Introduction

Dysdercus koenigii is an important cotton pest of Asian Countries including India. It damages cotton crop worth rupees in millions per year. It is an insect belong to phylum Arthropoda and order hemiptera.

As introduction part some information regarding Dysdercus, its life cycle and its age are given here the red cotton bug, Dysdercus koenigii is easy to rear in laboratory conditions and handy and amenable for experimental procedure. To build up the culture, adults and nymphs were collected from the cotton fields in the vicinity of The Banaras Hindu University and lady’s finger cultivated fields in the nearby villages.

The insects were raised in glass jars in a BOD incubator set at 28°C + 1°C, 16 hr. photoperiod and 75 % RH. They were fed on water soaked cotton seeds and water provided in small glass vials plugged with cotton. A strip of blotting paper folded into fan-like fashion was slipped around the inner wall of the culture jars to enable the insects to climb and descend in the folds and to get a semblance of natural environment. The jars were covered with cotton cloth and rubber-band. The food and water were changed every day.

The sexes mated in due course and laid clutches of eggs on the floor of jars. The eggs are spherical creamish in colour and about 1 m.m. in diameter. As the embryos develop inside the shells, the eggs turned somewhat reddish due to the colour of the developing embryo inside. The eggs hatch in 7 days time producing tinny
[1 mm] red colour nymphs resembling the adults in all respects except in their small size and lack of genitalia and wings. The first through fifth instars respectively took 7-9, 4-5, 4-5, 4-5 and 6 days [25-30 days in total] to moult into adults. The sexes of the nymphs could be recognized from the slender abdomen with a dark subgenital [gonopore] area in last segment of the male and relatively broader abdomen and conspicuously lighter gonopore area in the female. The sexual dimorphism becomes more perceptible in later instars.

The adult Dysdercus koenigii are brick-red in colour with their antennae and thoracic scutellum dark-coloured, abdominal sterna white-banded and terga uniformly red. The female adults are larger [15 mm] than the males [12 mm]. The mating occurs on the second day of adult emergence and lasts for a duration varying from 72 to 96 hrs. The adult has a laboratory life-span of 30-35 days in which it undergoes 6 egg-cycles; the first egg-cycle takes 8 days and the rest 4-5 days each.

With experience, different nymphal instars can be recognized by their size. The newly emerged nymphs and adults are more slender and distinctly paler than the older ones. Such insects were removed to separate jar/petridish and their age reckoned as “0” hr. the I, II, III and IV instars were excluded from the present hematological studies due to either too small to yield adequate blood sample or not necessary for the present work.

The fifth [V] instars and adult insects of both sexes were sorted out and their haemocyte counts, total and differential, were taken at 24 hr intervals up to day 5 and 8 [up to first egg cycle] in fifth instars nymphs and adults respectively. Blood
samples were obtained from insects heated in water at 50°C for 5 minutes [fixed blood] and from live insects [unfixed blood].

A drop of unfixed blood obtained near the edge of a slide by amputating the antenna was drawn into a thin uniform film by pulling the edge of an inclined slide backward. The film was air-dried and stained.

Blood smears were stained panoptically with overnight incubation in ethanol and then in May-Grunwald solution for 6 min. and Giemsa-Romanowski one [1:3, solution: water] for 45 min. and haemocytes were differentiated from at least 100 cells/insect. In this method, the air-dried blood smear slide is flooded with 0.1% stock solution of May-Gurnwald stain [sigma] in menthol for 3 minutes, diluted with equal amount of distilled water and then allowed to stain for 2 minutes. Thereafter, the May-Gurnwald layer was discarded; the smear flooded with Giemsa stain [diluted to 40 times in distilled water] and allowed to stand for 10 minutes.

The slide was then washed in running water and mounted in DPX. For the total haemocyte count, the haemolymph was drawn into a Thoma blood-cells pipette up to its 0.5 mark and diluted up to the 11th mark with Tauber-yeager’s fluid. The pipette was 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 counted in its four corner and one central [1 mm2] squares under microscope.
If the distribution of cells in all the squares was not even, the sample was discarded and the procedure was repeated. The number of circulating haemocytes per cubic millimeter [mm3] was calculated using the formula of Jones.

To determine the DHC, at least 100 cells of each category chosen from random areas of the stained blood smear were counted on a laboratory blood cells counter under microscope and the percentage of cell types was calculated. Newly emerged [0 hr] fifth instars [both females and males] were sorted out and kept starved in separate jars for four days in a BOD incubator set as described earlier. The THC and DHC of starving fifth instars were recorded at 24 hr interval up to day 4. Thereafter, no blood could be obtained.

For these experiment “0” hr fifth instars of both sexes were sorted out from the culture jars. For this experiment, they were sorted out in two lots. One lot was kept in an incubator/refrigerator maintained at 4 C for day 3 and other lot was kept at the room temperature 28 C as controls.

The THC and DHC of both lots are taken at 24 hr intervals up to the aforesaid days. As chilling [4° C] ‘0’hr fifth instars were sorted out and divided into two lots. One lot was kept at 15° C and other lot at 28° C [room temperature] as controls in incubator.

The THC and DHC of both lots were taken at 24 hr intervals up to day 5. Newly emerged ['0'hr] fifth instars of both sexes were sorted out from the culture jars. Fifth [0 hr] instars divided into two groups. One group kept under high temperature and other at 28° C [room temperature] as controls in an incubator. The THC and DHC of the both groups were taken at 24 hr intervals up to days 5.
Injection of 10 micro liter saline [insect Ringer] solutions was injected in ‘0’hr fifth instars the sternum between the meta thoracic legs with the help of a micro syringe. Controls were injected with equal amount of distilled water. The THC and DHC of both experimental and controls were taken at 24 hr intervals up to day 5.

The ‘0’hr fifth instars were sorted out from the culture jars. Water-narcotized insects were positioned in a specially devised operating clamp under a binocular microscope. A transverse slit was made in the neck membrane with a razor-blade-scalpel and the fat body behind the brain was removed to expose the retrocerebral-endocrine aortal complex [Nerve]. The nerve or arota or neurohaemal organ was raised a little by means of a hooked needle and severed behind the CA [corpora allata] by a pair of micro scissors.

A drop of penicillin-streptomycin [1:1 by weight] mixture dissolved in insect Ringer was introduced in the slit to compensate for the loss of the haemolymph and also to protect the wound from infection. The insect was then unclamped and placed on a blotting paper to revive.

The revived insects were kept in separate jars provided with food and water in vials. Controls were treated in a similar manner but their aortal complex was raised not severed.

The THC and DHC of nervectomised and controls were recorded at 24 hr intervals up to days 5. For this experiment ‘0’hr of adults of both sex were sorted out from culture jars. Narcotized them in water and then placed in specially devised operating clamp under a binocular microscope. A transverse slit was made in the week membrane with a razor-blade-scalpel and the fat body behind the brain was
removed to make a serve wound. Controls were treated in similar manner but there was no wound.

The insects were then unclamped and placed on a blotting paper to revive. The revived insects were kept in separate jars provided with food and water in vials. The THC and DHC of experimental and controls were recorded at 24 hr intervals up to days 8.

For this experiment ‘0’hr adults of both sex were sorted out from culture jars. The adults were divided into two lots. One experimental and other control. Both lots of insect were treated as previous experiment i.e. effect of wound. The experimental insects treated with antibiotics [streptomycin and penicillin] and controls were without treatment of antibiotics.

