Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(1): 126-133

Received: 20-11-2016
Accepted: 21-12-2016

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Toxicity of Dimethoate and Chlorpyriphos on haemocyte count in male *Platynotus belli* Fairmaire (Coleoptera: Tenebrionidae)

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Abstract
The toxicity of Dimethoate 30% EC and Chlorpyriphos 20% EC organophosphate insecticides in field concentration was observed against the haemocyte counts of *Platynotus belli* Fairmaire beetle. In these studies, seven types of haemocytes were identified viz., Prohaemocyte, Plasmatocyte, Granulocyte, Oenocytoid, Coagulocyte, Adipohaemocyte and Spherulocyte in the haemolymph of *P. belli*. The untreated specimen of control group *P. belli* beetle total haemocyte count (THC) were 26233.33 ± 251.66 cells/mm3. The Dimethoate and Chlorpyriphos treatment showed the differences cell number as compared to control group. The three concentrations 400ppp, 600ppm and 800ppm showed maximum increased in cell number which is inversely proportional to the concentration of insecticide i.e. 37150 ± 175.796; 34183.33 ± 175.599 and 27403.67 ± 352. 406 respectively in Dimethoate treated beetle. While, in Chlorpyriphos treatment also increased the cell number which is inversely proportional to the concentration of insecticide i.e. at 200ppm 35383.33 ±539. 289; 400pmm 26300 ± 100 and at 600ppm 11383.33 ± 534. 633. Both insecticides showed the decreased number of cells when concentration was increased.

Keywords: Dimethoate 30% EC, Chlorpyrifos 20%, haemocytes, *Platynotus belli*

1. Introduction
Haematological studies are very important in insect physiology. In insect open type of circulatory system is present. It circulates pale colored fluid called haemolymph. It contains various types of cells called as haemocytes. The haemocyte performs various physiological functions in the body of insects. They provide direct nutrients to various tissues and stored them also. Haemocytes provides phagocytosis, encapsulation of foreign bodies in the insect body cavity, coagulation to prevent loss of blood, nodule formation and transport of food materials and may be hormones and detoxification of metabolites and biological active materials [40]. Arnold and Sohi [5] studied five categories of haemocytes *viz*., Prohaemocyte, Plasmatocyte, Granulocyte, Spherulocyte and Oenocytoid in the fresh blood of *Malacosoma disstria* H. In red pumpkin beetle, *Aulacophora foveicollis* Mall and Gupta [35] identified five classes of haemocytes, *viz*., Prohemocyte, Plasmatocyte, Granulocyte and Adipohemocyte.
The most common types of haemocytes are Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes, Adipohaemocytes, Coagulocyte and Oenocytoids. The characteristic feature is slightly differing in various insect species [23, 47]. Due to economical and ethical problems with the use of vertebrates in biomedical studies, insects have been suggested as alternative biomodels for toxicological preclinical studies [13, 21]. In addition, insects have been widely used in other fields of biomedical research, such as neuroscience [15, 17]. Haematology is an integral part of preclinical studies on animals and good knowledge of haemocytes, therefore is required before insects can reach a level of importance similar to that of vertebrate models in terms of comparative clinical pathology [11].
Recently, some information has been gathered on effect of insecticides on the haemocyte morphology and their populations. The notable contributions include the work of different scientist [42, 20, 6, 4, 29, 55, 24, 18, 19], etc.
The research and development of pesticides has brought a large number of chemicals in the protection of crops against insect pests. These insecticides are also toxic to nontarget organisms [54]. Now a day's green revolution use of different pesticides get reduced but, Chlorpyriphos and Dimethoate pesticides till today in grand use.

Platynotus belli is a black stout ground beetle mostly found in dry places. They are of moderate size many of them are bulk and weight. The body is hard often flattened globular, the elytra fitting closely and soldered together. It is a non flyer.
As there is no such information available on the haemolymph of present beetle, *P. belli* under study. Therefore, in present work, attempts are made to study the morphology and effect of two most commonly used insecticides on populations of circulating haemocytes in the adults of *P. belli* beetle.

