Effect of Nomolt on Differential and Total Haemocytes in the Desert Locust *Schistocerca gregaria* Forskal (Orthoptera:Acrididae)

Salam S. Teleb

Zoology Department, Faculty of Science, Zagazig University, Egypt
salamteleb@gmail.com

Abstract: The insecticidal action of Nomolt at various dosages against *Schistocerca gregaria* was employed for its effects on total haemocyte count (THC)and differential haemocyte count (DHC). The total counts after application of different concentrations from Nomolt were significantly decreased at 1,3, 5 days on 5th nymphal instar and up to 1day old adult males and females comparing with those of the untreated. Changes in DHC have been assessed in relation to application of graded concentrations of Nomolt. The haemocyte profile was determined 1 day, 3 days, 5 days post-treatment as well as 1day in the adult males and females. Different types of haemocytes registered a dose-dependent response by either exhibiting increase or decrease in their relative proportions. The percentage of prohaemocytes, oenocytoids, plasmatocytes, and granulocytes of increased in the treated blood smears as compared to the control while spherulocytes was decreased in the percentage of these cells in treated blood smears. Spherulocytes was the most sensitive cells to the Nomolt whereas the oenocytoids showed least affected cells. However, there was a consistent increase in the proportion of *disintegrated* cells in accordance with increase in concentration of Nomolt applied. Extreme pathological symptoms were observed in cell membrane, distortion of the cytoplasmic and nuclear membrane, and abnormal staining of the haemocytes.

[Salam S. Teleb. **Effect of Nomolt on Differential and Total Haemocytes in the Desert Locust *Schistocerca gregaria* Forskal (Orthoptera:Acrididae).** *J Am Sci* 2011;7(11):479-484]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 60

Keywords: Nomolt, total haemocytes, differential haemocytes, *Schistocerca gregaria*

1. Introduction

The desert locust, *Schistocerca gregaria* Forsk. (Acrididae: Orthoptera) is one of the most important pests, because of its polyphagous, attacks on a wide range of plants including agricultural crops. The desert locust is an international pest. Efforts have been made to control this cosmopolitan insect through the International Locust Control Organization of the Food and Agriculture Organization (FAO). The insecticide used in this connection is agro insecticide (Nomolt15% Sc). The Nomolt15%Sc (active ingredients :Teflubenzuron(IUPAC: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6- difluorobenzoyl urea)is a group of insect growth regulators (IGRs) comprises juvenile hormone analogs (JHAs), once regarded as, third generation insecticides" (Williams, 1967), as well as benzoyl phenyl ureas (BPUs). IGRs generally interfere with the normal morphogenesis and reproduction (Retnakaran, *et al*., 1985).

Insect haemocytes, categorized into several types circulate in the haemolymph. Their primary functions are coagulation, phagocytosis, encapsulation, detoxification, and storage and distribution of nutritive materials. Our present knowledge of insect haemocytes is limited to studies of not more than 200 insects species in about 100 genera (Arnold, 1979). Haemocytes have been studied mostly in Lepidoptera, Hymenoptera, Coleoptera, and Diptera (Gupta, 1985). There is an inherent variability of haemocytes within a species as well as among closely related species (Arnold, 1979; Gupta, 1979). Haemocyte picture of various types have been investigated in the German cockroach *Blatella germanica* both in the nymphs and adults (Chiang *et al.,* 1988). The haemocytes of desert locust, *Schistocerea gregaria* Forsk. were first studied by Mathur and Soni (1936). They recorded four distinct types of haemocyte i.e., mother cells, proleucocytes, granular leucocytes and phagocytes. They also recorded the total haemocyte count from the adult of desert locust and it was 6500 cells/mm3.

The objective of the present research is to evaluate the effect of Nomolt 15%Sc on differential and total haemocyte counts in the 5th nymphal and adult stages of desert locust, *Schistocerea gregaria* F.

