

## Effect of sublethal doses of Cypermethrin on the haemocytes of *Periplaneta americana* (Dictyoptera: Blattidae)

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**Abstract:** The American cockroaches, *Periplaneta americana* (Linnaeus) are omnivorous insects and spread a great number of diseases world-wide. Hematological studies are very important in insect physiology because the haemocyte performs various physiological functions in the body. Cypermethrin exhibited the enormous changes to total and differential haemocyte count after 24 hours exposure. The action of Cypermethrin at various sublethal doses (0.008%) and (0.02%) against American cockroaches was introduced to study Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC). Six different types of haemocytes had been observed in the haemolymph, including Prohaemocytes (PRs), Plasmocytes (PLs), Spherulocytes (SPs), Oenocytoids (OEs), Adipohaemocytes (ADs), Coagulocytes (COs). The average number of blood cells counted was higher in male as compared to female. The number of blood cells counted under the microscope after the use of 0.008% and 0.02% of insecticides were significantly decreased in adult male and female cockroaches. It had been observed that the numbers of blood cells greatly reduced after the application of 0.02% Cypermethrin (10% EC) as compared to 0.008% on the haemolymph of adult cockroaches.

**Keywords:** *Cypermethrin, haemocytes, American cockroaches.*

**Introduction:** *Periplaneta americana* (Linnaeus) (Dictyoptera: Blattidae) are commonly known as the American Cockroaches. They are usually omnivorous insects, feed on great variety of food items and are found almost in everywhere. The haemocytes are cells that circulate in the haemolymph of insects, providing quick and efficient response against pathogens that invade the haemocoel (de Andrade et al., 2010). They are motile and phagocytic with lysosomal vesicles, and play important role in the immune system of invertebrates. Cypermethrin is a synthetic, pyrethroid insecticide that has high insecticidal activity, low avian and mammalian toxicity, and adequate stability in air and light (Kaufman et al., 1981, and U.S.D.A., 1995). Cypermethrin is a stomach poison as well as a contact insecticide (Jin and Webster, 1998). Various literatures reported on effect of Lambda Cyhalothrin and Deltamethrin on the haemocytes of desert locust by Khalid and Anjum (2001); haemocyte types and total and differential counts of Dipteran larvae by Silva et al. (2002); the haemocyte types, differential and total count on Lepidoptera by Jalali, & Salehi, (2008); effect of Methoprene on total haemocyte counts and histopathology of haemocytes on Lepidoptera by Sendi and Salehi (2010); effects of the pyrethroid insecticide Deltamethrin on the haemocytes of Lepidopteran larvae by Kurt and Kayış (2015); toxicity of some insecticides to the haemocytes of giant honeybee by Perveen and Ahmad (2017); histopathological effect of Deltamethrin on the mid-gut of American cockroach by Majumdar et al. (2016).

Hematological studies are very important in insect physiology because the haemocyte performs various physiological functions in the body. The primary functions of haemocytes are: coagulation to prevent loss of blood, phagocytosis, encapsulation of foreign bodies in the insect body cavity, nodule formation, detoxification of metabolites and biological active materials and distribution of nutritive materials to various tissues and stored them also and may be hormones (Garcia and Rosales, 2002; Zhou *et al.*, 2004; Ling and Yu, 2006; Ribeiro and Brehelin, 2006; Siddiqui and Al-Khalifa, 2012; Chavan *et al.*, 2017).

Environmental pollutants such as insecticides, pesticides and heavy metals cause huge structural changes to haemocytes. These changes can be used to characterize the genotoxic, physiological, and biochemical effects of pollutants. Therefore, *P. americana* can be treated as laboratory model organisms in endocrinology, entomology, reproductive physiology because of their availability, ease of rearing, short life cycle and can be able to show results within few days.

The present study deals with the effect of most commonly used insecticide Cypermethrin (10% EC) on the haemolymph of *P. americana*. It aims to observe the total and differential haemocyte counts of adult male and female cockroaches and also to record the abnormalities caused by the insecticide on the haemolymph of adult *P. americana*.