2. Materials and methods

P. belli is a black stout ground beetle mostly found in dry places. They are of moderate size many of them are bulk and weight. The body is hard often flattened globular, the elytra fitting closely and soldered together. It is a non flyer.
The adults of *P. belli* collected from the different field sites during the year 2014-2015 and kept in the laboratory condition by providing the food and water. After collecting the 50 beetles they were kept in plastic container having aeration. In nature beetle under study are scavengers but in laboratory condition they were provided with wheat flour and for water supply given that the cotton balls soaked with water. In laboratory condition beetles fed voraciously and they took water directly from the cotton balls provided. After acclimatization the beetles were used for the experimental purpose. The research studies were carried out in Physiology laboratory, P. G. Department of Zoology, S. G. M. College, Karad.

P. belli maintained in laboratory

Adult *P. belli*

Organophosphate insecticides Chlorpyriphos 20% EC and Dimethoate 30% EC were used at its recommended concentration for the field and above the field trial and below the field trial. Three concentrations of Chlorpyriphos 20% were prepared i.e. 200ppm, 400ppm and 600ppm. While for Dimethoate 30% three concentrations i.e. 400ppm, 600ppm and 800ppm were prepared and then used for experimental purpose. Seven groups were prepared; one group was kept as control without any application of insecticide. Each group containing 5 beetles kept in separate petriplate and then insecticides were applied dorsally with the help of micropipette adjusted at the rate of 20µl and after each application of insecticide a new tip was replaced. Effect of insecticides was noted after 30 minutes from each group in terms of abnormalities found in the haemocyte count and the changes in the total and differential haemocyte count.

2.1 Total Haemocyte Count mm^3 (THC)

The total haemocyte count was achieved by using Neubauer haemocytometer. The haemolymph was collected by pricking needle in the abdomen and quickly drawn in to a white blood cell diluting pipette up to 0.5 mark and then diluted 20 times with versene ringer up to II mark. A drop of diluents was placed near the edge of the cover slip. Neubauer ruling automatically filled the counting chamber by its capillary action. Both the upper and lower chambers were used and four white cell squares in each chamber were counted after 5 minutes from discharging the diluent. The total haemocyte count was computed by using the formula suggested by Jones (1962). Three observations were recorded for this purpose.
For THC used the following formula,

$$\text{THC} = \frac{\text{No. of cells counted} \times 10 \times 20}{\text{Number of squares counted}}$$

Where, 10 = Depth factor of chamber (constant),
20 = Dilution.

2.2 Differentials Haemocyte Count (DHC)

For making DHC a drop of fixed haemolymph was taken over clean microscopic slide in a clean calibrated drop of versene ringer. The smear was air dried and stained by wright's stain for 15 minutes then washed with distilled water. Differential counting of haemocytes was done under an oil immersion Olympus microscope (10x X 100x). Each time, 100 cells were counted and percentage of various classes was determined. The experiment was repeated four times using Completely Randomised Design (CRD) and data was subjected to the standard statistical analysis.

3. Results

The results obtained in present investigation of control group and after application of insecticides depicted in Tables1, 2, and 3 and also graphically showed in Fig. 1, 2, 3 and 4.
In present study 7 types of haemocytes in the haemolymph of *P. belli* were found. The following classes and types (based on the size and shape of the cells, their staining characteristic) were recognized: [23, 28].

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.

Plasmatocytes: Plasmatocytes were observed as round, fusiform and spindle shaped with a relatively smaller nucleus.

Granulocytes: These were rounded and ovoid in shape. The centrally located nucleus was found to be relatively small, round, elongated and surrounded by abundant of cytoplasmic granules.

Oenocytoids: These are large cells, may be oval or elongated in shape, with occasional rounded form. The nucleus is often eccentrically positioned.

Coagulocytes: Coagulocytes are large in size, rounded and nucleus is centrally placed and cytoplasm is transparent

Adipohaemocytes: Adipohaemocytes were spherical and oval cells. Compared with that of the plasmatocytes, the nucleus was small, rounded and eccentrically located. The cytoplasm contains small refringent fat droplets and vacuoles.