2. Material and Methods

1-Origin of population:

The stock colony of *Schistocerea gregaria*. was maintained for several generations at constant room temperature (32±2ºC) and (75-80RH). The insects were reared under crowded breeding conditions outlined by Hunter-Jones (1961) and Hassanein (1965). Newly hatched hoppers were kept in wooden cages with wire-gauze sides (40 x 40 x 60 cm) and small door in the upper-side to allow the daily feeding and cleaning routine. The bottom was covered with 20 cm layer of sterilized sand. Cages were equipped internally with 60 W electric bulb for lightening (17:7 LD) and warming. Successive generations were raised before obtaining the nymphs for the present experimental work. Fresh

food plant was lettuce (*Lactuca sativa*) along the period of study except few weeks every year, because of the absence of this plant species. During these weeks, insects were fed on *maize*. All experiments were conducted with *Lactuca sativa* only.

2- Nomolt 15%Sc (Teflubenzuron)

The IGR Teflubenzuron was obtained from Shoura chemical**.**

Chemical name: IUPAC: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea

Synonyms: DART; DIARACT; NEMOLT; NOMOLT; CL 291,898; SAG

Formulations: Teflubenzuron is available as suspension concentrates containing 150 g ai/l (NOMOLT, NEMOLT, and DART SC 15) or 50 g ai /1 (NOMOLT and DART SC 5).

3- Experimental insects:

The First day of the 5th nymphal instar of *S. gregaria* were treated with different concentrations of Nomolt (500,100 and 50 ppm) The Feeding technique was applied using fresh lettuce (*Lactuca sativa*) after dipping for 3 minutes in each concentration. Feeding on treated food plant was allowed for 24 hrs for the newly moulted 5th instar nymphs. Control insects had been allowed to feed on untreated food plant and kept under the same laboratory conditions. Five replicates (10 nymphs/rep.) were carried out for each treatment. Each individual nymph was kept in a suitable glass vial provided with a thin layer of sterilized sand. The vials were carefully located in a cage supported with a suitable electrical bulb .

4- Total haemocyte count (THC).

The haemolymph was drawn into a Thoma white blood cell pipette up to 0.5 mark and diluted up to the 11 mark with Toissin's solution (NaCl =1.0 gm, Na_2SO_4 = 8.0 gm, neutral glycerin = 20 ml, methyl violet = 0.025 gm and distilled water = 160 ml) up to mark II (Mahmood and Yousaf, 1985). The pipette was then shaken for several minutes and the first three drops were discarded. A double line with improved Neubauer ruling Haemocytometer was filed with diluted haemolymph and the haemocytes counted in its four corner and one central (1mm2) squares under a microscope (Olympus, Japan). If the distribution of cells in all the squares were not even, the sample was discarded. The number of circulating haemocytes per cubic millimeter (mm3) was calculated using the following formula of Jones (1962).

$$\frac{\text{Haemocytes in five 1mm2} \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}}$$

Where dilution = 20 times, Depth factor of the chamber = 10 (constant) and No. of squares counted = 5 for blood smear slide preparation.

5- Differential haemocyte count (DHC**).**

Regarding DHC a drop of fixed haemolymph was taken over a clean microscope slide and smear was made by drawing second slide across the first one at 45° angle. The smear was air-dried and stained by Wright's stain for four minutes. A freshly prepared buffer solution (Na2HPo4= 3.8 gm; KH2Po4 = 5.47 gm and distilled water = 1 liter) of pH 6.6 was applied for 15 minutes to neutralize the haemocyte contents for differential staining. Differential counting of haemocytes was done under oil immersion, phase microscope (10X x 100X) .Each time, 100 cells were counted and percentage of various classes was determined (Mahmood and Yousaf, 1985). The experiment was repeated five times .

3. Results and Discussion

1-Total haemocyte count (THC)

