough endoplasmc reticulum. The normal (control) granulocytes appeared as spherical- or oval-shaped cells with basophilic cytoplasm (deeply stained) which contained large number od acidophilic granules. Also, the granulocyte had an ecentric nucleus occupying 58.3-66,6% of the cell volume. Some granulocytes contained fillopodia. The coagulocytes appeared as spherical or oval-shaped cells with pale hyaline cytoplasm containing scattered granules.

D) Descriptive disorders of haemocytes and their inclusions:

As obviously seen in Plate (2), haemocytes of nymphs and adults of *S. gregaria* were morphologically affected by the *F. bruguieri* extracts. Nymphal treatment with n-butanolic extract resulted in the appearance of some small darkened granulocytes as well as lysed granulocytes. Also, some coagulocytes were lysed and appeared with ruptured cell membrane and extruding cytoplasmic contents after treatments with methanolic and n-butanolic extracts.

Photomicrographs in Plate (3) clearly show the formation of numerous vacuoles in some plasmatocytes after nymphal treatments with methanolic or n-butanolic extract. Also, n-butanolic extract dangerously influenced some granulocytes because various vacoules appeared in their nuclei and cytoplasm. In addition, little vacoules were formed in the cytoplasm of some coagulocytes.

Discussion

1) Recognition of Haemocytes types in S. gregaria:

Gupta (1979) recognized 7 main haemocyte types in various insect orders namely: prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipohaemocytes, coagulocytes and oenocytoids but he merged the category coagulocytes with that of granulocytes (Gupta, 1994). Five types of haemocytes (prohaemocytes, plasmatocytes, granulocytes, adipohaemocytes and spherulocytes) were recognized by Gupta (1979) in Hemiptera and then confirmed by Sanjayan *et al*. (1996) in the hemipteran *Spilostethus hospes*. On the other hand, only three

principal haemocyte types (prohaemocytes, plasmatocytes and granulocytes) were observed in the hemipteran *Rhyncocoris kumarii* (George, 1996; George and Ambrose, 2004). The haemocytes were reduced to two only in *Drosophila* species: lamellocytes and crystal cells (Ribeiro and Brehelin, 2006). The most common haemocyte types, prohaemocytes, granulocytes and oenocytoids, have been described from species in diverse orders including: Lepidoptera, Diptera, Orthoptera, Blattaria, Coleoptera, Hymenoptera, Hemiptera and Collembola (Ahmad, 1988, 1992; Joshi and Lambdin, 1996; Hernandez *et al*., 1999; de Silva *et al*., 2000).

In the present study, only three types of haemocytes were distinguished in nymphs and adults of the desert locust, *S. gregaria* : plasmatocytes, granulocytes and coagulocytes. None of the individual methods for studying the various morphological types of haemocytes was entirely satisfactory for all types of cells within a given insect (George, 1996). Differences in techniques have often led and are likely to continue to lead to conflicting view until systematic comparative studies are forthcoming. Various techniques often yield profound different information about types, number, distribution and functions of haemocytes (George and Ambrose, 2004). Also, the haemocyte classification, types and morphology, are often affected by some factors affecting the haemolymph physical properties or biochemical composition: diet, temperature, disease (Carrel *et al*., 1990), physiological condition of the insect (Chapman, 1998) and the developmental stages (Jones, 1977). Classification of the insect circulating haemocytes is a subject of controversy, and the terminology used to designate each cellular type is often different from one species to another. However, a survey of the literature on insect haemocytes suggests that there are resemblances for most of the cell types and functions, in different insect species (Ribeiro and Brehelin, 2006).

2) Disturbed total haemocyte population in S. gregaria:

Because the cellular defense, in the insect immune system, refers to haemocyte-mediated immune responses like phagocytosis, nodulation and encapsulation (Strand and Pech, 1995; Schmidt *et al*., 2001), the present study comprised the total haemocyte count (THC) in the nymphs and adults of *S. gregaria*. With no exception, THC in haemolymph of the early-aged nymphs increased, irrespective of the extract or concentration level. Reversely, THC in the haemolymph of nymphs at mid- and late-ages was remarkably dropped, regardless to the extract or concentration level. Concerning the adults both the methanolic and petroleum ether extracts exhibited an increasing effect on THC while the n-butanolic extract exerted a serious prohibitory effect on it.

