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CHANGES IN HAEMOCYTE COUNT IN HAEMOLYMPH OF DIFFERENT LARVAL STAGES OF ERI SILKWORM ON APPLICATION OF DIMETHOATE, AN ORGANOPHOSPHOROUS PESTICIDE

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ABSTRACT

In the haemolymph of 4th and 5th instar larvae of the Eri Silkworm (*Philosamia ricini*) five types of haemocytes have been identified. Changes in the Differential Haemocyte Counts (DHCs) have been assessed in relation to application of graded concentrations of Dimethoate 30EC solution. The haemocyte count was done on 1st , 2nd and 3rd days of post-treatment of sub lethal doses of dimethoate (i.e. 0.5% and 1%) on 4th and 5th instar larvae. Different types of haemocytes registered a dose-dependent response by either exhibiting increase or decrease in their relative proportions. The adipohaemocytes and prohaemocytes were the most sensitive cells to the pesticidal stress. The plasmatocytes showed least damage on low concentration (0.5%) of dimethoate. Furthermore, the treated insects apparently responded by releasing more granulocytes, into the circulation as evident by increase in the percentage of these cells in treated blood smears compared to the normal.

INTRODUCTION

The research and development of pesticide has brought a large number of chemicals in the protection of crops against insects pests.These insecticides are also toxic to non target organisms Nath *et al.*, 1997; Suh *et al.*, 2000).In the present scenario of green revolution although the application of different pesticides tend to decrease but dimethoate, an organophosphorous pesticide, till today in a grand use. It was reported by different scientists that its application decreases the number of haemocytes (Ayesha *et al.*, 2007).Insect haemocytes present in the haemolymph are categorized into several types. Haemocyte classification is still far from perfect and one will see different names used for the same blood cell types. They perform various physiological functions like phagocytosis, encsapsulation, detoxification and storage and distribution of nutritive materials. Present knowledge of insect haemocytes is limited to studies of not more than 200 insect species in about 100 genera (Arnold 1979). Haemocytes have been studied mostly in Lepidoptera, Hymenoptera, Coleoptera and Diptera (Gupta 1985). Knowledge of normal haemocytes of an insect is necessary to physiologist, toxicologist and biochemists, as alteration in structure, types and number of cells reflects changes in physiological and biological processes. Insect haemocytes respond to internal changes during development (at ecdysis) and to conditions such as starvation, wounding, parasitism, diseases, chemicals including insecticides. Plasmatocytes and granular cells are described as the main cell types involved in all defense mechanisms (Beaulaton and Monpeyssin 1977; Ratcliffe et al.1985; Ratcliffe and Rowley 1987; Wiesner and Götz 1993). Phagocytosis is considered the first barrier against pathogens and it has been described in the hemolymph of many insect species against biological (Ratcliffe and Rowley 1979; Ratcliffe *et al.*,1985; Götz and Boman 1985; Ratcliffe 1986) and non-biological agents (Wiesner 1991, 1992; Slovák *et al.*,1991).Several studies have shown that many sub lethal doses of insecticides limit the development, survival and growth of parasitoid wasps either by direct chemical contact or by ingestion of treated prey (Simmonds *et al.*,2002; Sak *et al.*,2007).The possible involvement of insect haemocytes in curbing the biological and chemical control efforts of pests is also apparent due to their implication in the detoxification of insecticides (Lackie 1988).A number of toxicological studies have examined the effects of insecticides on the chemical changes in insect haemolymph (Nath *et al.*,1997; Serebrov *et al.*,2001). In the present work, an attempt has been made to determine the changes in the differential haemocyte count(DHC) after treated with dimethoate, an organophosphorous pesticide, on 4th and 5th instar larvae of Eri silkworm (*Philosamia ricini*).

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MATERIALS AND METHODS

The eri silkworms were collected as experimental animal from local sericulture farm.The eri silkworms were reared by feeding the insects with a diet of castor leaves. The 4th and 5th instar larvae of Eri silkworm were taken for study.