**Materials and Methodology:** The adult male and female American cockroaches were collected during February-March, 2019 from the manhole in Kolkata (22.5880° N, and 88.3680° E), West Bengal. They were kept under suitable environmental conditions and the photoperiod of 12 hours light and 12 hours dark were also maintained. They were kept in plastic jars and upper side of the jars was perforated so that they could get oxygen. The cockroaches were allowed to eat bread crumbs. Daily feeding and cleaning were also maintained. Cypermethrin is a synthetic pyrethroid insecticide and is used to control wide variety of house-hold pests, spiders, flies, cockroaches, pests of fruit and vegetable crops. The chemical name of Cypermethrin (10% EC) is IUPAC: [Cyano- (3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate. Cypermethrin concentration applied on the adult male and female cockroaches during experimentation is shown in Figure 1.



Fig1. Cypermethrin concentration on the adult male and female Cockroaches (*Periplaneta americana*)

**Preparation of different insecticidal concentrations:-**

0.1% stock solution of Cypermethrin (10% EC) was prepared in distilled water. Two experimental concentrations i.e. 0.02% and 0.008% were prepared from stock solution freshly through diluting stock solution in distilled water.

**Application of insecticidal concentrations:-**

Bread crumbs were thoroughly mixed with 2.5 ml of two different concentrations of insecticide (0.02% and 0.008%) in each Petri-dish. The Petri-dishes were placed in two separate plastic jars. Parallel to these control set up was also maintained.

**Total Haemocyte Count (THC):-**

The haemolymph of cockroach was collected into Thoma white blood cell pipette up to the 0.5 mark and it was then diluted up to 11 mark with Toissin's solution (NaCl = 1.0 gm, Na<sub>2</sub>SO<sub>4</sub> = 8.0 gm, neutral glycerin = 20 ml, Methyl violet = 0.025 gm and Distilled water = 160 ml) upto the mark 11 (Mahmood and Yousaf, 1985). The pipette was shaken several minutes and the first 3-4 drops were discarded to avoid errors. Two drops of this solution were placed near the edge of the cover slip. Neubauer haemocytometer then automatically filled up the counting chambers by the capillary action. Bubbles should be avoided. Haemocytes counted in the four corners and one central chamber under the microscope. The haemocytes must be evenly distributed in the hemocytometer, if not, the sample was discarded. This process was repeated several times to obtain results. THC of both normal and treated cockroaches (0.02% and 0.008%) was studied under microscope. The number of circulating haemocytes of male and female cockroaches per cubic millimeter (mm<sup>3</sup>) was calculated using the formula, given by Jones (1962):

$$\frac{\text{Number of haemocyte counted per chamber} \times \text{dilution} \times \text{depth factor}}{\text{Number of 1 mm squares counted}}$$

Where, dilution = 20 times, Depth factor of the chamber = 10 (constant) and No. of squares counted = 5 for blood smear slide preparation.

**Differential Haemocyte Count (DHC):-**

Haemolymph preparation was done, according to Arnold and Hinks (1979). The cockroach was hold with fingers and the thoracic leg was amputated from the base of the femur by using sharp scissor. Haemolymph oozed out through the amputated leg, and the ooze was collected on the center of the clean glass slides by touching its leg. A thin blood smear was made by drawing a second slide over the first slide at 45° angle. The slides were then allowed to dry for 1 minute, and fixed for 2 minutes with drops of absolute methyl alcohol. Fixed cells were

stained with Giemsa’s solution (diluted 1 : 20 in distilled water) for 20 minutes, washed several times with tap water, and dipped into distilled water. The stained smears were air-dried and mounted in DPX with cover slip. The differential counting of haemocytes was observed under microscope (10X x 100X). DHC was studied in both control and treated cockroaches. The percentages of haemocytes were calculated by using the formula (Ghoneim et al., 2017) given below-

$$\frac{\text{Number of each haemocyte type}}{\text{Total number of haemocytes examined}} \times 100$$

Depending on the cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus, the free haemocytes in the haemolymph of cockroaches had been identified and distinguished in the present study.