The total haemocyte count of 5th nymphal instar of desert locust, *Schistocerea gregaria* Forsk. was counted before and after the application of Nomolt. The results of these experiments are presented in Table (1) **.**It is obvious that THC in untreated 5th nymphal instar of desert locust possess 7800±130.4 (cells/mm3) at one day old and increase in the 3rd and 5th days (8890±710and 9380±128.1 cells/mm3 respectively)while the total haemocyte count in one day old adult males and females were10520±198.5and 8560±231.5 respectively .After the application of Nomolt , there was a gradual decline in the total haemocyte count (THC) after1day old 5th nymphal instar post treatment at the different concentrations(50,100 and 500 ppm).these were 6900±89.4, 5240±220.5 and 3740±397.5 cells/mm3 respectively. Further declined to 6840±120.8, 4920±96.9 and 3300±371.5 cells/mm3 at3days for 50,100 and 500 ppm respectively. However, at 5 days THC declined to 7320±267.6, 5460±160.0 and2340±274.9 cells/mm3 for 50,100 and 500 ppm respectively. In the males emerged from treated nymphs the THC. decline to 6850±263.6 ,and 4330±86.0 cells/mm3 at 50and 100ppm and sharply decline in females to 5262±316.9 and 3760±146.9 cells/mm3at 50 and 100 ppm respectively.

At 500ppm there is no males or females emerged from treated nymphs.

Statistical analysis for the results after applications, revealed that effect of Nomolt on the blood cell counts is significant ($P < 0.05$) after 1,3and 5days post treatment with 50,100 and 500ppm and up to 1day old adult females and males .

It is interesting to note that the THC in males was higher than that of the female *Schistocerea gregaria* . and it remained so even after the treatment. Nomolt which has a chitin inhibiting activity probably functions like toxins (Lim and Lee, 1982) .Nomolt inhibiting the formation of chitin in insects might utilize the haemocytes from haemolymph thus causing decline in THC. Alhariri and Anjum (2001) stated that the total counts after application of Lambdacyhalothrin

and Deltamethrin were significantly increased comparing with those of the untreated females of *Schistocerca gregaria*. The insecticides also showed an increase in plasmatocytes and granulocytes percentage; whereas, a decrease in percentage took place for Oenocytoids and Spherulocytes. Abnormalities caused by insecticides to the haemocytes were: agglutination, denucleation and enlargement of cells, distortion of the cytoplasmic and nuclear membrane, and abnormal staining of the haemocytes**.** Prakash *et al*.,(2007) reported that Penfluron seems to cause a great reduction in haemocytes in both sexes at 72 hours and 96 hours after treatment against *Dysdercus koenigii*.

2- **Differential haemocyte count (** DHC**).**

After one day, old 5th nymphal instar (Table 2), the density of prohaemocytes increased with increasing concentrations. However, with 500ppm Nomolt there was approximately two half times(28.6±0.2) more cells in the smear compared to control(11.8±0.4%) The plasmatocytes were highly affected cells, percentages of plasmatocytes decreased from normal by46.8±1.1% to 33.2±0.4, 23.0±0.3and8.0±0.3% at 50, 100 and 500ppm, respectively. showing a statistically significant reduction(P<0.05). Similarly, the spherulocytes were also affected cells, percentages of spherulocytes showed reduction as the concentrations of Nomolt. increased . The population of granulocytes increased to 12.0±0.7% and 16.4±0.5% following treatment with 50 and 100ppm Nomolt as compared to 9.2±0.6% in the control. However, by the higher concentrations (500ppm) the population of granulocytes was reduced to 1.8±0.3% compared to control (9.2±0.6%). Subsequent to the application of 50, 100and 500ppm concentrations the relative percentage of oenocytoids was progressively increased .However, only a few oenocytoids showed damage and most of these cells were recognizable even in the smears of 500ppm Nomolt affected nymphs. The proportion of damaged and disintegrated cells was enhanced with increase in concentration of Nomolt. The highest concentration caused destruction of 29.4±0.4% haemocytes compared to 0.0% in control showing a statistically significant increase(*P*<0.05) .

