Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 533-537

Received: 01-03-2019
Accepted: 05-04-2019

Muhammad Irfan
1. Department of Agri-Entomology, University of Agriculture, Faisalabad, Faisalabad, Pakistan
2. Department of Entomology, University of Sargodha, Sargodha, Pakistan

Muhammad Altaf Sabri
Department of Agri-Entomology, University of Agriculture, Faisalabad, Faisalabad, Pakistan

Asad Abdullah
Department of Entomology, University of Sargodha, Sargodha, Pakistan

Muhammad Jaffar Hussain
Department of Entomology, University of Sargodha, Sargodha, Pakistan

Muhammad Younus Ahmdani
Department of Agronomy, University of Sargodha, Sargodha, Pakistan

Correspondence
Asad Abdullah
Department of Entomology, University of Sargodha, Sargodha, Pakistan

Quantitative changes of heamocytes in *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) larvae in response to different insecticides

Muhammad Irfan, Muhammad Altaf Sabri, Asad Abdullah, Muhammad Jaffar Hussain and Muhammad Younus Ahmdani

Abstract
Quantitative studies concerning abnormalities and hemolytic activity was performed under laboratory conditions for larvae of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Four insecticides (decarafluron 50EC, emamectin benzoate 1.9EC, chlorpyrifos 40EC and abamectin 1.8EC) were studied for the immunosuppressive activity of 5th instar larvae of *S. litura*. Immunosuppression was assessed by examining changes in total heamocytes count and differential heamocytes count. Newly ecdysed 5th instar larvae (normal) total heamocytes count of 1.6x10^3 cells/mm^3. But a great decline in total heamocytes count was observed by the application of decarafluron and drastically increase due to the application of abamectin. From five different types of heamocytes, proheamocytes were found the most sensitive to insecticidal stress and rupturing of the cell wall was identified as the most common abnormality. The observations suggest that insecticides are capable of inducing multiple forms of cell death in *S. litura* larvae.

Keywords: *Spodoptera litura*, differential heamocytes, total heamocytes, insecticides

Introduction
Common cutworm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) is considered as one of the most damaging and economic pests of many agricultural areas because of its high destruction and heavy losses to the cultivated crops [16]. It is a polyphagous insect [9] and has a wide host range like cotton, okra, potato, tomato and pumpkin [25]. The larvae feed on the undersurface of the leaves and gregariously skeletonize them leaving only the midrib and veins in severe cases as well as damage flowers, and squares causing significant losses [5]. *S. litura* can cause crop losses at the critical stage of the crop and due to its high level of infestation in the field [3].

Most of the farmers use synthetic insecticides to suppress the population of *S. litura* in Pakistan. However, the application of insecticide also affect the hemolytic activity in the insect body and the response of heamocytes can vary to diverse pesticides with the passage of time and at the applied dose [24, 21, 2]. The resistant mechanism of arthropods which contains cellular and humoral protection abilities; later on, comprise the encapsulation and phagocytosis [15, 22]. The insect blood has considered a crucial pathway for the transmission of resources to different organs in the body. The heamocytes count in insect hemolymph may affect due to both internal as well as by external factors that depend on oldness, level, and gender detection technique for various body part and structures with respect to the approaches and devotions [10]. Previously, findings indicate the seven main classification of heamocytes and later suggested six [8]. These are known as plasmatocytes, proheamocytes, granulocytes, plasmatocytes spherulocytes, podocytes, oenocytoids, and granular plasmatocytes. Vermiform and podocytes cells were categorized in a similar category as the podocytes [13] due to the morphologically converted in sphere-shaped these cells having extensions through the evolution. These cells are dissimilar to the plasmatocytes due to the characteristics [12]. Due to having spreading properties on the glass slide granular plasmatocytes known as plasmatocytes. Due to variation in studying heamocytes, there was a difference in cell counting and in the method of detection [20] time period and sexual characteristics of various insects [6, 16].

