CHAPTER THREE

HAEMOCYTE TYPES AND TOTAL AND DIFFERENTIAL COUNTS IN UNPARASITIZED AND PARASITIZED HELICOVERPA ARMIGERA LARVAE.
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HAEMOCYTE TYPES AND TOTAL AND DIFFERENTIAL COUNTS IN UNPARASITIZED AND PARASITIZED HELICOVERPA ARMIGERA LARVAE.

3.1 Introduction

Different types of haemocytes have important role in the protection of insects against invading pathogens and parasitoids. Hence, identification of various types of insect blood cells based on the structure and function is important.

The hematological studies are important in the field of insect physiology because certain vital activities are performed by haemocytes (Nahla et al. 2010). Insect haemocytes categorized into several types of circulating in the haemolymph. They have been studied mostly in Lepidoptera, Hymenoptera, Coleoptera and Dipetra (Gupta 1985). The prohaemocytes are specialized for division, plasmatocytes, specialized for phagocytosis, granulocytes, sperulocytes and oenocytiod specialized for secretion and storage (Brehelin and Zachary 1986). There is inherent variability of haemocytes within a species depending on the developmental and physiological stages (Sanjayan et al., 1996; beetz et al., 2008).

An endoparasitoid must avoid haemocyte-mediated immune responses to develop in the host larvae. Many parasitoids have adapted to this mechanism by suppressing one or more components of host immune defense system (Carton et al., 2008). These endoparasitoids wasps use various strategies such as polydnavirus (PDV), venom, teratocytes for manipulating the physiology of their host and disrupt the host immune system by ovipositing eggs (Jones et al., 1993; Vinson 1990). Polydnavirus causes
developmental arrest in *Spodoptera littura* when parasitized by *Chelonus. inanitus* (Soller and Lanzrein, 1996). The ovarian secretions of braconid endoparasitoids contain polydnavirus, venom and soluble proteins. When polydnavirus is delivered to the hemocoel of lepidopteran hosts during parasitization, hemocytes are efficiently infected although other tissues are also infected. Polydnaviruses don’t replicate in the parasitized host (Asgari et al. 1996; Beckage and Gelman 2004; Turnbull and Webb 2002; Webb and Strand 2005; Strand et al. 1992; Theilmann and Summers 1986) although transcription of viral genes occurs in infected cells. Virus infection and expression of polydnavirus-encoded proteins is required for successful parasitization and induces pathologies such as altered host development (Cusson et al. 2000; Pennacchio et al. 1998; Soller and Lanzrein 1996; Kent et.al., 1999), reduced humoral immunity (Shelby et al. 2000), and abrogation of cellular immunity (Cui et al. 2000; Strand 1994; Matthew W. Turnbull 2004). The hemocytes are cells that circulate in the hemolymph, providing quick and efficient response against pathogens that invade the hemocoel (Lavine and Strand, 2002).

Several parasitoids show different types of effects on the host body like reduction in the total number of haemocytes (THC) (Stettler et al.,1998), inhibition of haemocyte spreading, apoptosis in circulating haemocytes (Strand and Pech, 1995) and effect on hematopoiesis in the hematopoietic organs by suppressing the cell cycle (Teramoto and Tanaka, 2004). Tiwari and Shukla (2000) and Pandey et. al. (2008) reported the hematological changes of insect in response to the foreign invaders. Impact of metyrapone and nucleopolyherdo virus on morphology of haemocytes of *Spodoptera littorals* and *Helicoverpa armigera* has studied by Gelbic et.al.(2006) and Kalia et.al. (2001) respectively. Also alteration in both total and differential haemocyte counts
(DHC) has been reported in the case of fungal (Hung et al., 1979), bacterial (Narayanan & Jayaraj, 1974), viral (Narayanan, 1979), and parasitoid infection (Narayanan and Jayaraj, 1973.

