

# Toxic Effects of Endosulfan 35 EC and Deltaphos 350 + 10 EC on the Haemocytes of Red Cotton Bug, *Dysdercus koenigii* (Fb.) (Pyrrhocoridae: Hemiptera)

M. RIZWAN-UL-HAQ<sup>1</sup>, A. RASHID<sup>†</sup> AND MUHAMMAD ALTAF SABRI

Department of Agricultural Entomology and <sup>†</sup>Directorate of Research, University of Agriculture Faisalabad–38040, Pakistan

<sup>1</sup>Corresponding author's e-mail: [mian\\_rizwan15@yahoo.com](mailto:mian_rizwan15@yahoo.com)

## ABSTRACT

The toxicity of endosulfan 35 EC and Deltaphos 350 + 10 EC on the total and differential haemocyte count and abnormalities in the haemocyte caused by these poisons in the adult of *Dysdercus koenigii* Fb. were studied. Five types of haemocytes i.e. plasmatocytes, granulocytes, prohaemocytes, oenocytoids, spherulocytes, were identified with percentages of 37.75, 16.25, 19.25, 2.25 and 0.75%, respectively after the treatment with endosulfan 35 EC. In case of Deltaphos 350 + 10 EC the percentages of these five haemocytes varied as 42.50, 17.50, 19, 5.25 and 0.50%, respectively. In case of endosulfan the total haemocyte count (THC) increased (19123 cells/mm<sup>3</sup>) just after the application of insecticide, while decreased (10531 cells/mm<sup>3</sup>) after half an hour and increased tremendously again (23738 cells/mm<sup>3</sup>) after one hour of application from the normal count (17000 cells/mm<sup>3</sup>). In case of Deltaphos 350 + 10 EC the total haemocyte count (THC) increased (18068 cells/mm<sup>3</sup>) soon after the application of insecticide, while it decreased (11804 cells/mm<sup>3</sup>) after half an hour and decreased again (8603 cells/mm<sup>3</sup>) after one hour of application from the normal count (17000 cells/mm<sup>3</sup>). The abnormalities recorded in the haemocytes were distortion of the shape, rupturing of the wall, abnormal staining of the cells, and displacement of nuclei on one side of the cell.

**Key Words:** Study of toxicity; Effect of endosulfan35EC; Deltaphos350+10EC; Haemocytes

## INTRODUCTION

Cotton crop is backbone for the economy of Pakistan. This crop is a necessity for the industry of Pakistan as it provides raw material to over 400 textile mills, 1035 ginning factories and 5000 expellers of Pakistan (Anonymous, 2000). In the year 2002 - 2003, the area under this crop was 3.125 million hectares (Anonymous, 2003).

Cotton provides shelter food and employment for large proportion of agricultural and industrial labour of the country (Khan & Khan, 1995). So there is a dire need to produce a successful and healthy crop. Many steps have been taken to increase the per acre yield by introducing high yielding varieties, quality seed, and advanced plant protection techniques.

Red cotton bug (*Dysdercus koenigii* Fb.) is important heteropteran (Pyrrhocoridae: Hemiptera) insect pest of cotton, reproduces rapidly in the field, so it has fast and efficient egg development (Venugopal *et al.*, 1994). A few years ago it was a minor pest but now becoming serious with its increasing population. It is also called as “cotton stainer”. The main damage of red cotton bug is the injection of fungal spores (Nematospores) in to the boll. The developing fungus stains the lint (Hill & Waller, 1990).

Insects have only extra-cellular fluid, haemolymph, in contrast to vertebrates, which have two such fluids, blood and lymph. It is not surprising that the haemolymph, in

general, serves the functions of both blood and lymph in insects. Thus the fluid fraction (plasma) is the transport system for nutrition, hormones and metabolic wastes and contains elements of the immune system, while the cellular components are haemocytes (Gillot, 1995). The number of the cells present in the circulating blood of various adult species varied from 1000 per cubic mm to about 100 times of this number (Tauber & Yeager, 1936). The numbers of various types of haemocytes in different time intervals provide useful information that how resistant and susceptible insect species are against different insecticides. The percentage of phagocytes in differential counting of haemocytes may provide useful information about the immunity of insects against insecticides. The knowledge of effects of insecticides on the insect haemocytes can be useful in determining the control recommendations against that species. The aim of the present study was to examine the effect of endosulfan 35 EC and Deltaphos 350 + 10 EC on the total haemocyte count, differential haemocyte count, the abnormalities caused by these insecticides in the haemocytes, and to determine that, which one of these produce better results against red cotton bug (*Dysdercus koenigii* Fb.).

## MATERIALS AND METHODS

The adults of red cotton bug were collected from

cotton field located at University of Agriculture Faisalabad and brought to the laboratory for the present research. endosulfan 35 EC and Deltaphos 350 + 10 EC were applied with 0.80 and 0.60% concentrations (recommended doses), respectively on the thorax, with the help of micro-applicator at the rate of 8 µL per application. There are total three applications i.e. at 0 min, after 30 min, after 60 min of application, after each application of insecticide, a new tip was replaced. Effect of insecticide was noted just after application, after 30 min and 60 min and in terms of abnormalities found in the haemocytes and the changes in the haemocyte count. The following techniques and apparatus was used to note total haemocyte count, differential haemocyte count and abnormalities in the haemocytes.

