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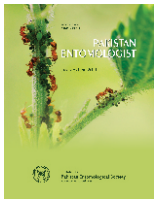

EFFECT OF TRIFLUMURON AND DIAFENTHURON ON THE HAEMOCYTES OF AMERICAN BOLLWORM, *HELICOVERPA ARMIGERA* (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The effect of triflumuron and diafenthuron (LC_{40}, LC_{50}, LC_{60}) on total and differential haemocyte was evaluated on 3rd and 5th instar larvae of *Helicoverpa armigera*. The total haemocyte count just after the application of LC_{50} of triflumuron in 3rd instar larvae increased (78900cells/mm^3), while decreased (83800 cells/mm^3) after half an hour, and increased again (90200 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3). The percentage of prohaemocytes decreased from normal (42% to 26%), whereas the percentage of plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 29%, 32%, 4% and 10% respectively after the application of insecticide. Similar results were recorded with 5th instar larvae. The total haemocyte count just after the application of LC_{50} of diafenthuron in 3rd instar larvae increased (82400 cells/mm^3) from the normal count, while decreased (79900 cells/mm^3) after half an hour, and increased again (84300 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 24%, 30%, 3% and 9% respectively), whereas the percentage of prohaemocytes, decreased from normal (42%, to 34% respectively) after the application of insecticide in 3rd instar larvae .

Keywords: Diafenthuron, *Helicoverpa armigera*, haemocyte, triflumuron

INTRODUCTION

Helicoverpa armigera is one of the most important and destructive pest of many crops that had a wide geographical distribution (Cunninghaml *et al*., 1999). It is widely distributed in Asia, Europe, Australia, Canada, Africa, Manitoba, Mexico, USA, Brazil, Peru, Argentina, and (Gowda, 2005; Shah and Shahzad, 2005). This polyphagous pest can damage more than 182 plant species including cotton, sunflower, chickpea, sorghum, groundnut, tobacco, alfalfa, clover, flax, maize and various vegetable crops like tomato, pepper, okra, lettuce, cabbage, eggplant, broccoli, beans, pea, fruits i.e. strawberry, watermelon and forest trees. (Ahmed *et al*., 2004; Gowda, 2005; Annonymous, 2007). Chemical control is the most commonly used method against this pest in Pakistan. These insecticides greatly affect the different systems of the insects especialy haemocytes (Haq *et al*., 2005).

Haemocytes play important role in insect defence system and have various physiological functions in the body due to which insect show immunity to foreign bodies and pathogens. One of the physiological function is the chemicals circulation within the insect body by these cells, thus certain chemical directly affect the defensive mechanisms of insects. An insecticide or a chemical applied on an insect will affect defence system by altering its haemocytes number. Different insecticides applied on different insects have been shown to affect the circulating cell types and shapes (Pandey *et al*., 2008; Qamar and Jamal, 2009; El Mohandes *et al*., 2010). For example, penfluron a chitin inhibitior makes an insect defenceless (Pugazhvendan & Soundararajan, 2009), but El Mohandes *et al*. (2010) reported that different type of pollen directly influence the defence mechanism of honey bee by altering the haemocytes number. Neem based insecticide

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applied on *Danais chrysippus* had been shown to reduce the phagocytic activity of immune system by a remarkable reduction in blood cells (Pandey *et al*., 2008). Acephate directly affected the immune system of *Dysdercus cingulatus* with the change in blood cell types (Qamar and Jamal, 2009). Nicotinyl insecticides were used against *Dysdercus koenigii* and different types of haemocytes and their varying number due to these insecticides was also observed (Haq *et al.*, 2005). Sabri and Tariq (2004) observed the abnormalities of the haemocyte cells after the application of some insecticides on the red pumpkin beetle. He observed cells rupturing and also cracking of cytoplasmic walls, abnormal staining of cells, denucleation, cells enlargement and blood cell deformation in the blood. Three different types of plant oils were used against *H. armigera* and observed that all the three oils affected the multiplication of haemocytes (Padmaja and Rao, 2000).

