

Effect of Aldrin and Dipterex on the Haemocytes of Red Cotton Bug, *Dysdercus cingulatus* Fabr. (Hemiptera: Pyrrhocoridae). Zaheer S. ZAIDI and Mumtaz A. KHAN (Department of Zoology, Section of Entomology, Aligarh Muslim University, Aligarh, 202001 India) Received May 29, 1977. *Botyu-Kagaku*, 42, 141, 1977.

21. アカホシカメムシの血球に対する Aldrin と Dipterex の作用 Zaheer S. ZAIDI and Mumtaz A. KHAN (Aligarh Muslim University) 52. 5. 29受理

アカホシカメムシの雌雄成虫の血球は、Aldrin と Dipterex の経皮投与で病的な影響を受ける。低濃度の Aldrin の作用は、Dipterex より弱く、血球の損傷は少ない。Dipterex の2%溶液は、たいていの血球は破壊してしまう。脂肪血球、顆粒血球はともに壊れ易く、脂肪血球が最も弱い。また遊凝球は、破壊されにくい。細胞質血球は、病的な損傷を受けても恢復し易い。

Histological effects of chemicals and insecticides on different tissues of insects were studied in a variety of insects as reviewed in detail by Hoskin (1940)⁷⁾, Winteringham and Lewis (1959)¹⁵⁾, Brown (1963)³⁾, Perry (1964)¹⁰⁾ and Patton (1963)⁹⁾. But the pathological effects of insecticides on the haemocytes were only investigated in *Calliptamus italicus* (Tareeva and Nanjikov, 1931)¹⁴⁾, *Periplaneta orientalis* (Shull et al., 1932)¹³⁾, *Locusta migratoria* (Pilat, 1935)¹¹⁾, *Prodenia eridania* (Yeager and Munson, 1942)¹⁶⁾, *Leptinotarsa decemlineata* (Arvy, et al., 1950)²⁾, *Ephistia kuhniella* (Arnold, 1952)¹⁾, *Pediculus humanus humanus* (Hopp, 1953)⁸⁾, and *Periplaneta americana* (Gupta and Sutherland, 1968)⁶⁾ by contact and stomach poisons as well as by fumigation.

However, this aspect of insect haematology was not studied in any hemipterous bug. Therefore, *Dysdercus cingulatus*, a pest of cotton and other Malvaceae plants was used to investigate the effect of the topical application of Aldrin (a chlorinated hydrocarbon) and Dipterex (a broad spectrum organophosphorus compound) on different types of haemocytes of adult.

Materials and Methods

A stock culture of *Dysdercus cingulatus* was maintained at 29°C±1°C and 70% to 80% R. H. The insects were fed on fresh, soaked and healthy cotton seeds.

Different concentrations (0.25%, 0.5%, 1.0%, 1.5% and 2.0%) of O, O, dimethyl-(2, 2, 2-trichloro-1-hydroxyethyl phosphonate, commonly called "Trichlorfon" and patently known as

Dipterex (gifted by Bayer, India) were prepared by dissolving the technical grade in acetone. Similar dilutions of the technical Aldrin (1, 2, 3, 4, 10, 10-hexachloro-1:4, 5: 8-diendomethano-1, 4, 4a, 5, 8a-hexahydronaphthalene (gifted by Shell, Nederland) were also prepared.

From each dilution of these insecticides 0.0005 ml was applied on the prothoracic tergum of 4-day-old *D. cingulatus* of each sex by a micro-applicator. 25 adults of each sex were treated with each dilution of the respective chemicals and kept at the above mentioned conditions of temperature and humidity.

Blood smears were prepared from the treated adults of each sex at intervals of 6 hour upto 24 hours and thereafter every 24 hours upto 72 hours following the treatment. The blood smears were stained with both Giemsa's and Lieshman's stains for histopathological observations of the haemocytes. Similarly, the blood smears of adults of the corresponding age treated with only acetone were prepared as control for comparison.

Results

In the blood smears of normal males and females of *D. cingulatus* five types of haemocytes were observed and described as prohaemocytes plasmotocytes, adipohaemocytes, granular haemocytes and oenocytoids (Zaidi and Khan, 1974)¹⁷⁾. The normal blood picture is shown in Fig. 1 and 2. Since the object of the present investigation was to observe the histopathological conditions of different haemocytes as a result of the topical application of Aldrin and Dipterex on *D. cingulatus*

and it was not the purpose to observe the toxicity of these insecticides leading to mortality or knockdown property. Therefore, determination of lethal doses (LD) of these insecticides based on the percentage of mortality was not recorded. Further, for the histopathological observations on the haemocytes, death of the insect was confirmed on the physiological basis when all sorts of body movements even by touch stimuli ceased and haemolymph dried up. Therefore, pharmacological and external symptoms of the treated adults were also studied at regular intervals following treatment to select out the physiologically live insects for observing the blood picture.

