



EFFECT OF CHITIN SYNTHESIS INHIBITOR, FLUFENOXURON ON HAEMOCYTES OF *SPODOPTERA MAURITIA* (BOISD). (LEPIDOPTERA: NOCTUIDAE)


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**ABSTRACT:** Larvae of *Spodoptera mauritia* (Lepidoptera: Noctuidae) were treated with 2.5 ppm and 5 ppm flufenoxuron and their haemogram were investigated after 24 and 48 hours of post- treatment. The haemogram of *Spodoptera mauritia* comprised eight distinct classes of haemocytes. They are Plasmatocytes, Granulocytes, Prohaemocytes, Spherulocytes, Adipohaemocytes, Oenocytoids, Vermicytes and Podocytes. In the normal day1 sixth instar larvae, THC populations of  $1.3860 \pm 3.1199$  cells/mm<sup>3</sup> and in day2 larvae,  $1.2540 \pm 4.1548$  cells/mm<sup>3</sup> in haemolymph were recorded. The percentage of DHC number of plasmatocytes was the highest  $73.50 \pm 4.062$ , compared to untreated larvae ( $1.3860 \pm 3.1199$  cell/mm<sup>3</sup> of haemolymph). THC steeped down to  $1.2320 \pm 3.3122$  cells/mm<sup>3</sup> of haemolymph and  $1.000 \pm 3.5925$  cell/mm<sup>3</sup> of haemolymph at 24 hrs after treatment of the flufenoxuron with 2.5 ppm and 5 ppm, respectively. The haemocytes decreased during 24 hrs and 48 hrs after the treatment with 2.5 ppm and 5 ppm of flufenoxuron, compared with the control. Various abnormalities were evidenced in the haemocyte after 24 and 48 hours, post treatments of flufenoxuron (2.5 ppm and 5 ppm) on the sixth instar larvae of *Spodoptera mauritia*. Major changes were observed in granulocytes and plasmatocytes. These data imply that these two concentrations (2.5 ppm & 5 ppm) of flufenoxuron could strongly interfere with the differentiation of haemocytes and thereby decrease the capability of larval immune defense.

**Key words:** haemocytes, *Spodoptera mauritia*, flufenoxuron, plasmatocytes, haemolymph.

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## INTRODUCTION

Extensive use of fumigants and conventional insecticides has resulted in increased resistance among insect pests and negative public reaction to their use. These factors have prompted a search for alternative control measures which are effective against the pests, are safe to the mammals and have minimal impact on the environment. Insect growth regulators are one of the potential alternatives to conventional control measures for pests.

Insect growth regulators (IGR) also called third generation insecticides, are compounds that affect insect growth via interference with metabolism or development. The chitin synthesis inhibitors (e.g.: Diflubenzuron, Triflumuron and Flufenoxuron) prevent the formation of chitin, which is a vital component of insect cuticle. These compounds have been tested successfully against several insect species [1]. The haemocytes perform various physiological functions in the body of insect. Knowledge on the impact of insect growth regulator, especially chitin synthesis inhibitor in insect on the hematological parameters is comparatively few. Hence in the present study, an attempt has been made to determine the changes in the differential haemocyte count (THC) after treatment with flufenoxuron, on sixth instar larvae of *Spodoptera mauritia*.

## MATERIALS AND METHODS

**Collection and maintenance of insect culture:** The adult moths of *S. mauritia* were collected at night using fluorescent lights. They were kept in glass beakers covered with muslin cloth and were fed with a dilute solution of honey and maintained at laboratory conditions of  $28\pm 2^{\circ}\text{C}$ , RH  $90\pm 30\%$  under 12 hour light: 12 hour dark photoperiod.

**Chemical compound:** Flufenoxuron: (Pestanal) analytical grade.

**Treatments:** The larvae 6<sup>th</sup> instar (day 0) used for the experiments were taken from the laboratory stock colony, reared and maintained as described above. The age of the larvae were abbreviated as day n where day 0 denotes the day of ecdysis of a particular instar. Larvae showing synchronous development from single egg mass were utilized for various experiments in order to avoid variation on intermoult duration. 10 individuals of 6th instars day zero larvae were topically applied with 5  $\mu\text{L}$  of desired concentrations of compound (flufenoxuron). Larval food (*Ischaemum aristatum*) was given for the test larvae. Each tested concentration, was four times replicated. The control was set up by taking 5  $\mu\text{L}$  of acetone.