A pilot experiment is done to find out LD_{50} values of dimethoate for both 4th and 5th instar larvae of eri silkworm. Two sublethal concentrations- 0.5% and 1% of dimethoate 30EC solution were selected for experimental purpose and injected to the 4th and 5th instar larvae of Eri silkworm. For smear preparation, at first the larvae were kept at 50^0C water bath for 2 minutes to fix the haemolymph. A small amount of haemolymph was collected by cutting the proleg on the 7th abdominal segment and a smear was prepared on the slide. The dried smear was then stained with Giemsa stain and kept for 15 minutes and after rinsing in distill water, mounted by DPX. For differential haemocyte count, cell categories were counted in 100 cells in each smear using a phase contrast microscope. The relative numbers of plasmatocytes, granulocytes, adipohaemocytes, prohaemocytes and sperulocytes of affected larvae were determined at 24 hour, 48 hour and 72 hour following the treatment with selected sub lethal concentrations of dimethoate. Differential haemocyte counts of treated larvae were compared with that of the normal. The values were statistically analyzed following standard statistical procedure. Students't' test was done to determine the significance of variation.

RESULTS AND DISCUSSION

In the present study the LD_{50} value for 24 hrs for 4th instar larva was determined as 1.6% and for 5th instar 2% of dimethoate 30EC solution. 5 different haemocyte types were identified in the 4th and 5th instar larval haemolymph of Eri Silkworm (*Philosamia ricini*).

Haemocyte types

a) **Plasmatocytes:** Plasmatocytes were observed as round, fusiform and spindle shaped with a relatively smaller nucleus.
b) **Granulocytes:** These were rounded and ovoid in shape. The centrally located nucleus was found to be relatively small, round, elongate and surrounded by abundant of cytoplasmic granules.
c) **Adipohaemocytes:** Adipohaemocytes were spherical and oval cells. Compared with that of the plasmatocytes, the nucleus was relatively small, rounded and eccentrically located. The cytoplasm contains small refringent fat droplets and vacuoles.
d) **Prohaemocytes:** These were found to be small, round, oval and elliptical cells with variable sizes. The nucleus was larger compared with other haemocyte types and centrally located.
e) **Spherulocytes:** Cells were observed as oval with a small nucleus.The cytoplasm was thick and homogeneous with a number of spherules present around the nucleus.

Differential haemocyte count (DHC)

The differential haemocyte count profile during the different stages (i.e. on 4th and 5th instars)of eri silkworm showed some variations. The granulocytes were the most abundant haemocytes followed by plasmatocytes, spherulocytes and prohaemocytes.The adipohaemocytes were numerically less abundant. On injection of sub lethal 0.5% and 1% concentrations of dimethoate 30EC solution, the haemocytes in the haemolymph of eri silkworm larvae showed reduction in the number of plasmatocytes, adipohaemocytes, prohaemocytes and spherulocytes. But the numbers of granulocytes were found to increase in the dimethoate treated larval haemolymph.

The Differential haemocyte count of 4th and 5th instar larvae treated with dimethoate and its comparison with the cell count of the controlled ones at three different time intervals are shown in the following Tables (1, 2, 3 and 4). It was seen that the injected doses of dimethoate (0.5% and 1%) affect all the larval haemocytes more or less.

Effects of dimethoate on 4th instar larval haemocytes

After application of 0.5% of dimethoate 50EC solution on the 4th instar larvae of eri silkworm, the percentage of plasmatocyte found in the haemolymph of normal larvae showed a linear decrease from 24th hr to 72nd hrs which was not significant statistically ($P>0.05$). But in case of 1% sublethal concentration of dimethoate, it showed a significant reduction ($P<0.01$). (Table-2) Granulocyte constituted 29.2% of haemocyte in normal larvae which subsequently increased with the increasing time intervals and also with increase in concentration of dimethoate which was statistically significant ($P<0.01$). Following the application of 0.5% and 1% (Table-1&2) concentrations of dimethoate, it was seen that the number of adipohaemocyte decreased with the increased concentration which was not statistically significant at 24th hour but at 48th and 72nd hrs, it showed significant decrease in number ($P<0.01$). The prohaemocyte density which was 13% in normal, decreased with time intervals and at 72nd hr it was 6.4% for sublethal 0.5% and 3% for sublethal 1% concentration of dimethoate showing a significant reduction statistically ($P<0.01$) but for sublethal 0.5% at 24th hr the reduction of prohaemocyte was found to be not significant ($P>0.05$). The percentage of spherulocyte in the normal larvae was 29.4% but after application of sublethal concentration of dimethoate - 0.5% and 1%, it started decreasing gradually and the affect was seen more with the increasing concentration showing statistically significant values ($P<0.01$). (Table-1&2)

