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Effect of Thiacloprid and Imidacloprid on the Haemocytes of American Bollworm, *Helicoverpa armigera*

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Abstract: The effect of thiacloprid and imidacloprid on total and differential haemocyte was evaluated on 3rd and 5th instar larvae of *Helicoverpa armigera*. The total haemocyte count just after the application thiacloprid in 3rd instar larvae increased (81300 cells/mm^3), while decreased (72800 cells/mm^3) after half an hour and increased again (84000 cells/mm^3) after one hour of application. The differential haemocyte count, plasmatocytes increased from normal 18% to 32%, whereas the percentage of prohaemocytes and granulocytes decreased (42% and 8.25% to 23% and 5% respectively) and percentages of spherulocytes and cystocytes, increased from normal (29% and 2.75% to 34% and 6.5% respectively. Similar results were recorded with 5th instar larvae. The total haemocyte count just after the application of thiacloprid and imidacloprid in 3rd instar larvae increased (76800 cells/mm^3), while decreased (46200 cells/mm^3) after half an hour and increased again (80000 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3).The differential haemocyte count, plasmatocytes increased from normal 18% to 39%.The percentage of prohaemocytes, spherulocytes, cystocytes and granulocytes decreased from normal 42%, 29%, 2.75% and 8.25% to 34%, 21%, 1% and 5% respectively after the application of thiacloprid and imidacloprid in 3rd instar larvae.

Key words: *Plasmatocytes · Prohaemocytes · Spherulocytes · Cystocytes · Granulocytes* Armyworm

INTRODUCTION

Immunosurvillence cells (haemocytes) play pivotal role in various physiological processes and insect immunity. These cells through wound signals contribute in wound healing [1], aid in the transport of nutrients to different tissues and can also store them [1]. Phagocytosis, encapsulation, nodule formation and coagulation are also important function [2]. Phagocytosis is an important function through which micro-organisms and foreign partials are engulfed. Among various other cell types which are responsible for phagocytosis, plasmatocytes play most important role. Much large particles that cannot be engulfed are encapsulated by a large number of the haemocyte types. These cells accumulate around the foreign body and become the part of haemocoel [3]. These cells are also involved in detoxification mechanisms, hormones secretion helping for growth stimulation, proteinsynthesis, phenol metabolism and nutrients storage [4].

The number of circulating haemocytes present in the blood varies considerably with time. Due to changes in the blood, the haemocyte number in unit volume is also influenced. Before moulting the number of haemocytes increases considerably and again decreases after the process of molting [5]. Haemocytes play an important role in insecticide detoxification in the transport action of hormones in endocrine system. These hormones are carried through haemocytes to the target organs and tissues [4]. Insecticides applied on an insect effect on the

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defense system by altering its haemocytes number. Different insecticides applied on different insects have been shown to affect the circulating cell types and shapes [5-7]. For example, penfluron a chitin inhibitor makes an insect defenseless [5, 8] reported that different type of pollen directly influence the defense mechanism of honey bee by altering the haemocytes number. Neem based insecticide applied on *Danaus chrysippus* had been shown to reduce the phagocytic activity of immune system by a remarkable reduction in blood cells [7].

Helicoverpa armigera is one of the most important and destructive pest of many crops that had a wide geographical distribution [10]. It is widely distributed in Asia, Europe, Australia, Canada, Africa, Manitoba, Mexico, USA, Brazil, Peru, Argentina and America [11]. 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 [10, 11]. Chemical control is the most commonly used method against this pest in Pakistan. These insecticides greatly affect the different systems of the insects especially haemocytes [13].

The objective of the present study was to examine the effect of thiacloprid and imidacloprid 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 (Agar 9.6g, Granular tapioca 4.8g, Chickpea powder 2.4g, Ascorbic acid 2.4g, Sorbic acid 1.2g, Dried active yeast, 1.2g, Methyl-para-hydroxy-benzoate 0.096g, Vitamin mixture 0.0048g, Formaldehyde 400ml). The 3rd instar larvae of *H. armigera* were used in the experiments.

Insecticides: Two different insecticides, Calypso® 240SC, (Thiacloprid, Bayer Pakistan Pvt. Ltd.) and Confidor® 20SL, (Imidacloprid, Bayer Pakistan Pv.t Ltd.) were used with 60.9 and 2.58 ppm concentrations respectively on the thorax of 3rd instar larvae with the help of micro applicator at the rate of 3µl of insecticide. These concentrations (60.9 and 2.58 ppm) are the LC_{50} values of Imidacloprid and thiacloprid for 3rd instar larvae of *H. armigera*

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 minutes. 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 [14].

Total Haemocyte Count: Neubauerhaemocytometer 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 Neubauerhaemocytometer. 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. Actual number of cell per cubic mm = Average number of cell counted per square millimeter × depth × dilution per square millimeter.

RESULTS AND DISCUSSION

Total and differential Haemocyte Count in untreated larvae On an average there were 68175 blood cells/mm in the haemolymph of 3rd larval instar of *H. armigera*. Similarly, in 5th instar larvae on an average 7428.5 blood cells/mm^{3}haemocytes were recorded (Table 1).