**Smear preparation and Staining:** A drop of fresh haemolymph was collected by puncturing proleg on the abdominal segment of the larva (with the help of 70% ethanol sterilized needle) and mixed well with anticoagulant. A thin uniform smear of haemolymph was spread on the slide by rubbing the edge of an inclined slide backward. Stock solution of Giemsa stain was prepared as per protocol [2]. A portion of it was diluted 10 times with double distilled water (DDW). Then, air dried smear was stained for 20 min and thereafter rinsed with DDW and mounted in DPX.

**Differential and Total Haemocyte Count (DHC and THC):** DHC was conducted by counting of different categories of randomly selected cells from stained smears of 10 individuals.

For THC, the haemolymph was drawn in to a thoma blood cell pipette up to its graduated mark of 0.5 and diluted up to the 11<sup>th</sup> mark with Tauber-Yeager's fluid [2], then shaken for several minutes and the first three drops were discarded. A double line with improved Neubauer ruling haemocytometer was filled with diluted haemolymph and the haemocytes were counted at four corners and one central ( $1\text{mm}^2$ ) square. When the distributions of cells in all squares were not even, the sample was discarded and the procedure was repeated. The number of haemocytes per cubic millimeter ( $\text{mm}^3$ ) was calculated using the formula of Jones [3]:

$$\frac{\text{Haemocytes in five } 1\text{mm}^2 \times \text{Dilution} \times \text{Depth factor of chamber}}{\text{No. of squares counted}}$$

No. of squares counted

Where dilution = 22 times, depth factor of the chamber = 10(constant) and the number of square counted = 5.

### Statistical Analysis

All data were presented as mean  $\pm$  SE. Statistical significance was determined using the one-way analysis of variance (ANOVA) and separated by a least significant difference multiple range test, and a probability level  $p < 0.05$  was considered statistically significant by SPSS version 16.0 [4].

## RESULTS AND DISCUSSION

During the present study, eight distinct classes of haemocytes were recognized in the last instar larvae of *S. mauritia*. They are Plasmacytes, Granulocytes, Prohaemocytes, Spherulocytes, Adipohaemocytes, Oenocytoids, Vermicytes and Podocytes. The THC of normal day1 sixth instar larvae were  $1.3860\pm 3.1199$  cells/ $\text{mm}^3$  and in day2 larvae,  $1.2540\pm 4.1548$  cells/ $\text{mm}^3$  in haemolymph and were recorded (Table.1).

The data given in Table 2, revealed that the number of plasmacytes was the highest  $73.50\pm 4.062$  followed by other cells. The data presented in Table 3 revealed that the percentage of DHC in day 2 sixth instar larvae of *Spodoptera mauritia* were Plasmacytes at the highest  $63.20\pm 34.227$ , followed by other cells. Circulating haemocytes have important functions in the immune system, metabolism, and detoxification that eventually play a crucial role in the defense of xenobiotics or microbial infection.

Sharma *et al* [5] showed that the number and proportion of different haemocytes were beneficial for insects to develop environmental fitness. Earlier many studies were envisaged on the effects of various toxins, such as insecticide's stress factors on the haemocyte of various insects. But only, limited work has been carried out on the role of insect growth regulators (IGR), particularly Chitin Synthesis Inhibitor (CSI) on the hematology of insects.

**Effect of flufenoxuron on the THC of sixth instar larvae of *Spodoptera mauritia*:** When compared to untreated larvae ( $1.3860 \pm 3.1199$  cell/ml<sup>3</sup> of haemolymph), THC steeped down to  $1.2320 \pm 3.3122$  cells/ml<sup>3</sup> of haemolymph and  $1.000 \pm 3.5925$  cell/ml<sup>3</sup> of haemolymph after 24 hr treatment of the compound with 2.5 ppm and 5 ppm, respectively. Similarly, compared to untreated larvae ( $1.2540 \pm 4.1548$  cell/ml<sup>3</sup> of haemolymph), THC reached to  $0.9460 \pm 2.5509$  cell/ml<sup>3</sup> of haemolymph and  $0.9020 \pm 2.1877$  cell/ml<sup>3</sup> of haemolymph, at 48 hr after treatment with 2.5 ppm and 5 ppm, respectively (Table.1). The results of present findings are in agreement with the works of several authors. Sabri and Tariq, [6] noticed that THC decreased after application of 20EC, Tracer 240EC and Deltaphos-R on *Aulacophora foveicollis* Lucas. Similar results were reported by Fareed [7] when used Tracer 480 SC on the spotted boll worm, *Earias* spp and noted that hemocyte number decreased after half an hour.