**Results and Discussions:** Haemolymph of insect consists of fluid plasma and haemocytes. Numbers and sizes of circulating haemocytes vary in relation to age and life cycle stages of insect. Different types of haemocytes have important role in the protection of insects against invading pathogens and parasitoids. Tiwari and Shukla (2000) and Pandey et. al. (2008) reported the hematological changes of insect in response to the foreign invaders. Cypermethrin acts as a contact poison as well as stomach poison in invertebrates. Higher dose of Cypermethrin immediately affects voltage dependent sodium channels by inhibiting ATPase enzymes in the nerve membrane of central nervous system (Jones, 1995).

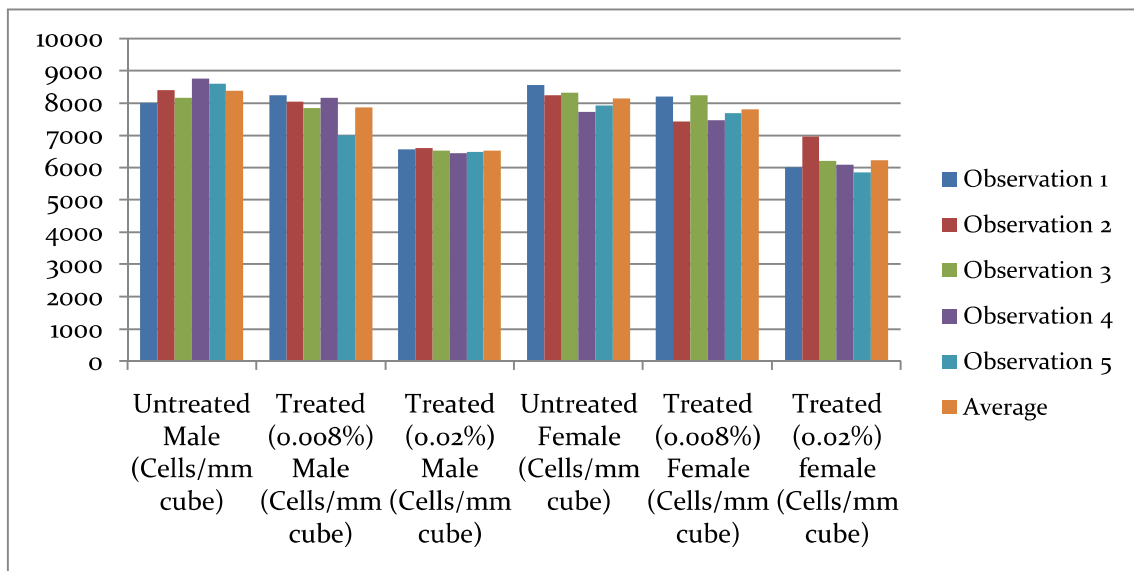


Fig 2: Comparative study of Cypermethrin on the Total Haemocyte Count (THC) of the adult *Periplaneta americana* after 24 hours exposure.

**Total Haemocyte Count (THC)**

Figure 2 shows the comparative study of Cypermethrin on the Total Haemocyte Count (THC) of the adult *Periplaneta americana* after 24 hours exposure. The total haemocyte count of

adult male and female *P. americana* was observed under microscope before and after the application of insecticide (Cypermethrin).