The objective of the present study was to examine the effect of triflumuron and diafenthuron, on the total haemocyte count, differential haemocyte count, and to observe the abnormalities caused by these insecticides in the haemocytes of *H. armigera.*

MATERIALS AND METHODS

Collection and rearing *of H. armigera*

A field population of *H. armigera* (third to fifth instar larvae) was collected from the fields of cotton, tomato in August 2011. This population was cultured at 25 ± 2 °C, 60 ± 5% RH with 16:8 (light :dark) cycle. The larvae were provided with artificial diet until pupation. The pupae were shifted in a plastic box lined with tissue paper. On adult emergence, individuals were transferred to transparent rearing jars and fed with 10% sugar solution. Nappy liner strips were hanged in the rearing cages for egg laying. Eggs were collected on daily basis. After hatching, neonates were shifted to the artificial diet. Third and fifth instar larvae of *H. armigera* were used in experiments.

Insecticides

Two insecticides, Alsystin® 480SC (triflumuron; Bayer Pakistan Pvt Ltd) used for borer and Polo®500SC (diafenthuron; Syngenta Pakistan Pvt Ltd) used for sucking insect were used in the experiment.

Toxicity Bioassay

Topical bioassays were conducted with 3rd and 5th instar larvae. Five concentrations of each insecticide were prepared in distilled water with Triton X-100 (50 µg / ml) as an additional surfactant. A control containing only water was also used. Each concentration was applied on individual insect with the help of micro-applicator at the rate of 3µl per insect. After the treatment one individual insect were shifted in a Petri dish containing artificial diet. Mortality data was noted after 7 days. Larvae that failed to respond to gentle contact with a fine brush was considered as dead. LC_{40}, LC_{50} and LC_{60} values were calculated by using the R software (R version 2.9.0).

The 3µl of LC_{40}, LC_{50} and LC_{60} concentrations of each insecticide were applied topically to 3rd and 5th instars larvae with the help of a microapplicator for heamocytes studies. Effect of these insecticides was observed immediately after application, after 30 minutes and after 60 minutes in terms of abnormalities in total haemocyte count and differential haemocyte count.

Differential Haemocyte Count

DHCs were estimated by taking a drop of haemolymph on a clean glass slide and preparing the smear. The air-dried smears were dip in methyl alcohol for 5-7 minuts. The smears were then air dried. Immerse the dried film in the Wright's stain for 15 minutes. Then Wash the slides with distilled water at pH 6.8. Neutralize the haemocyte contents in freshly prepared buffer solution of pH 6.6 for 15 minutes and air dried the slides. Counting was done with the help telecounter under Phase contrast microscope 10X. A minimum of 200 cells were counted each time and the percentage of various classes were calculated as described by Mahmood and Yousaf (1985).

Total Haemocyte Count

Neubauer haemocytometer was used for total haemocyte counting. Standard sampling of the haemolymph was carried out with a Thoma white blood cell diluting pipette. Haemolymph from the abdominal leg was collected on a glass slide and then quickly drawn into Thoma white blood cell diluting pipette upto mark 0.5. This was diluted 20 times with Toisson's solution (NaCl = 1.0 gm, Na_2SO_4 =8.0 gm, neutral glycerine = 30ml, Methyl violet =0.025 gm and distilled water =160ml) and Thoma white blood cell diluting pipette was filled upto mark II (Mahmood and Yousaf, 1985). This solution was properly stained with staining shaker for 5 minute it prevents the blood to coagulate. Three initial drops of haemolymph mixture were disposed off and one drop of haemolymph mixture was placed near the edge of the coverslip of the Neubauer haemocytometer (Jones, 1962). The counting chamber was filled automatically by capillary action. Haemocytometer was left for 5 minutes so that the blood cells could settle down and then observed the haemocytes under Phase contrast microscope 10X. The four corner squares of both chambers of haemocytometer were counted under low power followed by high power of microscope focusing the counting chamber. Cells of each group of 16 squares, touching bordering the bottom left hand side and the central line were counted. Cells touching the central line in the top and right hand side were not included in the count. Total haemocyte counting (THC) was done following the formula described by Jones (1962).