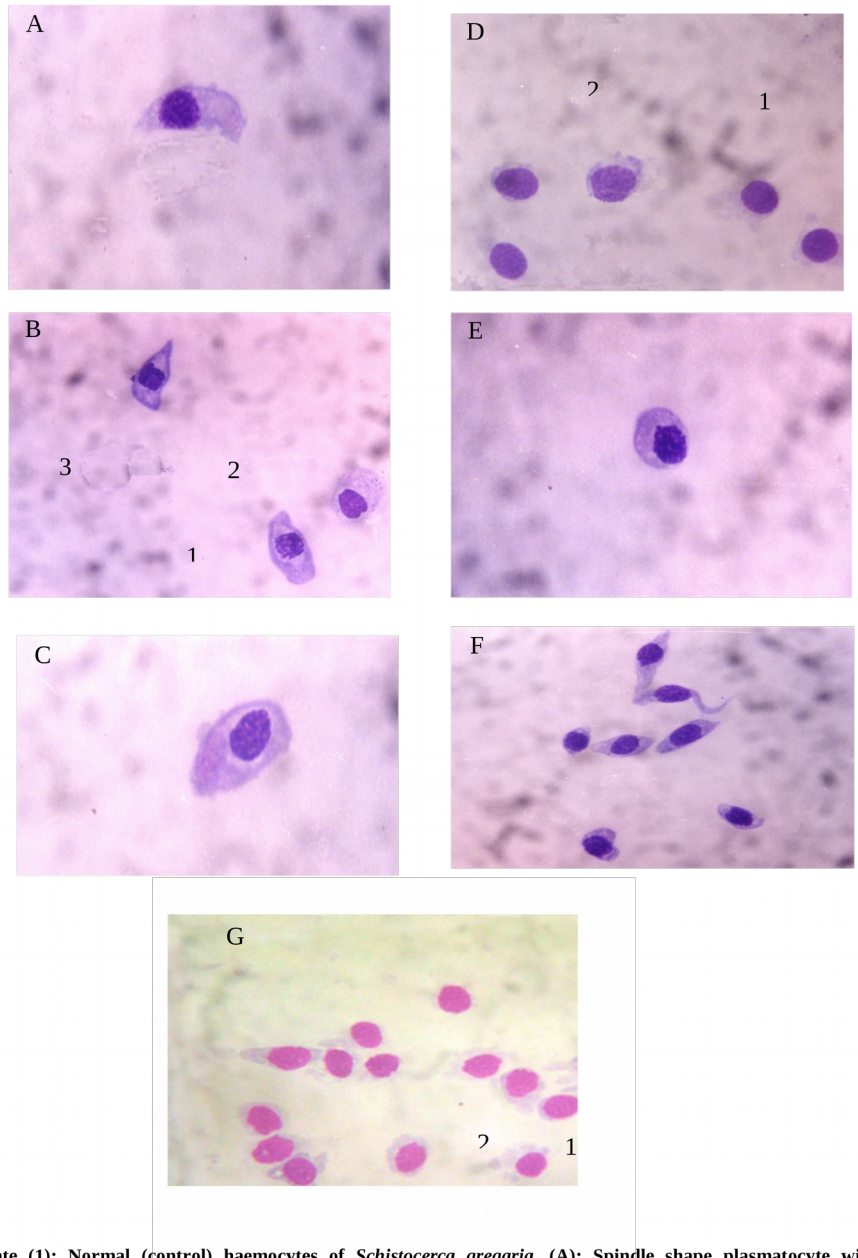
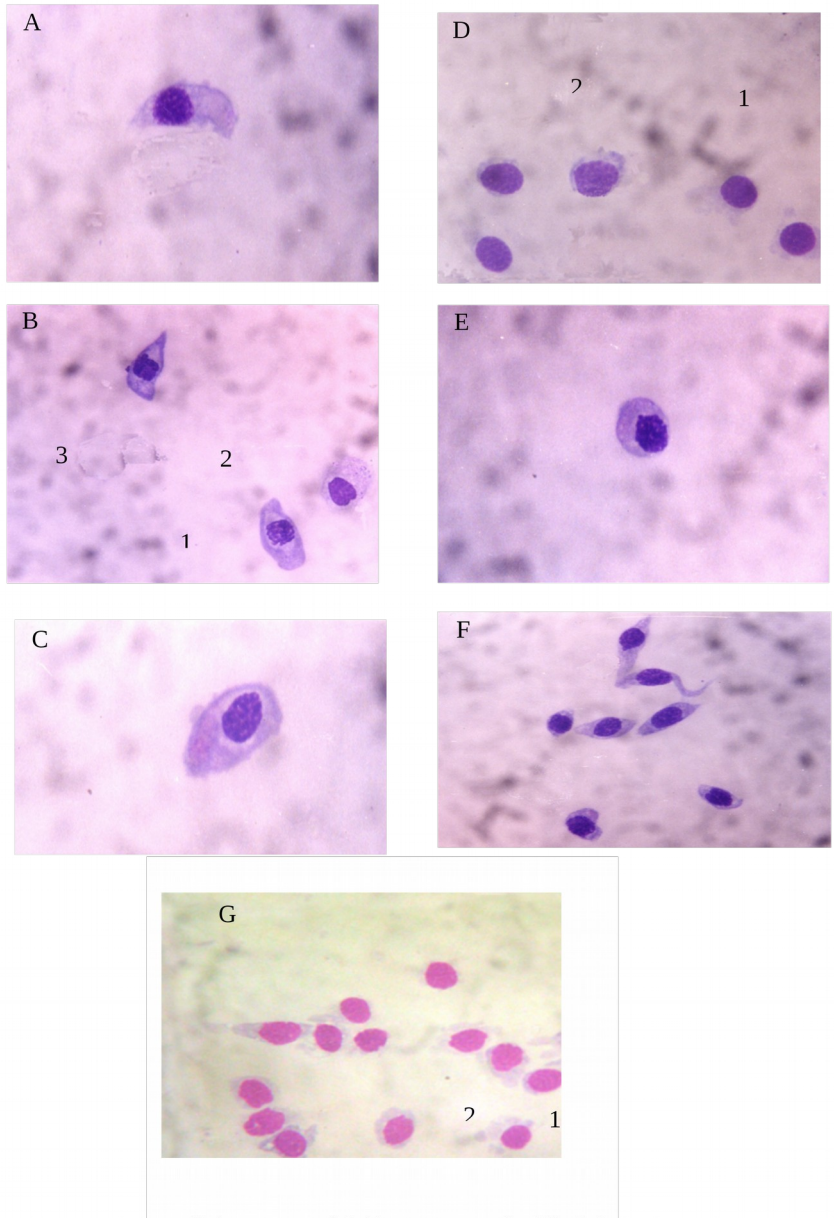