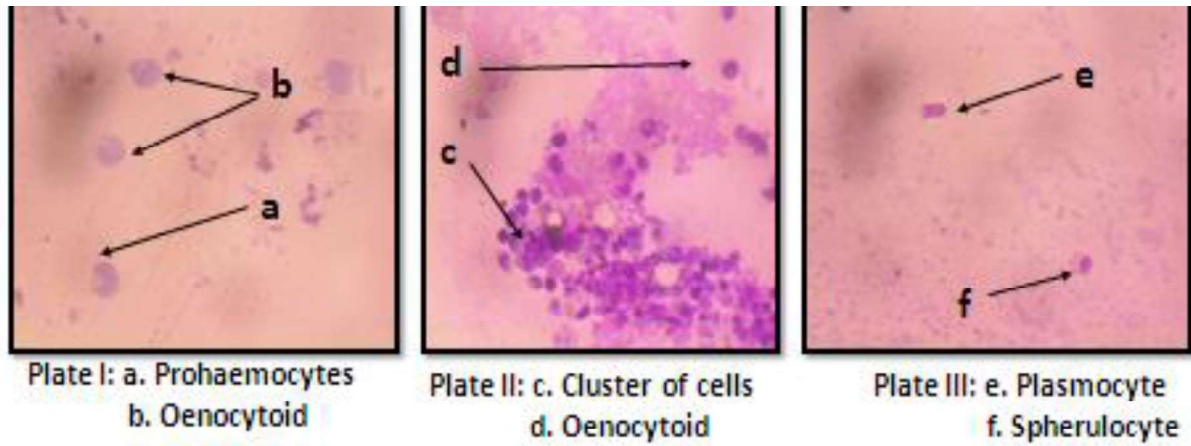


Fig.3. (Plate I; Plate II; Plate III): Different haemocytes in untreated cockroaches

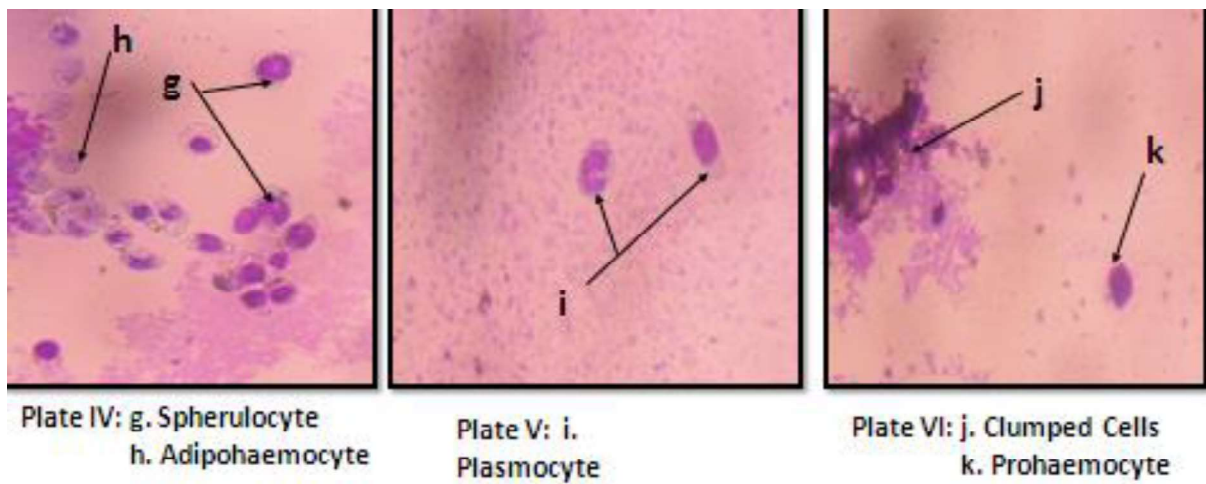


Fig 4. (Plate IV; Plate V; Plate VI): Different haemocytes in treated (0.008% Cypermethrin) cockroaches