*Chelonus blackburni* (Hymenoptera: Braconidae) is an egg – larval uniparental endoparasitoid of *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Raj et al., 1999). This parasitoid completes larval development within the body cavity of the host insect causing developmental arrest in the prepupal stage. This parasitoid was used to overcome the cellular immune system employed by the differentiation and haemocyte counting from the parasitized larvae of *H. armigera*. A better understanding of these mechanisms may provide us basic data about reasons for success of *C. blackburni* in insect haemolymph and might leads to highlights in biocontrol or insect growth regulators to control *H. armigera* or give us an idea to improve strategies of using both of them in integrated pest management programme (IPM). The changes in total haemocyte counts (THCs) and differential haemocyte counts (DHCs) of *H. armigera* larvae against *C. blackburni*, are important criteria, determining cellular immune reactions. The success of the immune response depends on the role of the haemocytes in this process (Russo et.al. 2001, Andrade 2010).

Although the biology of this pest has received a lot of attention, little is known about the immune system of *H. armigera* when infected by *C. blackburni*. The alterations in the number of the hemocytes in response to the parasitization in hemolymph and consequently the role of these cells in defense mechanisms are so far not studied. This work aims to study the role of the hemocytes in antiviral immunity through the
quantitative alterations in these cells of *H. armigera* larvae when infected by the parasitoid *C. blackburni*.

### 3.2 Materials and methods

**Haematology:**

Parasitized and unparasitized larvae (10-12 days old) were individually chilled on ice. They were surface sterilized with 75% ethanol and then bled on to a piece of parafilm by cutting their anterior region with sterile micro scissor. Haemolymph (10 µl) was collected in eppendorf tubes and stained by using Giemsa (4%). This mixture was transferred on to three haemocytometers (Neuberger chamber) allowed to settle for a few minutes following which total haemocyte counts were made with phase contrast microscopy.

5.2.2. Differential haemocyte counts (DHC):

Parasitized and unparasitized larvae were bled directly on to a slide by cutting the forelegs with the help of micro scissors. A smear made from the droplet of haemolymph was allowed to air dry and fixed in absolute methanol for 4 min, stained with 4% Giemsa stain for 2-3 min and subsequently washed with distilled water. The slide was air-dried and mounted in DPX. The smear was examined and visualized with a phase contrast microscope at 40X magnification and images were acquired with a digital camera. Ten such slides were prepared from parasitized and unparasitized larval haemolymph. The haemocyte types were identified by using established morphological characters as described by Gupta (1985). The average size of the haemocyte types were estimated by measuring the length and width of twenty cells of each type.
3.3 Results

Haematology:

The effect of parasitisation of *C. blackburni* on *H. armigera* revealed prominent changes in the total haemocytes count (THC). There is a significant decrease in THC in parasitized larvae of *H. armigera* than in unparasitized larvae. Number of cells counted per microlitre at the end of 12 days was significantly lower in parasitized larvae. The THC of unparasitized larvae was significantly higher being 9143±410 as compared to 2440±104 in parasitized larvae (Fig. 9).

![Figure 9. THC of larvae of *H.armigera* parasitised by *C. blackburni* and unparasitised larvae.](image-url)
Differential haemocyte counts (DHC):

Six primary types of haemocytes were observed in haemolymph of parasitized and unparasitized larvae of *H. armigera*: prohaemocytes, plasmatocytes, granulocytes, sperulocytes, adipohaemocytes and oenocytoids.

**Prohemocytes (PR):**

These cells are either round, oval or elliptical with variation in size (16.98 µm to 14.03 µm). The nucleus is large, round and occupies most of cell cytoplasm which forms a very thin layer around the nucleus, i.e., cells have a high nuclear cytoplasmic ratio. The nucleus size is variable (11.68 µm to 10.52µm) and these cells are characterised by a thin or dense homogenous and intensive basophilic layer of cytoplasm that surrounds the nucleus. PRs are generally found in groups and appear indistinguishable from young and small plasmatocytes.

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**Plasmatocytes (PL):**

PLs are highly polymorphic cells. Their shapes range from spindle, with a very pointed end, to oval with a large centrally located nucleus. They could be grouped as spherical,
oval and irregular plasmatocytes. The irregular shapes are due to cytoplasmic extension. PLs are significantly larger than the PRs. When they are spherical in shape their size is varies from 35.6µm to 10.96 µm. When oval, their average size is 20.70µm to 10.26µm. The nuclei can be spherical (10.06 µm to 7.19µm) or oval (12.49µm to 6.6µm). After Giesma staining, the cytoplasm stains blue colour (basophilic) while the nucleus stains dark as observed in prohaemocytes (Figure 10 B).