**Differential haemocyte count.** Differential haemocyte count was made in untreated adults of *Dysdercus koenigii* Fb., and also after the application of endosulfan 35 EC and Deltaphos 350+10 EC using the following procedure. The haemolymph was fixed by glacial acetic acid vapours for 5 - 10 min in a small desiccator at 40°C. One of the thoracic legs was pricked by needle and the exuding haemolymph was drawn in to Thoma white blood cell diluting pipette. A small drop of this blood was placed on the clean white grease free 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 Wrights stain for four min. A freshly prepared buffer solution (Na<sub>2</sub>HPO<sub>4</sub> = 3.8 g, KH<sub>2</sub>PO<sub>4</sub> = 5.47 g and distilled water 1 L) of pH 6.6 was applied for 15 min to neutralize the haemocyte contents for differential staining. Differential counting of haemocytes was under oil immersion phase microscope (10 x 100 X). Each time 100 cells were counted and the percentage of various classes was computed (Mahmood & Yousaf, 1985).

**Total haemocyte count.** The total haemocyte count was made from the haemolymph of untreated *Dysdercus koenigii* Fb. and also after treatment with endosulfan 35EC and Deltaphos 350+10 EC using the following procedure.

The fixed haemolymph was collected on a clean glass slide by pricking the needle in to the thoracic leg or by puncturing the abdomen and quickly drawn in to Thoma white blood cell diluting pipette up to 0.5 mark and then diluted 20 times with Toisson's solution (NaCl = 1.0 g, Na<sub>2</sub>SO<sub>4</sub> = 8.0 g, neutral glycerine = 20 mL, Methyl

violet = 0.025 g and distilled water = 160 mL) up to mark II (Mahmood & Yousaf, 1985). After shaking properly and discarding the first three drops (Jones, 1962) a drop of mix was placed near the edge of the cover slip of the haemocytometer and the chamber was automatically filled by capillary action and to let the blood cells settle down. The haemocytometer was left for 5 min (Tonapi, 1994) and after that cells were counted in the four corners ruled squares in each of the two chambers. The experiment was repeated four times for each insecticide.

## RESULTS AND DISCUSSION

**Total haemocyte count in the un-treated specimen of red cotton bug (*Dysdercus koenigii* Fb.).** The results obtained from the present study that have been given in Table I indicates that on an average there are 17000 cells/mm<sup>3</sup> in the haemolymph of un-treated adult of *Dysdercus koenigii* Fb.

**Differential haemocyte count in the un-treated specimen of red cotton Bug (*Dysdercus Koenigii* Fb.).** The differential haemocyte count was done with the object to find out the percentage of various classes of haemocytes in the haemolymph of adult of red cotton bug *Dysdercus koenigii* Fb. under normal conditions. The data given in Table I reveal that the percentage of plasmatocytes was the highest 39.75% followed by granulocytes 32%, prohaemocytes 22%, oenocytoids 4.25% and spherulocytes 2%.

**Effect of endosulfan 35 EC on the total haemocyte count (THC).** The total haemocyte count (THC) increased (19123 cells/mm<sup>3</sup>) soon after the application of insecticide, while decreased (10531 cells/mm<sup>3</sup>) after half an hour and increased considerably again (23738 cells/mm<sup>3</sup>) after one hour of application from the normal count (17000 cells/mm<sup>3</sup>) as shown in Table II.

**Effect of endosulfan 35 EC on the differential haemocyte count (DHC).** The percentages of plasmatocytes, granulocytes, prohaemocytes decreased from normal 39.75, 32, 22, 4.25 and 2% to 37.75, 16.25, 19.25, 2.25 and 0.75%, respectively after the application of endosulfan 35 EC as shown in Table II.

**Effect of deltapos 350 + 10 EC on the total haemocyte count (THC).** The data given in Table III reveal that the total haemocyte count (THC) increased (18068 cells/mm<sup>3</sup>) soon after the application of insecticide, while it decreased (11804 cells/mm<sup>3</sup>) after half an hour and further decreased again (8603 cells/mm<sup>3</sup>) after one hour of application from the normal count (17000 cells/mm<sup>3</sup>).

**Effect of deltapos 350 + 10 EC on the differential haemocyte count (DHC).** The data in Table III show that percentages of, granulocytes, prohaemocytes and spherulocytes decreased from normal, i.e. 32, 22 and 2% to 17.50, 19 and 0.50%; whereas, the percentage of plasmatocytes and oenocytoids increased from normal 39.75 and 4.25% to 42.50 and 5.25%, respectively.