The THC and DHC were recorded at 24 hr intervals up to days 8. The ‘0’hr adults were sorted out from culture jars. The metathoracic leg of one side was amputated near sternum. The amputated spot covered with antibiotics. The metathoracic leg of control were just tilted but not amputated. The THC and DHC of both the experimental and controls were taken at 24 hr intervals up to day 8. The ‘0’hr adults of both sex were sorted out from culture jars.

The adults were grouped into two lots. One lot considered as experimental and other lot as controls. The experimental were exposed to an antigen [JHA, R-394, ethyl-9-cyclohexyl-3, 7-dimethyl-2, 4-noadienoate] dissolved in acetone and it topically applied in the dose of 10 micro gram/micro liter acetone on the thorax of ‘0’hr adults. Controls received 1 micro liter acetone alone. The THC and
DHC of both lots were taken at 24 hr intervals up to day 8. The '0' hr adults of both sex were sorted out and were kept in separate jars to prevent mating.

These adults were also at 2 hr regular intervals disturbed up to day 1. The control adults were allowed to mate normally and they were not disturbed at 2 hr regular intervals up to day 1. The THC and DHC of both experimental and controls were taken at 24 hr intervals up to day 8. The body cavity of insect is called haemocoel in which extracellular fluid or blood flows as like vertebrates, in insects it is called haemolymph.

In haemolymph number of different types of cells present as like blood corpuscles of vertebrates and they are called haemocytes. These haemocytes are involves in intermediary metabolism, transport of nutrients, transport of hormones, phenol oxides [PO] activity, connective tissue formation, growth, egg maturation, moulting, wound healing and in defense or general immunity of insects.

The development of haemocytes and their differentiation into different types from its first report has been a subject of investigation no doubt number of workers worked on them, the reports of these workers not uniform therefore a single conclusion not is come into mind. Recently a worker reported the development of haemocytes and their differentiation based on some processes occurring during embryonic development the workers investigated this aspect into an insect drosophila.

In this context here given the findings of present work in form of as a brief introduction that discussed in the later chapters of discussion and also its some aspects in the light of introduction part in the current chapter.
The thesis deals with the study of effects of stresses and antigen on blood cells [THC and DHC] of Dysdercus koenigii. The thesis is divided into three parts. The part first includes the informations on Dysdercus koenigii and the present work. The part second includes the informations regarding THC and DHC of fifth instars [both females and males] and adults [both females and males]. The part third includes the experimental studies i.e. the effects of stresses and antigen on THC and DHC of fifth instars and adults of both sex. Dysdercus koenigii is a serious cotton pest of Asian countries including India.

It damages cotton crop worth rupees in millions per year. It is an insect belong to phylum Arthropoda and order hemiptera. The adult Dysdercus koenigii are brick-red in colour with their antennae and thoracic scutellum dark-coloured, abdominal sterna white-banded and tegra uniformly red. The female adults are larger [15 mm] than the males [12 mm]. The mating occurs on the second day of adult emergence and lasts for a duration varying from 72 to 96 hrs. The sex mates and laid clutches of eggs. The eggs hatch in 7 days time producing tinny [1 mm] red colour nymphs. The first through fifth instars respectively took 7-9, 4-5, 4-5, 4-5 and 6 days [25-30 days in total] to moult into adults.

The insects were raised in glass jars in a BOD incubator set at 28 C + 1 C, 16 hr photoperiod and 75 % Relative humidity. They were fed on water soaked cotton seeds and water provided in small glass vials plugged with cotton. The adult has a laboratory life-span of 30-35 days in which it undergoes 6 eggs-cycles. The first egg-cycle takes 8 days and the rest 4-5 days each. Like blood and blood cells of vertebrates, in insect body cavity there is haemolymph with haemocytes
which flows. The haemocytes are various types. In the present insect, six types of haemocytes are recognized. These are Prohaemocytes [PRs], Plasmatocytes [PLs], Granulocytes [GRs], Adipohaemocytes [ADs], Oenocytes [OEs] and Vermicytes [VEs].

These haemocytes are involves in many functions of insect’s physiology e.g. in intermediary metabolism, transport of nutrients, transport of hormones, phenoloxidase [PO] activity, in general immunity, growth, egg maturation and connective tissue formation. Informations related to normal haemocytes and its counting i.e. Total Haemocyt Count [THC] and Differential Haemocyte Count [DHC] gives much knowledge of an insect because as alterations in haemocyte structure, types and number of cells reflects changes in physiological and biochemical processes.

Therefore, the present study has been under taken for the Ph.D thesis. The present study may helps in finding out a biological means against the conventional insecticides and drawbacks of Bt cotton, insect pests, the use of which caused an environmental imbalance, problem of insect resistance and environmental pollution.

The findings of the present work may also helps in searching of clues of some haemocytes on which more experiments to be carried out in study of immunity and disease related problems of vertebrates including human being. In the fifth instars and adults, six haemocyte classes have been identified on the basis of their distinctive morphological and cytological characteristics as studied under light microscope [LM]. They are PRs, PLs, GRs, ADs, OEs, and VEs. The PRs are small rounded cells.
The PLs are pleomorphic [many shaped] but largely spindle and in fusiform shape. The GRs are rounded cells with fine granules in cytoplasm. The ADs are large with number of fat globules in the cytoplasm.

The OEs are rounded cells having eccentric nucleus with dense and homogenous cytoplasm. The VEs are greatly elongated and narrow cells with tapering ends.

The THC steadily increases from day 0 to reach at maximum peak on day 3 after that it declines up to day 5 and moulting occurs. The trends of THC are same in both sex. The higher THC reported in females than males. The PRs from day 0 to day 2 increases after that decreases.

The PLs steadily increases from day 0 to day 5 i.e. just before moulting reaching at maximum. Its population dominated among all other haemocytes. The GRs from day 0 to decreases up to day 5. The ADs slightly increasing trend from day 2, population lowers in comparison to others.

The OEs appear from day 2 to increases up to day 5 but population lowers to ADs. The VEs only appear on day 4 it population remains lowest. The trends of PRs, PLs, GRs, ADs, OEs, and VEs are same. In males PLs, GRs, slightly lower in comparison to female, while ADs, OEs, and PRs slightly higher than female.

The THC in the adult female steadily increases from day 0 up to 2 declining thereafter up to day 5. It increases significantly. The THC in the male remain at a lower than that of the female.

The PRs population in the female is maximum in the newly emerged adult. It gradually decline reaching its lowest on day 7 before first egg-
cycle. The PLs population starts at a lowest percentage in the day 0, attaining its maximum count on day 6, declines thereafter. The GRs starts at a lowest percentage in the day 0, gradually increases attaining its maximum count on day 8.

The ADs starts with a maximum percentage in day 0 adults, decline reaching its lowest in day 6. The OEs are seen on day 1, gradually increase attaining maximum on day 3 then decline reaching the lowest count in day 8. The VEs are seen occasionally on day 6, their population remain smaller.

The difference between DHC of male and female is statistically insignificant for all other haemocytes except ADs which are significantly higher in newly emerged male adult than that of female adult. The effect of stress like starvation, chilling [4°C], low temperature [21°C], high temperature [35°C], nervectomy and saline injection were observed on THC and DHC of fifth instars [both females and males] while effects of stresses like wound, antibiotic, irritation, antigen and amputation of leg were observed on THC and DHC of adults [both females and males]. The fifth instars were starved for 4 days and effects were observed on different days. The starvation caused drastic reduction in THC on day 4. Starvation caused drastic decrease in PLs population on day 3. But increase in population of GRs, OEs, and VEs.