Granulocytes (GR):

GRs are variable in shape and size. They may be either small or large and vary in shape from spherical to oval. The average size of large GRs is (24.27µm to 21.13µm) and small GRs is (14.66 µm to 12.62µm) and are easily distinguished by the presence of vacuoles in the cytoplasm. These cells are characterised by a small nucleus and large amounts of different size granules. The nucleus is basophilic and generally occupies a central position and its size varies from 7.61µm to 6.90 µm in small GRs to 10.28µm to 9.51µm in large GRs (Figure 10 C). These type of cells show intermediate features between plasmatocytes and granulocytes.

Spherulocytes (SP):

SPs are variable in shape (polymorphic) with a small nucleus which is generally small, central or eccentric with nucleoli surrounded by little cytoplasm and large vesicles with membrane bound vacuoles in the cytoplasm. The cytoplasm is characterised by the presence of highly basophilic or acidophilic spherules and small spherical vacuoles. The average size of SP varies from 18.13µm to 16.27µm and the average nucleus size is 4.95
µm to 4.15 µm (Figure 10 D). These cells show intermediate characteristics between plasmatocytes and spherulocytes.

**Adipohaemocytes (AD):**

Adipohaemocytes are very few when compared with other haemocytes. These cells are polymorphic and can be large or small in size, oval or irregular in shape. The nucleus is relatively small, round, or slightly elongated and centrally or eccentrically located. The nucleus size varies from 5.10 µm to 4.19 µm and average cell size is between 15.95 µm to 13.88 µm (Figure 10 E). The nucleus appears to be concave, biconvex, punctate or lobate. After Giesma staining, the cytoplasm shows a high basophilic and variable numbers of large refringent fat droplets and other nonlipid granules and vacuoles.

**Oenocytoids (OE):**

These cells are large, spherical or oval in shape and the nucleus is generally small, round, elongated and generally eccentrically located. The average size of these cells varies from 17.10 µm to 14.27 µm. They maintain their shape throughout observations without any pseudopods or filopods. The nucleus size may vary. The nucleus is more basophilic than the cytoplasm. Occasionally two nuclei are present having a general shape of the cell. The average nuclear size varies from 4.45 µm to 4.30 µm (Figure 10 F). After Giesma staining, OEs show moderate acidophilic and reveal a homogeneous cytoplasm containing fine and weak acidophilic granulations. These cell types show intermediate characteristics between GRs and OEs.
Figure 10. Morphology of haemocytes of *H. armigera* (unparasitized) after Giemsa staining. Prohemocyte (A), Plasmatocyte (B), Granulocyte (C), Sperulocyte (D), Adipohaemocyte (E), Oenocytoid (F).

The haemocytes differential count in the parasitized and unparasitized larvae of *H. armigera*:
Table 6.2.1: The haemocytes differential count in the parasitized and unparasitized larvae of *H. armigera*

<table>
<thead>
<tr>
<th>Cellular type (%)</th>
<th>PR</th>
<th>PL</th>
<th>GR</th>
<th>SP</th>
<th>AD</th>
<th>OE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Larvae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unparasitized</td>
<td>46</td>
<td>15.36</td>
<td>14.60</td>
<td>26.44</td>
<td>1.91</td>
<td>0.33</td>
</tr>
<tr>
<td>Parasitized</td>
<td>55.39</td>
<td>12.85</td>
<td>21.42</td>
<td>8.57</td>
<td>1.18</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Larvae of *H. armigera* parasitized by *C. blackburni* reveal a significant increase in percentages of prohaemocytes and granulocytes while in unparasitized larvae, the percentage of plasmatocytes and sperulocytes significantly increases. There are no significant differences observed in the percentage of adipohaemocytes and oenocytoids in either parasitized or unparasitized larvae.

![Cell aggregation of Plasmatocytes in parasitized Helicoverpa armigera larvae](image)

Figure 11. Cell aggregation of Plasmatocytes in parasitized *Helicoverpa armigera* larvae
In parasitized larvae of *H. armigera* plasmatocytes amount was lower but cell aggregation and spreading of these cells appeared in many places.