Three days following treatment with50 and 100ppm nomolt, the percentage of prohaemocytes exhibited an increasing trend .However, with 500ppm concentration there was slight fall in their population compared to lower concentrations in the smears of control nymphs, plasmatocytes constituted 33.0±0.4% of total cell population. Subsequent to the application with50 , 100ppm,and500ppm concentration, the respective percentage of these haemocytes was 29.4±0.4,25.4±0.5and 10.4±1.1 showing reduction with increasing concentrations of Nomolt which was statistically significant at 100 and 500ppm(*P*<0.05).The spherulocytes were most fragile cells and were highly damaged even by lower concentrations. Following 100ppm Nomolt, the population of spherulocytes was reduced to one third. Moreover, by 500ppm the spherulocytes was completely damaged. Similarly, the population of granulocytes progressively increased to nearly 14.4±0.5 by 100ppm concentration. However, following the highest concentration these cells were not found in the smear. Oenocytoids were the least affected cells in the smear. Although, the number of these cells remained constant in the treated insects their relative percentage showed an increase due to disintegration of other types of cells. Following the highest concentration of Nomolt the population of these cells increased to 21.8±0.9 compared to 3.0±0.3% in control showing increase with increase in concentrations (*P*<0.05). There was a consistent increase in the proportion of damaged/ disintegrated cells in accordance with the increase in concentration of Nomolt. Thus a maximum of 35.2±0.7%(*P*<0.05),cells were found damaged/disintegrated with the highest concentration of Nomolt showing statistically significant increase (Table3)

After 5 days of the treatment with different concentrations of Nomolt (Table4) prohaemocytes showed an increase in their population, highest being in 500ppm Nomolt treated nymphs. Plasmatocytes were severely affected by 500ppm concentration comprising only 4.2.±0.6% of total cells showing statistically significant (*P*<0.05) reduction in their population compared to control (34.4±0.5%). Spherulocytes were significantly less (23.8±0.4 %) in 100ppm Nomolt affected smears (*P*<0.05), however, these cells were completely damaged by 500ppm. Similarly, granulocytes, too, were unidentifiable in smears affected with higher concentration500ppm. The population of oenocytoids showed a steady increase with the increase in concentration of Nomolt . The relative percentage was almost four and a half times higher in 500ppm Nomolt treated nymphs19.0.±0.7% as compared with control 5.6±0.5. % thereby showing a statistically significant reduction (*P*<0.05).

After application of 500ppm Nomolt 50.4±1.1% cells unidentifiable compared to 0.2±0.1% in control thereby showing statistically significant increase (*P*<0.05).

In one-day-old males (Table-5), the prohaemocyte population showed statistically insignificant increase with the application of lower concentrations .Plasmatocyte population inconsistently decreased showing statistically insignificant reduction. Similarly, spherulocytes showed insignificant reduction by the application of different concentrations of Nomolt. Granulocytes and oenocytoids too followed the same trend. Likewise, population of damaged cells showed statistically insignificant change.

In one-day-old females emerged from treated nymphs (Table5) only prohaemocytes showed

statistically significant increase by 100ppm Nomolt (p<0.05) compared to control. Besides that all the other haemocyte types exhibited inconsistent and statistically insignificant change.

In the present study with Nomolt , there was found, significant reduction in the THC of the treated 5th nymphal instar and in adult males and females of *Schistocerea gregaria* post treatment of Nomolt and a number of abnormalities caused to haemocytes. were also observed. hence, this reduction could be due to the death of pathological cells by degeneration of the cells: cell membrane, cytoplasm and nucleus. These changes, interestingly , are similar to those produced by some of the insecticides (Gupta and Sutherland, 1968, Zaidi and Khan, 1977; Hassan, 1985, Mahmood and Yousaf,1985 Azam and Ilyas, 1986 , Younes *et al.,* 1999 and Haq *et al.*, 2005) These results are in partial accordance with those of Ambrose and George (1996), Ayub (1996) who studied the effect of Quinalphos and endosulfan, Fyfanon 50 EC and Perfekthion 40 EC and Methyl parathion 50 EC on the differential haemocyte count of *Acanthaspis pedestris* Stal., and *Drosicha stebbingi* Green., respectively and observed that the percentage of prohaemocyte increased from untreated . Gupta and Sutherland, 1968). Injection of various doses of B-ecdysone to *Spodoptera litura*, caused an increase in the prohaemocyte and plasmatocyte population and decline in the adipohaemocyte. Gupta (1985) injected a juvenoid into the last nymphal instar of cockroach and found a 50% reduction of haemocytes in the adult. Since the adult haemocyte count tallied with that of the nymphal count, he postulated that the analogue had a juvenilizing effect on these cells.. Alhariri (2000) who studied the effect of carbamates, pyrethroids, Decis 2.5 EC, Karate 2.5 EC, Lorsban 40 EC and Basudin 60 EC, respectively on haemocyte count of *Schistocera gregaria* Forsk and noted that the total haemocyte count increased just after application of insecticides. Qamar and Jamal(2009) showed that changes in the differential haemocyte counts have been assessed in relation to application of graded concentrations of Acephate against females. *Dysdercus cingulatus*.The haemogram profile was determined 6 hrs, 1 day,3 days, 5 days post-treatment as well as post-moulting. Different types of haemocytes registered a dose-dependent response by either exhibiting increase or decrease in their relative proportions. The adipohaemocytes were the most sensitive cells to the insecticidal stress whereas the oenocytoids showed least damage to their cellular integrity. However, there was a consistent increase in the proportion of damage/unidentifiable blood cells in accordance with increase in concentration of acephate applied.Sendi and Salehi, (2010) showed that at the low dose of methoprene caused THC reduction in general and of PLs, ADs and SPs in Particular. . However, the high dose (100µg/µL acetone) produced THC declined considerably The hitspathological symptoms were observed as changes in PLs form.