The trend of THC and DHC are insignificant in comparison of males and females. The fifth instars of both sex were placed in refrigerator/incubator at 4°C for 3 days and THC and DHC observed at 24 hr intervals. Temperature at low 4°C caused drastic lowering of THC in both females and males. The PRs start with maximum
percentage in day 0 fifth instars than decline. No significant difference is detected in them in respect to controls.

The PLs also as like to PRs. PLs population is lower to the controls. The population of GRs higher in respect to controls. More ADs reported to the controls. The OEs population appears on day 2 and still lower. The VEs population not appeared. Fifth instars [both females and males] were placed in incubator at 15° C for 5 days. At this temperature THC slightly increase from day 0 to day 3 than decline in female fifth instars.

The THC remains lower to the controls. In female higher THC recorded in comparison to male. The PRs population starts with a maximum in the day 0 fifth instars declines reaching its lowest in the day 5. The PLs population slightly increases from day 0 to attain maximum on day 4. Most populated population among all. The GRs also follows increasing trends. The ADs slightly better to the controls.

The OEs lowers in comparison to the controls. The VEs appeared on day 5 and lowest in count. Fifth instars [both females and males] were placed in incubator 35° C for 5 days. At high temperature, the THC starts with lowest count in day 0, significantly increases up to day 2, than it fluctuated. The higher THC were recorded in fifth instars of both sex to the controls.

The PRs population increases from day 0 to its maximum on day 2, declining thereafter up to day 5. The PRs significantly higher to the controls. In female it is better than male. The PLs population significantly increases from day 0, attaining a maximum on day 3. Its population significantly higher in comparison to the
controls. The GRs population starts with a maximum percentage in the day 0 fifth instars, decline gradually reaching lowest on day 3, thereafter it increases. The ADs population lowers to the controls. The OEs appeared on day 2 and increase up to day 5. Its population higher to the controls.

The VEs appeared on day 4 and significantly improved. Its population significantly higher to the controls.

The 10 micro liter saline [Insect Ringer] injection caused no significant effect on THC and DHC of fifth instars of both sex in comparison to the controls. By surgeries from the neck region retro-cerebral-endocrine aortal complex [nerve] pulled and cut behind corpora allata.

The THC and DHC were recorded at 24 hr intervals up to day 5. The THC in the nervectomised females steeply decline from day 0 to day 3 after that improvements in THC recorded. The THC in controls is significantly higher. No significant difference is found in the THC between the nervectomised males and females except that the THC in the former is higher to those of the latter. The PRs population starts with a maximum on day 0 but gradually declines reaching to its lowest in the day 5.

The PRs population in these females is more or less similar to the controls except that its population is higher in day 2 nervectomised females. The PLs population starts with a maximum in day 0, increasing gradually up to day 2, then after declines. The PLs population in nervectomised female fifth instars higher to the controls. The GRs from day 0 to declines up to day 2, thereafter increasing. The ADs population of ADs not better in nervectomised fifth instars in comparison to the controls.
Less population of OEs reported in experimental to the controls. Some more population of VEs reported to the controls.

A severe wound was made near neck region of adults [both females and males] and at 24 hr intervals the THC and DHC were recorded up to day 8. The wound not treated with antibiotic. THC in affected insects were significantly decline from day 1 to 6 but after that THC return there as normal in comparison to the controls. THC trends are same in both females and males. The PRs population in the females is maximum in the newly emerged adult. It gradually declines reaching its lowest on days 7. Its population always lowers in respect to the controls. The PLs population from day 1 to increasing up to day 6 and it significantly higher in respect to the controls.

The GRs population also follows the increasing trends from day 1 to day 6. Its population higher to the controls. The ADs population follows decreasing trends from day 1 to day 6. Its population significantly lowers in comparison to the controls. The OEs appeared from day 1 to continue up to day 8. Its population always below to 10 % and is lower to the controls.

The VEs appeared on day 2 and only recorded on next day after that not seen. Its population below to 3 %.

The difference between DHC of male and female is statistically insignificant for all other haemocytes except ADs and PLs are lower in male adult than that of female adult. The Newly emerged adults [0 hr] were collected and then by surgery a severe wound was made near neck region after that mixture of antibiotics [Pencilline-Streptomycine] dissolved in insect ringer was given to affected adults.
The THC and DHC at 24 hr interval were recorded up to day 8. THC insignificantly lower to the re controls THC from day 1 to increasing up to day 8. The PRs population in the female is maximum in the newly emerged adult. It gradually declines reaching its lowest on day 7. Its population always lowers in respect to the controls.

The PLs population from day 1 to increasing up to day 6 and it significantly higher in respect to the controls. The GRs population also follows the increasing trends from day 1 to day 6. Its population higher to the controls. The ADs population follows decreasing trends from day 1 to day 6. Its population significantly lowers to the controls. The OEs appeared from day 1 to continue up to day 8. Its population lowers to the controls.

The VEs are lowest in count and only seen on day 2 and 3. It not recorded in controls on day 2 and 3. The ‘0’hr adults of both sex were collected and kept in separate jars to prevent mating and they also at 2 hr regular intervals disturbed up to day 1.

The THC and DHC were recorded at 24 hr intervals up to day 8. The THC in the virgin and disturbed females gradually increases from day 0 to attain its maximum on day 2, thereafter decline gradually up to day 5. After that again shows increasing trends. THC in such females lowers to the controls. THC in the male is slightly higher than in mating and not disturbed controls throughout observed period but it remains always lower to that of the female.
The PRs population in the female gradually declines from day 0 to day 2, improves on day 3 and declines again reaching its lowest on day 5, thereafter increases again up to day 8. The PRs population in the virgin and disturbed females is more or less similar to the controls. The PLs population starts at lowest on the day 0, attaining its maximum on day 7 and declines on day 8. In virgin and disturbed females the PLs population is significantly lowers than the controls.

The GRs population from day ‘0’ to gradually increases attaining its maximum on day 6. No significant variation in the GRs population is detected in the irritating [virgin and disturbed] females than the controls. The ADs population is higher in the irritating females than the controls. The OEs population remains low throughout the observed period. No any significant variation in OEs population is noticed between the virgin and disturbed females to the controls.

The variation in population of the different haemocytes in male adults that forcibly prevents form mating and disturbed is insignificant to those of the virgin and disturbed females [irritating]. Adults [0 hr] of both sex were collected and they were exposed an antigen [JHA, R-394, ethyl 9- cyclohexyl-3, 7- dimethyl-2, 4- nonadioenoate] dissolved in acetone and it topically applied in the dose of 10 mg/ml acetone on the thorax.

The THC and DHC were recorded at 24 hr intervals up to day 8. In affected females THC significantly declines up to day 3, thereafter THC improves up to day 6. It was insignificant to the controls. In the affected males THC trend was same as females. The PRs population gradually declines from day 0 to day 8. Its value slightly
higher to the controls. The PLs population up to day 3 higher in affected females to the controls.

Thereafter PLs declines up to day 4 thereafter again improve. The GRs population declines up to day 3 after that improved up to day 8, it lowers in comparison to the controls. The ADs population fluctuating throughout observed period. The OEs populations higher in affected females then to the controls.

The VEs appeared on day 3 and continue up to day 8. Its populations marginally higher to the controls. The DHC in males almost like to females except slight variation in PRs and ADs were recorded. The ‘0’hr adults of both sex were sorted out and the metathoracic leg of one side was amputated near sternum. The amputated spot covered with antibiotics.