**Table I. Total and differential haemocyte count in the un-treated adult of *Dysdercus koenigii* Fb.**

No. of observations	Total haemocytes/mm <sup>3</sup>	Differential haemocyte count				
		no. of PL %	GR%	PR%	OE%	SP%
1	17375	43	37	27	5	1
2	18785	37	33	20	8	4
3	16740	41	36	26	3	2
4	15100	38	22	15	1	1
Average	17000	39.75%	32%	22%	4.25%	2%

**Table II. Effect of endosulfan 35 EC on the total and differential haemocyte count of adult of *Dysdercus koenigii* Fb.**

No. of observations	Total no. of haemocytes			Differential haemocyte count				
	Just after application	After 30 minutes	After 60 minutes	PL%	GR%	PR%	OE%	SP%
1	19375	12419	20948	37	20	26	2	0
2	16223	8837	24893	40	14	20	4	2
3	19537	9725	25347	35	18	14	0	1
4	21357	11143	23764	39	13	17	3	0
Average	19123	10531	23738	37.75	16.25	19.25	2.25	0.75

**Table III. Effect of Deltaphos 350 + 10 EC on the total and differential haemocyte count of adult of *Dysdercus koenigii* Fb.**

No. of observations	Total no. of haemocytes			Differential haemocyte count				
	Just after application	After 30 minutes	After 60 minutes	PL%	GR%	PR%	OE%	SP%
1	17693	13251	8799	42	17	19	6	0
2	16800	10458	9856	49	13	25	3	2
3	19235	10964	7534	45	24	17	7	0
4	18544	12543	8223	34	16	15	5	0
Average	18068	11804	8603	42.50	17.50	19	5.25	0.50

**Analysis of variance.** Statistical analysis of the data from the view point of total haemocyte count (THC) after the application of these insecticides at three levels of time interval are presented in Table IV. It is evident that various time intervals are significant, while the insecticides and interaction among the insecticides and time intervals are significant (As there are three time intervals of application of insecticides while two insecticides were used. The haemocytes respond differently after the application of insecticides at these three intervals as compared to untreated specimen).

**Table IV. Anova Table**

S.O.V	d.f	S.S	M.S	F. Cal
Insecticides	1	148344592.667	148344592.667	60.4235**
Time interval	2	229562181.333	114781090.667	46.7525**
Interaction IxT	2	315258965.333	157629482.667	64.2054**
Error	18	44191444.000	2455080.222	
Total	23	737357183.333		

\*\*significant (prob<0.01)

## DISCUSSION

**Effect of endosulfan 35 EC on the total and differential haemocyte count.** The data presented in Table II revealed that the total haemocyte count (THC) increased (19123 cells/mm<sup>3</sup>) just after the application of insecticide, while decreased (10531 cells/mm<sup>3</sup>) after half an hour and increased tremendously again (23738 cells/mm<sup>3</sup>) after one hour of application from the normal count (17000 cells/mm<sup>3</sup>).

These results are coinciding with Mahmood and Yousaf (1985), who studied the effect of some insecticides on the haemocyte count of *Gryllus bimaculatus* de Geer. and observed that total haemocyte increased significantly as compared to un-treated larvae.

Regarding differential haemocyte count (DHC), the

data given in Table II indicate that the percentages of plasmatocytes, granulocytes, prohaemocytes, oenocytoids and spherulocytes, decreased from normal 39.75, 32, 22, 4.25 and 2%, to 37.75, 16.25, 19.25, 2.25 and 0.75%, respectively.

These results are in accordance with those of Sexana and Tikku (1990), who studied the effect of plumbagin on the haemocyte count of *Dysdercus koenigii* Fb. and noted that the percentage of plasmatocyte decreased in the treated insect. These results are also in accordance with Khan (1994), who studied the effect of Curacron 500 EC on *Leucinodes orbonalis* (Guen.) and observed that the percentage of granulocytes and oenocytoids decreased from the normal.

**Effect of deltapfos 350 + 10 EC on the total and differential haemocyte count.** The data given in Table III revealed that the total haemocyte count (THC) increased (18068 cells/mm<sup>3</sup>) just after the application of insecticide, while it decreased (11804 cells/mm<sup>3</sup>) after 30 min and again decreased (8603 cells/mm<sup>3</sup>) after one hour of application from the normal count (17000 cells/mm<sup>3</sup>).

These results are in accordance with Shukla and Bahadur (1986) as they also noted the smooth decrease in the haemocytes.

Regarding differential haemocyte count (DHC) the data shown in Table III revealed that percentages of plasmatocytes and oenocytoids, increased from normal, i.e. 39.75, 4.25 to 42.50 and 5.25%, while the percentages of granulocytes, prohaemocytes, spherulocytes reduced from normal 32, 22 and 2% to 17.50, 19 and 0.50%, respectively after the treatment.

These results are in conformity with Sabri and Rauf (1995), who noted the increase in the percentage of plasmatocytes and oenocytoids from the normal.

**Abnormalities caused to haemocytes.** The following abnormalities recorded in the haemocytes of red cotton bug, after the application of endosulfan 35 EC and

Deltaphos 350 + 10 EC were enlargement of haemocytes, distortion of the shape of haemocytes, rupturing of wall of haemocytes, denucleation of cells, abnormal staining of the cells, displacement of nuclei on one side of the cell. The abnormalities found are in conformity with those of Mamood and Yousaf (1985), Khan (1994) and Sexana and Tikku (1990).

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