Abnormalities caused to haemocytes. The following abnormalities were recorded in the haemocytes of 5th nymphal instar and in adult males and females of *Schistocerea gregaria* post treatment of Nomolt; distortion of the shape of haemocytes, rupturing wall of the cells, denucleation of cells, and abnormal staining of the haemocyte.The present results are in conformity with the findings of previous workers (Hassan, 1985; Mahmood and Yousaf,1985).

Table 1: Effect of Nomolt on total haemocyte count (Cells/mm3) in the 5th nymphal instar and adults of *Schistocerea gregaria* .

Conc.(ppm)	Total Haemocyte Count(Mean ±S.E)**				
	1 day Old 5th	3days Old 5th	5days Old 5th	1 day old males	1 day old females
Control	7800±130.4	8890±710	9380±128.1	10520±198.5	8560±231.5
50	6900±89.4*	6840±120.8*	7320±267.6*	6850±263.6*	5262±316.9*
100	5240±220.5*	4920±96.9*	5460±160.0*	4330±86.0*	3760±146.9*
500	3740±397.5*	3300±371.5*	2340±274.9*	0.0	0.0

* Significant $P < 0.05$

**Results are the means of 5 replicates

Table 2: Differential haemocyte counts determined after 1day following the treatment of various concentrations of Nomolt on 5th nymphal instar of *Schistocerea gregaria.*

Concentrations (ppm)	**Prohaemocytes**	**Plasmatocytes**	**Spherulocytes**	**Granulocytes**	**Oenocytoids**	**Disintegrated Cells**
	(% ± S. E.)*					
Control	11.8±0.4	*46.8*±1.1	*24.6*±0.5	*9.2*±0.6	*7.6*±0.2	*0.0*
50	15.2±0.6	*33.2*±0.4	*21.0*±0.3	*12.0*±0.7	*10.8*±0.4	*7.8*±0.4
100	21.6±0.2	*23.0*±0.3	*16.0*±0.7	*16.4*±0.5	*13.6*±0.5	*9.4*±0.3
500	28.6±0.2	*8.0*±0.3	*1.4*±0.2	*1.8*±0.3	*30.8*±0.4	*29.4*±0.4

Table 3: Differential haemocyte counts determined after 3days following the treatment of various concentrations of Nomolt on 5th nymphal instar of *Schistocerea gregaria*

Concentrations (ppm)	Prohaemocytes	Plasmatocytes	Spherulocytes	Granulocytes	Oenocytoids	Disintegrated Cells
	(% ± S. E.)*					
Control	15.8±0.6	*33.0*±0.4	*31.2*±0.4	*10.4*±0.5	*3.0*±0.3	*6.6±0.3*
50	21.2±0.4	*29.4*±0.4	*24.2*±0. *4*	*12.6*±0.5	*4.6*±0.3	*8.0*±0.4
100	26.2±0.4	*25.4*±0.5	*20.4*±0.5	*14.4*±0.5	*7.8*±0.4	*9.4*±0.3
500	32.6±0.7	*10.4*±1.1	*0.0*	*0.0*	*21.8*±0.9	*35.2*±0.7