**Effect of flufenoxuron on DHC of sixth instar larvae of *Spodoptera mauritia*:** The percentage of Plasmacytes, Granulocytes, Prohaemocytes, Spherule cells, Podocytes, Vermicytes, Oenocytoids and Adipohaemocytes decreased from  $65.20 \pm 5.884$ ,  $62.30 \pm 8.287$ ,  $12.50 \pm 2.953$ ,  $19.60 \pm 3.062$ ,  $11.00 \pm 2.708$ ,  $6.80 \pm 2.70$ ,  $17.80 \pm 4.341$  and  $13.40 \pm 3.921$  to  $43.0 \pm 4.784$ ,  $41.80 \pm 2.974$ ,  $8.30 \pm 2.214$ ,  $11.30 \pm 2.497$ ,  $7.40 \pm 2.547$ ,  $3.20 \pm 1.687$ ,  $12.90 \pm 4.202$  and  $9.80 \pm 3.425$  respectively, during 24 hour after the application of treatment with 2.5 ppm and 5 ppm of flufenoxuron, as when compared with the control (Table 2). The percentage of Plasmacytes, Granulocytes, Prohaemocytes, Spherule cells, Podocytes, Vermicytes, Oenocytoids and Adipohaemocytes decreased considerably as compared with control from  $45.80 \pm 4.894$ ,  $42.70 \pm 4.715$ ,  $9.60 \pm 3.340$ ,  $14.70 \pm 2.627$ ,  $6.60 \pm 1.838$ ,  $10.10 \pm 3.573$ ,  $6.60 \pm 1.838$  and  $1.80 \pm 1.033$  to  $41.00 \pm 3.944$ ,  $38.60 \pm 2.366$ ,  $7.00 \pm 1.491$ ,  $11.30 \pm 2.11$ ,  $6.30 \pm 1.494$ ,  $8.70 \pm 2.627$ ,  $5.10 \pm 1.853$  and  $1.00 \pm 0.667$  respectively, after 48 hr of treatment with 2.5 ppm and 5 ppm of flufenoxuron as shown in (Table 3). Also significant variations in the decline of haemocyte population were noticed at 48hours, than 24hours.

**Table 1: Haemogram of total haemocyte count at 24 hours and 48 hours of flufenoxuron treated (control, 2.5 ppm & 5 ppm) larvae of *Spodoptera mauritia*.**

Time intervals	Control	2.5 ppm	5 ppm
24 hrs	1.3860±3.1199	1.2320±3.3122a	1.1000±3.5925b
48 hrs	1.2540±4.1548c	0.9460±2.5509a	0.9020±2.1877b

Mean ±standard deviations. Means followed by the same letter in the same column are not significantly different ( $p < 0.05$ ).

**Table 2: Haemogram of 6<sup>th</sup> instar larvae of *Spodoptera mauritia* after 24 hours of treatment of flufenoxuron.**

Haemocyte type	Control	2.5 ppm	5 ppm
Plasmacytes	73.50±4.062	65.20±5.884a	43.0±4.784a
Granulocytes	71.50±12.508	62.30±8.287b	41.80±2.974a
Prohaemocytes	14.10±4.012	12.50±2.953d	8.30±2.214c
Spherulocytes	21.50±3.536	19.60±3.062b	11.30±2.497a
Podocytes	13.10±3.348	11.00±2.708b	7.40±2.547b
Vermicytes	8.90±2.644	6.80±2.70b	3.20±1.687b
Oenocytoids	20.20±3.938	17.80±4.341b	12.90±4.202c
Adipohaemocytes	16.30±4.191	30.40±3.921c	9.80±3.425c

Mean ±standard deviation. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. \*Significant at  $P < 0.05$  level.