The THC and DHC at 24 hr intervals were recorded up to day 8. In females the THC marginally declines up to day 2 which was insignificant to the controls. The THC trend was similar to the normal female adults as described earlier. The THC in males the trend was same. In males THC lower to the females. The PRs population declines from day 0 up to day 8.

The PLs population increases from day 0 up to day 6. Thereafter decline. The PLs slightly higher in the females to the controls and respective males. The GRs population increases from day 0 up to day 8. The GRs marginally higher in the females to the controls. The ADs population significantly declines from day 0 up to day 6, thereafter slightly improved. ADs population was insignificant in the females to the controls.
The OEs appeared on day 1 continue up to day 8. Its population almost constant throughout recorded period always near 5%. The VEs only recorded on day 6 and its population lowest in count. The DHC in males as like to females some insignificant variation in the PRs, PLs, ADs and OEs were noticed. The formations related to normal haemocytes and its counting i.e. Total Haemocyte Count [THC] and Differential Haemocyte Count [DHC] gives many knowledge of an insect because as alterations in haemocyte structure, types and number of cells reflects changes in physiological and biochemical processes.

Insect haemocyte respond to internal changes during postembryonic development and to conditions such as starvation, wounding, parasitism, diseases, chemicals including insecticides. Therefore, in present time number of biochemists, toxicologists, physiologists and biotechnologists are chosen the haemocyte for the study.

These study for control of insecticides resistance, environmental imbalances, options of Bt cotton, control of pest with biological means, as experimental materials for trials of medicines and study of immune responses. Many literatures are available on different aspects of insects including haemocytes. Number of them are also available on the present insect i.e. Dysdercus koenigii.

These literatures mostly related to its bionomics, postembryonic development, stresses, haemocyte types and haemocyte counting. The literatures related total haemocyte count [THC] and differential haemocyte count [DHC] on starvation, chilling, low temperature, high temperature, saline injection, nervectomy in
fifth instars [both females and males] and on wound, antigen, antibiotic, irritation, amputation of leg in adults [both females and males] either lacks or doubtful.

Therefore, the present investigation in the red cotton bug, Dysdercus koenigii, an important cotton pest has been undertaken, such studies may help in finding out a biological means against the conventional insecticides and drawback of Bt cotton, insect pests, the use of which caused an environmental imbalance, problem of insect resistance and environmental pollution. In the present study the surprising and interesting findings in the part of abstract introduction given here which provide the basic informations to the researcher and academicans of the related field.

THC in the fifth instars and adults, six haemocyte classes have been identified on the basis of their distinctive morphological and cytological characteristics as studied under light microscope [LM]. They are PRs, PLs, GRs, ADs, OEs, and VEs. The Total Haemocyte Count [THC] in Fifth instars, The THC steadily increases from day 0 to reach at maximum peak on day 3 after that it declines up to day 5 and moulting occurs. The trends of THC are same in both sex. The higher THC reported in females than males.

The THC in the adult female steadily increases from day 0 up to 2 declining thereafter up to day 5. It increases significantly. The THC in the male remains at a lower level than that of female. The Differential Haemocyte Count [DHC] in fifth instars in females the PRs- From day 0 to day 2 increases after that decreases.
The PLs steadily increases from day 0 to day 5 i.e. just before moulting reaching at maximum. Its population dominated among all other haemocytes. The GRs from day 0 to decreases up to day 5. The ADs slightly increasing trend from day 2, population lowers in comparison to others. The OEs appear from day 2 to increases up to day 5 but population lowers to ADs. The VEs only appear on day 4 it population remains lowest. In males the trends of PRs, PLs, GRs, ADs, OEs, and VEs are same.

In males PLs, GRs, slightly lower in comparison to female, while ADs, OEs, and PRs slightly higher than female. Starvation caused drastic reduction in THC on day 4. Starvation caused drastic decrease in PLs population on day 3. But increase in population of GRs, OEs, and VEs. Temperature at low 4 °C caused drastic lowering of THC in both females and males.

At high temperature, the THC starts with lowest count in day 0, significantly increases up to day 2, than it fluctuated. The higher THC was recorded in fifth instars of both sex to the controls. The 10 micro liter saline [Insect Ringer] injection caused no significant effect on THC and DHC of fifth instars of both sex in comparison to the controls.

THC in the nervectomised females steeply decline from day 0 to day 3 after that improvements in THC recorded. The THC in controls is significantly higher. No significant difference is found in the THC between the nervectomised males and females except that the THC in the former is higher to those of the latter.
The effect of antibiotic, the newly emerged adults [0 hr] was collected and then by surgery a severe wound was made near neck region after that mixture of antibiotics [Pencilline-Streptomycin] dissolved in insect ringer was given to affected adults. The THC and DHC at 24 hr interval were recorded up to day 8.

The THC insignificantly lower to the re controls. THC from day 1 to increasing up to day 8. On DHC, PRs- The PRs population in the female is maximum in the newly emerged adult. It gradually declines reaching its lowest on day 7. Its population always lowers in respect to the controls. The PLs population from day 1 to increasing up to day 6 and it significantly higher in respect to the controls. The GRs population also follows the increasing trends from day 1 to day 6. Its population higher to the controls.

The ADs population follows decreasing trends from day 1 to day 6. Its population significantly lowers to the controls. The OEs appeared from day 1 to continue up to day 8. Its population lowers to the controls. The VEs Lowest in count and only seen on day 2 and 3. It not recorded in controls on day 2 and 3.

The effect of antigen, the adults [0 hr] of both sex were collected and they were exposed an antigen [JHA, R-394, ethyl 9-cyclohexyl-3,7-dimethyl-2, 4- nonadioenoate] dissolved in acetone and it topically applied in the dose of 10 mg/ml acetone on the thorax. The THC and DHC were recorded at 24 hr intervals up to day 8.

In affected females THC significantly declines up to day 3, thereafter THC improves up to day 6. It was insignificant to the controls. In the affected males
THC trend was same as females. On DHC, The PRs population gradually declines from day 0 to day 8. Its value slightly higher to the controls. The PLs population up to day 3 higher in affected females to the controls. Thereafter PLs declines up to day 4 thereafter again improve.

The GRs population declines up to day 3 after that improved up to day 8, it lowers in comparison to the controls. The ADs population fluctuating throughout observed period. The OEs population higher in affected females then to the controls. The VEs appeared on day 3 and continue up to day 8. Its populations marginally higher to the controls. The DHC in males almost like to females except slight variation in PRs and ADs were recorded. The present study also helps in finding of clues of some haemocytes on which more experiments to be carried out in study of immunity and disease related problems of vertebrates including human beings.

As part of introduction of review of literature, a brief and condensed review of literature incorperated here. The blood cells or haemocytes in insects were first described as transport globules. They originate from the haemopoietic tissues in the embryo and then differentiate into distinctive functional types. Based on their morphological and histological characteristics, haemocyte are classified in to six types: proleucocytes, phagocytes, granular leucocytes, adipoleucocytes and oenocytes and spherule cells.

After then some worker studied haemocytes of southern army worm, Prodenia eridania and classified them into nine types. Pioneered the study of blood cells in-vitro with phase contrast microscope and compared the new information with earlier works on fixed and stained cells [Lavine and Strand, 2002, Strand 2008, Pandey and Tiwari 2012].
The identification and classification of insect haemocytes have been recently based on their ultrastructural features [Brehelin, 1993] and immunochemical identification [Elizabeth et al, 1994; Charalambidis et al., 1996]. The important reviews on haemocyte classification are those of [Pathak 1993, HF Greeny et al.2012, Pandey and tiwari 2012]. The total haemocyte count [THC] was first studied in Orthoptera, Odonata, Hemiptera and Homoptera, after then some other workers included other orders like Neuroptera, Coleoptera, Lepidopiera and Hymenoptera. Their method based on the mammalian method of blood cell count has been adopted by other workers over the year [Berger et al, 2003].