Plate (1): Normal (control) haemocytes of *Schistocerca gregaria*. (A): Spindle shape plasmatocyte with eccentric nucleous. (B 1): Oval shaped plasmatocyte with centric nucleous. (B 2): Round shaped plasmatocyte with eccentric nucleous. (B 3): Oval shaped plasmatocyte with centric nucleous. (C): Oval shaped plasmatocyte with eccentric nucleous. (D 1): Round shaped granulocyte. (D 2): Oval shaped granulocyte. (E): Round shaped granulocyte with clear eccentric nucleous and without granules. (F): Granulocyte with fillopodia. (G 1): Round shaped coagulocyte. (G 2): Oval shaped coagulocyte.



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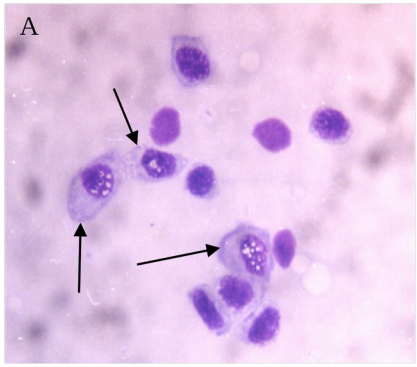

Plate (3): Vacuole formation after treatment of *Schistocerca gregaria* with *Fagonia bruguieri* extracts. (A): Vacuoles in the nucleus of plasmatocyte. (B): granulocyte with a vacuole. (C): Lysis of granulocyte with vacuoles. (D): coagulocyte with vacuoles.