Effects of Dimethoate on 5th instar larval haemocytes

After application of 0.5% of dimethoate 30EC solution on the 5th instar larvae of eri silkworm, the percentage of plasmatocyte found in the haemolymph of normal larvae showed a linear decrease from 24th hr to 72nd hrs which

Table 1 Showing Differential Haemocyte count (%) of Normal larvae and the larvae treated with sub lethal dose of dimethoate 30EC solution (0.5%) in 4th instar.

	Normal	0.5% Dimethoate Treated		
cell types	Mean%±S.D.	24 hrs Mean%±S.D.	48 hrs Mean%±S.D.	72 hrs Mean%±S.D.
Plasmatocyte (pl)	34.2 ± 2.7	33.6 ±2.67 NS	31.9±2.46 NS	30± 2.21 NS
Granulocytes(gr)	29.2± 4.39	44.5 ±2.05**	44.7±2.27**	48.1±1.79**
Adipohemocyte(ad)	1.5 ± 1.58	0.9±1.28 NS	0.5± 0.7**	0.5± 0.7**
Prohaemocyte(pr)	13±3.61	11.6±3.29 NS	8± 1.58**	6.4± 1.14**
Spherulocyte(sp)	29± 3.64	28.6± 1.95**	25.4± 3.78**	18.6± 2.4**

Mean±S.D followed with NS=not significant (p>0.05), *=significantly different (p<0.05), **=highly significantly different (p<0.01), df=38

Table 2 Showing Differential Haemocyte count (%) of Normal larvae and the larvae treated with sub lethal dose of dimethoate 30EC solution (1%) in 4th instar

	Normal	1% Dimethoate Treated		
cell types	Mean %±S.D.	24hrs Mean%±SD	48hrs Mean%±SD	72hrs Mean%±SD
pl	34.2±2.7	25.4±2.43**	18.7±2.11**	18.4±2**
gr	29.2±4.39	54.5±5.56**	58.85±6.69**	44.6±7**
ad	1.5±1.58	0.3±0.43 NS	0.2±0.38**	0.2±0.8**
pr	13±3.61	6.3±2.63**	3.2±1.87**	3±1.78**
sp	29.4± 3.64	22.4±2.71**	14.5±1.57**	13±1.38**

Mean±S.D followed with NS=not significant (p>0.05), *=significantly different (p<0.05), **=highly significantly different (p<0.01), df=38

Table 3 Showing Differential Haemocyte count (%) of Normal larvae and the larvae treated with sub lethal dose of Dimethoate solution (0.5%) in 5th instar.

	Normal	0.5% Dimethoate Treated		
cell types	Mean%±S.D.	24 hrs Mean%±S.D.	48 hrs Mean%±S.D.	72 hrs Mean%±S.D.
pl	22.7±3.35	22 ± 3.54 NS	21.9± 2.46*	16±3.16**
gr	34.9±5.66	40± 3.54**	54.2 ±3.19**	60.8±3.70**
ad	2.6±2.07	1.2±0.44*	1±0.707**	0.6±0.55**
pr	12.9±2.6	11.2±2.59*	10.6± 3.85**	5.2±1.64**
sp	31.1±2.37	22.2±3.70**	20.8±3.11**	13.2±3.27**

Mean±S.D followed with NS=not significant (p>0.05), *=significantly different (p<0.05), **=highly significantly different (p<0.01), df=38

Table 4 Showing Differential Haemocyte count (%) of Normal larvae and the larvae treated with sub lethal dose of Dimethoate solution (1%) in 5th instar

	Normal	1% Dimethoate Treated		
cell types	Mean %±S.D.	24hrs Mean%±SD	48hrs Mean%±SD	72hrs Mean%±SD
pl	22.7±3.35	20.4±3.2**	16.6±2.41**	12±3.39**
gr	34.9±5.66	47.4±2.97**	61±3.67**	68.2±2.86**
ad	2.6±2.07	1±1*	0.4±0.58**	0.2±0.45**
pr	12.9±2.6	4.2±1.30**	2±1**	0±0**
sp	31.1±2.37	21±2.92**	14±1.58**	13±1.38**

