INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2001/03–1–81–84
http://www.ijab.org

Effect of Lambdacyhalothrin and Deltamethrin on the Haemocytes of Desert Locust, *Schistocerca gregaria* Forsk

M. KHALID AL HARIRI AND ANJUM SUHAIL†
Department of Plant Protection, Ministry of Agriculture, Damascus, Syria
†Department of Agriculture Entomology, University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT

Five haemocyte classes (Prohaemocytes, Plasmatocytes, Granulocytes, Oenocytoids and Spherulocytes) were distinguished in the adult of desert locust, *Schistocerca gregaria* Forsk. The total counts after application of insecticides were significantly increased comparing with those of the untreated females. Lambdacyhalothrin (Karate 2.5EC) caused more increase in the total counts than that caused by Deltamethrin (Decis 2.5EC) comparing with the total counts of untreated adult female. 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.

Key Words: Lambdacyhalothrin; Deltamethrin; Haemocytes; Acididae; Orthoptera

INTRODUCTION

The desert locust, *Schistocerca gregaria* Forsk. (Acrididae: Orthoptera) is one of the most important pests, because of its polyphagous nature, 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 insecticides used in this connection are likely to cause an extensive damage to the blood by which toxic chemicals are conveyed from the site of absorption to the organs, where they are metabolised and excreted (Welling & Paterson, 1985).

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/mm^3. The objectives of the present research were to study the differential and total haemocyte counts in the adult stage of desert locust, the effect of two pyrethroid insecticides recommended by FAO to control locusts, i.e., Karate 2.5EC (Lambdacyhalothrin) and Decis 2.5EC (Deltamethrin) on the total and differential haemocyte counts of the adult female of desert locust, and the abnormalities caused to the haemocytes by these insecticides.

MATERIALS AND METHODS

Specimens of desert locusts were collected from Cholistan desert (Bahawalpur) and kept in cages (46 cm x 46 cm x 46 cm) and put in growth chamber adjusted at temperature of 30°C and 60% R.H. and photoperiod of 12 hrs light and 12 hrs dark. Cabbage, wheat and grasses were offered as a diet. For fixing the haemocytes, the desert locust insects were exposed to glacial acetic acid vapour in a small desiccator at 40°C for 5-10 minutes. The metaleg was removed and the exuding haemolymph was drawn into a Saaringia white blood-cell diluting pipette. A small drop of this blood was placed on a slide and smeared by drawing another slide over it. These blood smears were stained in wright's stain for four minutes. A freshly prepared buffer solution (Na_2HPO_4 = 3.8 gm, KH_2PO_4 = 5.47 gm and distilled water = 1 litre) of pH 6.6 was applied for 15 minutes to neutralize the haemocyte contents for differential staining.

Total counting of haemocytes was done with a Neubauer haemocytometer. The blood was drawn into a Saaringia white blood-cell diluting pipette up to 0.5 mark and then diluted 20 times with Toisson's solution (NaCl = 1.0 gm, Na_2SO_4 = 8.0 gm, neutral glycerine = 20 ml, methyl violet = 0.025 gm and distilled water = 160 ml) up to II mark. A drop of this diluent was placed near the edge of the coverslip. Neubauer ruling automatically filled the counting chamber by its capillary action. Both the upper and lower chambers were used and four white-cell squares in each chamber were counted after five minutes from discharging the diluent. The total haemocyte count was computed by using the formula suggested by Jones (1962). Five observations were recorded for this purpose. Differential counting of haemoyctes was done under an oil immersion phase microscope (10_x x 100_x). Each time 100 cells were counted and the percentage of various classes was determined.

Two pyrethroid insecticides were used for this research study i.e., Karate 2.5EC and Decis 2.5EC in 0.04 and 0.03%, respectively. Eight micro-litres of the insecticide solution was applied topically on the dorsal side of the

abdomen of the adult female of desert locust and the total haemocyte counts were calculated for three time intervals, viz., soon after application, 30 minutes and 60 minutes after application. Five replications were conducted for each insecticide and for each time interval, using the Completely Randomized Design (CRD) and data was subjected to the standard statistical analysis using techniques of analysis of variance.