**Table 3: Haemogram of 6<sup>th</sup> instar larvae of *Spodoptera mauritia* after 48 hours of treatment of flufenoxuron.**

Haemocyte type	Control	24 Hrs	48 Hrs
Plasmatocytes	63.20±34.227c	45.80±4.894a	41.00±3.944a
Granulocytes	48.80±1.874a	42.70±4.715a	38.60±2.366ab
Prohaemocytes	10.80±3.120a	9.60±3.340a	7.00±1.491c
Spherulocytes	19.30±2.058b	14.70±2.627b	11.30±2.111a
Podocytes	9.80±1.229b	6.60±1.838b	6.30±1.494b
Vermicytes	12.90±3.143a	10.10±3.573a	8.70±2.627a
Oenocytoids	7.10±1.449c	6.60±1.838c	5.10±1.853a
Adipoaemocytes	21.10±3.143a	1.80±1.033c	1.00±0.667b

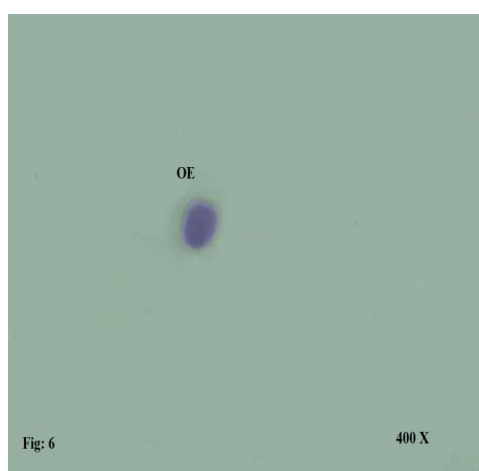
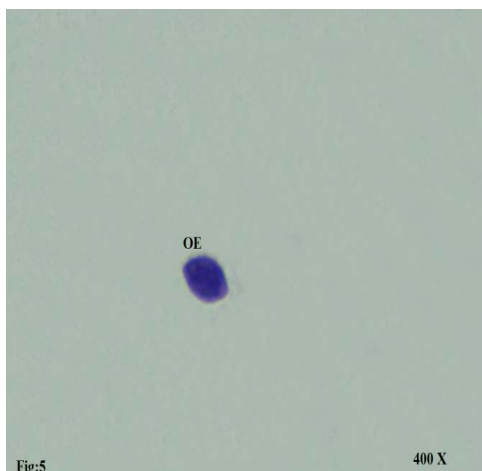
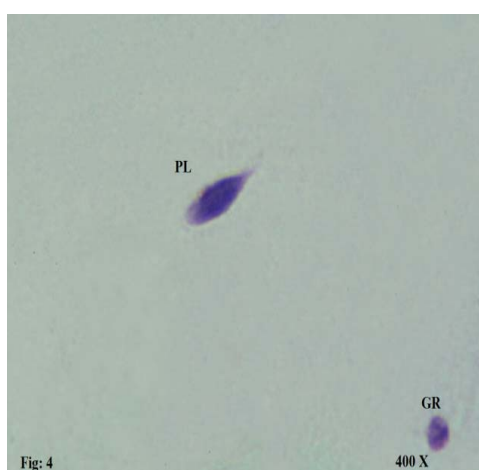
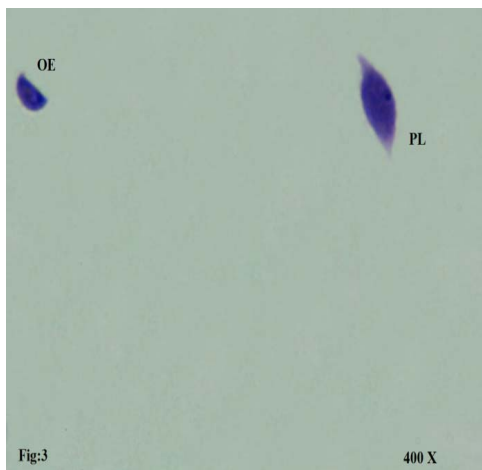
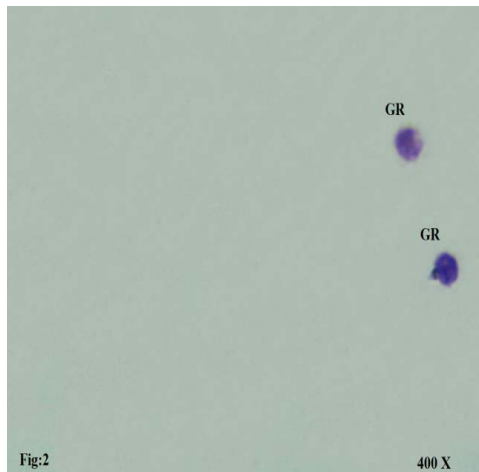
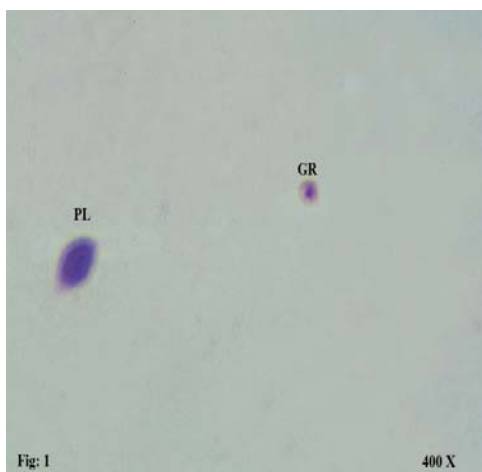
Mean ±standard deviation. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. \*Significant at  $P < 0.05$  level.