Mean±S.D followed with NS=not significant (p>0.05), *=significantly different (p<0.05), **=highly significantly different (p<0.01), df=38

was not significant statistically at 24th hr. Whereas the reduction is significant at 48th (p<0.05) and 72th (p<0.01) hrs. But in case of 1% sublethal concentration of dimethoate, it showed a significant reduction (P<0.01). (Table-2) Granulocyte constituted 29.2% of haemocyte in normal larvae which subsequently increased with the increasing time intervals and also with increase in concentration of dimethoate which was statistically significant (P<0.01). Following the application of 0.5% and 1% (Table-1&2) concentrations of dimethoate , it was seen that the number of adipohaemocyte decreased with the increased concentration which was statistically significant at 24th hour (p<0.05) and at 48th and 72nd hrs, it showed highly significant results (P<0.01).

The prohaemocyte density which was 13% in normal, decreased with time intervals and at 72nd hr it was 5.2% for sublethal 0.5% and 0% for sublethal 1% concentration of dimethoate showing a significant reduction statistically (P<0.01) but for sublethal 0.5% at 24th hr, the reduction of prohaemocyte was found to be significant at P<0.05.

The percentage of spherulocyte in the normal larvae was 29.4% but after application of sublethal concentration of dimethoate -0.5% and 1%, it started decreasing gradually and the affect was seen more with the increasing concentration showing statistically significant values (P<0.01). (Table-1&2)

George and Ambose (2004) studied the impact of five organophosphorous insecticides, viz. monocrotophos,

dimethoate, methylparathion, quinalphos and endosulfan on the differential haemocyte counts (DHC) of *Rhynocoris kumarii*. All of the insecticides except endosulfan initially reduced both prohaemocytes and plasmatocytes, increased the granular haemocytes, and altered the percentage of cystocytes and oenocytoids. On the contrary, endosulfan initially increased the prohaemocytes and plasmatocytes, decreased the granular haemocytes. The highest impact on the DHC was caused by methylparathion, monocrotophos, and the least impact by endosulfan but this study showed that except granulocyte other four haemocyte types of eri silkworm were decreased.

In the present study, the reduction in PLs revealed that dimethoate was toxic and lethal to the cellular defensive system of 4th and 5th instar larvae of *P.ricini.* Tikku *et al.,* (1992) and George & Ambrose (2004) also recorded reduction percentage in PLs of adult *R. kumarii* due to tested organophosphate insecticides that were found to be highly toxic to the treated organism.

The pesent study also depicts the decrease in the percentage of PRs after the application of dimethoate as investigated by Tikku *et al.,*(1992)and George & Ambrose (2004) in the adult of *Dysdercus koenigii* F. (Pyrrhocoridae: Hemiptera) against azadirachtin and *R. kumarii* against malathian respectively. The PRs serve as stem cells in the haemolymph (Silva *et al.,*2002) and reduction in PRs could be correlated to the greater transformation of PRs into other type of cells which play their role in phagocytosis (Bhatti, 2002; Saxena& Srivastava, 2001) or destruction of haemopoietic organs, responsible for the production of PRs (Tiwari *et al.,*2002).Here in the 5th instar larval stage after application of sublethal 1% of dimethoate solution,at 72nd hr the prohaemocytes were totally damaged and its percentage became zero. The present study showed that the increase in the number of granulocytes which are involved in the detoxification of chemicals and killing of microorganism through encapsulation and phagocytosis (Saxena & Srivastava, 2001; Chapman, 1998; Steinhaus, 1949). The increase observed in GRs after exposure to azadirachtin and abamectin might be attributed to the greater transformation of PLs and PRs into GRs during detoxification (George & Ambrose, 2004) and correlated with greater role played by GRs in detoxification through phagocytosis (Jose & Martin, 1989; Kurihara *et al.,* 1992).

George & Ambrose (2004) and Sharma *et al.,*(2003) also recorded the greatest increase in GRs in methyl parathion-exposed individuals of *R.kumarii* and in azadirachtin-exposed sixth-instar larvae of *Spodoptera Litura* F. (Noctuidae:Lepidoptera).

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