RESULTS AND DISCUSSION

Haemocyte classification

The following classes and types (based on the size and shape of the cells, their staining characteristics and type, number, size and staining affinities of their inclusions) were recognized:

Prohaemacytes (PRs). These are small, round cells, a thin layer of cytoplasm surrounds a centrally located nucleus, and the nucleus is almost filling the cell. The cytoplasm is normally homogenous, lacking any granules or vacuoles. They were representing 37.2% of the total haemocyte population in the adult female.

Plasmatocytes (PLs). These are medium to large in size, polymorphic cells i.e., rounded, ovoid or spindle-shaped with round or elongate nucleus, and the nucleus is centrally located. They were representing 16.4% of the total haemocyte population in the adult female.

Gronulocytes (GRs). These are round, oval or fusiform in shape. The nucleus is round or elongated and centrally positioned. Cytoplasm is filled with an immense number of small granules. They were representing 18.9% of the total haemocyte population in the adult female.

Oenocytoicls (OEs). These are large cells, may be oval or elongated in shape, with occasional rounded form. The nucleus is often eccentrically positioned. The cytoplasm is distinguished by an elaborate system of filaments that fills the cytoplasm, in addition to small granules. They were representing 9% of the total haemocyte population in the adult female.

Spherulocytes (SPs). These are oval cells and packed with large spherical granules, which tend to obscure the cytoplasm and the nucleus. They were representing 18.6% of the total haemocyte population in the adult female.

During the present study, five distinct classes of haemocytes were found in desert locust, *Schistocerca gregaria* Forsk. These findings are in accordance with those of Arnold and Sohi (1974), Price and Raticliffe (1974), Costin (1975) and Ayub (1996) who worked on different insect species. The present results are in partial accordance with those of Mathur and Soni (1936), Hoffmann (1967), Akai and Sato (1979), Mahmood and Yousaf (1985) and Masconi *et al*. (1989) who studied the haemocyte of desert locust, *Locusta migratoria*, *Locusta migratoria*, *Gryllus bimaculatus* and *Periplaneta Americana,* respectively and recognized four types of haemocytes. Mall and Gupta (1979) and Khan (1994) recorded five types, but the only difference is that Adipohaemocytes recorded instead of Spherulocytes. Meranpuri *et al*. (1991) observed only two major types of haemocytes in migratory grasshopper, *Melanoplus sanguinipes*, i.e., plasbsatocytes and granulocytes. These differences might be attributed to the differences in insect species used, procedures used for identification and the characters adopted by other workers. In the current study the lines given by Gupta (1993) were adopted. He classified the haemocytes into six main types, as he designated both cystocytes and coagulocytes as one type i.e., granulocytes and considered that vermicytes and podocytes are variant forms of plasmatocytes.

Total haemocyte count (THC)

The total haemocyte count of adult male and female of desert locust, *Schistocerea gregaria* Forsk. was counted before the application of insecticides, and the THC of the adult female was counted after the application of insecticides. The results of these experiments are presented below:

Normal insects. It is obvious from Table I that males possess more THC than that of females. The average figures are 10330 and 8690 cells/mm^3 in males and females, respectively. These findings are in accordance with those of Webley (1951), Muhammad (1961) and Hoffmann (1970) who studied the hoemocyte number in *Locusta migratoria migratoriodes* Reiche and Fair-Maire, three local grasshoppers and *Locusta migratoria*, respectively. The present results differ from those of Tauber and Yeager (1935), Chatha (1963), Akram (1970) and Mahmood and Yousuf (1985), who studied the haemocyte numbers in *Gryllus assimilis* L., *Chrotogonus trachypterus* Blonohard, *Acheta domesticus* L. and *Gryllus bimaculatus*, respectively, as they found that females possess more THC than males of their respective test insects.