To a great extent, the increasing THC in the haemolymph at certain ages of nymphs and adults as a response to the action of some or all *F. bruguieri* extracts agrees with those reported results in several insects after treatment with different chemical compounds, pathogens or botanicals. Increasing THC was reported in *Manduca sexta* by bacterial injection (David and Peter, 1982), *Spodoptera littoralis* after larval treatment with the chitin synthesis inhibitor Dimilin (Osman *et al*., 1984), *Orgyia leucostigma* by yeast injection (Guzo and Stoltz, 1987), *Acanthaspis pedestris* after treatments with the insecticides monocrotophos, dimethoate and methylparathion (Ambrose and George, 1996), *Parasarcophaga surcoufi* after treatment with azadirachtin (Azt), *S. littoralis* after treatments with Azt or the neem preparation Margosan-0 (Rizk *et al*., 2002), *Agrotis ipsilon* after treatment with acetonic extract from *Melia azadirach* (El-Shiekh, 2002), *S. gregaria* by *Bacillus thuringiensis* injection (Barakat *et al*., 2002), *R. kumarii* after treatments with the

insecticides methylparation, monocrotophos, dimethoate and quinalphos (George and Ambrose, 2004) and *S. littoralis* after treatments with the chitin synthesis inhibitors flufenuxuron and chlorfluazuron (Zohry, 2006).

Otherwise, the decreasing THC in the haemolymph of nymphs of some ages after treatment with *F. bruguieri* extracts, or of the newly emerged adults after nymphal treatment with n-butanolic extract, is in accordance with those results reported for *Locusta migratoria* after injection with *B. thuringiensis* (Hoffmann *et al*., 1974), *Galleria mellonella* after injection with certain species of bacteria (Chain and Anderson, 1982), *S. gregaria* after injection with the fungal toxin laminarin (Abou El-Magd, 1992), *Cyrtacanthacris tatarica* after injection with Azt (Peter and Ananthakrishan,1995), *R. kumarii* after treatment with the insecticide endosulfan (George and Ambrose, 2004) and for *S. littoralis* after treatment with flufenoxuron (Zohry, 2006).

The increasing THC in nymphs or adults of *S. gregaria* as a response to the action of *F. bruguieri* extracts, in the present study, may be attributed to the defensive action of haemocyte detoxification of these extracts (George, 1996) or to decreased blood volume (Feir, 1979). Also, the increase of THCs may be due to the release of sessile haemocytes and the activation of mitotic division of the haemocytes, because many insect species possess populations of sessile haemocytes (Ratcliffe and George, 1976) which might be activated in response of injection or treatment with some insecticides or plant extracts. Moreover, the increase in THC could be considered as an immune response against pathogen or any foreign body such as the introduced plant extracts (Chu *et al*., 1993; Anderson *et al*., 1995; Ordas *et al*., 2000). It may be important to mention that the brain endocrine complex is involved in haemocyte accumulation following some initial stimulus (Nappi, 1974). Jones (1967) suggested that ecdysteroids can regulate the number of haemocytes. Also, the prothoracic glands stimulate the production of haemocytes in *L. migratoria* last instar nymphs (Hoffmann, 1970). Because the neem extract, Azt, could be responsible factor for the modification of haemolymph ecdysteroid titers (Redfern *et al*., 1982; Barnby and Klocke, 1990), *F. bruguieri* extracts, in the present study on *S. gregaria*, may acted as an antiecdysteroid materials promoting to the increasing THC.

On the other hand, the decreasing THC in the haemolymph of certain nymphal ages or after treatment of certain *F. bruguieri* extracts, in the present study, may be correlated with the decrease of some types of haemocytes and may be due to the involvement of haemocytes in phagocytosis and nodule formation, which always accompanied with the death of defensive haemocytes or may be due to the action of released toxins (Gagen and Ratcliffe, 1976 & Zohry, 2006).