Insects have only extracellular fluid, hemolymph, in contrast to vertebrates, so it severs the functions of both blood and lymph [8].

The different type of heamocytes performs very important services in the insect structure. They provided direct nutritional material to different muscles and also work as a food reservoir. The most frequently mentioned phagocytosis enclosed the imported elements in the insect body, clotting the injured part to prevent hemolymph loss, node formation, transport of nutritional material carrying hormones, transport of food element and possibly hormones for the detoxification of metabolism [15]. The study of hemolytic activity in response to the insecticidal application can be useful to check the resistance and susceptible insect species to those insecticides and at a different time interval. Similarly, %age of phagocytes in various heamocytes count could deliver important facts for the insect resistance for pesticides. The study related to the impact of insecticides on the hemolytic activity of insects is important to determine the control recommendations against that insect species. Keeping in view the differentiation in hemocyte categories and their counting due to insecticidal pressure, the experiment was conducted for the evaluation of THC, DHC and the deformities produced in *S. litura* larvae.

Materials and Methods

Insect

Collection of *S. litura* larvae was carried out from the experimental site department of Entomology, University of Agriculture Faisalabad. These insects were reared in sterilized cages diameter (46cm x 46cm x 46cm) at 32±2°C temperature and 65±5% relative humidity in the ecotoxicology laboratory of Entomology, where they were fed on fresh cotton leaves to get full grown larvae for research. The larvae were fed daily obtained molting success and the next generation of larvae was used for the further experiment.

Application of insecticides

Four different insecticides were used under the recommended concentrations in the field condition (Table 1). The fully grown (5th instar) caterpillars were placed in Petri dishes, and pesticides were applied typically on the thoracic region of larvae with a micro-applicator. The effect on total heamocytes count (THC), differential heamocytes count (DHC), and in terms of deformities was noted just after application, after 30 and 60 minutes of the insecticides exposure. Samples of hemolymph were dissected with a sterilized micro-needle syringe by removing the abdominal leg carefully with a sterilized pair of scissors [10]. The thin film after the air-dried treated for five to seven minutes in methyl alcohol with second time drying. The thin layers were absorbed in the Giemsa stain for 15 minutes and clean it with sanitized H_2O. Finally, the slides were attached with Canada balsam to fix the blood.

Differential heamocytes counts (DHC)

The fixed hemolymph was examined under oil immersion phase microscope and was identified the different types of heamocytes and counted by tele-counter battlement method [16]. The smear was divided into three transverse fields along the edge, two fields up, two in the center of the smear and two down, starting from the thin end. The percentages of different types of heamocytes were calculated from 200 examined cells from these fields [10].

Total heamocytes count (THC)

The pesticides application carried out on the full-grown larvae in a petri dish, first and second drops of hemolymph were drawn on slide [11] and then quickly dipped in Thoma White Blood Cell Pipette up to 0.5 marks and then diluting solution (Glacial acetic acid, one ml; distilled water, 100 ml; gentian violet, 0.3 percent) was added in pipette up to mark II, thus making the 20 time dilution of the solution. After discarding the first three drops, one-two drops of the solution were placed on the Neuberger's chamber (Thoma-Zeiss hemocytometer) with a coverslip for total heamocytes counts (THC). The cells' counting was started from the corners containing 16 cubes (white cell) for each division of two chambers. THC was calculated by using the formula suggested by Jones [10]. Five counts were made as replication for each treatment to assess the increase or decrease in THC in standard environments at different time intervals.

The experiment was repeated twice with four replications for each under the arrangement of completely randomized design (CRD). The data were subjected to statistical analysis using analysis of variance technique to check the significance of insecticides application at different time interval on insect hemolytic activity.