(a) *Effect of Aldrin*

The haemocytes remain unaffected during the first 24 hours following the treatment with 0.25, 0.5, 1.0 and 2.0 per cent Aldrin. However, some pathological effects appeared in the haemocytes after 48 hours following the application of 0.5 per cent. Such haemocytes only showed abnormal vacuoles in a few cells. Even following the treatment by 1.0 per cent Aldrin, the haemocytes remained unaffected except some plasmatocytes which become fusiform. However, the blood smears of treated adults by 1.5 per cent Aldrin indicated occasional changes in the shape of other types of haemocytes as well. Besides there was abnormal vacuolization in many haemocytes especially in the plasmatocytes.

Following 72 hours after the treatment with

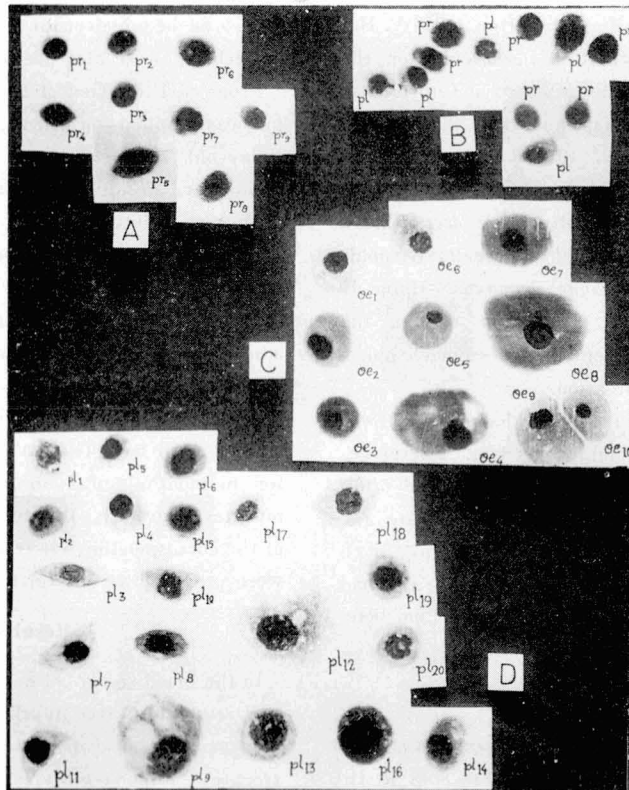


Fig. 1. Photomicrographs of different types of haemocytes in *D. cingulatus*.

- A. Prohaemocytes (pr₁ to pr₉)
- B. Prohaemocytes (pr) and Plasmatocytes (pl)
- C. Oenocytoids (Oe₁ to Oe₁₀)
- D. Plasmatocytes (pl₁ to pl₂₀)

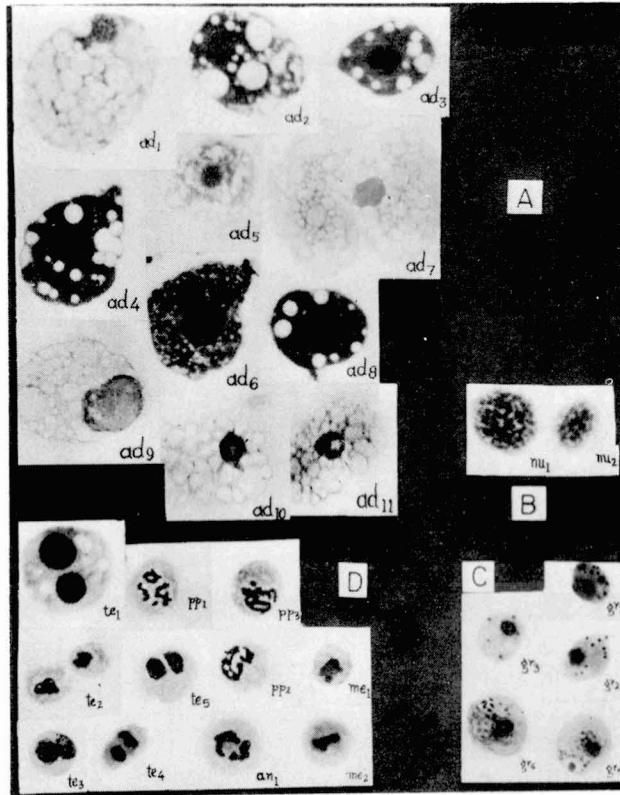


Fig. 2. Photomicrographs of different types of haemocytes of *D. cingulatus*.