3) Changed Differential Haemocyte Population in S. gregaria:

The primary functions of haemocytes are: coagulation, phagocytosis, encapsulation, detoxification and storage and distribution of nutritive materials (Sanjayan *et al*., 1996). Phagocytosis is an important innate immune response against microbial infections (Garcia-Garcia and Rosales, 2002; Hart *et al*., 2004) and effective mechanism to eliminate apoptotic cells demonstrated that the insect haemocytes have distinct functions in phagocytosis of foreign particles and self dead cells (Hart *et al*., 2004; Zhou *et al*., 2004). Ling and Yu (2006). Also, the increasing counts of some haemocyte types and decreasing counts of others may be due to the transformation of some types into others for achieving the phagocytic function or other tasks in the battle against the biotic targets like bacteria, yeast and apoptic bodies and abiotic materials such as particles of Indian ink (Hernandez *et al*., 1999; de Silva *et al*., 2000) or chemical compounds of plant extracts.

Hence the present study included the investigation of the effects of *F. bruguieri* extracts on the differential haemocyte counts (DHC) in *S. gregaria.* **A**s a response to the methanolic extract, plasmatocyte counts significantly decreased in the early- and mid-aged nymphs, but significantly increased in the late-aged ones. Also, plasmatocyte counts in haemolymph of adults were remarkably regressed. After treatment with petroleum ether extract, plasmatocyte counts were unexceptionally reduced in nymphs and adults. In addition, a drastic prohibiting action of n-butanolic extract was exerted on plasmatocyte counts in all nymphs, except late-aged ones, and in adults. **A**fter treatment with methanolic extract, increasing granulocyte counts were observed in haemolymph of the early- and late-aged nymphs , while mid-aged nymphs had slightly decreased counts or no changed count. After treatment with petroleum ether extract, considerable increments in the granulocyte counts were observed, irrespective of the stage, age, or concentration level. Reversely, granulocyte counts decreased as a response to the effect of n-butanolic extract in

nymphs and adults. **V**aried effects of the methanolic extract were distinctively recorded in coagulocyte counts, depending on the nymphal age. In addition, remarkably increased counts were determined in haemolymph of adults. As a response to petroleum ether extract, significantly decreasing coagulocyte counts in the late-aged nymphs were estimated, but pronouncedly increasing counts were measured in nymphs of other ages and in the adults. The coagulocytes were induced by n-butanolic extract because increasing counts were determined in the haemolymph of all nymphs, except the late-aged ones and adults.

As clearly shown, an inhibitory effect of *F. bruguieri* extracts was generally detected on the plasmatocyte count in the nymphs and adults of *S. gregaria*. In contrast, a major promoting effect of the present extracts on both the granulocytes and coagulocyte counts was recorded, with few exceptions. However, the decrease of plasmatocyte counts in the present study might be due to the transformation of these haemocytes into other haemocyte types (Beaulation and Monpeyssin, 1976; George, 1996). In other words, the decreasing plasmatocyte counts could be attriuted to the fact that the plasmatocytes are highly polymorphic and might be converted into other types of haemocytes (Gupta and Sutherland, 1966).

The n-butanolic extract from *F. bruguieri* exceptionally induced the plasmatocyte count in the late-aged nymphs and newly emerged adults of S. gregaria, in the present study. The increasing plasmatocyte count was reported for some different insects by some other chemicals or botanicals. Increasing plasmatocyte counts were recorded in the fourth instar larvae of *S. littoralis* after treatment with Margosan-0 (Rizk *et al*., 2002), in *A. ipsilon* larvae after treatment with *M. azedarach* extract (Abou El-Ghar *et al*., 1995), in *S. littoralis* last instar larvae after treatment of the insecticide flufenoxuron (Zohry, 2006). However, such increase in plasmatocyte counts may be due to their principal sharing in the phagocytosis because they are the most important phagocytic cells in the haemolymph (Jones, 1962) and are implicated also in the encapsulation of necrotic tissues (Essawy, 1990) as well as the detoxification of the compounds contained in the n-butanolic extract from *F. bruguieri*. In addition, this increase could be attributed to the resting plasmatocytes to enter the circulating haemocytes and the rapid transformation of the prohaemocytes (stem cells) into plasmatocytes (Osman *et al*., 1984).

With regard to the granulocyte counts, in the present study, both methanolic and petroleum ether extracts from *F. bruguieri* induced to increasing cell population in the nymphs and adults of *S. gregaria* but n-butanolic extract exhibited a reverse effect in all developmental stages. These results agree with those reported results by Rizk *et al.* (2002) for *S. littoralis* after treatment with Margosan-0 and George and Ambrose (2004) for *R. kumarii* after treatment with some insecticides. However, the increasing count of granulocytes may be explained by the transformation of some haemocytes into granulocytes (Gupta and Sutherland, 1966) which reveals their role in the detoxification of the toxic compounds in the present plant extracts (Jose and Mortin, 1989; Kurihara *et al*., 1992; George and Ambrose, 2004).