### Effect of flufenoxuron on haemocyte morphology:

Various abnormalities were evidenced in the haemocyte after 24 hour post treatment of flufenoxuron (2.5 ppm) on the sixth instar larvae of *Spodoptera mauritia*. Major changes observed are in granulocytes and plasmatocytes. The other notable changes observed among the cells are: granulocytes are with cytoplasmic projections, oenocytoids with a shallow appearance at one side, followed by the rupture of the cell wall, loss of cytoplasmic compactness of the barrel shaped plasmatocytes (Plate:1 Figs:1-6). Observations were recorded after 48 hours of application of 2.5 ppm flufenoxuron resulting in clumping of haemocytes, followed by rupturing of wall of haemocytes, distortion of the shape of haemocytes especially including granulocytes, podocytes and vermicytes.

5 ppm flufenoxuron post administration after 24 hours on the sixth larval instar of *Spodoptera mauritia*, caused damage in most of the cellular types. The observed abnormalities are following, spindle shaped plasmatocytes with a cytoplasmic projection, loss of cytoplasmic compactness of pseudopods of plasmatocytes, the oenocytoids with shallow pit at one side with thinning of cytoplasm, eccentrically pushed nuclei and most of these cells are with irregular cell boundaries (Plate:2 Figs7- 11). Noticeable changes were observed after 48 hour post treatment of the 5 ppm of compound inducing clumping of haemocytes in *Spodoptera mauritia* larvae (Plate:3 Fig:12,13). Mainly the dose induced damage of oenocytoids, granulocytes, and plasmatocytes. Structural malformations were clearly observed, shape of oenocytoid with numerous cytoplasmic projections, loss of cytoplasmic compactness of pseudopods and plasmatocytes, enlargement and deformation of the shape of granulocytes were other evident changes. In our previous study on the late developmental stages of *S. mauritia*, the observed eight haemocytes are very much distinguished with respect to their cytological stained features [8]. Bhagawathi and Mahantha, [9] also recorded a reduction in percentage of prohaemocytes with larvae of Eri silk worm against dimethoate. These findings are similar to George and Ambrose, [10] against *Dysdercus koeginii* after application of azadirachtin and malathion respectively. Batti, [11]; Saxena and Srivasthava, [12] depicted about destruction of haemopoetic organs, that are eventually responsible for the production of prohaemocytes. Tiwari et al [13] and Nahala et al [14] also reported that, sub lethal doses of dimilin decreased the proportion of prohaemocytes in *Agrotis ipsilon*

The two (2.5 ppm & 5 ppm) concentrations of flufenoxuron not only significantly inhibited chitin synthesis of *Spodoptera mauritia*, but also affected change in the total number and the proportion of the circulating haemocytes, thereby affecting immune function and survival of the individuals. The residual activity of insecticides can enhance selection for insect resistance. Therefore, predicting the overall effects of insecticidal use, including mortality and sub-lethal effects in insects, can facilitate the development of truly selective insecticides that can be employed in integrated pest management strategies. In conclusion, the impact of chitin synthesis inhibitor, flufenoxuron exhibited a decrease in capability of larval immune defense, there by altering the hormonal triggers in the treated individuals.