Actual number of cell per cubic mm = Average number of cell counted per square millimeter × depth × dilution per square millimeter.

Statical analysis

Lc_{40}, LC_{50} and LC_{60} values of the insecticides were determined with R version 2.9.0 (R Development Core Team, 2009).

RESULTS

Toxicity to 3rd and 5th instar larvae of *H. armigera*

Lc_{40}, LC_{50} and LC_{60} values of triflumeron against 3rd instar larvae were 8.67, 11.02 and 14.15 ppm respectively (Table 1). Silmilarly LC_{40}, LC_{50} and LC_{60} values for 5th instar larvae were 55.14, 138.37 and 365.03 ppm respectively (Table 1). LC_{40}, LC_{50} and LC_{60} values of diafenturon against 3rd larval instar were 134.28, 145.47 and 156.02 ppm respectively (Table 1). Similarly LC_{40}, LC_{50} and LC_{60} values for 5th instar larvae were 186.79, 206.43 and 221.40 ppm respectively (Tables 1).

Total Haemocyte Count For 3rd and 5th Instar Larvae Of untreated *H. armigera*

On an average there were 68175 blood cells/mm in the haemolymph of 3rd larval instar of *H. armigera* were observed. Similarly, in 5th instar larvae on an average 7428.5 blood cells/mm^3 haemocytes were recorded.

Differential Haemocyte Count For 3rd and 5th Instar Larvae Of untrated *H. armigera*

Percentages of differential haemocyte counts (DHC) in control larvae of 3rd instar were prohaemocyte (42.00%), spherulocytes (29%), plasmatocytes (18.00%), cystocytes (2.75%) and granulocytes (8.25%). The percentage of prohaemocyte is the highest (41.00%) followed by spherulocytes (25.75%), plasmatocytes (15.00%), oenocytoids (16.00%), cystocytes (3.5%) and granulocytes (2.25%) in 5th instar larvae.

Total and differential haemocyte counts in 3rd and 5th instar larvae of *H.armigera* treated with triflumeron.

Effect at Lc_{40}

The total haemocyte count just after the application of triflumuron in 3rd instar larvae increased (68175 cells/mm^3), while decreased (79900 cells/mm^3) after half an hour, and increased again (84600 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3) (Table 2). The percentage of prohaemocytes decreased from normal (42% to 34%), whereas the percentage of plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 24%, 30%, 3.5% and 9% respectively after the application of insecticide in 3rd instar larvae (Table 3). The total haemocyte count just after the application of triflumuron in 5th instar larvae increased (12247.5 cells/mm^3), while decreased(14195 cells/mm^3) after half an hour, and increased again (18000cells/mm^3) after one hour of application from the normal count (7428.5 cells/mm^3) (Table 2). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (15%, 25.75%, 3.5% and 2.25 to 30%, 31.5, 4 and 6 respectively), whereas the percentage of prohaemocytes and oenocytoids, decreased from normal (41% and 16% to 38% and 12% respectively) after the application of insecticide in 5th instar larvae (Table 3).