Moreover, the inhibitory effect of n-butanolic extract from *F. bruguieri* on the granulocyte count in nymphs and adults of *S. gregaria* , in the present study, come to an agreement with those results obtained for caterpillars of *Trichoplus* spp. after infection with *Nosema* (Laigo and Paschke, 1966), *Rhodnius prolixus* nymphs after wounding (Lia-Fook, 1968), *M. sexta* caterpillars after injection with two species of bacteria (Horohov and Dunn, 1982), *P. surcoufi* larvae after treatment with Azt (Ayaad *et al*., 2001), *G. mellonella* larvae after injection with *Bacillus cereus* (Barakat, 1997), *S. gregaria* nymphs after injection with *B. thuringiensis* (Barakat *et al*., 2002), and for *S. littoralis* larvae after treatment with flufenoxuron (Zohry, 2006). Such decreasing population of granulocytes, in the present study, may be due to the use of them in phagocytosis (Barakat *et al*., 2002).

In connection with the differential coagulocyte population in the present study, all *F. bruguieri* extracts stimulated the count of these haemocytes in nymphs and adults of *S. gregaria*, with an exception of n-butanolic extract which prohibited them in adults only. Unfortunately, no rational interpretation of this increasing coagulocyte population as a response to the *F. bruguieri* extracts, is available rightnow because this type of haemocytes is scarcely mentioned in the literature but it may be attributed to the role of these haemocytes in phagocytosis (Brehelin and Hoffmann, 1980).

4) Qualitative haemocyte profile in S. gregaria:

Changed characterization of haemocytes was based on: changes in the plasma membrane (erosion and extrusion of their cytoplasmic contents), vacoulization and degeneration of the cytoplasm, nuclear changes (pycnosis, karyorrhexis, granulosis

and division of nuclei) were reported as pathological features of insect haemocytes by the action of insect growth regulators, chitin synthesis inhibitors or plant extracts in the larvae of *Pieris rapae* (Miselyunene, 1976), larvae of *Plodia interpunctella* (El-Kattan, 1995), nymphs of *S. gregaria* (Barakat *et al*., 2002) and larvae of *S. littoralis* (Zohry, 2006).

Haemocytes of the nymphs and adults of *S. gregaria*, in the present study, were morphologically affected by both the methanolic and n-butanolic extracts from *F. bruguieri* because some small darkened granulocytes and lysed granulocytes appeared. Also, lysed coagulocytes, with ruptured cell membrane and extruding cytoplasmic contents, were observed. These morphological disorders of some haemocytes may be attributed to the action of the present plant extracts on the 'actin' which localized in the lamellar extensions of the cells as interpreted for *Drosophila melanogaster*, *S. litura* and *Plutella xylostella* by Anunradha and Annadurai (2008) who concluded that Azt or any naturally originating pesticidal molecule may exert its activity by targeting actins.

A number of dangerous intracellular changes was observed in the present study owing to the effect of *F. bruguieri* extracts. Some plasmatocytes, granulocytes and coagulocytes appeared with various vacuoles in the cytoplasm. Moreover, some granulocyte nuclei appeared with vacuoles. The formation of similar vacuoles were reported in the cytoplasm of plasmatocytes of *S. littoralis* after treatment of larvae with Margosan-0 (Rizk, 1991). Not the formation of cytoplasmic vacuoles, but some other cytoplasmic components of granulocytes were reported in the last instar larvae of *P. surcoufi* after treatment with Azt (Ayaad *et al*., 2001). The exact mode of action of the present plant extracts on the haemocyte constituents is unfortunately available rightnow. Further cytological and ultrastructural investigations should be needed to disclose or explicate such serious intracellular disorders.

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التغيرات الحاصلة في هيموجرام الجراد الصحراوي *شيستوسركا جريجاريا* (مستقيمات الأجنحة: الجراديات) بفعل مستخلصات مختلفة من النبات البري *فاجونيا بروجيري* (زيجوفيلليسي).

