aqueous extract shows maximum inhibition (15.6 mm) against silkworm pathogen *B. subtilis*. Choudhury *et al.* (2002) evaluated ethyl acetate extract of *A. sativum* for antibacterial activity against silkworm pathogen *B. thuringiensis*. Ankri and Mirelman (1999) explained that wide range of micro organisms including bacteria, fungi, protozoa and viruses were sensitive to crushed *A. sativum* preparations. Koch and Lawson (1996) analysed that the *A. sativum* cloves had the presence of unusual concentration of sulfur containing compounds (1-3 %).

Huges and Lawson (1991); Garau (1994); Gould (1994) and Graham (1998) described that thiosulfimates and other secondary metabolites of garlic including 7-glutamyl peptides, seordinins, steroids, terpenoids, flavonoids and other phenols might be responsible for the range of therapeutic effects for *A. sativum*. Reuter *et al.* (1996) had studied the antibacterial effects of *A. sativum* against *Aerobacter*, *Aeromonas*, *Bacillus*, and *Escherchia*. Dankert *et al.* (1979) and Singh and Shukla (1984) had found the complete lack of resistance of bacteria to *A. sativum*. Dewitt *et al.* (1979) reported that as a result of bactericidal activity of *A. sativum*, toxin production by the bacteria is prevented. Cavallito and Bailey (1944) had demonstrated that *A. sativum* juice diluted to one part in 1,25,000 inhibits the bacterial growth of *Staphylococcus*, *Streptococcus* and *Bacillus*. Jezpwa *et al.* (1966), Adetumbi and Lau (1983), Khan *et al.* (1985), Ahsan and Islam 1996, Tsao *et al.* (2003), Zhou (2003) and Chattopadhyay and Bhattacharyya (2007) revealed that preparation of fresh *A. sativum* and vaccum dried powdered *A. sativum* preparations were effective against *S. aureus,*
S. viridians, E. coli, B. subtilis, and B. thuringiensis. Ankri and Mirelman (1999) had reported that, allicin one of the active principles of freshly crushed A. sativum homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit antibacterial activity against a wide range of gram-negative and gram positive bacteria. The main antimicrobial effect of allicin is due to its chemical reaction with thialgroup of various enzymes. In the present study, S. trilobatum exhibited inhibitory effect (12.5 mm) against B.subtilis. This result was in corrraberation with Banso and Adeyemo (2007), whom reported that the leaves of S. trilobatum possessed the highest antibacterial activities against a number of microorganism. In the present study three botanicals viz., A.cepa, A.sativum and S.trilobatum were used to test against B.subtilis affected B.mori larvae. A.cepa exhibits more antibacterial activity.
CHAPTER - III

INTRODUCTION

Diseases are major yield limiting factors in silkworm crop. Yield loss due to various silkworm diseases is estimated to be around 30-40% in India (Chitra et al., 1995). Insects show defense response through cellular and humoral components (Dunn, 1986 and Gupta, 1986). Humoral reactions involve slow synthesis of antibacterial, antiviral principles and require several hours for full expression. When cells participate in the protection of an insect against infectious diseases, the resistance is called cellular immunity. Some authors prefer the term cellular factor in the haemolymph rather than cellular immunity (Boman and Hultmark, 1987; Dumphy and Thurston, 1990).

The free haemocytes in the haemolymph of insects are responsible for the defense reactions against foreign agents that penetrates the haemocoel (Vey and Gotz, 1986; Tepass et al., 1994; Falleiros and Gregario, 1995; Gillespie et al., 1997; Inoue et al., 2001; Watanabe, 2002 and Narayanan, 2004). The free haemocytes of lepidopterous insects have been studied in a wide range of species like southern armyworm, Prodenia eridamin (Yeager, 1945); Antheraea yamamai G. (Akai and Sato, 1978); Noctuidae (Arnold, 1982); Galleria mellonella (Ashhurst, 1982); Plodia incerpunctella (Beeman et al., 1983); Heliothis armigera (Essawy et al., 1985); Lambdina fiscellaria fiscellaria (Boiteau and Perron, 1997); Anticarsia gemmatalius (Andrade et al., 2003); B. mori (Dohrn, 1876; Akai
and Sato, 1973; Krishnan et al., 2002 and Saran et al., 2002); *P. demoleus* (Jalali and Salehi, 2008) and tasar silkworm (Singh et al., 2008).