Table 4: Differential haemocyte counts determined after 5 days following the treatment of various concentrations of Nomolt on 5th nymphal instar of *Schistocerea gregaria.*

Concentrations (ppm)	Prohaemocytes	Plasmatocytes	Spherulocytes	Granulocytes	Oenocytoids	Disintegrated Cells
	% ± S. E.*					
Control	7.4±0.5	*34.4*±0.5	*38.0*±0.9	*14.4*±0.5	*5.6*±0.5	*0.2±0.1*
50	11.4±0.5	*29.5*±0.9	*32.8±0.9*	*13.2*±0.5	*6.2*±0.6	*7.3*±0.4
100	15.4±0.5	*25.0*±0.4	*23.8±0.4*	*12.0*±0.3	*7.8*±0.4	*16.0*±0.5
500	26.4±0.9	*4.2.*±0.6	*0.0*	*0.0*	*19.0.*±0.7	50.4±1.1

Table 5: Differential haemocyte counts after 1 day old Males and females following the treatment of various concentrations of Nomolt on 5th nymphal instar of *Schistocerea gregaria* .

es		Plasmatocytes		Spherulocytes		Granulocytes		Oenocytoids		Disintegrated	
		% ± S. E.*									
Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
±0.4	40.6±0.9	51.4±0.6	46.2±1.1	32.4±0.5	6.8±0.4	5.4±0.5	2.5±0.5	1.6±0.2	0.5±0.1	1.4±0.2	
±0.3	35.0±0.7	52.6±0.6	42.8±0.4	26.6±0.5	7.2±0.4	6.7±0.5	4.2±0.6	2.1±0.2	6.6±0.4	2.4±0.2	
6±0.3	32.4±0.9	53.2±0.6	40.2±0.9	22.8±0.4	9.2±0.4	7.2±0.4	7.7±0.4	2.4±0.2	5.8±0.5	3.8±0.2	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	

* Results are the means of 5 replicates

Corresponding author
Salam S. Teleb
Zoology Department, Faculty of Science, Zagazig University, Egypt
salamteleb@gmail.com

References

1. Alhariri, M.K., (2000): Toxicity of some insecticides on the haemocytes of desert Locust, *Schistocerca gregaria* Forsk. M.Sc. Thesis, Department of Agri. Entomology, University of Agriculture, Faisalabad–Pakistan .
2. Alhariri, M.K. and Anjum S.(2001):Effect of Lambdacyhalothrin and Deltamethrin on the Haemocytes of Desert Locust, *Schistocerca gregaria* Forsk. International Journal of Agriculture & Biology, 1560–8530 /03–1–81–84.
3. Ambrose, D.P. and P.J.E. George (1996): Effect of Quinalphos and Endosulfan on the differential and total haemocyte count of *Acanthaspis pedestris* Stal (Heteroptera: Reduviidae). *Fresenius Environ. Bull.,* 5: 207–12.
4. Arnold J.W. (1979) : The Haemocytes of insects.In Rockstein, M. (2nd Ed.), vol. 5, *The Physiology of Insecta*, Academic Press, New York and London.
5. Ayub, S., (1996): Effect of some Organophosphates on the haemocytes of Mango mealy bug, *Drosicha stebbingi* Green. *M.Sc. Thesis,* Department of Agri. Entomology, University of Agriculture, Faisalabad–Pakistan .
6. Azam, A. F. and Ilyas, M.(1986):The effects of BHC on the hemocytes of *Dysdercus cingulatus* (Pyrrhocoridae: Hemiptera). Biologia (Lahore), 32: 23-28.
7. Chiang, A. S., Gupta, A. P. and Han, S. S., (1988): Arthropod immune system: I. Comparative light and electron microscopy accounts of immunocytes and other hemocytes of *Blattella germanica* (Dictyoptera: Blattellidae). J. Morphol., 198: 257-267.