Results

Built on size and shape of nucleus and soma, presence of cytoplasm, type, size, and number of inclusion, five different heamocytes [granulocytes (GR), proheamocytes (PR), oenocytoids (OE), spherulocytes (SP) and plasmoheamocytes (PL)] were identified in hemolymph of *S. litura* larvae. Plasmatocytes were small to large polymorphic cells. The nucleus may be round or elongate and centrally located. Spherulocytes (SP) cells were rounding generally larger than granulocytes and nucleus were small, centrally or eccentrically located. Prohaemocytes (PR), was oval, round or elliptical and were smaller than the other cells. The nucleus was compact, large in relation to cell size. Oenocytoids (OE) were varied in size and shape. The cytoplasm was granular, thick and homogeneous. Granulocytes (GR) were small to large, oval or spherical cells and cytoplasm was granular.

There was a significant variation in the hemolytic movement of *S. litura* larvae after application of different insecticides after a different time interval. There was also a significant interaction between the treatment and time interval (Table 2).

Heamocytes in normal larvae

There were 16450 blood cell/mm^3 of total heamocytes count in normal *S. litura* larvae (Table 2). Differential heamocytes count in normal *S. litura* larvae showed the maximum 44.5% of proheamocytes, tracked by proheamocytes 31.88%, granulocytes 14.5%, oenocytoids 6.8% and spherulocytes 2.31% (Figure 1).

Toxicity of decarafluron on THC and DHC of *S. litura*

Total hemocyte count (THC) decreased to 10450 blood cell/mm^3 immediately after insecticide exposure and continuously decreased to 8850 cells/mm^3 and 8050 cell/mm^3 after 30 and 60 minutes of exposure (Table 3). The %age proportion of proheamocytes, granulocytes, and oenocytoids was maximum than the average i.e., 44.5%, 14.5% and 6.81% to the percentage of 62.25%, 18%, and 9.18% respectively after exposure of decarafluron (Table 4).

Toxicity of chlorpyrifos on THC and DHC of *S. litura*

Total hemocyte count (THC) increased to 19775 cell/mm^3 immediately after application of chlorpyrifos and this increasing trend continued with the passage of time. After 30

minutes, THC reached up to 18987.5 cell/mm^3 and increased more to 21012.5 cell/mm^3 after 60 minutes of exposure (Table 3). The percent amount of plasmatocytes, oenocytoids, and spherulocytes increased by the common i.e., 31.8%, 6.81% and 2.31% to the %age of 37.12%, 8.81%, and 2.93% respectively after chlorpyrifos exposure (Table 4).

Toxicity of emamectin benzoate on THC and DHC of *S. litura*

After exposure to emamectin benzoate, THC decreased to 15000 cell/mm^3 immediately and reached 13250cells/mm^3 and 12450 cell/mm^3 after 30 and 60 minutes respectively (Table 3). The percent prohaemocytes, granulocytes, with the increase of oenocytoids than the average amount i.e., 44.5%, 14.5% and 6.81% to the percentage of 50.56%, 18.94%, and 10.68% respectively after exposure of emamectin benzoate (Table 4).

Toxicity of Abamectin on THC and DHC of *S. litura*

THC drastically increased to 24187.5 cell/mm^3 immediately after exposure and decreased slowly to 20550cells/mm^3 and 18267.5 cell/mm^3 after 30 and 60 minutes of exposure but THC was higher as compared to normal larvae containing 16450 cell/mm^3 (Table 3). Only the percentage of plasmatocytes and oenocytoids was maximum to 40.13% and 11.06% from normal having 31.88% and 6.81% respectively. The percent amount of proheamocytes, granulocytes, and spherulocytes decreased to 36.5% 11.43% and 0.87% as compared to normal larvae having 44.5%, 14.5%, and 2.31% respectively (Table 4).