**5.2.10. The morphometric analysis of the different haemocytes types found in the haemolymph of unparasitized and parasitized *H. armigera* larvae.**

Table No.6.3.1: The morphometric analysis of the different haemocytes types found in the hemolymph of unparasitized *H. armigera* larvae.

Cellular Types of Unparasitized haemocytes of *H. armigera* (mean±SD)

<table>
<thead>
<tr>
<th>Cellular Types</th>
<th>PR (Spherical)</th>
<th>PL (Oval)</th>
<th>GR (Large)</th>
<th>GR (Small)</th>
<th>SP</th>
<th>AD</th>
<th>OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.D. (µm)</td>
<td>Max 3.42±0.8 8</td>
<td>3.83±0.98 1</td>
<td>2.57±0.8 0</td>
<td>4.35±1.3 0</td>
<td>0.70±0.2 4</td>
<td>4.32±1.1 0</td>
<td>5.31±1.3 0</td>
</tr>
<tr>
<td></td>
<td>Min 1.84±0.4 7</td>
<td>1.66±0.43 8</td>
<td>1.51±0.4 8</td>
<td>3.68±1.1 0</td>
<td>1.78±0.6 3</td>
<td>4.20±1.0 0</td>
<td>3.11±0.8 0</td>
</tr>
<tr>
<td>N.D. (µm)</td>
<td>Max 2.64±0.6 8</td>
<td>2.77±0.71 1</td>
<td>1.64±0.5 1</td>
<td>2.31±0.7 3</td>
<td>0.82±0.2 9</td>
<td>1.93±0.4 9</td>
<td>1.12±0.2 9</td>
</tr>
<tr>
<td></td>
<td>Min 2.35±0.6 0</td>
<td>1.85±0.47 4</td>
<td>2.35±0.7 4</td>
<td>2.66±0.8 4</td>
<td>1.26±0.4 4</td>
<td>1.86±0.4 8</td>
<td>1.11±0.2 8</td>
</tr>
<tr>
<td>C.A. (µm²)</td>
<td>Max 5.43±0.1 36</td>
<td>5.93±3.95 2</td>
<td>3.26±0.3 2</td>
<td>12.68±1.81</td>
<td>1.21±0.1 5</td>
<td>14.25±0.95</td>
<td>13.93±0.92</td>
</tr>
<tr>
<td></td>
<td>Min 4.90±0.3 2</td>
<td>4.20±0.28 1</td>
<td>3.12±0.3 1</td>
<td>4.85±0.4 8</td>
<td>0.85±0.1 0</td>
<td>2.83±0.1 8</td>
<td>0.98±0.0 06</td>
</tr>
<tr>
<td>N.A. (µm²)</td>
<td>Max 0.52±0.0 4</td>
<td>1.73±3.67 1</td>
<td>0.13±0.0 1</td>
<td>7.82±1.3 3</td>
<td>0.35±0.0 5</td>
<td>11.42±0.77</td>
<td>12.94±0.91</td>
</tr>
</tbody>
</table>

Cellular diameter (C.D.), nuclear diameter (N.D.), cellular area (C.A.), nuclear area (N.A.), cytoplasmatic area (Cy.A.).

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Table No.6.3.2 The morphometric analysis of the different haemocytes types found in the haemolymph of parasitized *H. armigera* larvae.

<table>
<thead>
<tr>
<th>Cellular Types of parasitized haemocytes of <em>H. armigera</em> (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C.D. (µm)</strong></td>
</tr>
<tr>
<td>Max</td>
</tr>
<tr>
<td>Min</td>
</tr>
<tr>
<td><strong>N.D. (µm)</strong></td>
</tr>
<tr>
<td>Min</td>
</tr>
<tr>
<td><strong>C.A. (µm²)</strong></td>
</tr>
<tr>
<td><strong>N.A. (µm²)</strong></td>
</tr>
<tr>
<td><strong>Cy.A. (µm²)</strong></td>
</tr>
</tbody>
</table>

Cellular diameter (C.D.), nuclear diameter (N.D.), cellular area (C.A.), nuclear area (N.A.), cytoplasmatic area (Cy.A.).
Figure 12. The morphometric analysis of the different haemocytes types found in the haemolymph of unparasitized and parasitized *H. armigera* larvae.