In insects several types of haemocytes were observed in the haemolymph. Dohrn (1876) was the first to describe embryonic blood cells in *B. mori*. Seven types of blood cells are in silkworm, *B. mori* (Raichoudry and Sengupta, 1959) and Six types (Nittono, 1960; Gupta, 1979; Arnold, 1979; Jones, 1979; Russo et al., 1993; Butt and Shields, 1996 and Jalali and Salehi, 2008). Differential haemocyte count (DHC) was studied in *B. mori* (Gupta, 1979; Arnold, 1979; Wago, 1980 and Balavenkatasubbaiah et al., 2001), in *G. mellonella* (Lea, 1985) and in *A. mylitta* (Saran et al., 2002). The chief defensive cells are the plasmatocytes and the granular cells in *B. mori*, which take part in phagocytosis, encapsulation and nodulation reactions in response to bacterial infection (Gupta, 1986; Krishnan et al., 2000 and Ottaviani, 2005). The number of circulating cells may greatly vary on the technique used, age, stage and the physiological status of the insects (Jones, 1962). There was a great deal of references available on the ultrastructure of haemocytes and their multiplication in insects like *B. mori* (Devauchelle, 1971; Akai and Sato, 1973; Sato et al., 1976; Jalali and Salehi, 2008; Sharma et al., 2001 and Thomas and Nair, 2010). The haemocyte counts are normally highly variable within the same species as well as among species (Jones, 1979; Lea, 1985; Bahadur and Pathak, 1991; Saran et al., 2002 and Malikarjuna et al., 2002).
A variety of functions like mechanization and immobilization of invading organism by encapsulation and/or phagocytosis, wound repair, coagulation have been reported for haemocytes (Jones, 1962; 1964 and 1970; Lai-Fook, 1970; Wago and Ichikawa, 1979; Horohove and Dunn, 1983; Gupta, 1986; Ratcliffe, 1993; Russo et al., 1993 and Pech et al., 1995). The total haemocyte count (THC) and differential haemocyte count (DHC) may indicate the susceptibility status of the insect. In the present study, the relationship of THC and DHC with regard to susceptibility status of silkworm to flacherie infection and the influence of phytoextract of A. cepa on the haemocyte count in the infected silkworm was investigated.
MATERIALS AND METHODS

*A. cepa* belonging to the family *Alliaceae* known for their antibiotic properties (Didry *et al.*, 1987; Kim, 1997 and Chaithradhyathi *et al.*, 2009) and available locally was selected and procured to test their efficacy against bacteriosis in mulberry silkworm, *B. mori* (race: PM × CSR2) and its influence on haematological defense. 24 hr. after third moult, the larvae were allowed to feed on mulberry leaves treated with 1 %, 5 % and 10 % aqueous extract of *A. cepa*. Silkworm larvae of *B. mori* (race: PM × CSR2) were orally inoculated (by feeding food plant leaf smeared with *B. subtilis* suspension with $1 \times 10^5 \mu g/ml$ inoculum). Six replications with 50 silkworm larvae were maintained for each treatment to observe the effect of phytoextracts on bacteria in the infected larvae. The experiment was carried out for 8 days.

In another set, larvae were inoculated with *B. subtilis* and haemolymph was collected for estimation of total and differential haemocyte count. The above results were compared with that of same breed of larvae reared under normal conditions.

**Estimation of haemocytes count**

Everyday THC estimation in the haemolymph of all treated and control batches was determined following the method described by Tauber and Yeager (1934 and 1935) using haemocytometer.
For the THC the haemolymph was drawn into a thoma white blood cell pipette upto 0.5 mark and diluted upto 11 mark with Tauber-Yeager fluid. The pipette was shaken for several minutes and the first three drops were discarded. A double line with improved Neubaur ruling haemocytometer was filled with diluted haemolymph and the haemocytes were counted in their four corners and one central (1mm²) square. The number of circulating haemocytes per cubic millimeter (mm³) was calculated using the following formula of Jones (1962).

\[
\text{Haemocytes in five squares (1mm}^2\text{) } \times \text{dilution } \times \text{depth factor of chamber} = \frac{\text{No of squares counted}}{} \\
\]

Where dilution = 20 times

Depth factor of the chamber = 10(constant)

No. of squares counted = 5

The (DHC) was estimated by counting different haemocytes from a haemocyte population of 200. The haemocyte types were classified based on works of Jones (1962).
RESULTS