Table 1: Detail of insecticides and their concentrations used in the experiment

Trade Name	Dose (100 L of water/acre)	Concentration (%)
Decarafluron (Match 50EC)	250ml	0.25
Chlorpyrifos (Lorsban 40EC)	1000ml	1
Emamectin benzoate (Timer 1.9EC)	200ml	0.2
Abamectin (Cure1.8EC)	200ml	0.2

Table 2: Toxicity of different insecticides on heamolymph of *S. litura* larvae after a different time interval

S.O.V	d.f	F value	P value
Treatments (A)	4	460.6	*P<0.001*
Time interval (B)	2	36.7	*P<0.001*
A x B	8	12.4	*P<0.001*
Error	45		
Total	59		

P<0.001 showed the significance

Table 3: Toxicity of different insecticides on the total hemocyte count of *S. litura* larvae

Insecticides	Time Interval	THC (cells/ mm^3)
Decarafluron	Immediately	10450
	After 30 minutes	8850
	After 60 minutes	8050
Emamectin benzoate	Immediately	15000
	After 30 minutes	13250
	After 60 minutes	12450
Chlorpyrifos	Immediately	19775
	After 30 minutes	18987.5
	After 60 minutes	21012.5
Abamectin	Immediately	24187.5
	After 30 minutes	20550
	After 60 minutes	18267.5
Control		16450

THC=total heamocytes count

Table 4: Percentage of differential heamocytes count (DHC) of *S. litura* larvae after exposure to different insecticides

Insecticides	PR (%)	PL (%)	GR (%)	OE (%)	SP (%)
Decarafluron	62.25	9.5	18	9.18	1.06
Emmamectin benzoate	50.56	19	18.94	10.68	0.81
Chlorpyrifos	39.56	37.12	11.56	8.81	2.93
Abamectin	36.5	40.13	11.43	11.06	0.87
Control	44.5	31.88	14.5	6.81	2.31

PR= proheamocytes, PL= plasmoheamocytes, GR=granulocytes, OE=oenocytoids, and SP= spherulocytes

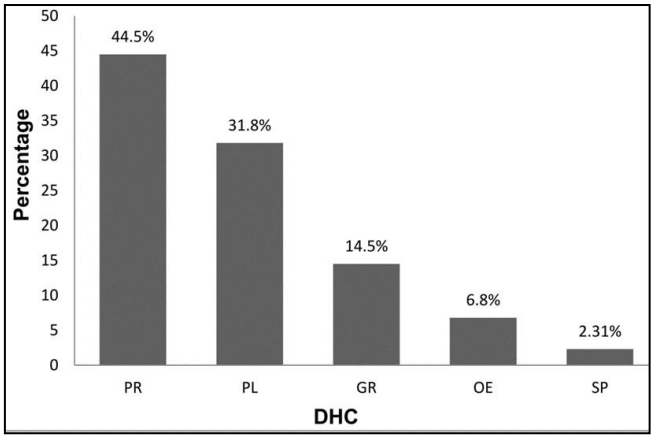

Fig 1: Differential heamocytes count in normal *S. litura* larvae, DHC=differential heamocytes count, PR= proheamocytes, PL= plasmoheamocytes, GR=granulocytes, OE=oenocytoids and SP=spherulocytes

Discussion

There were observed five various forms of *S. litura* larvae and the heamocytes differential counts fluctuated after insecticides exposure with the passage of time. For DHC, the only oenocytoids percentage increased from normal in all case of insecticides. Proheamocytes and granulocytes percentage was maximum after the applying of decarafluron and emamectin benzoate but decreased after application of chlorpyrifos and abamectin as compared to normal. However, the percent amount of spherulocytes recorded more than average only when applying chlorpyrifos while decreased in case of all other insecticides. Previously research done on the effect of insecticides on different insect species also showed the changes in DHC of different insect species. According to Sexana *et al*. [23] plumbagin decreased the plasmatocyte percentage in *Dysdercus koenigii* Fb. Similarly, Khan [11] observed that granulocytes and oenocytoids decreased from normal due to the application of curacron 500EC. Rizwan-ul-