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.

3. A] Untreated specimens of *P. belli* beetle

i] Total Haemocyte Count (THC)

During the present investigation the total haemocyte count from the haemolymph of *P. belli* is presented in Table no.1 and fig. 1and 2 on an average, there was 26233.33 ± 251.66 cells/mm^3 in untreated specimens (Control). The results can be correlated with the result of Hassan [26] in which he recorded haemocytes of *Tryporyza* species on an average 22475 cells/mm^3 and also in *Meladera Sp.* Male 22300 cells/mm^3 and in female 29100 cells/mm^3 recorded. These results differ from the Mall and Gupta [35] who studied the haemocyte of red pumpkin beetle and reported total haemocytes in an average of 5500 cells/mm^3. The present finding also differ from the Sabri and Tariq [49] who worked on red pumpkin beetle and observed 4372 cells/mm^3 of haemocyte in untreated species.

ii] Differentials Haemocyte Count (DHC)

In the present work, seven types of haemocytes were identified, viz., Prohaemocytes, Plasmatocytes, Granulocytes, Oenocytoids, Coagulocytes, Adipohaemocytes and Spherulocytes in the haemolymph of *P. belli*. The data given in the Table No. 2 and 3. The percentage of Granulocytes is the highest 30.67 ± 0.550 followed by Prohaemocytes 26.86 ± 1.513, Adipohaemocytes 21.19 ± 1.057, Oenocytoids 10.07 ± 0.438, Plasmatocyte 7.77 ± 0.408, Coagulocyte 3.17 ±0.306 and Spherulocytes 2.47 ± 0.438 respectively. The results obtained from the present study more or less similar with Saba and Tariq [49]. They also reported these types of haemocytes in their test beetle red pumpkin beetle and Japanese beetle respectively.

3. B] Treated specimens of *P. belli* beetle

i] Effect of Chlorpyriphos 20% EC on *P. belli*

a) Effect on Total Haemocyte Count (THC)

There are three concentrations of Chlorpyriphos 20% EC were used for experimental purpose as per field application. From that the 200 ppm concentration showed increased THC i.e. 35383.33 ± 539.58/mm^{3}as compared to control group 26233.33 ± 251.66/mm^3. While the concentration of 400 ppm and 600 ppm showed decreased THC as compared to the control i.e. 26300.00 ± 100/mm^{3}and 11383.33 ± 534.63 respectively.

From the above result it is clear that the low concentration of chlorpyriphos increase the THC indicates increase the immunity of *P. belli*. But increases concentration of chlorpyriphos insecticide affect cell number and therefore decreased THC and reduced the immunity of beetle.

b) Effect on Differential Haemocyte Count (DHC)

In DHC the chlorpyriphos affects all types of cells. The prohaemocytes, granulocytes, oenocytoides, adipohaemocytes and spherulocytes cell number was decreased as compared to control group. While in case of plasmatocyte and coagulocyte the number of cells increased. In case of plasmatocyte the concentration increased the number was decreased. At low concentration the plasmatocyte increased as compare to control group i.e. 34.37 ± 0.650 and 7.77 ± 0.408 respectively.

Relevant to above results the coagulocyte number was increased when the concentration was increased i.e. 200 ppm = 14.48 ± 0.445, 400 ppm =17.91 ± 1.000 and 600 ppm =19.31 ±0.601.

ii] Effect of Dimethoate 30% EC on *P. belli*

a) Effect on Total Haemocyte Count (THC)

The Dimethoate affects the cell number as compared to control group. From three different concentration 400 ppm concentration showed maximum increased cell number i.e. 37150 ±175.79/mm^3 while the minimum cell number was observed at 800 ppm concentration i.e. 27403 ±352.40 as compared to control group 26233.33 ±251.66.

The effect of Dimethoate shows increase in the concentration simultaneous decreases THC but this is maximum from the control group.

b) Effect on Differential Haemocyte Count (DHC)

The DHC percentage was decreased in Dimethoate 30% EC treated specimens of *P. belli.* The decreased number was observed in prohaemocyte, granulocyte, oenocytoid, adipohaemocyte except the plasmatocyte, coagulocyte and spherulocyte.