Effect at Lc_{50}

The total haemocyte count just after the application of triflumuron in 3rd instar larvae increased (78900cells/mm^3), while decreased (83800 cells/mm^3) after half an hour, and increased again (90200 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3) in 3rd

Table 1
Lc_{40}, LC_{50} and LC_{60} (ppm) Values of triflumuron and diafenthuron against 3rd and 5th Instar Larvae of *H. armigera.*

Insecticides	3rd instar				5th instar			
	LC_{40} (95%CI)	LC_{50} (95% CI)	LC_{60} (95% CI)	Slope± SE	LC_{40} (95% CI)	LC_{50} (95% CI)	LC_{60} (95% CI)	Slope± SE
triflumuron	8.74 (21.8- 3.5)	11.02 (24.1-5.03)	14.154 27.03-7.4)	1.65±0.52	55.14 (116- 26.21)	138.37 (568- 33.7)	221.40 (269- 182)	0.44±0.30
diafenthuron	134.28 (151- 119.4)	145.5 (161-132)	156.02 (165.5-147)	5.28±1.48	186.79 (206- 169.4)	206.43 (237- 180)	365.03 (666-200)	4.22±1.5

Table 2
Total number of haemocyte /mm in control and treated (with triflumuron) 3rd and 5th instars larvae of *H. armigera.*

Time Minutes	Control		LC_{40} (ppm)		LC_{50} (ppm)		LC_{60} (ppm)	
	Instars							
	3rd	5th	3rd	5th	3rd	5th	3rd	5th
0	68175	7428.5	76375	12247.5	78900	13525	87300	14125
30	68175	7428.5	79900	14195	83800	16575	92800	18400
60	68175	7428.5	84600	18000	90200	20450	99900	23525

instar larvae (Table 2). The percentage of prohaemocytes decreased from normal (42% to 26%), whereas the percentage of plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 29%, 32%, 4% and 10% respectively after the application of insecticide in 3rd instar larvae (Table 3).The total haemocyte count just after the application of triflumuron in 5th instar larvae increased (13525cells/mm^3), while decreased (16575 cells/mm^3) after half an hour, and increased again (20450 cells/mm^3) after one hour of application from the normal count (7428.5 cells/mm^3) (Table 2). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes and granulocytes increased from normal (15%, 25.75%, 3.5% and 2.25 to 34.5%, 318, 6 and 8 respectively), whereas the percentage of prohaemocytes and oenocytoids, decreased from normal (41% and 16% to 34% and 10% respectively) after the application of insecticide in 5th instar larvae (Table 3).

Effect at Lc_{60}

The total haemocyte count just after the application of triflumuron in 3rd instar larvae increased (87300cells/mm^3), while decreased (92800 cells/mm^3) after half an hour, and increased again (90200 cells/mm^3) after one hour of application from the normal count (99900 cells/mm^3) (Table 2). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 32%, 34%, 5% and 11% whereas the percentage of prohaemocytes, decreased from normal 42%, to 20% after the application of insecticide in 3rd instar larvae (Table 3). The total haemocyte count just after the application of triflumuron in 5th instar larvae increased (14125cells/mm^3), while decreased (18400 cells/mm^3) after half an hour, and increased again (23525 cells/mm^3) after one hour of application from the normal count (7428.5 cells/mm^3) (Table 2). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (15%, 25.75%, 3.5% and 2.25 to 38%, 40%, 7.5% and 8.5% respectively), whereas the percentage of prohaemocytes and oenocytoids, decreased from normal (41% and 16% to 30% and 8% respectively) after the application of insecticide in 5th instar larvae (Table 3).

Total and differential haemocyte counts in 3rd and 5th instar larvae of *H. armigera* treated diafenthuron

Effect at Lc_{40}

The total haemocyte count just after the application of diafenthiuron in 3rd instar larvae increased (82400 cells/mm^3) from the normal count, while decreased (79900 cells/mm^3) after half an hour, and increased again (84300 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3) (Table 4). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 24%, 30%, 3% and 9% respectively), whereas the percentage of prohaemocytes, decreased from normal (42%, to 34% respectively) after the application of insecticide in 3rd instar larvae (Table 5). Similarly, the total haemocyte count just after the application of diafenthiuron in 5th instar larvae increased (19020 cells/mm^3), while decreased (18172.5 cells/mm^3) after half an hour, and increased again (20135 cells/mm^3) after one hour of application from the normal count (7428.5 cells/mm^3) (Table 4). The differential haemocyte count, plasmatocytes, spherulocytes and cystocytes increased from normal (15%, 25.75% and 3.5% to 31%, 26.25% and 3.75% respectively), whereas the percentage of prohaemocytes and oenocytoids, decreased from normal (41% and 16% to 38% and 12% respectively) (Table 5).