Haq *et al*. [18] indicated the reduction of all DHC from normal of *D. koenigii* Fb. after application of endosulfan 35EC. After insecticide exposure, the increase in heamocytes count might be due to the recovery of the immune system to hazards [19, 4]. Furthermore, the decline in hemocyte counts might depend on test insect, different instar of insects, insecticide, and concentration tested [24]. Such variation in different heamocytes count under exposure to different test insecticides was also observed by Al-Hariri & Suhail, [1], Fatima *et al*. [4]. Plasmatocytes and granulocytes are considered as important constituent among all heamocytes in cell-mediated immunity, while there was an interaction between other hemocyte categories with plasmatocytes and granulocytes that participate in the immunity reaction [14]. Together plasmatocytes and granulocytes accomplish the immunity function linked with phagocytosis and encapsulation in most of lepidopterous and some coleopterous insects [15, 17]. Generally, findings for THC revealed an instant decline with decarafluron and emmamectin benzoate and while the maximum was in others remain. After half an hour, THC also decreased in decarafluron and emmamectin benzoate and increased for other pesticides. Similar results for THC were observed after one hour of insecticides exposure. The changes in the counting of cells may depend on the procedure of heamocytes reviewing, an inspection of permanent and changed heamocytes [20] and different instars of insects [6]. To increase the heamocytes count under insecticides stress indicates their capability to endure the exterior conservational pressures [16, 9]. Heamocytes as sensitive objects in the hemolymph of insect have a variable response to a different type of insecticides. It may oscillate increased or decreased and showed the response to a resistant mechanism for the pesticides stresses [15]. The abnormalities observed later on the applying of various pesticides were bursting of the cell wall, cohesion of the cells, cells enlargement, cells denucleation and displaced the nucleus on one side of the cell. Most of the researchers Ayub [2], Al-Hariri & Suhail, [1]. Confirmed abnormalities in hemocyte under insecticide stress. Iqbal [27], Sabri and Tariq [28] documented prohaemocytes sufficient quantity in the larvae blood, these results are in accordance with the current study aginst the larvae. Alhariri [29], Sharma *et al*. [30], Gelbi *et al*. [31] who reported the similar classifications of insect blood cells for particular individuals. Zhang *et al*. [26] also supported the current study they concluded that heamocytes in the pool of hemolymph are complexed portions. Gad and Abdel-Megeed [34] who recorded decreased in heamocytes total and differential counts by the spinosad and emamectin benzoate against the *S. littoralis*. These results are also in agreement with Zibaee *et al*. [33], Sayah [35]. That after the application of pyriproxyfen the reduction in the haemocytes circulation occurred including the maximum quantity of plasmatocytes and granulocytes. These findings are also in line with Abdel-Rahman and Abou-Taleb [32]. They revealed that lufenuron and chlorfluazuron showed significant results against *S. litura* 2nd instar larvae when counting the heamocytes.

Conclusions

The impact of insecticides on total and differential heamocytes counts and abnormalities in *S. litura* larvae showed variable response when detected immediately, thirty and sixty minutes of exposure. THC showed an immediate decrease when decarafluron and emmamectin benzoate was applied while increased in all other insecticides. However, THC value was greater than normal after application of chlorpyrifos and Abamectin, even after one hour. Intended for differential hemocyte counts, only oenocytoids exposure was maximum to all applied pesticides. Furthermore, different abnormalities such as agglutination of the cells, cells enlargement and cells denucleation were observed. Bases on this study it is concluded that the insecticides significantly affect the hemolymph and immune system of insects and cause abnormalities in the insect body. The current experiment findings can be advantageous to select an appropriate insecticide for the management of *S. litura.*

Abbreviations

THC : total heamocytes count
DHC : differential heamocytes count
PR : proheamocytes
PL : plasmoheamocytes
GR : granulocytes
OE : oenocytoids
SP : spherulocytes

