Review of Literatures

The Haemocytes:

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 into 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].
Haemocyte counts – Total and Differential

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 Pseudaletia 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 mouth, Ephesia 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.

**Stress Effects:**

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. 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-Streptomycin] 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 is 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 it’s 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 under taken, 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 moultng 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.

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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.

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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.

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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 incorporated 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.

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*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 convince 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, Coagutocytes 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 leucocytes further distinct into Neutrophils, Basophils, Eosinophils, types, while agranular leucocytes distinct into lymphocytes, monocytes. In embryonic stage first red blood cells form from yoke cells later on rudimentary liver become the first haemopoeitic 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 arota.

In insects neurohaemal organ are arota. 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 insects perform various functions e.g. egg maturation, cuticle formation, phenoloxidase activity [PO], defense, resistance, growth and moulting. The different types of haemocytes 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 morphology 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 aggressive 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 lipopolysaccharides 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.

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.

Injection of salt solution has been found both to include accumulation and depletion of NSM in the NSCs. whereas, some workers observed increase in THC after injection of saline in *Euproctis* and *Schistocerca*, but some workers reported that the percentage of PLs and PRs increased while ADs decrease in *Tenebrio* larvae following injection of saline.

While a number of workers find no significant decrease in THC and DHC of the sexes the findings of many others are variable, for example, studied a number of insect orders and reported that the average count in the females was higher than that from the males. Some workers also found the same, noticing higher percentages of the PLs and PRs and lower of the ADs in females than in the males. Some workers observed a higher cell count in the males than the female adults. Pathak and Kulshreshtha [1993] stated that in *Blattella germanica*, the PRs were present in the
freshly moulted [sexually immature] adults but absent in copulating/Post coital adults and females after deposition of oothecae. The POs, on the other hand, were present in the haemolymph of adults of all stages except sexually immature adults of both the sexes and females carrying oothecae. Similarly, the SNs were noted in all stages except the sexually immature ones. The VEs were noted only in the haemolymph of copulatory females. For antigens effects there is also variation in reports of authors [Delclos K. B. et al. 2001, Castle and Thrasher 2002, Park et al. 2003, Josef Berger et al. 2003, Schmid-Hempel P. 2003, Gottstein et al. 2003].

**Injection-materials**, 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 consists phagocytosis of aggressive 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].

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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 consider 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].