- A. Adipohaemocytes-(ad₁ to ad₁₁)
- B. Nuclei of adipohaemocytes-(nu₁ and nu₂)
- C. Granular haemocytes-(gr₁ to gr₅)
- D. Mitosis : Prophase (pp₁ to pp₃),
Metaphase (me₁ and me₂),
Anaphase (an₁),
Telophase (te₁ and te₅) in plasmatocytes, te₂ in
oenocytoid, te₃ and te₄ in prohaemocytes.

0.5 and 1.5 per cent Aldrin, there was no significant development of pathological damage in the haemocytes. Mitotic figures appeared normal. However, 2.0 per cent Aldrin caused more pathological damage in the haemocytes than those of the lower concentrations and its effect became apparent. Within 24 hours following the treatment the cells especially the plasmatocytes became fusiform and showed abnormal vacuolization with rough cell boundary in some cells. There was a tendency in the cytoplasm to be structureless and full of abnormal vacuolization. However, the

oenocytoids remained unchanged. After 48 hours, about 25 per cent haemocytes were normal, whereas the rest did not possess distinct cell boundary and cytoplasmic structure. The cell membrane became indistinct and there was an increase of abnormal vacuolization. Some of the oenocytoids changed their spherical shape and had larger nucleus as compared to the size of the normal one. The nuclei of some cells were pushed towards the periphery.

Maximum pathological conditions of the haemocytes were observed after 72 hours when the