The variations in THC amongst insects have been reviewed by worker [Andrade FG et al, 2003]. Most of them reported constant increase in THC throughout larval development and decrease during the pupal development [Tiwari et al, 1999]. Other workers have found gradual increase in THC throughout the insect’s life cycle [Bowers MD 1990, 1991, 1993, 2003]. Witting [1995] reported decrease in THC after V instar moult which continued until prepupation in Pseudoaletia unipuncta. But other workers have found no differences in THC in the larva, pupa and adult of Leptinotarsa.

The differential haemocyte counts [DHC] in insects have been studied by Sonawane and More [1993]. Some scientist reported that PLs and GRs do not undergo any variation in their relative numbers during larval development of Ienebrio larva. In the mediterranean flour moth, Ephestia Kuhniella. Some workers found PRs to be numerous in the 1 instar declining in the following instars, the PLs increased from the mid-first instar to reach a maximum in the IV instar
declining thereafter, the OEs representing low number all through and the ADs, low in
the early instars increasing from the IV instar onwards.

In the flesh fly, *Sarcophaga bullata*. Some workers reports PRs, PLs, GRs, and SPs as principal haemocyte types. The PRs and PLs declined in number as the development progress whereas GRs increased but in the adult, the only haemocyte detected are the PLs. Some 90-95 percent haemocyte population in *Drosophila melanogaster* is the PLs and the remaining POs and Las which appear at the end of larval period or at pupation. In *Drosophila euronotus*, the haemocyte types found are the POs, LAs, and OEs, the last named appearing in the II larval instar increasing in the ultimate instar and declining markedly during the prepupal stage. Some workers found PRs, PLs, GRs and OEs as the recognizable categories in the V instar larva of *Pieris rapae crucivora* with their percentages as 1, 40, 54 and 4.4 respectively.

In *Heliothis virescens*, the PRs and PLs decreased in early larval life [5-8 days] and increased during pupation. The SPs increased within the same period from 38 to 59 percent and then decreased and OEs remained almost constant at 1-2 percent. In the ultimate larval instars of the two species of Lepidoptera.

*Euxoa compestris* and *Euxoa declarata*, the haemocyte types found are the PRs, PLs, GRs, SPs, and OEs with their percentages as 7, 30, 48, 12 and 3 respectively. In some advanced stages of the larval development, the GRs accumulate lipid droplets and so could be regarded as ADs.
In adult *D. cingulatus*, percentage of PRs is very small in comparison to that of the PLs and ADs. In a study of the haemocytes of an aphid, some workers found that the PRs were at low levels all through the larval adult stages, the PLs were the highest group until the end of the III instar and then they declined somewhat; the GRs were the low until the end of III instar and then they rose to be highest in numbers of all the types, the SPs and OEs remained at approximately the same levels throughout the study. In *Spodoptera litura*, the PRs, PLs, GRs, SPs, ADs, OEs, POs and VEs as the haemocyte types of which the PRs numerous during the IV and up to the early V instar declining thereafter, the SPs, GRs and ADs increased in size as well as in number in the pupal stage while the OEs were rarely observed. While studying the haemocyte population in III larval through pupal instars of *Spodoptera*. Found the PRs to be low in V instar and high in the old pupa; the PLs to rise in the IV instar declined thereafter; the GRs to be high in the III, low in the IV and V instars but rising in the early pupa and the SPs to be high in the V and low in other instars. The OEs, VEs and POs remain low all through [Pathak 1993]. The variations in THC and DHC during the moult have been investigated by a number of workers. While some of them found a decrease in the THC during moulting [Swain and Behura 1996], some others reported increase particularly at metamorphosis [Mishra and Tiwari 2005].

Some workers reported that filling of the PLs by India ink in V instar *Rhodnius* temporarily delayed the moulting. Some workers found no change in the proportion of PRs, PLs and GRs at ecdysis in the same insect. The changes in the THC and DHC in starved insects have been studied by many workers. While some workers reported an increase in the THC during starvation [Begum R and Gohain R
1996, Chisholm J.R S and V.J. Smith, 1994], others found a decrease [Sujatha and Dutta –Gupta, 1991] and still others found no change.

Regarding changes in DHC, the views of the authors are not uniform. Workers carried out a series of experiments on the effect of starvation on the haemocytes of last instar *Tenebrio* larvae and noted increase in number of GRs and decrease in number of PLs. Some workers noted same result in same insect after prolonged starvation. Some workers found that starvation reduced the number of fusiform haemocytes in Bombay larvae and reported that the relative proportion of SPs increased as the starvation period decreased.

Some workers found significant increase in the relative number of ADs and degenerating cells but decrease in the number of fusiform PLs in starved IV instar of *Anagasta*. Some worker studied haemocyte population changes in *Rhodnius* and concluded that great differences occurred between fed and unfed nymphs on the fifth day after ecdysis. In fed nymphs, the percentage of PLs and OEs increased greatly. In adult females following a blood meal, PLs were increased and lysing GRs decreased. Some workers found decrease in number of PLs in *Galleria* larvae during the first 3 days.

Some workers found a decrease in PLs and increase in GRs population during starvation of adult *Periplaneta Americana*. As high temperature, an increase in THC has been found by many workers [Shu-sheng liu et al 1995, Morales-Ramos, J.A. and J.R. Cate 1993] while at low temperature a decrease [Shu-sheng liu et al 1995].
Some others have found no change in THC at low temperature. Further, very little information is available regarding effect of temperature on DHC for the convenience of the study for the researchers and academicians, those are interested to carry out or investigate the fascinating aspect of haemocytes and the related topics, in the present chapter of the thesis given the detailed introduction on haemocytes i.e. its types, morphology, development, functions and its applications.

Blood circulation in insects have open circulatory system means in the blood cavity of insect the blood vessel not always continues inform of different branches of vessels that ends in pumping structure i.e. heart instead of this the blood vessels ends into sinuses or lacunae or open space of the body cavity so it is called open circulatory system. This system is not efficient as the close circulatory system, which are the characteristic features of vertebrates. Insect’s body cavity is called haemococel because it closely associated to blood flow, the narrowing of body spaces, in advance stage behave like blood channels or vessels or lacunars tube.

The haemococel is the type of true coelom, i.e. the cavity formed by splitting of mesoderm. In the haemococel the blood or haemolymph, flows. The haemolymph contains number of cellular structures. These are collectively called haemocytes. These haemocytes are actually blood cells of insects.

As like mammalian or vertebrate blood cells, these haemocytes plays important roles in insect’s life. In the present insect i.e. Dysdercus koenigii number of haemocytes are present viz Prohaemocytes, Plasmatocytes, Granulocytes, Adipohaemocytes, Oenocytes and Vermicytes. Our findings are agreed with number of workers.
But some workers doubt on presence of Vermicytes and at place of this reported Sphreulocytes and spinocytes. In general text of research papers, abstracts, thesis and books these haemocytes represented few letters code or common name of the haemocytes e.g. Prohaemocytes represented by PRs, Plasmatocytes represented by PLs, Granulocytes represented by GRs, Adipohaemocytes represented by ADs, Oenocytes by OEs, Vermicytes represented by VEs, Sphreulocytes represented by SPs, and Spinocytes by SNs. In other insects many more additional types of haemocytes are reported viz. Coagulocytes, Lamellocytes and Podocytes. The Coagulocytes represented by COs, Lamellocytes represented by LAs, and Podocytes represented by POs.