The percentage of plasmatocyte was highest in 400 ppm concentration as compared to control i.e. 25.32 ± 0.955 and 7.77 ± 0.408 respectively. But increase in the concentration of Dimethoate 30% simultaneous decreases the percentage of plasmatocyte for 400 ppm concentration 25.36 ± 0.955, 600 ppm concentration 24.96± 0.895 and for 800 ppm concentration it was 14.81 ± 1.114.

The prohaemocyte was also increased at low concentration i.e. 29.46 ± 0.406 at 400 ppm as compared to control 26.86 ± 1.513, but when concentration increased the percentage of prohaemocytes was decreased i.e. at 600 ppm 20.94 ± 0.864 and 800 ppm concentration 15.74 ± 0.77 as compared to control group 26.86 ± 1.513.

In case of coagulocyte the percentage of cell was increased in all treated group as compared to control group. For 400 ppm concentration it was 7.23 ± 0.556, 600 ppm concentration 27.80 ± 0.746 and for 800 ppm concentration it was 19.41 ± 0.373.

The spherulocyte was also increased in all groups. The highest increase was observed at 800 ppm concentration i.e. 7.54 ± 0.585 as compared to other concentration 400 ppm and 600 ppm 5.44 ± 0.713 and 5.11 ± 0.600 respectively.

Table 1, 2 and 3 shows that the DHC is also changed in treated beetles and the change is somewhat in similar proportion for both insecticides.

Table 1: Effect of Chlorpyriphos 20% and Dimethoate 30% on total haemocyte count mm3 (THC) of *Platynotus belli* F

Differential Haemocyte Countin % (DHC)				
	Control	Chlorpyriphos 20%		
Types of cell		200ppm	400ppm	600ppm
PRs	26.86± 1.513	24.02± 0.072	22.09± 0.081	19.35± 0.469
PLs	7.77± 0.408	34.37± 0.650	27.77± 0.780	21.43± 0.590
GRs	30.67± 0.550	12.04± 0.496	16.95± 0.356	21.18± 0.770
OEs	10.06± 0.438	3.13± 0.321	2.71± 0.027	5.50± 0.465
COs	3.17± 0.306	14.48± 0.445	17.91± 1.000	19.31± 0.631
ADs	21.19± 1.057	10.01± 0.032	7.44± 0.263	10.36± 0.535
SPs	2.47± 0.438	2.67± 0.493	2.80± 0.331	7.06± 0.337

Table 2: Effect of Chlorpyriphos 20% on differential haemocyte count (THC) of *Platynotus belli* F.

Total haemocyte count mm3 (THC)						
Control	Chlorpyriphos 20%			Dimethoate 30%		
	200ppm	400ppm	600ppm	400ppm	600ppm	800ppm
26233.33 ± 251.661	35383.33 ± 539.289	26300 ± 100	11383.33 ± 534.633	37150 ± 175.793	34183.33 ± 175.594	27403.67 ± 352.406

Table 3: Effect of Dimethoate 30%on Differential haemocyte count (THC) of *Platynotus belli* F.

Types of cell	Differential Haemocyte Count in % (DHC)			
	Dimethoate 30%			
	Control	400ppm	600ppm	800ppm
PRs	26.86± 1.513	29.46± 0.406	20.94± 0.864	15.74± 0.777
PLs	7.77± 0.408	25.32± 0.955	24.96± 0.895	14.81± 1.114
GRs	30.67± 0.550	13.19± 0.421	16.42± 0.506	20.74± 0.214
OEs	10.06± 0.438	4.12± 0.785	4.77± 0.167	4.92± 1.015
COs	3.17± 0.306	7.23± 0.556	27.80± 0.746	19.41± 0.373
ADs	21.19± 1.057	16.94± 0.458	18.42± 0.709	13.24± 0.661
SPs	2.47± 0.438	5.44± 0.713	5.11± 0.600	7.54± 0.585