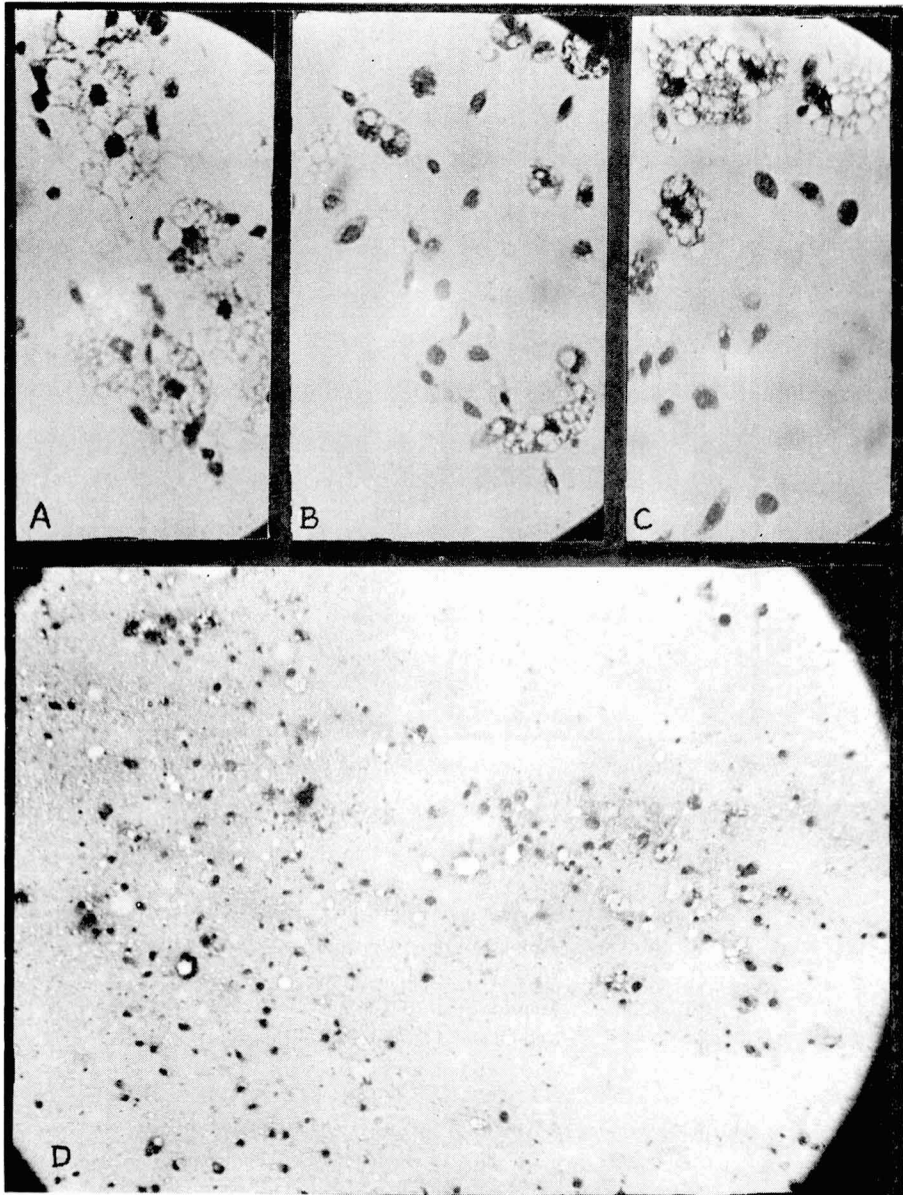


Fig. 3. Photomicrographs showing different types of pathological effects in different haemocytes of *D. cingulatus* by the topical application of Aldrin and Dipterex.

- A, B, C. Blood smear of insect treated with 2 per cent concentration of Aldrin, after 72 hours.
- D. Blood smear of insect treated with 2 per cent concentration of Dipterex after 72 hours.

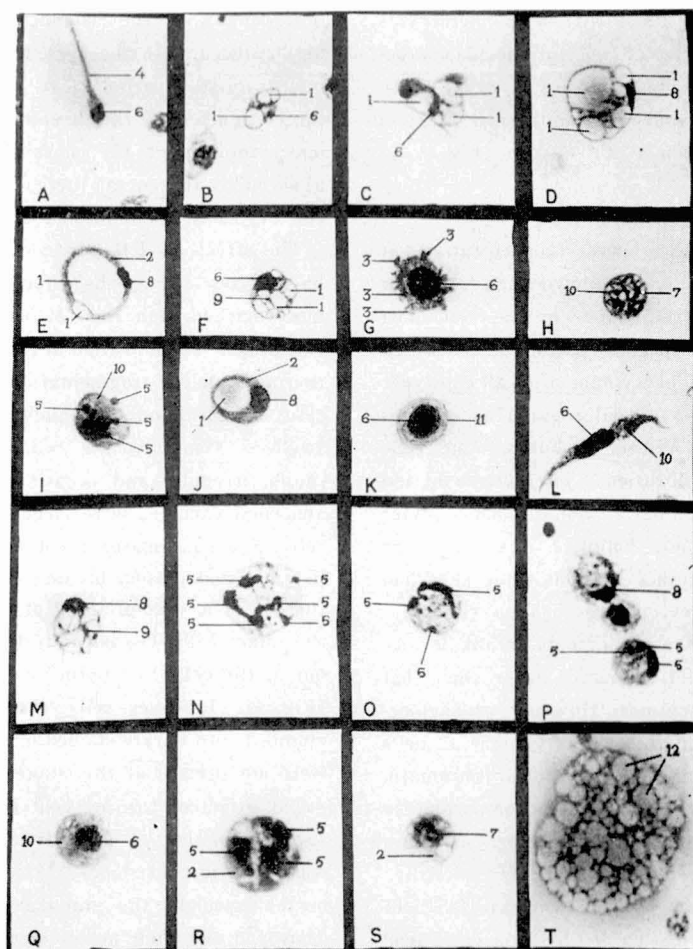


Fig. 4. Photomicrographs showing important pathological effects in different haemocytes of *D. cingulatus* by topical application of Dipterex.

- A. J. Haemocyte with cytoplasmic extension.
- B to F and J. Haemocytes with abnormal vacuolization and their nuclei pushed towards periphery.
- F and M. Haemocytes showing ragged cytoplasm with their nuclei pushed towards periphery.
- G. Plasmatocyte showing cytoplasmic bulgings.
- H and S. Haemocytes showing vacuolized cytoplasm and nuclei showing abnormally prophase stage of mitosis.
- I, N, O, P and R. Showing haemocytes with fragmented nuclei.
- K. Haemocyte showing scattered achromophilic cytoplasm.
- L. Plasmatocyte that changed its shape from round to fusiform.
- Q. Oenocytoid with swollen nucleus.
- T. Adipohaemocyte without nucleus.

plasma became thick and destruction of many other types of cells along with adipohaemocytes occurred. The nuclei of most of the plasmatocytes and prohaemocytes were destroyed by either fragmentation or expulsion from the cells. Mitotic figures were not seen at this stage (Fig. 3. A, B, C).