Percentages of differential haemocyte counts (DHC) in control larvae of 3rd instar were prohaemocyte (PR) (42.00%), spherulocytes (SP) (29%), plasmatocytes (PL) (18.00%), cystocytes (SC) (2.75%) and granulocytes (GR) (8.25%). The percentage of prohaemocyte is the highest (41.00%) followed by spherulocytes (25.75%), plasmatocytes (11.5 %), oenocytoids (16.00%), cystocytes (3.5%) and granulocytes (2.25%) in 5th instar larvae (Table 2).

Total and Differential Haemocyte Counts Treated Larvae with Thiacloprid: The total haemocyte count just after the application of thiacloprid in 3rd instar larvae increased (81300 cells/mm^3) from normal (68175 cells/mm3) while decreased (72800 cells/mm^3) after half an hour and increased again (84000 cells/mm^3) after one hour of application (Table 1).The application of plant oils, *Azadirachta indica, Artemisia annua* and *Ageratum conyzoides* on the last instar of *H. armigera* effect the number of total haemocytes count when compared with control larvae [15]. The differential haemocyte count, plasmatocytes increased from normal 18% to 32%, whereas the percentage of prohaemocytes and granulocytes decreased (42% and 8.25% to 23% and 5% respectively) and percentages of spherulocytes and cystocytes, increased from normal (29% and 2.75% to 34% and 6% respectively (Table 2). The total haemocyte count just after the application of thiacloprid in 5th instar larvae increased (20650 cells/mm^3) from normal (68175 cells/mm3) while decreased (18600 cells/mm^3) after half an hour and increased again (23470 cells/mm^3) after one hour of application. (Table 1) The percentage of prohaemocytes and oenocytoids decreased from normal (41% and 16% to 40% and 13% respectively), whereas the percentage of plasmatocytes, spherulocytes, cystocytes and granulocytes increased from normal (15%, 25.75%, 3.5% and 2.25 to 37%, 28%, 8% and 4% respectively (Table 2).

Table 1: Total number of haemocyte /mm in control and treated (with thiacloprid) 3rd and 5th instars larvae of *H. armigera*

	Control		Treated	
Time	3rd instars larvae	5th instars larvae	3rd instars larvae	5th instars larvae
0 Minutes	68175	7428.5	81300	20650
30 Minutes	68175	7428.5	72800	18600
60 Minutes	68175	7428.5	84000	23470

Table 2: Differential haemocytes (%) in control and treated with thiacloprid 3rd and 5th instar larvae of *H. armiger*

Differential haemocytes %	Control		Treated	
	3rd	5th	3rd	5th
Prohaemocyte (PR)	42	41	23	30
Spherulocytes (SP)	29	25.75	34	28
Plasmatocytes (PL)	18	11.5	32	30
Cystocytes (SC)	2.75	3.5	6	8
Granulocytes (GR)	8.25	2.25	5	4
Oenocytoids (OE)	0	16	0	13

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

	Control		Treated	
Time	3rd instars larvae	5th instars larvae	3rd instars larvae	5th instars larvae
0 Minutes	68175	7428.5	76800	10222
30 Minutes	68175	7428.5	46200	5194.25
60 Minutes	68175	7428.5	80000	13290.5

Table 4: Differential haemocytes (%) in control and treated with imidacloprid3rd and 5th instar larvae of *H. armigera*

Differential haemocytes %	Control		Treated	
	3rd	5th	3rd	5th
Prohaemocyte (PR)	42	41	34.00	29.75
Spherulocytes (SP)	29	22.25	21	17.5
Plasmatocytes (PL)	18	15	39	27.75
Cystocytes (SC)	2.75	3.5	1	10.25
Granulocytes (GR)	8.25	2.25	5	1.5
Oenocytoids (OE)	0	16	0	13.25

Total and Differential Haemocyte Counts in Treated Larvae with Imidacloprid: The total haemocyte count just after the application of insecticide in 3rd instar larvae increased (76800 cells/mm^3), while decreased (46200 cells/mm^3) after half an hour and increased again (80000 cells/mm^3) after one hour of application from the normal count (68175 cells/mm^3) (Table 3). Our results are in contradiction with the findings of Rizwan [16] who