**Plate: 1- Effect of flufenoxuron (2.5 ppm) after 24 hours of post treatment on the haemocytes of sixth instar larvae of *S.mauritia*.**

Fig: 1- Ruptured cell wall of granulocyte (GR) and plasmatocytes (PL).

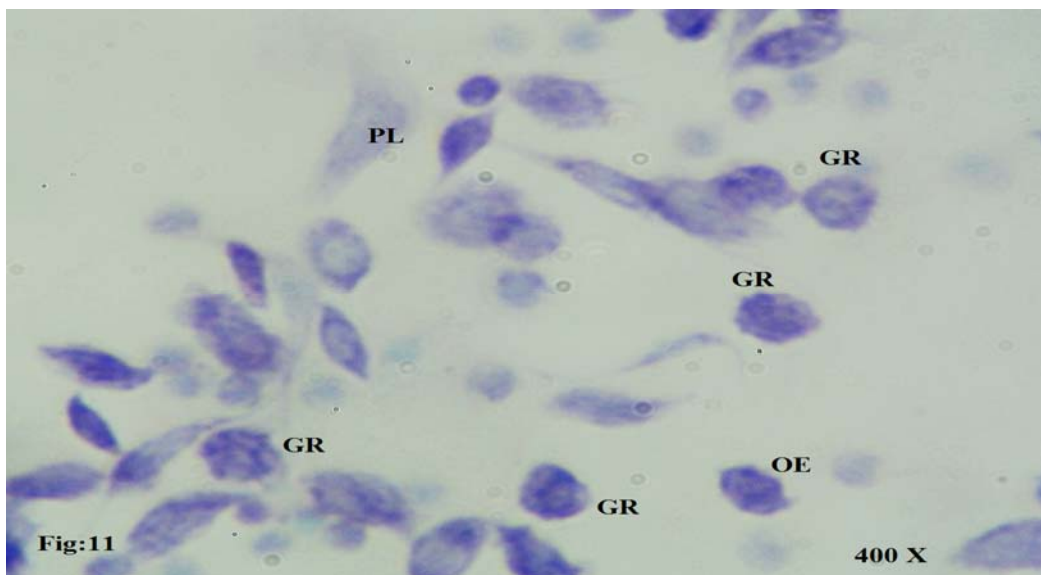
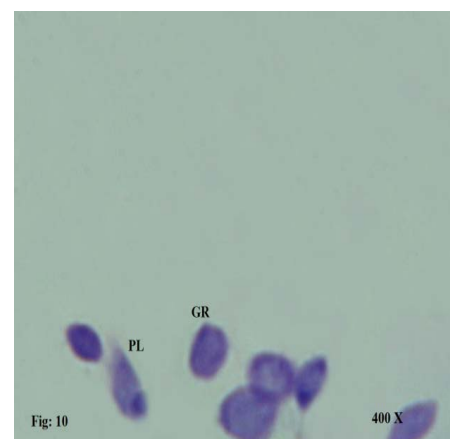
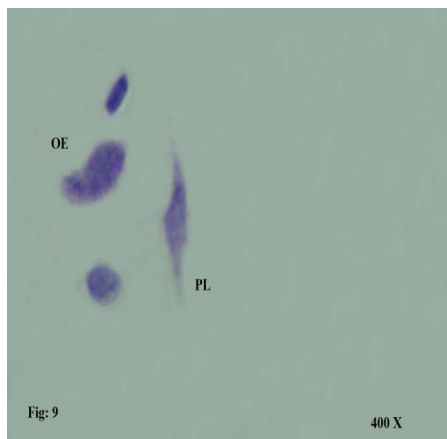
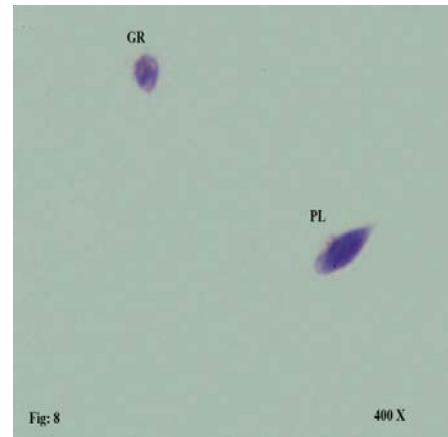
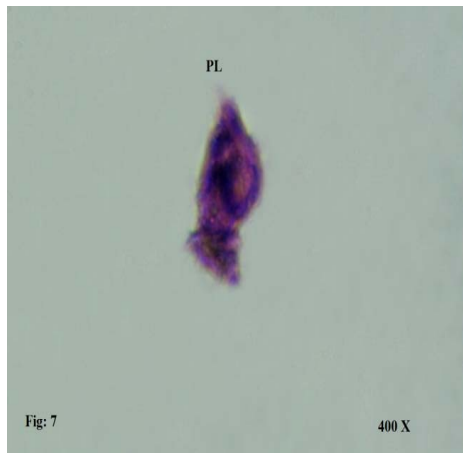
Fig: 2- Granulocyte (GR) with cytoplasmic projection.