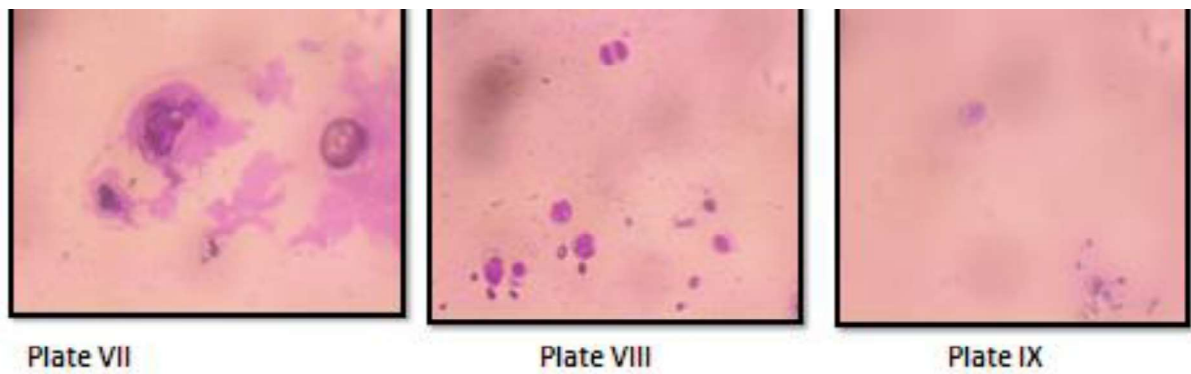


Fig 5 (Plate VII; Plate VIII; Plate XI): Different damaged haemocytes in treated (0.02% Cypermethrin) Cockroaches



**Untreated cockroaches:**The haemocytes were observed more in adult male cockroaches compared to that of female cockroaches. The highest number of haemocytes found in male cockroaches about 8760 cells per millimeter cube while the maximum number of haemocytes found in female cockroaches about 8560 cells per millimeter cube. The average numbers of haemocytes were observed in male 8384 cells/mm cube but in female the average haemocytes were 8152 cells/mm cube. These results differ from those of Pugazhvendan and Soundararajan (2012) who studied quantitative changes of Total Haemocytes Count during metamorphosis and reproduction in Hemipteran species.

**Treated cockroaches:** The effect of Cypermethrin (0.02% and 0.008%) after 24 hours exposure, on the blood cell count in haemolymph of the adult male and female *P. americana*, was greatly significant (Table 1). The highest number of blood cells observed and counted under microscope after the application of 0.008% Cypermethrin (10% EC) was significantly higher than the cells were counted after the application of 0.02% Cypermethrin (10% EC). The average number of blood cells counted under the microscope after the use of 0.008% insecticide was 7856 cells/mm cube in male whereas 7808 cells/mm cube in female which was significantly lower than the data obtained from untreated male and female cockroaches. It had been seen that the number of blood cells greatly reduced after the application of 0.02% Cypermethrin in female cockroaches, it greatly varied from 5840 to 6960 cells/mm cube than male which varied from 6440 to 6600 cells/mm cube on the haemolymph of adult *P. americana*. The similar trends were also observed by the findings of Kurt and Kayis (2015) who observed the effects of the pyrethroid insecticide Deltamethrin on the haemocytes of moth species. These results differed from those Al Hariri and Suhail (2000) who observed the

Table 1: Effect of Cypermethrin on the Total Haemocyte Count (THC) of adult *Periplaneta americana* after 24 hours exposure.

No. of Observations	Untreated Cockroaches		Treated Cockroaches with 0.008% Cypermethrin (10% EC)		Treated Cockroaches with 0.02% Cypermethrin (10% EC)	
	Male (Cells/mm cube)	Female (Cells/mm cube)	Male (Cells/mm cube)	Female (Cells/mm cube)	Male (Cells/mm cube)	Female (Cells/mm cube)
1.	8000	8560	8240	8200	6560	6000
2.	8400	8240	8040	7440	6600	6960
3.	8160	8320	7840	8240	6520	6200
4.	8760	7720	8160	7480	6440	6080
5.	8600	7920	7000	7680	6480	5840
<b>Average</b>	<b>8384</b>	<b>8152</b>	<b>7856</b>	<b>7808</b>	<b>6520</b>	<b>6216</b>

effect of Lambda Cyhalothrin and Deltamethrin on the Haemocytes of desert locust.

### **Differential Haemocyte Count (DHC)**

Different haemocytes in untreated, treated (0.008% Cypermethrin) and different damaged haemocytes in treated (0.02% Cypermethrin) Cockroaches are shown in Figure 3, Figure 4 and Figure 5 respectively.