Effect at Lc_{50}

The total haemocyte count just after the application of diafenthiuron in 3rd instar larvae increased (85200 cells/mm^3), while decreased (84080 cells/mm^3) after half an hour, and increased again (85980 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3) (Table 4). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 25%, 32%, 4.5% and 9.5% respectively), whereas the percentage of prohaemocytes, decreased from normal (42% to 29% respectively) after the application of insecticide in 3rd instar

Table 3
Differential haemocytes in control and treated with triflumuron 3rd and 5th instar larvae of *H. armigera.*

Differential haemocytes (%)	Control		LC_{40} (ppm)		LC_{50} (ppm)		LC_{60} (ppm)	
	Instars							
	3rd	5th	3rd	5th	3rd	5th	3rd	5th
PR	42	41	34	38	26	34	20	30
SP	29	25.75	30	31.5	32	38	34	40
PL	18	15	24	30	29	34.5	32	38
CO	2.75	3.5	3.5	4	4	6	5	7.5
GR	8.25	2.25	9	6	10	8	11	8.5
OE	0	16	0	12	0	10	0	8

Table 4
Total number of haemocyte/mm in control and treated with diafenthuron 3rd and 5th instars larvae of *H. armigera.*

Time (Minutes)	Control		LC_{40} (ppm)		LC_{50} (ppm)		LC_{60} (ppm)	
	Instars							
	3rd	5th	3rd	5th	3rd	5th	3rd	5th
0	68175	7428.5	82400	19020	85200	20122	87800	22512.5
30	68175	7428.5	79900	18172.5	84080	19452.5	82900	18912.5
60	68175	7428.5	84300	20135	85980	21075	88040	23235

Table 5
Differential haemocytes in control and treated with diafenthuron 3rd and 5th instar larvae of *H. armigera.*

Differential haemocytes %	Control		LC_{40} (ppm)		LC_{50} (ppm)		LC_{60} (ppm)	
	Instars							
	3rd	5th	3rd	5th	3rd	5th	3rd	5th
PR	42	41	34	38	29	36.5	23	34
SP	29	25.75	30	26.25	32	28	34	38
PL	18	15	24	31	25	33.5	28	36.5
CO	2.75	3.5	3	3.75	4.5	4.25	5	6
GR	8.25	2.25	9	3.25	9.5	5.25	10	6.75
OE	0	16	0	12	0	10.5	0	10

larvae (Table 5). Similarly, The total haemocyte count just after the application of diafenthiuron in 5th instar larvae increased (20122cells/mm^3), while decreased (19452.5 cells/mm^3) after half an hour, and increased again (21075cells/mm^3) after one hour of application from the normal count (7428.5 cells/mm^3) (Table 4). The differential haemocyte count, plasmatocytes, spherulocytes and cystocytes increased from normal (15%, 25.75% and 3.5% to 33.5%, 28% and 4.25% respectively), whereas the percentage of prohaemocytes and oenocytoids, decreased from normal (41% and 16% to 36.5% and 10% respectively) in 5th instar larvae (Table 5).