These haemocytes were identified on the basis of cytological characteristics, morphology of cell. In earlier workers mostly classified haemocytes on the basis of study those carried under light microscope [LM]. Later on number of workers carried study under scanning electron microscope [SEM]. In the present time some worker used Monoclonal antibody/Particular protein i.e. Antigen-Antibody reaction for the classification of haemocytes.

Still in the present time for the classification of haemocytes not a single criteria has been in practice, with the study of number of research papers, it is noticed that the criteria of cytological characteristics and morphological features are may be the best way to classified the haemocytes in Prohaemocytes, Plasmatocytes, Granulocytes, Adipohaemocytes, Oenocytes and Vermicytes in the Dysdercus koenigii.

In other insects Lamellocytes, Podocytes, Spinocytes, Coagulocytes and Spherlocytes. The formations of blood cells
are called haemocytes, in vertebrate haemopoiesis start during embryonic development. During embryonic development the mesoderm layer splits and latter on differentiate into precursors of blood vessels, bone marrow and haemocytoblast which further differentiate and forms different types of blood cells i.e. Erythrocytes [Red blood corpuscles] and leucocytes [White blood corpuscles].

The precursors of leucocytes in advance stage divide in to granular leucocytes and agranular leucocytes. Granular lucocytes further distinct into Neutrophils, Basophils, Eosinophils, types, while agranular luocytes distinct into lymphocytes, monocytes. In embryonic stage first red blood cells form from yoke cells later on rudimentary liver become the first haemopoetic site for the production of temporary blood. In adults the long bones are the principal site for the production of blood. Likewise the blood cells i.e. haemocytes formation not as complicated as of vertebrates. In insect during embryonic development the mesoderm layer splits in form of patches which are called somites. These somites are the actually progenitors of all the haemocytes.

During embryonic development the mesoderm splited patched actually pleuripotent in nature, due to cytoplasmic movement of fertilized egg and some other triggering stimulates, the receptive mesoderm patches start to production of specific m-RNA that translate a specific protein, in the presence of this specific protein a cascade mechanism now begins that ends in the differentiation of haemocytes.

The differentiated haemocytes collected at some specific places including the progenitors of haemocytes. Such places are called
haemopoeitic organs or haemopoeitic sites. These haemopoeitic sites are lymph glands, wingsbud, near space of aorta.

In insects neurohaemal organ are aorta. These structure playing important roles in transport of hormones from the brain to the body and also send appropriate stimulus to the brain from body as results brain's NSCs [Neuro Secretory Cells] do good work in production of NSM [Neuro Secretory Material] i.e. brain hormones, under the influence of brain hormones, the other endocrine glands e.g. corpora cardiaca [CC], corpora allata [CA] and prothrocic glands [PTG] stimulated and secreted the hormones that directly affected the physiology of insect including production and differentiation of haemocytes.

The haemocytes in insect performs various functions e.g. egg maturation, cuticle formation, Peholoxidase activity [PO], defense, resistance, growth and moulting. The different types of haemocyte are assigned to specific functions but it is very common observations during the present work and also by the literature review.

The haemocytes are not specific for specific functions. No doubt with the study of haemocytic parameter i.e. Total haemocyte Counts [THC] and Differential haemocyte Count [DHC], a clear cut indications and possibility has been correlated to the different haemocytes for example just prior to moulting increases in the population of plasmatocytes [PLs] indicated their roles in tissue tear and wear i.e. involves in moultings. Likewise in “0”hr Age of nymphs or adults has more population of Prohaemocytes [PRs] clearly indicated their role in population or conversion in other haemocytes, it is more evident because in advancing age of nymphs or adults the
population of Prohaemocytes [PRs] gradually declines. The other haemocytes e.g. Granulocytes [GRs] shows possible roles in defense and protection or phagocytosis.

The Adipohaemocytes [ADs] shows their roles in storage of nutrients because before to egg maturation its population increases but during possible days of egg maturation its population declines. The Oenocytes [OEs] associated to stress physiology it is evident with the present work because in extreme stress conditions the Oenocytes [OEs] population appears more in comparison to their controls. The Vermicytes [VEs] are not shows any clear idea about its functioning, it always shows a narrow range of population, in stress of high temperature its population shows a significant jumps on late hours indicate they are also involves in stress physiology.

The morophology of the different haemocytes also confirms their functioning capabilities, before to clearance of this statement. First the descriptions of the different haemocytes are given those based on study of light microscope in present study. The Prohaemocytes are small rounded cell. Nucleus seen in the centre it occupies most of the area of the cytoplasm as a result in surrounding of nucleus a clearly visible ring like structure appeared. During observations of Prohaemocytes number of times Prohaemocytes was seen in division phase. The size of the Prohaemocytes smallest to the all other haemocytes. The Plasmatocytes [PLs] are varied in forms but mostly they are found in spindle shapes, but its fusiform, oval shapes also common.

After the Prohaemocytes division phases are frequent in the plasmatocytes. The Granulocytes [GRs] are large oval shaped cells; it is characterized with the presence of
fine granules in the cytoplasm. Due to the presence of granules in cytoplasm nucleus not very clear.

The Adipohaemocytes [ADs] are the oval shaped cells; cytoplasm filled with fat droplets, so its cytoplasm very much heterogeneous and appears like patches. The size of adipohaemocytes is more than to Prohaemocytes, plasmatocytes and granulocytes.

The Oenocytes [OEs] are oval and large size cells the nucleus is clear and prominent and present at one side of cell. The cytoplasm of oenocytes filled with fine granules as result cytoplasm of oenocytes looks darken in appearance but this darkness present in homogenous in nature.

The Vermicytes [VEs] are elongated narrow creeping like cells, the nucleus is small rounded but present in the centre of vermicytes. The both ends of vermicytes are tapered. The developments of haemocytes and their differentiations are a very interesting phenomenon. The related worker stated this phenomenon associated to gene expression and a fine level of signal transduction activities.

The haemocytes development and its differentiation activities are initiated during late embryonic development. The mesoderm patch or somites are responsible for the formation of haemocytes and associated circulatory system in insects.

When an appropriate stimulus either cytogenic or surrounding effect including both extrinsic and intrinsic, reach on the complementary receptor site of responsive mesodermal somites, a cascade reaction mechanism start with
involvements of series of enzyme which finally express a particular gene that transcribe m-RNA and this m-RNA comes in to cytoplasm and translate a particular or specific protein. This protein responsible for differentiation of haemocytes. Similar to vertebrates, insect have a capable immunity against microbial infections exposing in their environment.

This immunity based on involved components known as cellular and humeral defenses [Beckage, 2008]. Cellular immunity consist phagocytosis of aggresive microorganisms by haemocytes, nodule formation and encapsulation. Humoral responses comprises factors related to the recognition of invading microorganisms, melanization and coagulation as well as killing factors such as antimicrobial peptides [AMPs], reactive oxygen species and reactive nitrogen intermediates, including nitric oxide, prostaglandins and eicosanoids [Boman, 1998; Stanely, 2006; Beckage, 2008].