observed that immediate after the application of Curacron 500 EC, the haemocytes increased and after 30 minutes decreased but again decreased after 60 minutes. These results are in accordance with the observations of Fareed [17] who noted that after the application of Reldan 500EC haemocytes increased and decreased after 30 minutes but increased after 60 minutes. The differential haemocyte count, plasmatocytes increased from normal 18% to 39%. The percentage of prohaemocytes, spherulocytes, cystocytes and granulocytes decreased from normal 42%, 29%, 2.75% and 8.25% to 34%, 21%, 10.25% and 5% respectively after the application of insecticide in 3rd instar larvae (Table 4). Similarly, The total haemocyte count just after the application of s in 5th instar larvae increased (10222 cells/mm^3), while decreased (5194.25 cells/mm^3) after half an hour and increased again (13290.5cells/mm^3) after one hour of application from the normal count (7428.5 cells/mm^3) (Table 3). In another study Abbas [18] noted that percentages of plasmatocytes decreased after the application of Match50 EC and Abamectin 1.8 EC when treated against *Papiliodemoleus* L. The percentage of plasmatocytes increased from normal, 15% to 27.75%, whereas the percentage of prohaemocytes, oenocytoids, cystocytes and granulocytes decreased from normal, 41%, 16%, 3.5% and 2.25% to 29.75%, 13.25%, 1% and 1.5% respectively in 5th instars larvae (Table 4). These result are in accordance with the previous study which report six different types of differential cells in the haemolymph of brinjal fruit borer and also reported a decreased in the total cells just after the application of insecticide [19]. Similar results were reported in another study in which total haemocytes increased just after the application and then decreased after half an hour and again increased after one hour of application of acetamaprid against *Dysdercuskoenigii*a dults [13]. Abdin *et al.* (2002) [20] reported five different types of haemocytes in the haemolymph of lacewing larvae and observed that percentage of granulocytes, spherulocytes and plasmatocutes increased after the insecticide application while the percentages of oenocytoidsand prohaemocytes decreased. He observed that total haemocytes number increased just after application of Nimbokil and then again increases after half an hour and decrease (not from the normal) after an hour. These finding are also in conformity with the study, which reported that in *Coccinella septempunctata* treated with spinosad, abamectin and azadirachtin the total haemocyte count (THC) and type of haemocytes varied with the application of insecticide as well as with different time intervals [21]. The result of present study could be use to select the appropriate insecticide.

REFERENCES

1. Yamashita, M. and K. Iwabuchi, 2001. Bombyx mori prohemocyte division and differentiation in individual microcultures. J. Insect Physiol., 47: 325-331.
2. Sabri, M.A. and B. Tariq, 2004. Toxicity of some insecticides on the haemocytes of red pumpkin beetle, Aulacophorafoveicollis Lucas. Pak. Entomol., 26(1): 109-114.
3. Chapman, R.F., 1998. The insects structure and function. 4th ed. Cambridge University Press, Cambridge, UK.
4. Parakash, M., 2008. Insect Physiology.Encyclopaedia of Entomology-3. pp: 216-257. Discovery Pub. House PVT. LTD. New Delhi, India.
5. Patnaik, B.D., 2002. Physiology of Insects. Dominant Pub. and Distrib. New Delhi, India, pp: 149-150.
6. 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 Apismellifera L. Egypt. Acad. J. Biol. Sci., 3(1): 213-220.
7. Pandey, J.J., R.K. Tiwari and D. Kumar, 2008. Reduction in haemocytes mediated immune response in Danaischrysippusfollowing treatments neem based insecticides. J. Entomol., 5(3): 200-206.
8. Qamar, A. and K. Jamal, 2009. Differential haemocyte counts of 5th instar nymphs and adults of Dysdercuscingulatus Fabr. (Hemiptera: Pyrrhocoridae) treated with acephate, an organophosphorus insecticide. Biol. Med., 1(2): 116-121.
9. Pugazhvendan, S.R. and M. Soundararajan, 2009. Effect of penfluron on total haemocyte count of Chrysocorispurpureus. Middle-East. J. Sci. Res., 4(4): 338-340.
10. 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. Entomol. Res., 89: 201-207.
11. 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.
12. 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.

13. 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., pp: 1813-2235.
14. Mahmood, A. and N. Yousaf, 1985. Effect of some insecticides on the haemocytes of *Gryllus bimaculatus* de Geer. Pak. J. Zool., 17(1): 71-84.
15. 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(1): 112-116.
16. Rizwan-ul-haq, M., A. Sabri and M.A. Rashid, 2005. Toxicity of Nicotinyl Insecticides on the Haemocytes of Red Cotton Bug, *Dysdercus koenigii* (Fb.) (Pyrrhocoridae:Hemiptera). J. Agri. and Soc. Sci., 1(3): 339-341.
17. 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. Entomol., Univ. Agric., Faisalabad.
18. Abbas, M., 2008. Haemocyte studies on lemon butterfly Papiliodemoleus L. (Papilionidae: Lepidoptera), larvae in response to application of different insecticides. M.Sc., Thesis, Univ. Agri., Faisalabad.
19. 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, Leucinodesorbonalis (GUEN). Pak. Entomol., 24(2): 115-119.
20. Abdin, Z., A. Suhail, J. Iqbal, U. Waseem and A. Nawaz, 2002. Toxicity of Bio-insecticide Nimbokill (Azadirachtin) to the haemocytes of green lacewing, Chrysoperla carnea Steph. (Chrysopidae: Neuroptera). Pak. Entomol., 24: 143-146.
21. Suhail, A., M.D. Gogi, M.J. Arif, M.A. Rana and M. Sarfraz, 2007. Effect of various treatments of azadirachtin, spinosad and abamectin on the haemogram of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Pak. Entomol., 29(2): 151-164.