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سجّل البحث الحالي تأثيرات بعض مستخلصات نبات "خشيات" البري: *فاجونيا بروجيري* ، في التعداد الإجمالي لخلايا (كريات) الهيموليمف ، والتعدادات التمييزية لها، وكذلك الأضرار الحاصلة في الشكل الظاهري للخلايا ومحتوياتها الداخلية، وذلك في حوريات الدور الأخير واليافعات حديثة البزوغ للجراد الصحراوي *شيستوسركا جريجاريا*. تزايد التعداد الإجمالي لخلايا الهيموليمف في الحوريات حديثة العمر، بلا استثناء، بصرف النظر عن نوع المستخلص أو مستوى تركيزه. وعلى العكس، هبط التعداد الإجمالي لخلايا الهيموليمف هبوطا حادا في الحوريات متوسطة العمر والحوريات متأخرة العمر، بصرف النظر عن نوع المستخلص أو مستوى تركيزه. وفيما يتعلق باليافعات، فلقد أبدى كل من المستخلص الميثانولي ومستخلص الإثير البترولي تأثيرا حافزا في التعداد الإجمالي لخلايا الهيموليمف ، بينما أثّر المستخلص البيوتانولي تأثيرا تثبيطيا خطيرا فيه.

في الدراسة الحالية تمّ التعرّف على ثلاثة أنماط فقط من خلايا هيموليمف حوريات ويافعات الجراد الصحراوي، في هذه الدراسة : الخلايا الجبلّية، والخلايا المحبّبة، وخلايا التخثر. **الخلايا الجبلية**: استجابة لتأثير المستخلص الميثانولي تنازل تعداد هذه الخلايا تنازلا ملحوظا في هيموليمف الحوريات حديثة العمر والحوريات متوسطة العمر، لكنه تصاعد تصاعدا واضحا في هيموليمف الحوريات متأخرة العمر. وإضافة إلى هذا، تراجع تعداد هذه الخلايا في هيموليمف اليافعات تراجعا معتبرا. واستجابة لتأثير مستخلص الإثير البترولي أختزل تعداد هذه الخلايا – بلا استثناء – في كل من الحوريات واليافعات. وأما المستخلص البيوتانولي ، فقد بذل فعلا اختزاليا شديدا في تعداد هذه الخلايا في هيموليمف جميع الحوريات – فيما عدا الحوريات متأخرة العمر – وكذا في اليافعات. **الخلايا المحببة**: بعد المعاملة بالمستخلص الميثانولي، تزايد تعداد هذه الخلايا في هيموليمف كل من الحوريات حديثة العمر والحوريات متأخرة العمر. وفي اليافعات، فقد تناقص تعداد هذه الخلايا تناقصا كبيرا. وأما مستخلص الإثير البترولي ، فقد شجع على تزايد تعداد هذه الخلايا تزايدا ملحوظا، بصرف النظر عن الطور أو العمر

أو مستوى تركيز المستخلص. كما تم الكشف عن تأثير تحفيزي للمستخلص البيوتانولي في تعداد هذه الخلايا بهيموليمف الحوريات من كافة الأعمار، ولكنه هبط هبوطا حادا في هيموليمف اليافعات. **خلايا التخثر**: أبدت مستخلصات النبات الحالي تأثيرات متباينة في تعداد هذه الخلايا، وذلك بحسب عمر الحوريات. كما تزايد تعدادها تزايدا كبيرا في هيموليمف اليافعات.

سجلت الدراسة الحالية، أيضا، عددا من التغيرات الشكلية لخلايا الهيموليمف في حوريات ويافعات الجراد الصحراوي، وذلك بفعل كل من المستخلص الميثانولي والمستخلص البيوتانولي ، وبدا تأثر خلايا الهيموليمف واضحا في ظهور بعض الخلايا المحببة بلون داكن وبأحجام صغيرة، عن الخلايا المحببة العادية، كما تحلل بعض الخلايا المحببة الأخرى. وكذلك، تحلل بعض خلايا التخثر التي ظهرت بأغشية متمزقة ومحتويات سيتوبلازمية بارزة إلى الخارج. كما ظهرت فجوات متنوعة في سيتوبلازم كافة خلايا الهيموليمف، إضافة إلى ظهور عدد من الفجوات في أنوية بعض الخلايا المحببة.