References

1. Al-Hariri MK, Suhail A. Effect of lambda-cyhalothrin and deltamethrin on the heamocytes of the desert locust, *Schistocerca gregaria* Forsk. International Journal of Agriculture Biology. 2001; 3:81-84.
2. Ayub S. Effect of some organophosphates on the heamocytes of mango mealy bug, *Drosicha stebbingi* Green. (M.Sc. thesis). Dept. Agri. Entomology, University of Agriculture, Faisalabad, Pakistan, 1996.
3. Dhir BC, Mohapatra HK, Senapati B. Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (Fab.). Indian Journal of Plant Protection. 1992; 20:215-217.
4. Fatima M, Tariq M, Gulzar A. Effect of triflumuron and diafenthuron on the heamocytes of American bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). Pakistan Entomologist. 2014; 36:51-56.
5. Ghumare SS, Mukherjee SN. Performance of *Spodoptera litura* Fabricius on different host plants: influence of nitrogen and total phenolics of plants and mid-gut esterase activity of the insect. Indian Journal of Experimental Biology. 2003; 41:895-899.
6. Gilliam M, Shimanuki H. Progress report: studies of honeybee blood. American Bee Journal. 1967; 10:249-256.
7. Gupta AP, Sutherland DJ. Effect of sublethal doses of Chlordane on the haemocytes and midgut epithelium of *Periplaneta americana*. Annals of the American Thoracic Society. 1968; 61:910-918.
8. Gupta AP. Insect heamocytes: development, forms, functions and techniques. Cambridge University Press., New York, 1979, 614.
9. Holloway JD. The moths of Borneo: family Noctuidae, trifine subfamilies: Noctuinae, Heliothinae, Hadeninae, Acronictinae, Amphipyrinae, Agaristinae, Amphipyrinae, Agaristinae. Malayan Nature Journal. 1989; 42:57-226.
10. Jones JC. Current concepts concerning insect haemocytes. American Zoology. 1962; 2:209-246.
11. Khan MMS. Effect of some insecticides on the haemocytes of brinjal fruit borer, *Leucinodes orbonalis* (Guen.). Journal of Entomology. 1994; 41:30-223.
12. Kurihara Y, Shimazu T, Wago H. Classification of Heamocytes in the Common Cutworm, Spodoptera litura