(b) *Effect of Dipterex*

The effect of the lowest concentration of Dipterex within 24 hours following the treatment was apparent with the change in shape of most of the haemocytes especially plasmatocytes which became fusiform. The cytoplasm of all cell types except adipohaemocytes and granular haemocytes became granular. Within 48 hours, some cells had abnormal vacuolization. The cytoplasm and nuclei became granular. Some plasmatocytes developed cytoplasmic bulgings, extensions or pseudopodial appearance whereas some cells had clumping or their cytoplasmic content (Fig. 4). The cytoplasm of the oenocytoids shrank in size and thus their nuclei appeared larger than that of the normal oenocytoids. However, prohaemocytes were mostly unaffected. In general, all cells had the precipitation of their nuclear chromatin into granules. This condition was comparable to the beginning of the prophase of mitosis, although mitosis was not so frequent. Occasionally, advanced mitotic phases were also seen. In about 72 hours after application of this concentration, there was an apparent loss of cell number. Most of the cells had rough and in some cells broken membrane. In some cells the nuclei were pushed towards the periphery.

The haemocytes affected by 0.5 per cent Dipterex generally indicated similar effects as observed by the effect of 0.25 per cent. However, the changes in the haemocytes appeared earlier. After 24 hours following the treatment, most of the plasmatocytes became fusiform. Some cells became distorted. The cytoplasm of the most of the cells developed abnormal vacuoles. The adipohaemocytes and granular haemocytes were also affected. In some plasmatocytes, the nuclei became eccentric in position. The cytoplasm of nearly all the cells became highly granular. After 48 hours, the pathological effects extended further and covered more haemocytes. The

cellular content of a few cells became clumped. The density of the haemocytes in the smears was reduced. In the next 24 hours, there were more intense pathological conditions covering larger number of the haemocytes. Both nuclei and cytoplasm of the most of the cells were abnormal. In general, there was an increase in cell destruction and plasma became thick.

The effect of 1.0 per cent Dipterex on the haemocytes during the first 24 hours was not more marked than that of the first two concentrations. Vacuolization in the cytoplasm, distortion and nuclear fragmentation appeared in a few cells. After 48 hours, the nuclei of the oenocytoids swelled. Generally the cell membrane became rough, irregular and occasionally broken. The abnormal vacuoles were larger in size in some cells. Some plasmatocytes developed cytoplasmic bulgings and plasma became thick. In 72 hours, there was a precipitation of large granules in the intact cells. Occasionally the nuclei extruded out of the cells by rupturing the cell membrane (Fig. 4). In other cells, the nuclear material clumped into darkly stained mass. Mitotic figures were not present at this stage.

The effect of 1.5 per cent Dipterex was more severe than that of 1.0 per cent. The plasma was thick in consistency. Majority of the haemocytes especially the granular haemocytes were destroyed and their nuclei were generally clumped. However, the intact cells had eccentric nuclei and some of them were disfigured or fragmented with highly vacuolized cytoplasm (Fig. 4). After 48 hours the pathological conditions further developed. In most of the haemocytes, the cell membrane was either bulged or ragged. In most of the plasmatocytes and in some prohaemocytes, cytoplasmic processes were formed. Many haemocytes showed achromophilia as compared with the normal stains taken by these cells. After 72 hours, a few cells especially adipohaemocytes were completely destroyed whereas the identity of most of the cells was not possible. In most of the cells, especially in plasmatocytes, the nuclei became either crushed or fragmented.

Initially the effect of 2.0 per cent Dipterex was similar to that of 1.5 per cent. However, adipohaemocytes were completely destroyed and

their nuclei were only visible along with small portions of cytoplasm. But granular haemocytes completely disappeared. In most of the cells extrusion of the nuclei was in process and occasionally the haemocytes were without nuclei. The plasma became thick in consistency. After 48 hours, the identity of the cells was almost impossible due to the maximum pathological damage. The blood volume also decreased and plasma folded due to its thickness. Fragmentation of the cells was common and the remains of the broken haemocytes developed various degrees of achromophilia. The oenocytoids were recognizable although they also developed abnormal vacuolization, rough cell boundary and swollen nuclei. After 72 hours, pathological conditions of the haemocytes were maximum, when the plasma coagulated along with the destruction of all types of cells. Nuclei were destroyed either by fragmentation or expulsion from the haemocytes (Fig. 3, D). Blood smears had mostly cell debris of all types of cells. Thus the identity of almost all cells was impossible except a few plasmatocytes which appeared normal probably due to their recovery from the toxic effects of this insecticide.