Plate: I

Types of haemocytes observed in *P. belli*

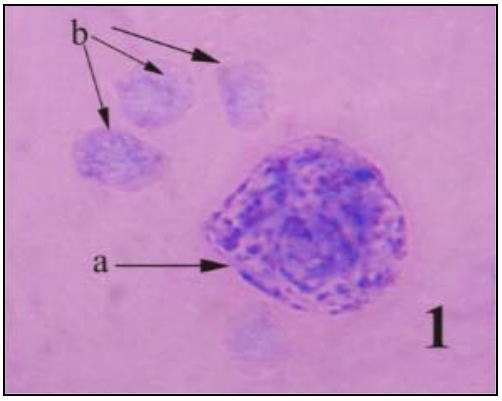

Fig 1: a. Granulocyte, b. Adipohaemocytes

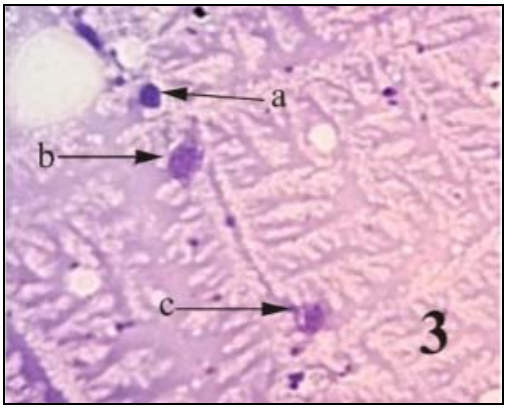

Fig 3: a. Prohaemocyte, b. Plasmatocyte and c. Spherulocyte

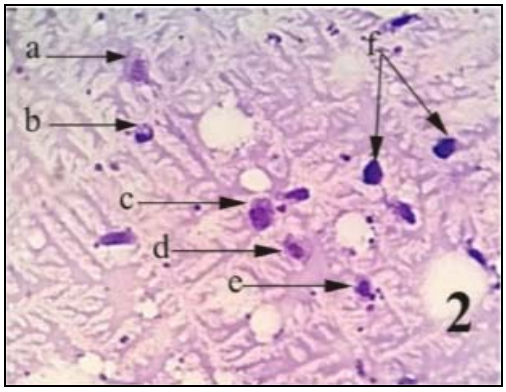

Fig 2: a. Coagulocyte, b. and c Oenocytoid, d and e Spherulocyte and f. Prohaemocyte