Mentioned immune reactions are initiated by pattern recognition molecules allowing insects to distinguish self-components from nonself-ones. Studies have been identified specific pattern recognition receptors responding to components in microorganisms such as peptidoglycans and lipopplysaccharides that are main compounds in the cell walls of bacteria and fungi [Theopold et al., 1999; Dziarski, 2004].

Peptidoglycan recognition proteins [PGRPs] have been identified in several insect species as activating cascade of melanization on invasive microorganisms [Rolff and Reynolds, 2010]. There are specific PGRPs for gram-positive, gram-negative and fungi in the hemolymph of insects.
Two signaling pathways namely Toll and Imd have been activated after recognition of gram-positive microorganisms as well as gram-negative ones, respectively [Rolff and Reynolds, 2010]. These signaling pathways lead to activation of cellular immunity and antimicrobial peptides via final Dif and Relish molecule in nucleus of haemocytes [Tzou et al., 2002; Leihl et al., 2006].

Different environmental factors can definitely affect immune reactions of insects that elucidation of these factors is a significant part to clarify various aspects of these mechanisms. Temperature, different ions and insecticides are some of the most important affecting factors [Zibaee et al., 2009].

In agriculture, combined tactics [as Integrated Pest management] are used to obtained efficient control of insects by considering the lowest disruption in environment. Several studies have been conducted to find combined effect of insecticides, highlighted by botanical materials, and microbial agents on insects. Results revealed that botanical compounds decrease immune ability of insects against microbial agents that describes in forward sections.

In insect immunity, circulating haemocytes have crucial roles in both cellular mechanisms and producing antimicrobial components. Five basic types of haemocytes have been identified as prohaemocytes, plasmatocytes, granulocytes, adipohaemocytes and oenocytoids [Lavine and Strand, 2002]. Prohaemocytes as the smallest one are the basic haemocytes that developed to
plasmatocytes and granulocytes when an infectious challenge appeared in the hemolymph.

They recognized as large central nucleus and narrow cytoplasm [Lavine and Strand, 2002; Zibaee and Bandani 2010]. Plasmatocytes and granulocytes are the important haemocytes in immune response to pathogens via phagocytosis [Granulocytes and relatively Plasmatocytes]; nodule formation and encapsulation [Strand, 2008]. They discriminate each other by spindle shape of haemocytes and rounded granulus granulocytes.

Adipohaemocytes contain lipid droplets so some literature considers them as fat bodies instead of haemocyte [Beckage, 2008]. Oenocytoids have two specific shape based on intact and immune challenged insects. In normal situation, oenocytoids are spherical cells with peripheral nucleus and crystalline inclusions without any granules. When an immune challenge occurred, nucleus is going to be smaller and granules appear showing their crucial roles in phenoloxidase [PO] cascade [Strand, 2008; Beckage, 2008].

Different environmental factors could be affect insect haemocytes both morphologically and functionally. For example, elevation of environmental temperature increases numbers of plasmatocytes and granulocytes up to 30-40 % in addition to their nodulation ability [Zibaee et al., 2009]. Also, different divalent cations have positive effect on haemocytes to provide a cellular network entrapping pathogens in the hemolymph [Willot et al., 2002; Willot and Tran, 2002; Zibaee et al., 2009c].
In addition of these positive factors on haemocytes of insects, several other factors, mainly insecticides, have negative effects on number and morphology of them. There are some reports on effects of plant products on the haemocytes such as *Periplaneta americana* L. [Blattodea: Blattidae], *Dysdercus koenigii* Fabricius [Hemiptera: Pyrrhoeoridae] [Saxena and Tikku, 1990; Tikku et al., 1992], *Cyrtacanthacris tatarica* L. [Orthopera: Acrididae] [Peter and Ananthakrishnan, 1995] and *Spodoptera litura* Fabricius [Lepidoptera: Noctuidae] [Sharma et al., 2001, 2003, 2008].

Studies by scan electron microscopy demonstrated the complete loss of filopods in plasmatocytes and cytoplasmic projections in granular haemocytes of *S. litura* larvae treated with Neem gold [Sharma et al., 2003], Sharma et al., [2008] also find similar results on effect *Artemisia calamus* oil on larvae of *S. litura* as loss of cytoplasmic projections in granular haemocytes.

Interestingly, they observed vacuolization in the cytoplasm and degeneration of the organelles, both in plasmatocytes and granular haemocytes, initiated by vacuolization and loss of firmness of organelles leading to degranulation and a degenerative transformation within a period of 48 h, subsequently resulting the total collapse of immunity-building mechanism of *S. litura* [Sharm et al., 2008]. *Atemisia annua* extract altered number of haemocytes and their phagocytic activity in *Eurygaster integriceps* Puton [Hemiptera: Scutelleridae]. Zibaee and Bandani [2010] reported the treatment of *E. integriceps* with *A. annua* extract affected the total number of haemocytes circulating in the hemolymph indicating that the responses could be due
to the toxic effect on the immune cells reducing number of haemocytes attached to fungal spores.

As part of introduction of discussion a concluding remarks are introduce here. Even though, much of the classification of haemocytes in insects is based on the classification proposed by Wigglesworth in a haemipteran, *Rhodnius prolixus*, not much haematological work has been done in the order haemiptera. [Review Pandey and Tiwari 2012; Greeney et al., 2012]. The five types described by Wigglesworth as Prohaemocytes [PRs], Plasmatocytes [PLs], Granulocytes [GRs], Adipohaemocytes [ADs], Oenocytes [OEs] have also been reported in the same and other haemipteran insects by several authors [Review of Pandey and Tiwari, 2012].

However, there are some other workers whose findings in haemiptera differs for instance someone reported Sphreulocytes [SPs] in *Dysdercus cingulatus* and Coagulocytes [COs] in *Rhodnius prolixus* in addition to the three of Wigglesworth’s types PRs, PLs, and GRs. Further some workers reported two other types Cystocytes [CSs] and Podocytes [PDs] in addition to the two of Wigglesworth’s type PRs and PLs. In *Cimax rotondatus*, Sonnawane and more [1993] have reported six types- PRs, PLs, GRs, SPs, OEs, and Thrombocytes [THs] of whom, thrombocytes is yet a new type not reported by any of the forgoing authors.

In the present study, in *D. koenigii* have come across with six haemocytes types are PRs, PLs, GRs, ADs, OEs, VEs earlier reported by Mishra [1999]. However, Salehi in year 1990 have reported the presence of Vermicytes [VEs] in lepidopterous insects. But they have not been recognized as distinct haemocytes types; instead they have been regarded as a variant of PLs that
appears only under certain specific physiological conditions [Pandey and Tiwari, 2012]. Surprisingly in the present study, they were noticed only in the late fifth instars nymphs [both females and males] and in the day 6 old adult i.e. prior to the moult and prior to the first egg cycle.

The findings of VEs in the present study supported the view of Mishra [1999] and Pandey and Tiwari [2012]. These workers believe in that the presence of VEs in late instars and just before first egg cycle in adults seems that those cells may have some role to perform in the adult stage and cannot be treated as sub type of PLs. Since the PLs are present in the adult stage including the nymphal stages if VEs were their sub types formed by the elongation of the cells, there they should have been visible in instars and adults as other five types of haemocytes.

Looking to their ability of creeping in between tissues/cells possibly transporting materials required at the specific time when they are seen. Here we differ to view of earlier workers in respect of VEs because in the present study we recorded VEs in stress conditions like wound, high temperature.

Therefore, VEs possible also involve with sbody defense of insect or related to stress physiology [Gupta and Mishra, 2014]. THC has been extensively studied both hemimetabola and holometabola [Salehi-1990, Tiwari et al., 1997, Mishra and Tiwari 2005].