#### **Untreated cockroaches**

Differential Haemocyte Count in *P. americana* revealed highest percentage of Prohaemocytes (12.57%), followed by Oenocytoids (9.96%), Spherulocytes (8.84%) respectively. Adipohaemocytes (4.85%) were frequently observed in the blood smear of untreated cockroaches. Plasmocytes (3.73%) were also recognizable. Coagulocytes (2.86%) were least observable haemocyte in normal cockroaches.

#### **Treated cockroaches (0.008% concentration of Cypermethrin)**

There was an increase in the percentage of disintegrated haemocytes after 24 hours treatment of 0.008% concentration of Cypermethrin. Plasmocytes were increased to 4.48% compared to control 3.73%. Oenocytoids showed a reduction (7.34%) in their population as compared to the control set up 9.96%. Adipohaemocytes (5.35%) were frequently observed. Spherulocytes were also reduced to 5.23% compared to control 8.84%. Coagulocytes were scarcely visible under microscope. A huge change was observed in Prohaemocytes and their percentage was significantly decreased from 12.57% to 8.59% in the haemolymph of adult *P. americana* 24 hours post treatment of Cypermethrin.

#### **Treated cockroaches (0.02% concentration of Cypermethrin)**

After 24 hrs treatment of 0.02% concentration of Cypermethrin, Prohaemocytes were greatly affected and their percentage decreased to 6.22% as compared to control 12.57%. The Plasmocytes were severely affected cells and their wall was also ruptured due to the effect of insecticide. There was a minor change in Coagulocytes and their population was slightly decreased from 2.86% to 2.49%. The percentage of Oenocytoid (6.47%) showed a decrease with an increase in the concentration of insecticide, although they were distorted little bit. Spherulocytes showed a reduction (6.10%) in their number as compared to control set up (8.84%). Abnormal staining of haemocytes (i.e. changes in cell shape) was observed in the blood smear of *P. americana* treated with 0.02% Cypermethrin.

Insect is a favored model organism for such studies because of its short life cycle, larval size, ease of rearing, and ability to show results within days (Cook and Mc Arthur, 2013). Therefore, haemocytes (via changes in cell number and development of structural abnormalities) are frequently used to demonstrate the cytogenetic damage caused by toxic chemicals (Yeh et al., 2005). Circulating haemocytes perform several biological functions in insect body including transport and storage of nutrients, nodule formation, detoxification, wound healing, coagulation and plasma precipitation, heat transfer and protection. Cypermethrin caused significant changes to total and differential haemocyte count after 24 hours exposure. The number of blood cells counted under the microscope after the use of

0.008% and 0.02% of insecticides were significantly decreased in adult male and female cockroaches. It had been seen that the number of blood cells greatly reduced after the application of 0.02% Cypermethrin (10% EC) as compared to 0.008% on the haemolymph of adult *P. americana*. Several abnormalities such as distortion of nuclear membrane, changes in the cytoplasmic ratio, formation of vacuoles inside the haemocytes were also observed. Haemocytes in insect haemolymph have variable responses to different insecticides exposure. Increase or decrease in the percentage of haemocytes depends on toxicity of the insecticide, insect immune system and certain environmental factors.

**Conclusion:** Different types of haemocytes have important role in the protection of insects against invading pathogens and parasitoids. Cypermethrin is a synthetic, pyrethroid insecticide that is extremely effective against a wide range of insect pests. Cypermethrin acts as a contact poison as well as stomach poison in invertebrates. Cypermethrin exhibited the enormous changes to total and differential haemocyte count after 24 hours exposure. The average number of blood cells counted was higher in male as compared to female. It had been observed that the number of blood cells greatly reduced after the application of 0.02% Cypermethrin (10% EC) as compared to 0.008% on the haemolymph of adult cockroaches. Several abnormalities such as distortion of nuclear membrane, changes in the cytoplasmic ratio, formation of vacuoles inside the haemocytes were also observed.

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