Effect at Lc_{60}

Similarly, the total haemocyte count just after the application of diafenthiuron in 3rd instar larvae increased (87800 cells/mm^3), while decreased (82900 cells/mm^3) after half an hour, and increased again (88040 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3) (Table 4). The differential haemocyte count, plasmatocytes, spherulocytes, cystocytes, and granulocytes increased from normal (18%, 29%, 2.75% and 8.25% to 28%, 34%, 5% and 10% respectively), whereas the percentage of prohaemocytes, decreased from normal (42%, to 23% respectively) after the application of insecticide in 3rd instar larvae (Table 5). Similarly, the total haemocyte count just after the application of diafenthiuron in 5th instar larvae increased (22512.5 cells/mm^3), while decreased (18912.5 cells/mm^3) after half an hour, and increased again (23235 cells/mm^3) after one hour of application from the normal count (7428.5 cells/mm^3) (Table 4). The differential haemocyte count, plasmatocytes, spherulocytes and cystocytes increased from normal (15%, 25.75% and 3.5% to 36.5%, 38% and 6% respectively), whereas the percentage of prohaemocytes and oenocytoids, decreased from normal (41% and 16% to 34% and 10% respectively) in 5th instar larvae (Table 5).

Abnormalities in the shapes of haemocytes after the insecticide application

After the application of insecticide following abnormalities were observed in the haemocytes of American bollworm i) Enlargement of haemocyte cells, ii) agglutination of haemocytes, iii) Denucleation of haemocytes, iv) Abnormal staining of the haemocyte cells, v) Deformation of the haemocyte shapes, vi) Rupturing of the cell wall.

DISCUSSION

In the present study, total and differential haemocytes were count in the haemolymph of 3rd and 5th instar larvae of *H. armigera*. In the untreated larvae of 3rd instar an average number of 68175 blood cells /mm^3 and in 5th instar 7428.5 blood cells/mm^3 haemocytes were recorded. The percentage of prohaemocyte in 3rd instar is (42.00%), spherulocytes (29%), plasmatocytes (18.00%), cystocytes (2.75%) and granulocytes (8.25%) while in 5th instar prohaemocytes are (41.00%) followed by spherulocytes (25.75%), plasmatocytes (15.00%), oenocytoids (16.00%), cystocytes (3.5%) and granulocytes (2.25%). Oenocytoids are not found

in 3rd instar.

Haemocytes responded toward the insecticide application. The total haemocyte count increased soon after application of difenthiron and triflumuron, then decreased after half an hour, and increased again after one hour. Similar results were reported by Fareed (2001) who used Tracer 480 SC on the spotted boll worm, *Earias spp*. and noted that haemocyte number increased after 0 minutes, decreased after half an hour and again increase after one hour. Similarly, Nawaz (2009) also observed the effect of Sevin 85 SP on the haemocytes of *Chrotogonus trachypterus* and reported that total haemocytes increased just after the application of insecticides again increase after half an hour and more increase was observed after an hour. Ftima *et al*. (2013) also reprted the similar effects of flubrndiamide and spiroteramat on the haemolymph of *H. armigera*. These results are in partial contradiction with the findings of Bibi (2001) who observed that after the application of Polo on adult *Apis mellifera* L. total haemocytes increased after 0 minutes, again increased after half an hour and decrease after an hour from the normal count.

The percentage of differential haemocytes fluctuated up and down from the normal count. This fluctuation may be due to the destruction caused by the application of insecticides. The percentage of plasmatocytes increased after the application of insecticide from the normal. These results are similar with the findings of Iqbal (2002) who reported that the percentages of plasmatocytes increased from normal when Tracer 240 SC was applied to the brinjal fruit borer *Leucinodes orbonalis* (GUEN).The plasmatocyres play more efficient role in phagocytosis. When the foreign particals were injected in the insects, these free blood cells increased in number, phagocytosed the foreign materials and enhanced the defensive system of the insect making it more resistant against insecticides. In conclusion triflumuron and diafenthiron affect the haemocytes in the haemolymph of *H.armigera*.

REFERENCES

Ahmed, S., K. Zia and N.R. Shah, 2004. Validation of chemical control of gram pod borer, *Helicoverpaarmigera* (Hub.) with new insecticides. Int. J. Agri. Bio., 6: 978-980.

Annonymous, 2007. Report of a pest risk analysis *Helicoverpa armigera* (Hübner, 1808). Plant Protection Services (Netherland) and Central Science Laboratory (UK) joint pest risk analysis of *Helicoverpa armigera*. pp. 1-18. http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/helicoverpa.pdf.