Discussion

The topical application of different concentrations of technical Dipterex on the prothoracic tergum of *D. cingulatus* adults causes pathological damage to all types of the haemocytes. The data clearly show that lower concentrations (0.25, 0.5 and 1.0 per cent) have less degree of damage in the haemocytes than that of the higher concentrations (1.5 and 2.0 per cent) over a period of 72 hours following the application. Further, the sequence of damage caused by lowest to highest concentrations of Dipterex was graded in nature. The most sensitive haemocytes to this insecticide are the adipohaemocytes and granular haemocytes. However, the oenocytoids appear to be more resistant to the effect of this insecticide because of their thickness. Thus they are less fragile as compared to other cells.

It is interesting to note that although all other types of cells are completely broken down by the effect of 2.0 per cent Dipterex over a period

of 72 hours, some of the plasmatocytes appear healthy. It may be possible that since plasmatocytes are phagocytic cells and they can resist to the toxic substances, therefore, a smaller percentage of plasmatocytes may recover before complete death ensues. The phagocytic cells are known to serve as resistant to foreign materials of the blood including the microorganisms.

It is clear that in contrast to the organophosphorus compounds, i. e., Dipterex, the chlorinated hydrocarbon, i. e., Aldrin is less effective to cause pathological damage to the haemocytes. The maximum damage caused by the application of 2.0 per cent Aldrin is much less than that of 2.0 per cent Dipterex. The oenocytoids remain unaffected even by the effect of 2.0 per cent concentration of Aldrin.

As regards the mode of action of Dipterex and Aldrin on the haemocytes of *D. cingulatus*, it is concluded that their topical application lead to their penetration through the integumental membrane into the circulating haemolymph whereby haemocytes come in contact with the chemical. However, the data suggest that the penetrating capacity of the two insecticides differs markedly. Further, it suggests that the penetration of Dipterex into the haemolymph is more significant because the damage of the haemocytes treated with this insecticide is more graded than those of Aldrin treated insects. The present information is therefore in contrast to that of Gerolt (1971)⁴⁾ who believes that penetration of the insecticides through the integument into the haemolymph does not take place at significant rate. However, Patton (1963)⁹⁾ and Reddy and Naidu (1967)¹²⁾ believe that the insecticides topically applied on the body route to the site of action through the haemolymph after dilution. The contact effect of insecticides through central nervous system may lead to the pharmacological and symptonic changes in the behaviour of the insects. However, the entry of the insecticide through the sense organs and its effect on the neuromusculature cannot explain the pathological changes in the haemocytes.

The pathological changes in the haemocytes of *D. cingulatus* can be explained by the fact that poisons react with cellular content, resulting in the precipitation of the cytoplasmic material and

making the cells fragile and finally these fragile cells undergo destruction. In mammals, especially in human beings, toxicity of certain drugs and narcotics is well known on the cells of liver and erythrocytes which become fragile and haemoglobin passed out of the cells (Houssay *et al.*, 1955)⁸⁾.

The present information on the pathological effects of insecticides (Dipterex and Aldrin) on the haemocytes of *D. cingulatus* is the first observation on a hemipterous pest. Further, the data on *D. cingulatus* are almost in conformity with observations on different insects belonging to different orders.

Summary

In both males and females of adult *Dysdercus cingulatus* Fabr. all types of haemocytes are pathologically affected by the topical application of the technical Aldrin and Dipterex. However, weaker concentrations of Aldrin do not cause any damage in comparison to those of Dipterex. In general Dipterex is more harmful to the haemocytes than Aldrin and 2.0% concentration of the former completely destroys most of the haemocytes. The adipohaemocytes and the granular haemocytes are more fragile cells than other and the former types of cells are most susceptible to dilute concentrations as well. But aenocytoids indicate greater resistance to the effect of these insecticides and these are mostly destroyed by the strongest concentration of Dipterex. However, some plasmatocytes indicate recovery from the pathological effects of Dipterex.

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