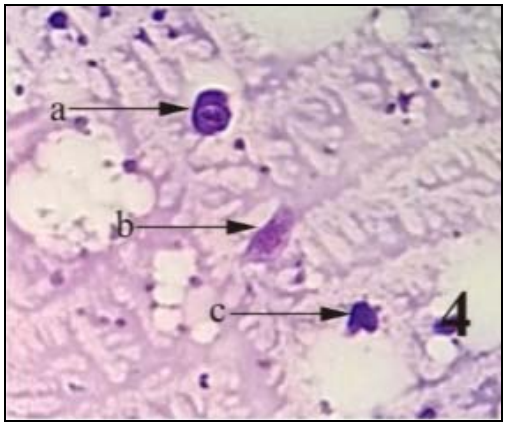

Fig 4: a. and c. Prohaemocyte, b. Plasmatocyte

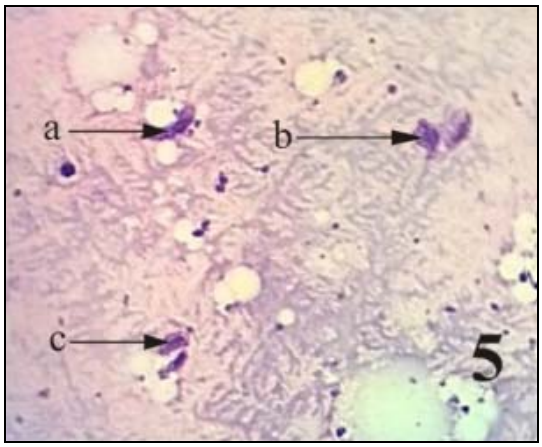

Fig 5: a. Plasmatocyte, b. and c. Coagulocyt**e**

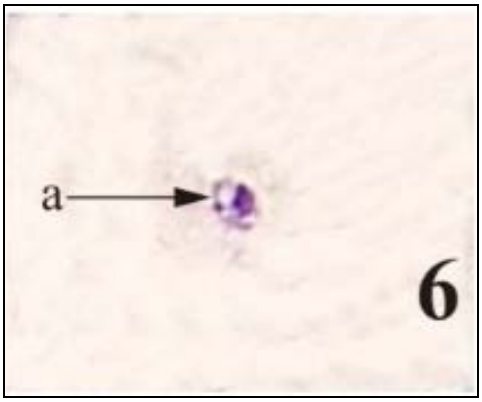

Fig 6: a. Oenocytoid

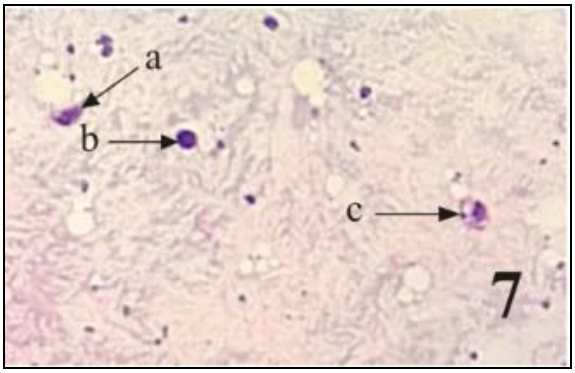

Fig 7: a. Plasmatocyte, b. Prohaemocyte and c. Oenocytoid

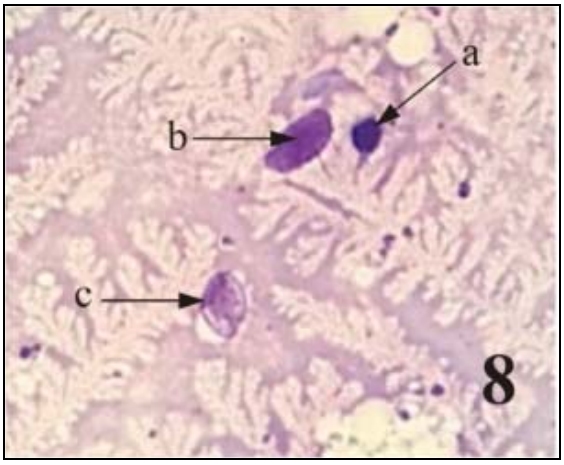

Fig 8: a. and b. Prohaemocyte and c. Coagulocyte

Plate: II

Types of haemocytes observed in *P. belli*

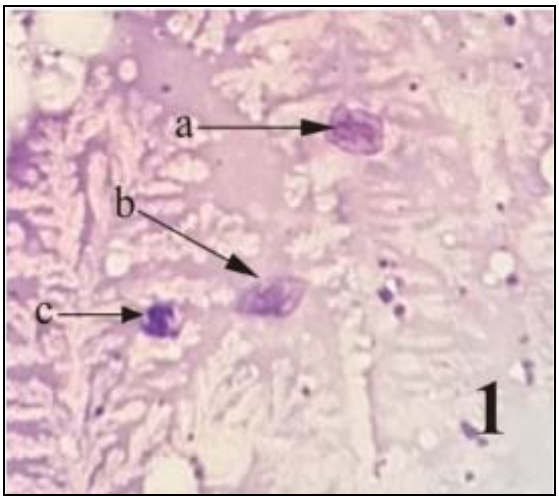

Fig 1: a. Coagulocyte b. Plasmatocyte and c. Granulocyte

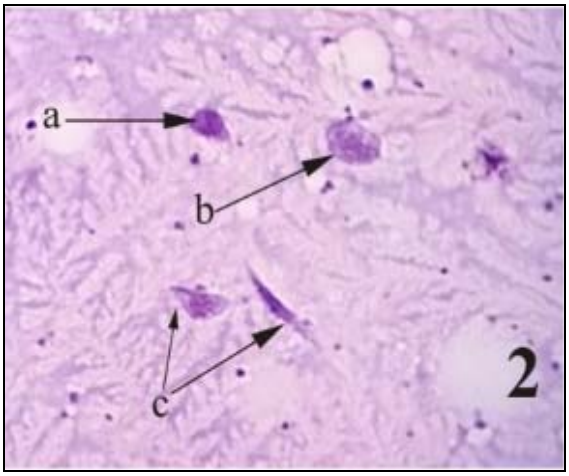

Fig 2: a. Prohaemocyte b. Coagulocyte and c. Plasmatocyte

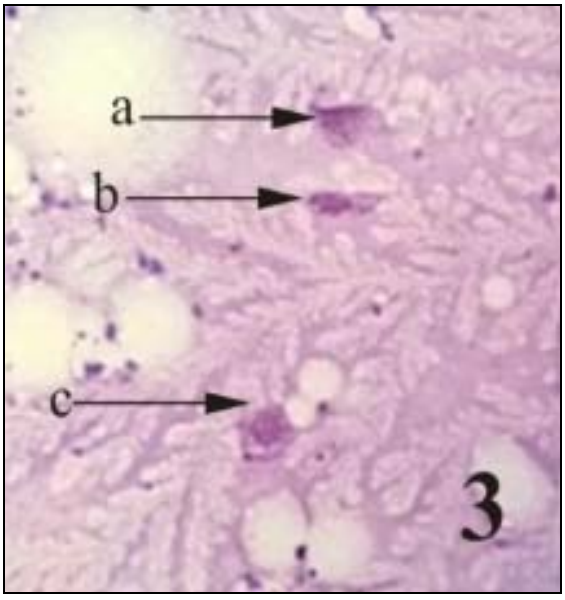

Fig 3: a. and b. Plasmatocyte, c. Coagulocyte

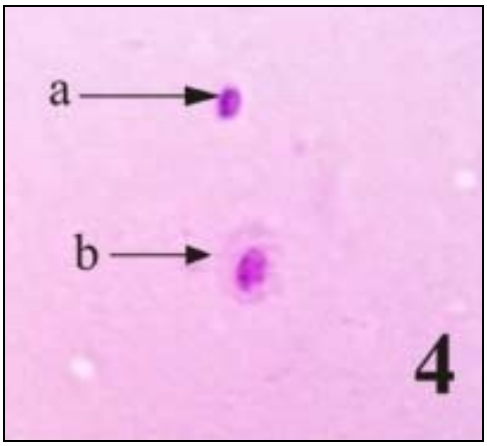

Fig 4: a. Prohaemocyte and b. Coagulocyte

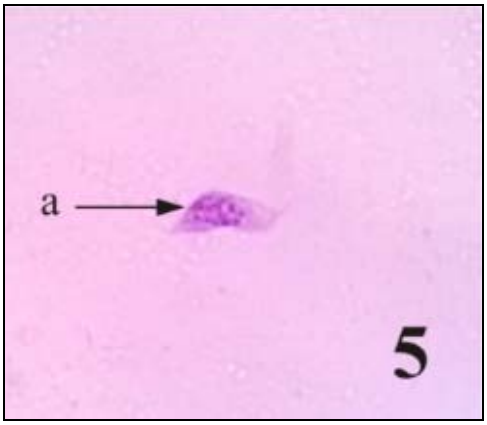

Fig 5: a. Plasmatocyte

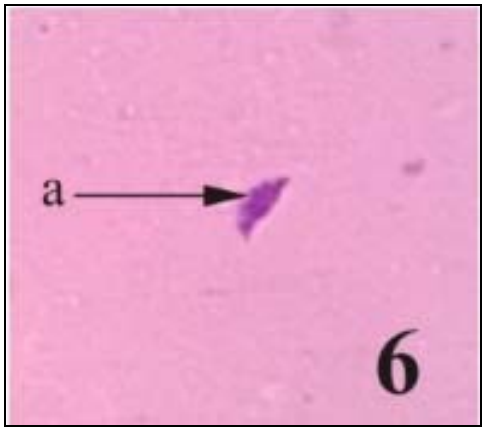

Fig 6: a. Plasmatocyte

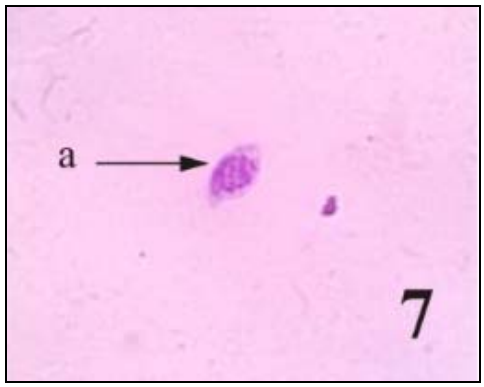

Fig 7: a. Plasmatocyte

Fig 8: a. Coagulocyte

4. Discussion

The results obtained from the present work after application of insecticide at low concentration total haemocyte count was increased, when compared with untreated *P. belli* beetle. These findings matched with Alhriri and Anjum [3]. He stated that the total counts after application of Lambdacyhalothrin and Deltamethrin were significantly increased comparing with those of the untreated female of *Schistocera gregaria.* These two insecticides also showed as increase in plasmatocyte, coagulocyte and spherulocyte.