Poisoned insects. Total haemocyte counts of poisoned insects (after the application of insecticides) are given in Table II and III. Statistical analysis for the results after insecticide applications, revealed that effect of insecticides on the blood cell counts is significant. The high number of the cells was counted after Karate 2.5EC application which was significantly higher than the number counted after Decis 2.5EC, which was, however, significantly greater than that of the control. When the total haemocyte numbers of the normal and the poisoned adult female are compared, it is seen that the counts after the application of insecticides are greatly enhanced as compared with those of normal adult females. These results are in accordance with Chatha (1963), Gupta and Sutherland (1968), Akram (1970),

Table I. Total haemocyte counts in adult male and female of desert locust, *Schistocerca gregaria* Forsk

No. of observations	Male	Female
1	10250	8425
2	10575	8125
3	10700	8375
4	9975	9025
5	10150	9500
Average	10330	8690

Table II. Effect of karate 2.5EC on the total haemocyte count of the adult female of desert locust, *Schistocerca gregaria* Forsk

No. of observations	No. of haemocytes mm^3		
	Just after application	After 30 minutes	After 60 minutes
1	14300	18900	11550
2	13025	21525	12075
3	14575	19300	11275
4	13675	19050	14700
5	13450	18225	12475
Average	13805	19400	12415

Table III. Effect of Decis 2.5EC on the total haemocyte count of the adult female of desert locust, *Schistocerca gregaria* Forsk

No. of observations	No. of haemocytes mm^3		
	Just after application	After 30 minutes	After 60 minutes
1	13075	12450	4175
2	11825	12425	4850
3	12525	11700	5425
4	11100	11575	7875
5	11850	11875	6900
Average	12075	12005	5845

Murtaza (1976), Hassan (1985), Mahmood and Yousaf (1985), Shukla and Bahadur (1986), Khan (1994) and Ayub (1996), who used *Chrotogonus trachypterus* Blanchard, *Periplaneta americana*, *Acheta domesticus*, *Dysdercus koenigii* Fb., *Tryporyza* sp., *Gryllus bimaculatus*, *Poekilocerus pictus*, *Leucinodes orbonalis* and *Drosicha stebbingi*, respectively. The present results differ from those of Muhammad (1961) and Patton (1961) who used three local species of grasshoppers and greyhouse cricket, respectively in their experiments and recorded that the application of insecticides resulted in a decrease in blood cell counts.

It was also seen during the present study that the blood cell counts increased rapidly just after application of the two pyrethroid insecticides. While THC went increasing after 30 minutes of Karate 2.5EC application, the THC faced very slight decrease after Decis 2.5EC application; whereas, after 60 minutes, the THC went decreasing below the normal count in case of Decis 2.5 EC, but kept over the normal count in case of Karate 2.5 EC. These results are in conformity with the results of Hassan (1985) and Khan (1994), and differ from those of Ayub (1996).

Regarding to DHC, the percentage of different classes of haemocyte have been affected after the insecticide applications, as the percentage of both plasmatocytes and granulocytes increased from 16.4 and 18.8 to 31.6 and 20% in case of Karate 2.5EC application and to 30.4 and 24.4 in case of Decis 25 application, respectively; whereas, the percentage of prohaemocytes, oenocytoids and spherulocytes decreased from 37.2, 9.0 and 18.6 to 27.8, 5.8 and 11.6% in case of Decis 2.5EC application, respectively. The percentage of ocnocytoids and sphenilocytes decreased from 9 and 18.6 to 3.4 and 7.2 after Karate 2.5EC application, respectively; whereas, the percentage of prohaemocytes increased slightly from 37.2 to 37.8 after Karate 2.5EC application.

Haemocyte abnormalities

The application of insecticides, in addition to abnormal counts, also caused great abnormalities to the type of blood cells. These abnormalities are, distortion of the cytoplasmic and nuclear membrane, rupturing of cell wall, agglutination of cells, denucleation of the cells, enlargement of cells and abnormal staining of the haemocytes. These findings are in conformity with the findings of Muhammad (1961), Chatha (1963), Akram (1970), Mahmood and Yousaf (1985) and Ayub (1996) either with full agreement or with slight contradiction.

It is remarkable to record the observations in behaviour of the desert locust after applying the insecticides, as they displayed nervous movement followed by rapid movement, almost fast running, wriggling movement and pumping movement of the abdomen after applying the two pyrethroid insecticides. As the effect of poisons increased, walking was reduced.

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(Received 10 December 2000; Accepted 20 December 2000)