Size of spherical plasmatocytes, sperulocytes, adipohemocytes and oenocytoids are significantly larger in unparasitized than in parasitized larvae (Cell diameter and nuclear diameter). Cell size of oval plasmatocytes and granulocytes do not show any significant difference in unparasitized and parasitized larvae.
3.4 Discussion

It is known that insects are beneficial as well harmful to human beings. To get healthier result there is need to endorse ‘beneficial insects and manage the harmful ‘insect pests. Generally, to improve the productivity of beneficial insects and to control the harmful insects, a number of parameters are being used by the researchers to see the impact of beneficial insects on harmful insects (pests). The ability to isolate and identify haemocytes is essential for studies in insect cellular immunity. It is reported that many kinds of stresses influence the insects at physiological level and are expressed through their poor survival and vigour (Pandey and Tiwari 2012). Haemocytes are important indicators for growth and metamorphosis of insects. They are found to show changes in their types, number and configuration under different stresses which finally affect the health and loss of insect (Kohlmaier and Edger, 2008).

Circulating haemocytes (“blood cells”) play vital role in defence mechanisms against microbes, parasitoids in the hemocoel. Although, in last 250 years, lot of research work has been conducted on haemocytes and their role in insect immunity (Schwammerdam, 1758; Cuenot, 1897; Kollmann, 1908; Wigglesworth, 1939; Paillot and Noel, 1928; Yeager, 1945; Jones, 1959, 1962; Gupta, 1979, 1985a; Charalambidis et al., 1996; Sorrentino et al., 2002; Tiwari et al., 2002; Pandey et al., 2003a, b, 2008a, b, 2010; Pandey and Tiwari, 2004, 2011; Ceraul et al., 2003; Pandey, 2004; Ling et al., 2005; Figueiredo et al., 2006; Gandhe et al., 2007; Merchant et al., 2008; Singh et al., 2008; Pandey and Tiwari 2012).

Haemocytes are very vital components of the insect immune system and are biochemically very sensitive having multiple functions such as nodule formation,
phagocytosis and encapsulation as defence mechanism; synthesis and transport of nutrients and hormones for proper growth and wound healing by way of connective tissue formation (Sorrentino et al., 2002; Ceraul et al., 2003; Ling et al., 2005; Figueiredo et al., 2006; Gandhe et al., 2007; Merchant et al., 2008; Singh et al., 2008; Pandey et al., 2008b, 2010). Although, the haemocyte are very responsive components of cellular immune responses and health indicator of insects will help in simple recognition of adversaries and also to see the impact of different parasitoids on pests. The impact of C. blackburni on haemocytes immune responses of H. armigera is not studied much. Present study will be useful to study the haemocyte immune response of H. armigera against C. blackburni insects. In several endoparasitoids, the parasitism results in a significant reduction of host haemocyte population, due to directly induced immunosuppression (Ibrahim and Kim 2006). C. blackburni parasitoids interfere with the function of haemocytes and our present study showed that haemocytes of H. armigera clearly affected by C. blackburni.

Endoparasitoids suppress, modify, or regulate the host immune-defense system by maternally derived secretions. Both maternal and embryonic immunosuppressive factors are present in some hymenopteran wasps, maternal factors include ovarian proteins, venom, and polydnavirus while embryonic factors include teratocytes (Theopold et al. 2000; Amaya et al. 2005; Asgari et al. 1996; Bae and Kim 2004; Basio and Kim 2005).

Successful parasitization by endoparasitic wasps in the hemocoel requires suppression of the immune system of the host to prevent encapsulation of the wasp’s egg, and developmental arrest of the host to divert host nutrients to support parasite development
Endoparasitoid belonging to the hymenopteran family. Braconidae and Ichneumonidae inject symbiotic virus called polydnavirus. This virus plays an important role in suppressing host immune responses and induces a developmental arrest in the prepupal stage so that development of the parasitoid can proceed (Kaeslin, 2005). Polydnavirus cause host immune suppression, allowing the parasitoid to mature without invoking a host immune response. The polydnavirus triggers apoptosis of host haemocytes, thus causing the host to be immune suppressed during the initial stages of parasitic infection (Strand and Pech, 1995; Webb et al. 2000).