(Lepidoptera: Noctuidae) I. Phase Microscopic Study. Japanese Society of Applied Entomology and Zoology. 1992; 27:225-235. DOI: 10.1303/aez.27.225.
13. Kroeger PT, Wingert RA. Using zebrafish to study podocyte genesis during kidney development and regeneration. Genesis. 2014; 52(9):771-792.
14. Kwon H, Bang K, Cho S. Characterization of the heamocytes in Larvae of *Protaetia brevitarsis* seulensis: involvement of granulocyte-mediated phagocytosis. PLoS ONE. 2014; 9(8):103620.
15. Lavine MD, Strand MR. Insect heamocytes and their role in immunity. Insect Biochemistry and Molecular Biology. 2002; 32:1309-1295.
16. Mahmood A, Yousuf M. Effect of some insecticides on the haemocytes of *Gryllus bimaculatus* de Geer. Pakistan Journal of Zoology. 1985; 17:71-84.
17. Manachini B, Arizza V, Parrinello D, Parrinello N. Heamocytes of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) and their response to *Saccharomyces cerevisiae* and *Bacillus thuringiensis.* Journal of Invert Pathology. 2011; 106:360-365.
18. Rizwan-ul-Haq M, Rashid A, Sabri, MA. Toxic effects of endosulfan 35 Ec and deltaphos 350+10 Ec on the haemocytes of red cotton bug, *Dysdercus koenigii* (Fb.) (Pyrrhocoridae: Hemiptera). Journal of Agriculture and Social Sciences. 2006; 2(1):29-32.
19. Rizwan-ul-Haq M, Sabri MA, Rashid A. Toxicity of nicotinyl insecticides on the haemocytes of red cotton bug, *Dysdercus koenigii* (Fb.) (Pyrrhocoridae: Hemiptera). Journal of Agriculture and Social Sciences. 2005; 1:239-241.
20. Rosenberger CR, Jones JC. Studies on total blood cell counts of the southern armyworm larva, *Prodenia eridania* (Lepidoptera). Annals of the Entomological Society of America. 1960; 53:351-355.
21. Sabir MA, Rauf A. The haemocytes of castor hairy caterpillar, *Euproctis fraterna* (Moore). Pakistan Entomologist. 1994; 17:40-42.
22. Schmidt O, Theopold U, Strand MR. Innate immunity and evasion by insect parasitoids. Bio Essays. 2001; 23:344-351.
23. Sexana BP, Tikku K. Effect of plumbagin on haemocytes of *Dysdercus koenigii* F. Proceeding: Animal Sciences. 1990; 99(2):24-119.
24. Shukla K, Bahadur J. Effect of dichlorovos on haemocytes morphology in the male *Poekilocerus pictus*. Folia morphology (Pargue.). 1986; 37:7-142.
25. Ullah MI, Arshad M, Afzal M, Khalid S, Saleem M, Mustafa I *et al*. Incidence of *Spodoptera litura* (Lepidoptera: Noctuidae) and its feeding potential on various citrus (Sapindales: Rutaceae) cultivars in the Sargodha region of Pakistan. Florida Entomologist. 2016; 99(2):192-195.
26. Zhang Z, Ye GY, Cai J, Hu C. Comparative venom toxicity between *Pteromalus puparum* and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) toward the heamocytes of their natural hosts, non-Target insects and cultured insect cells. Toxicon. 2005; 46(3):337-349.
27. lqbal M. Toxicity of some insecticides on the haemocytes of army worm, (*Spodoptera litura* F.). M. Sc. Thesis, Deptt. Agri. Entomol., Univ. Agri, Faisalabad, 2002.
28. Sabri MA, Tariq B. Toxicity of some insecticides on the haemocytes of red pumpkin beetle, *Aulacophora foveicollis* Lucas. Journal of Pakistan Entomologist. 2004; 26:109-114.
29. Alhariri MK. Toxicity of some insecticides on the haemocytes of desert locust, *Schistocerea gregaria* F. M. Sc. Thesis, Department of Agricultural Entomology, university of Agriculture, Faisalabad, 2000.
30. Sharma PR, Sharma P, Saxena BP. Effect of Neem gold on the haemocytes of the tobacco armyworm, *Spodoptera litura* F. (Lepidoptera; Noctuidae). Current Science. 2003; 84(5-10):690-695.
31. Gelbi J, kova S, Berger J. Influence of metyrapone on the morphology of haemocytes of the Egyptian cotton leaf worm *Spodoptera littoralis.* Toxicology. 2006; 45(3):371-377.
32. Abdel-Rahman SM, Abou-Taleb HK. Joint action of spinosad and spinet ram with certain IGR compounds against cotton leaf worm. Alexandria Journal of Agriculture and Research. 2007; 52:45-51.
33. Zibaee A, Zibaee I, Sendi JJ. A juvenile hormone analog, Pyriproxifen, affects some biochemical components in the hemolymph and fat bodies of Eurygaster integriceps Puton (Hemiptera: Scutelleridae). Pesticide Biochemistry and Physiology. 2011; 100:289-298.
34. Gad AA, Abdel-Megeed A. Effects of spinosad and emamectin benzoate on the blood picture and DNA structure of the cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Egyptian Science Magazine. 2006; 3:75-80.
35. Sayah F. Ultrastructural changes in the corpus allatum after azadirachtin and 20-hydroxyecdysone treatment in adult females of *Labidura riparia* (Dermaptera). Tissue Cell. 2002; 34:53-62.