Fig: 3- Oenocytoid (OE) with a shallow appearance at one side.

Fig: 4- Loss of cytoplasmic compactness of pseudopods of plasmatocytes (PL).

Fig: 5- A expanded oenocytoid (OE).

Fig: 6- A expanded oenocytoid (OE) with irregularity in the cell boundary.



**Plate: 2- Abnormalities in haemocytes profile as affected by treatment of flufenoxuron (5 ppm) after 24 hours on the sixth instar larvae of *S.mauritia*.**

Fig: 7- Lobed nucleus in plasmatocytes (PL) with cytoplasmic projection.

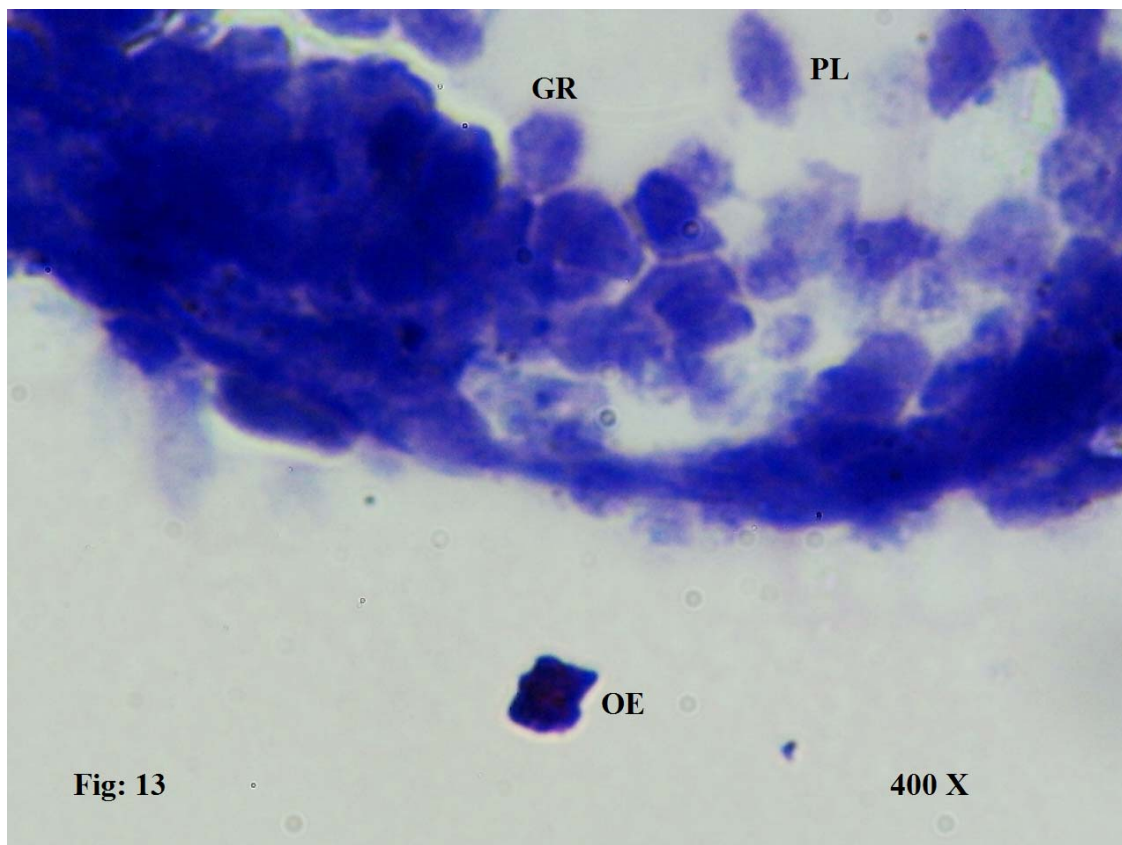
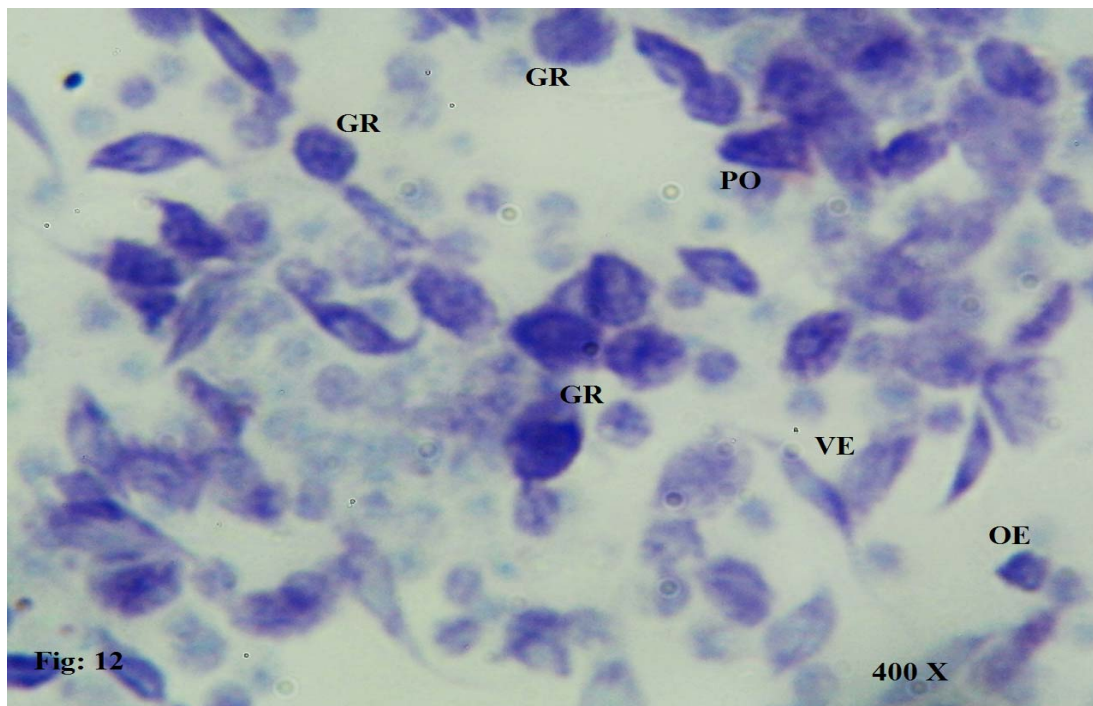
Fig: 8- Loss of cytoplasmic compactness of pseudopods of plasmatocytes (PL).

Fig: 9- Oenocytoid (OE) with shallow pit at one side and thinning of cytoplasm.

Fig: 10- Granulocyte (GR) with cytoplasmic projection.

Fig: 11- A group of cell with irregular cell boundary and eccentrically pushed nuclei.





**Plate: 3--Haemocyte contour of sixth instar larvae of *S.mauritia* after 48 hours of treatment of flufenoxuron**

Fig: 12- Abnormalities caused due to effect of 2.5 ppm flufenoxuron after 48 hour treatment on sixth instar larvae of *S.mauritia* induces the changes in haemocyte contour.

Fig : 13- Clumping of haemocyte population as effected by treatment of 5 ppm flufenoxuron.

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