In hosts of several larval parasitoids haemocytes have been shown to be a major site of expression of viral genes and for some a role in suppression of the host’s immune system expression levels were highest shortly after parasitization (reviewed in Webb, 1998; Schmidt et al., 2001). Kaeslin also found viral transcripts in haemocytes, fat body and nervous tissue but values being highest in haemocytes.

*C. blackburni* have a unique and complex association with their host, it develops in the haemocoel and fed on haemolymph of host larvae, deriving nutrients from the host thereby affecting growth and development of *H. armigera* (Jackson1978). Different types of haemocytes have important role in the protection of insects against invading pathogens and parasitoids. Hence, identification of various types of insect blood cells based on the structure and function is important for study the insect cellular immunity. Six types of haemocytes were observed in haemolymph of 3rd instar larvae of *H. armigera*, distinguishable in smears, after Giesma staining, namely prohaemocytes, plasmatocytes,
granulocytes, sperulocytes, adipohaemocytes and oenocytoids following the classification as described by Gupta (1979).

Prohaemocytes are smallest haemocytes and are easily identified by their small size and large nucleus-cytoplasm ratio (Gupta, 1979). Plasmatocytes are the most variable in shape as observed in the hemolymph smears of *H. armigera*. The irregular shapes are due to cytoplasmic extension (Amaral et.al 2010). In *H. armigera*, only plasmatocytes present cytoplasm expansions (Gianotti and Caetano, 1985).

Granulocytes of *H. armigera* have been reported by other investigators (Arnold and Hinks, 1976; Gianotti and Caetano, 1991). They are easily identified by their large size with the cytoplasm characteristically filled with basophilic granules in Giesma stained smears. In *H. armigera* larvae, emission of pseudopods was not observed in the granulocytes (Arnold, 1974). Spherulocytes are easily distinguished from the other haemocytes by the presence of small basophilic or acidophilic spherules distributed in the cytoplasm (Falleiros, 2003). Adipohemocytes in *H. armigera* cells are very few when compared with other haemocytes. These haemocytes having a large lipid like vesicle, sometimes large enough to deform the cell have been regarded as granulocytes variants by some authors (Chiang et al., 1988; Russo et al., 1994; Silva et.al 2002). Oenocytoids are large cells having a very low percentage among all types of haemocytes in *H. armigera*. They can be distinguished by their fine granulation (Barraco and Cestari, 1984). This type of haemocyte is not present in all insect species (Silva et.al 2002).

Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC) are influenced by *C. blackburni* in host *H. armigera*. After parasitization the total number of circulating haemocytes in *H. armigera* significantly decreases than unparasitized larvae. The
decrease in THC affected specimens indicates a severe and drastic decrease in the proportion of metabolically active cells. This reduces an ability of insect cellular defense system. Physiological mechanisms of phagocytosis, encapsulation and other related defense mechanisms primarily depend upon the availability of circulatory immune cells (Sanjayan et al., 1996). Ahmad (1991) reported that THC declined after treatments of different insecticides in \textit{H. armigera}.

In relation to circulating haemocytes counts, our results show an increase in numbers of some haemocytes in parasitized than in unparasitized larvae. These findings are in agreement with those obtained by Silva (2000) who also detected encapsulation process against parasitoids. Similar haemocyte reduction in the parasitism of \textit{C. kariyai} was caused by apoptosis of the circulating haemocytes and by histolysis of the haematopoietic organ (Teramoto and Tanaka 2004). Also decreases the haemocyte population in the parasitised \textit{D. saccharalis} shows haemocytes are functional elements involved in recognition and encapsulation of foreign objects by suppressing the host’s immune system.