Bibi, R., 2001. Toxicity of some insecticide on the haemocytes of honeybee (*Apis mellifera* L). M.Sc. Thesis, Deptt. Agri. Ento., Univ. Agri., Faisalabad.

Cunninghaml, J.P., M.P. Zalucki and S.A. West, 1999. Learning in *Helicoverpa armigera* (Lepidoptera: Noctuidae): a new look at the behaviour and control of a polyphagous pest. *B*ull. Entomol. Res., 89: 201–207.

El-Mohandes, S.S., E.A. Nafea and A.M. Fawzy, 2010. Effect of different feeding diets on the haemolymph of the newly emerged honeybee workers *Apis mellifera* L. Egypt. Acad. J. Biol. Sci., 3: 213-220.

Fareed, T., 2001. Toxicity of some insecticides on the haemocytes of spotted bollworm, *Earias spp*., and American bollworm, *Helicoverpa armigera* of cotton. M.Sc. Thesis, Dept. Agric. Ento., Univ. Agric., Faisalabad.

Fatima, M., M. Tariq and A. Gulzar, 2013. Effect of flubendiamide and spirotetramat on the haemocytes of American bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). Pak. Entomol., 35: 129-134.

Gowda, C.L.L., 2005. *Helicoverpa*-The global problem. In: *Heliothus/ Helicoverpa* management emerging trends and strategies for future reasearch. Sharma, H. C. (ed). pp 1-6. Sci. Pub, Inc. UK.

Haq, R.M., M.A. Sabri, and A. Rashid, 2005. Toxicity of nicotinyl insecticides on the haemocytes of red cotton bug, *Dysdercus Koenigii* (Fb.) (Pyrrhocoridae: Hemiptera). J. Agri. Soc. Sci., 1813–2235.

Iqbal, J., A. Suhail., Z. Abdin, and A.H. Anjum, 2002. Toxicity of Tracer 240SC on the haemocytes of last larval instar of brinjal fruit borer, *Leucinodes orbonalis* (GUEN). Pak. Entomol., 24: 115-119.

Jones, J.C., 1962. Current concepts concerning insect haemocytes. Amer. Zool., 2: 209- 46.

Mahmood, A. and N. Yousaf, 1985. Effect of some insecticides on the haemocytes of *Gryllus bimaculatus* de Geer. Pak. J. Zool., 17: 71 -84.

Nawaz, A., 2009. Effect of some insecticides on the haemocytes of *Chrotogonus trachypterus* Blanchard (Orthoptera: Pyrgomorphidae). M.Sc. Thesis, Dept. Agric. Ento., Univ. Agric., Faisalabad.

Padmaja, P.G. and P.J. Rao, 2000. Effect of plant oils on the total haemocyte count (THC) of final instar larvae of *Helicoverpa armigera* Hübner. J. Pestic Res., 12: 112-116.

Pandey, J.J., R.K. Tiwari, and D. Kumar, 2008. Reduction in haemocytes mediated immune response in *Danais chrysippus* following treatments neem based insecticides. *J*. Entomol., 5: 200-206.

Qamar, A. and K. Jamal, 2009. Differential haemocyte counts of 5th instar nymphs and adults of *Dysdercus cingulatus* Fabr. (Hemiptera: Pyrrhocoridae) treated with acephate, an organophosphorus insecticide. Biol. Med., 1 (2): 116-121.

Sabri, M. A. and B. Tariq, 2004. Toxicity of some insecticides on the haemocytes of red pumpkin beetle, *Aulacophora foveicollis* Lucas. Pak. Entomol., 26(1): 109-114.

Shah, Z.A. and M.K. , Shahzad, 2005. Population fluctuations with reference to differen developmental stages of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on chickpea and their relationship with the environment. Int. J. Agri. Bio., 7: 90-93.