8. Gupta, A. P. and Sutherland, D. J. (1968): Effects of sublethal doses of chlordane on the hemocytes and midgut epithelium of *Periplaneta americana*. Ann. Ent. Soc. Amer., 61: 910-918.
9. Gupta, A. P. (1979): Hemocytes types: their structure, synonymies, interrelationship, and taxonomic significance in insect hemocytes. Edited by A. P.
10. Gupta. pp.85-127. Cambridge University Press, Cambridge.
11. Gupta, A. P. (1985): Cellular elements in the hemolymph. In Comprehensive insect Physiology, Biochemistry and Pharmacology. Edited by G. A. Kerkut and L. I. Gilbert. 3: 401-451. Pergamon Press,Oxford.
12. Gupta, A. P. and Sutherland, D. J. (1968): Effects of sublethal doses of chlordane on the hemocytes and midgut epithelium of *Periplaneta americana*. Ann. Ent. Soc. Amer., 61: 910-918.
13. Haq, M. R., Sabri, M. A. and Rashid, A. (2005):Toxicity of nicotinyl insecticides on the hemocytes of red cotton bug, *Dysdercus koengii* (Fb.) (Pyrrhocoridae: Hemiptera). J. Agri. Soc. Sci., 3: 239-241.
14. Hassan, M.U., (1985): Studies on the effect of some pyrethroids on the haemocyte of *Tryporyza* sp. *M.Sc. Thesis,* Deptt. Agri. Ento., Univ. Agric., Faisalabad .
15. Hassanein, M.S., (1965): *Laboratory and Outdoor Cultures and Breeding of Locusts and Grasshoppers*, p: 10. FAO Publication 5/31901.
16. Hunter-Jones, P.,(1961): *Rearing and Breeding Locusts in Laboratory*, p: 12. Bulletin Anti-locust Research Centre London.
17. Lim, S.J. and Lee ,S.S. (1982) : The toxicity of diflurobenzuron to *Oxya japonica* (Willemse) and its effects on moulting. *Pestic. Sci*. **13:**537-544.
18. Mahmood, A. and M. Yousaf, (1985): Effect of some insecticides on the haemocytes of *Gryllus bimaculatus* De Geer. *Pakistan J. Zool.,* 17:71–84.
19. Mathur, C.B. and B.N. Soni, (1936): Studies on *Schistocerca gregaria* Forsk.IX. Some observations on the histology of the blood of the desert locust. *Indian J. Agri. Sci.,* 7: 317–25.
20. Prakash1 B, Sudhir B.and Kamlesh R. (2007*)*: Effect of Penfluron on Total Haemocyte Count of *Dysdercus koenigii Asian* J. Exp. Sci., 21(1): 151-154.
21. Qamar A, and Jamal .(2009): Differential haemocyte counts of 5th instar nymphs and adults of *Dysdercus cingulatus* Fabr (Hemiptera: Pyrrhocoridae) treated with acephate, an organophosphorus insecticide Biology and Medicine, 1 (2):116-121.
22. Retnakaran, A.; Granett, J. and Ennis, T. (1985): Insect growth regulators. In "Comprehensive Insect Physiology Biochemistry and Pharmacology" (Kertut G.A. and Gilbert, L.I., eds), Vol. 12, pp:529-601. Oxford: Pergaman Press.
23. Sendi, J. J. and Salehi, R. (2010): The effect of methoprene on total hemocyte counts and histopathology of hemocytes in *Papilio demoleus* L. (Lepidoptera). Munis Entomology &Zoology, 5 (1): 240-246]
24. Williams,c .M (1967):Third generation pesticides. Sci.Am., 217:13-17.
25. Younes, M. W. F., Abou El Ela, R. G. and El-Mhasen, M. A. (1999): Effect of certain insecticides on the hemocytes of the lesser cotton leafworm *Spodoptera exigua* (HB.) (Lepidoptera: Noctuidae). J. Union Arab. Biol, Cairo, 11: 435-457.
26. Zaidi, Z. S. and Khan, M. A.(1977) : Effects of aldrin and dipterex on the hemocytes of red cotton bug *Dysdercus cingulatus* Fabr. (Hemiptera: Pyrrocoridae). Botyu-Kagaku, 42: 141-148.

10/8/2011