In general our data were similar to the results reported in many other parasitoid/host systems such as \textit{Cotesia vestalis} (Haliday) (as \textit{Cotesia plutellae} Kurdjumov) \textit{Plutella xylostella} (Linnaeus) (Lepidoptera: Plutellidae) (Ibrahim and Kim 2006), \textit{Tranosema rostrale} (Brischke) (Hymenoptera: Ichneumonidae/\textit{Chorisstoneura fumiferana} (Clemens) (Lepidoptera: Torticidae) (Doucet and Cusson 1996), and \textit{Microplitis demolitor} Wilkinson (Hymenoptera: Braconidae)/\textit{Pseudoplusia includens} (Walker) (Lepidoptera: Noctuidae) (Strand and Pech 1995), in which parasitism variously increased or reduced the host’s haemocyte load. Our study showed that parasitism of \textit{H.}}
armigera by C. blackburni clearly affected the haemocytes and reduced the circulating haemocyte counts of H. armigera (Figure 6). In parasitized larvae, reductions were most evident in the numbers of the PLs and SPs. Unadherent PLs spread in the haemolymph and seen aggregated at many places. Their unstructured aggregation form multiple layer as a result of which the cell morphology changes. This unstructured aggregation may later be encapsulated by other haemocytes or by the cells that are released from aggregation (Nahla, 2010). Moreover, this study shows PLs is the major haemocytes in H. armigera and play the most significant roles in cellular immune reactions in H. armigera. Depending on the host-parasitoid complex, the host’s haemocytes may fail to spread (thereby inhibiting the encapsulation response) or undergo programmed cell death - apoptosis. In Manduca sexta larvae parasitized by Cotesia congregate, massive numbers of dead and dying haemocytes undergo clumping which are then removed from circulation soon after parasitisation, resulting in a dramatic drop in host’s total haemocytes (Amaya, 2005).

The percentage of SPs and PLs were more in unparasitized larvae, while PRs and GRs were higher in parasitized larvae of H. armigera. GRs are capable of adhering to foreign surfaces (cell mediated immune responses). There are might be a many reasons to decrease in PLs. In H. virescens larvae parasitized by T. nigriceps, the total number of circulating hemocytes transiently decreases, with hemocytes show different structural damages, with general morphological changes. These hemocyte alterations seem to be selectively induced in granulocytes. All these changes in hemocyte structure and function could be induced by venom and calyx fluid (Ferrarese 2005). In other studies, PLs are which are the immunocytes is a result of immune-depressant reaction of insecticides
which takes place on PLs (Saxena, et.al., 1990) and also, PLs are phagocytotic cells involved in removal of apoptotic cells during development as well as in the ingestion or encapsulation of pathogen (Hartensten, 2006). Tikku et al. (1992) and George and Ambrose (2004) also recorded reduction percentage in PLs of adult *R. kumarii* due to tested organophosphate insecticides that were found to be highly toxic to the treated organism. PLs and GRs which the insects need to utilize to feed and survive in the habitat (Sanjayan et al., 1996). The SPs (along with ADs) are considered a more mature form of GRs. The PRs serve as stem cells in the haemolymph (Silva et al. 2002). It is likely that PRs, either directly or indirectly aid in the multiplication of SPs. These SPs seem to be less fragile than GRS, which deregulate and disintegrate quickly, and are removed from circulation, under the influence of insecticide and similar stress conditions. Because of this nature, the numbers of SPs are less in parasitized larvae. Qualitative or quantitative changes in haemocytes are non-specific responses caused by the presence of foreign substances and/or stress conditions. Therefore, the difference in haemocytes population between unparasitized and parasitized *H. armigera* larvae indicates that the host defense system was activated (Nappi, 1981; Brehélin, 1982). Hemocytes play an essential role in defending invertebrates against pathogens and parasites that enter their haemocoel. The present study comprises the effect of certain stress-induced changes in haemocytes of *H. armigera*. Haematological investigations provide diagnostic keys as indicators of the physiological status of the insect. Hemocyte based understandings will be helpful in assessing the impact of parasitoids for better control of pest. Haemocytes represent a suitable cell type to analyze differentiation and their differential profile varies against parasitoid. Hence, haemocyte can be used as an indicator for change and hemocyte based
information may be used to taking preventative measures to save the economically important insects and control the insect pests.
3.5 References


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