The plasmatocytes and granulocytes cells are described as the main cell types involved in all defence mechanism [10, 45, 46], [58]. Phagocytosis is considered the first barrier against pathogens and it has been described in the haemolymph of many insect species against biological [45] and non-biological agents [56, 57, 53]. 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 [52, 53].

Abnormalities caused by insecticides to the haemocytes were: agglutination, denucleation and enlargement of cells, distortion of the cytoplasmic and nuclear membrane and abnormal staining of the haemocytes**.** Penfluron seems to cause a great reduction in haemocytes in both sexes at 72 hours and 96 hours after treatment against *Dysdercus koenigii* [43].

Increase in the concentrations of insecticides decreases the THC as compared to low concentration. But in the chlorpyriphos 20% EC at 600 ppm concentration the decreased THC was observed and these results matched with those of Abdin [1] and Iqbal [27] who noticed that THC decreased after the application of Sure (abamactin) and Lorsban. These findings are also similar with those of Mohmood and Yousaf [34], Khan [32], Awan [7] and who applied Diptrix, Curacron 500 EC and Nogos 100 EC on *Leucinodes orbonalis* Guon and *Pieris brassicae* L. respectively and observed that the THC increased just after application of these insecticides.

The application of insecticides in addition to abnormal counts also caused great abnormalities to the type of blood cells. These abnormalities are distortion of the cytoplasmic and nuclear membrane, rupturing of cell wall, agglutination of cells, denucleation of the cells and enlargement of cells and abnormal staining of the haemocytes. These findings are in conformity with the findings of Muhammad [37], Chatha [16], Akram [2], Mahmood and Yousaf [34] and Ayub [8] either with full agreementor with slight contradiction. Similar kinds of

observations were also reported by Arvey *et al.,* [6] in *C. decimlineata* and Gupta and Sutherland [24] in *P. americana*. However, regarding the cause of increase in cell number, Pilat [42], Fisher [20], Trehan and Pajni [55] expressed different views. They reported that the main cause behind increase in number is release of adhered haemocytes in general circulation and is in response to insecticide, as these cells are directly involved in defence mechanisms [23].

5. Conclusion

From the above discussion it can be concluded that some insecticides cause increase in total haemocyte count, which can be endorsed to the need of haemocytes for detoxification of poison, which is an inherent property of every organism to defend against the action of toxic substances.

The THC is increased after the application of both the insecticides. The highest increase of THC is observed at low concentration than the higher concentration. However, relatively it is more in Dimethoate 30% EC as compare to Chlorpyriphos 20% EC.

DHC is also altered in treated beetles and the changes are somewhat in similar proportion for both insecticides. The increase in THCs and changes in DHCs are attributed to the release of adhered haemocytes into the blood circulation due to the treatment of insecticide therefore, ultimately increased percentage of these cells.

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