The increasing trends of THC in fifth instars accordingly to some worker from day 0 to up to day of moulting like THC also increasing in adults from day 0 to day of completion of egg maturation. According some worker it in fluctuating i.e. increasing from day 0 to day 2 or 3 than decline also the fluctuation in
THC reported in adults so there are different views regarding trends of THC in ultimate fifth instars and in adults up to first egg cycle.

Therefore, in our present work the trends of THC recorded in fifth instars and adults of both sex. In the present study recorded the fluctuation in THC in fifth instars and in adults of both the sex are as follow in fifth instars THC increases from day 0 to day 3 than slightly decline up to day of moulting [day 5] and in adults from day 0 to day 6 THC was in increasing trends than decline up to day 8.

The fluctuation in THC in fifth instars correlated with the growth and tissue formation as these processes required more labour forces [haemocytes] for the transport of nutrients. Decline in THC possibly due to completion of growth and also due to inhibition of moulting i.e. restriction in wear and tear processes of tissues, so THC are extensively involves in these, due to this reason they not come in circulation as noticed in earlier days of fifth instars nymphs and adults.

In adults increase in THC probably due to beginning of egg maturation in females, number of workers reported the involvement of haemocytes in egg maturation, so the present findings support the earlier workers.

The slight decline in THC on day 7 may be due to degeneration of haemocytes, decrease in mitotic rates of PRs and also regression in haemopoiesis process at haemopoietic site.
These reasons evident within insects just before egg laying vital activities and level of ecdysone decreases. The reasons for the present findings support the view of Mishra and Tiwari [2005]. Such views also discussed with the following review of literatures. The response of haemocytes against biological agents as well as toxins has been studied in many insects. The resulting effects thereof are phagocytosis, encapsulation and distortion of cell contour or cellular disintegration (Saxena and Tikku, 1990).

The drastic reduction in THC in present insect following NBIs application is similar to the reports of Azambuja et al. (1991) in Rhodnius prolixus, Sharma et al. (2003) in Spodoptera litura and Tiwari et al. (2006) in Dysdercus koenigii. The decrease in THC number may be due to the clumping of haemocytes, the toxic effect of NBIs and/or to their inhibitory effect on endocrine glands. Sharma et al. (2003) reported about 50% reduction in THC after 72 h of oral feeding of neem gold in S. litura but 61% decline was seen in D. koenigii following topical application of neemazal (Tiwari et al., 2006).

It reveals that neemazal produced 59 and 56% reduction in THC after 24 and 48 h, respectively, which is more effective as compared to two other NBIs. It could be probably because of more azadirachtin content (1% in the former and 0.03% in the latter two). However, Figueiredo et al. (2006) found no significant difference in THC between azadirachtin treated R. prolixus nymphs and their controls. The maximum drop of PR percentage (53%) was followed by PLs (34%) and GRs (28%) respectively, 48 h after neemazal treatment. This indicates the maximum
participation of PRs in over-all decline in cell count more likely by the inhibition of their mitotic divisions (Salehzadeh et al., 2003).

The decline in PL- and GR-percentage was reported to be caused by their involvement in phagocytosis and nodule formation (Sharma et al., 2003). But the reduction in number of phagocytotic capsules in NBI treated D. chrysippus larvae led to suggest some other reasons for decline in their counts. Similar drastic reduction in encapsulation/phagocytosis response were also found in Drosophila larvae infected by eggs of parasitic wasps (Sorrentino et al., 2002) and azadirachtin treated R. prolixus nymphs (Figueiredo et al., 2006). Based on their studies, Figueiredo et al. (2006) have suggested for the first time that the phagocytosis is modulated by ecdysone.

The reduction in number of these cell types, therefore, seems to be due to the toxic effect of NBIs rather than their involvement in phagocytic actions. A large number of abnormalities observed in NBIs treated Danais larvae at cellular and developmental levels (see Results) have also been reported by earlier workers (Schmutterer, 1988; Singh, 1996; Sahayaraj and Paulraj, 2001; Sharma et al., 2003; Tiwari et al., 2006).

Further, the synthesis and release of PTTH by the brain was also reported to be deficient in azadirachtin treated R. prolixus nymphs (Garcia et al., 1990). The factors for these developmental and cellular defects are reported to lie in the endocrine system and the hormones secreted by them (Hoffmann, 1970; Schmutterer, 1990; Koul and Isman, 1991; Koul, 1996; Tiwari et al., 2006).
Further, our results reveal the reduction of body weight in NBIs treated larvae. This is suggestive of their antifeedant property as reported for azadirachtin treated cutworm, *Peridroma saucia* (Koul and Isman, 1991) and Aza treated larvae of the lemon-butterfly, *Papilio demoleus* (Pandey *et al*., 2006). The NBIs might act negatively on the prothoracic glands via brain thereby causing reduction in phagocytic response of haemocytes and reduction in body weight. Further, NBIs might have direct toxic effects on haemocytes leading to necrotic results. The over-all effect of the NBIs could be adverse on the immune activity of the present insect threatening its survival. The decline in THC in the present study after the expouser of antigen also support the view of workers of the above para but we differ in view of the above workers, because in the present finding after day 3 the THC were improved on day 4 that might be due to suppression of antigens and prevention of histological damages. In such suppression the different types of haemocytes playing roles. The fluctuation in population of PLs, GRs, OEs, and appearance of VEs correlated with these cells involvement in body defense and combat of antigens, support the Pandey *et al*., 2008; Gupta and Mishra, 2014b. The effect of antigens and other stresses of the present study as a single factor considered and the results discussed with following workers, which taken from review of literatures. The changes in the THC and DHC in starved insects have been studied by many workers. While some workers reported an increase in the THC during starvation [Begum R and Gohain R 1996, Chisholm J.R S and V.J. Smith, 1994], others found a decrease [Sujatha and Dutta –Gupta, 1991] and still others found no change.
Regarding changes in DHC, the views of the authors are not uniform. Workers carried out a series of experiments on the effect of starvation on the haemoytes of last instar *Tenebrio* larvae and noted increase in number of GRs and decrease in number of PLs. Some workers noted same result in same insect after prolonged starvation. Some workers found that starvation reduced the number of fusiform haemocytes in Bombay larvae and reported that the relative proportion of SPs increased as the starvation period decreased. Some workers found significant increase in the relative number of ADs and degenerating cells but decrease in the number of fusiform PLs in starved IV instar of *Anagasta*. Some worker studied haemocyte population changes in *Rhodnius* and concluded that great differences occurred between fed and unfed nymphs on the fifth day after ecdysis.

The identification and classification of insect haemocytes have been recently based on their ultrastructural features [Brehelin, 1993] and immunochemical identification [Elizabeth et al, 1994; Charalambidis et al., 1996]. The important reviews on haemocyte classification are those of [Pathak 1993, HF Greeny et al., 2012, Pandey and tiwari, 2012].

**OBJECTIVES**
1. To study number of stresses on D. koenigii to determine their relationship between physiology and haemocytes is much more than for any other single insect.

2. To study the effects of wound and antigen on the THC and DHC as investigated in the present study is totally lacking for any other insects.

3. To study relationship between physiology and haemocytes of the red cotton bug which is a serious pest of cotton causing a great economic injury to cotton grower of our country. Such studies will help in finding out a biological means against the conventional insecticides and drawback of Bt cotton insect pests, the use of which caused an environmental imbalance, problem of insect resistance and environmental pollution etc.