The results of the effects of aqueous extract of *A. cepa* on the total haemocyte count in *B. subtilis* infected silkworm *B. mori* was represented in Table 3.1 and Fig 3.1. In control the total haemocyte count was 8609, 9152, 9375, 10186, 10563, 11279, 11069, and 11202 (1 to 8 days) respectively. In all the treatments the total haemocyte counts was found to increase from 1\textsuperscript{st} day to 6\textsuperscript{th} day and decreased at 7\textsuperscript{th} and 8\textsuperscript{th} day. At 1\% concentration of *A. cepa* the THC was 8622±268/mm\textsuperscript{3} by the 1\textsuperscript{st} day and increased to 13477±208/mm\textsuperscript{3} by the 6\textsuperscript{th} day. On the 7\textsuperscript{th} day total haemocyte counts decreased to 12725±91/mm\textsuperscript{3}. On the 8\textsuperscript{th} day the count was increased upto 14468 ± 108/mm\textsuperscript{3}. Maximum THC was observed when the larvae were treated with 5\% *A. cepa*. The THC in the 1\textsuperscript{st} day was 9695 ± 103/mm\textsuperscript{3} but was 13621 ± 317/mm\textsuperscript{3} by the 6\textsuperscript{th} day. On the 7\textsuperscript{th} day THC decreased to 12482 ±146/ mm\textsuperscript{3}. On the 8\textsuperscript{th} day the count increased up to 13648 ± 91/mm\textsuperscript{3}. At 10\% concentration the THC was 8609 ± 105/mm\textsuperscript{3} on the 1\textsuperscript{st} day and increased to 13435 ± 124/mm\textsuperscript{3} by the 6\textsuperscript{th} day. On the 8\textsuperscript{th} day the count increased up to 13104 ± 53/mm\textsuperscript{3}. In bacterial inoculated group the count increased upto 2\textsuperscript{nd} day of infection. On the 1\textsuperscript{st} day the THC was 8816 ± 105/mm\textsuperscript{3}, and 9726 ± 209/ mm\textsuperscript{3} by the 2\textsuperscript{nd} day. On the 3\textsuperscript{rd} day the THC was 8693± 73/mm\textsuperscript{3} which again reduced to 3066 ± 48 /mm\textsuperscript{3} by the 8\textsuperscript{th} day (Table 3.1).
The observation with regard to DHC in silkworm *B. mori* after *B. subtilis* \((1 \times 10^{-5} \, \mu g/ml)\) inoculation and treatment with aqueous extract of *A. cepa* is depicted in Table 3.2 and Plate 3.1. Six types of cells were observed in the present study. They were prohaemocyte (PRs), plasmocytes (PLs), granulocytes (GRs), spherulocytes (SPs), oenocytoids (OEs) and degenerated cells (DEGs). The prohaemocyte decreased (18-13) up to 4\(^{th}\) day and increasing trend was observed from 5\(^{th}\) day onwards in all the treated groups. In control the number of PRs was increased from first day to eighth day (20, 23, 26, 32, 33, 34 and 35 respectively). The number of PRs increased (32) in bacterial inoculated group at the first day and decreased (10) at the 8\(^{th}\) day. The number of PRs appeared considerably higher (26, 24, 22, 21, 24, 28, 30 and 33) respectively in the 5\% *A. cepa* treated groups.

The number of PLs increased up to 3\(^{rd}\) day and decreased from 4\(^{th}\) day onwards in all the treated groups. As compared to control, the number of PLs was noticeably higher in *A. cepa* and bacterial inoculated groups. Their number increased after 24h in treatments on 3\(^{rd}\) day. Maximum number of PLs (44) was obtained when the larvae were treated with 5\% *A. cepa* on the third day.

The number of spherulocytes increased during developmental period from the 1\(^{st}\) day to 8\(^{th}\) day in all the treatments. The relative number of spherulocytes were considerably low in control group which ranged from (14, 16, 18, 20, 21, 27, 29, 32 respectively). In inoculated group the numbers decreased from 22, 21, 20, 18, 16, 15, 11, 8 when compared to control. In *A. cepa* treated groups the relative number of spherulocytes increased.
when compared to the control group (18, 21, 24, 27, 31, 36, 38, 42 from 1st day to 8th day respectively). The number of granulocytes increased up to 4th day and then decreased in all treatments. The THC indicates higher number of granulocytes followed by plasmatocytes and prohaemoocytes. The granulocytes ranged from 35, 37, 38, 43, 41, 40, 38, 37 in control group and 29, 30, 32, 34, 28, 20, 18, 11, in inoculated group. The granulocytes ranged from 38-50; 30-51 and 37-43 during 8 days respectively in 1%, 5% and 10% *A. cepa* treated groups.

The number of oenocytoid decreased up to 5th day and increasing trend was observed from 6th day onwards in all the treatments. Oenocytoid numbers were less in all treatments. The number increased slowly in control groups (4-15), but decreased in bacterial treated groups (11, 10, 8, 8, 6, 6, 5, 3 from 1st day to 8th day respectively). The same trend was followed in *A. cepa* treated group (5, 4, 5, 5, 8, 9, 10, 11 from 1st day to 8th day respectively). The number of degenerated cells increased from 1st day to 8th day in all the treated groups. In the control group the number of degenerated cell was comparatively less (8, 10, 11, 12, 8, 7, 6, 6, from 1st day to 8th day respectively) than the plant extract treatment (22, 24, 25, 24, 23, 18, 15, 14 from 1st day to 8th day respectively) and inoculated control (18, 20, 21, 25, 29, 31, 32, 40 from 1st day to 8th day respectively).
DISCUSSION

Insects possess a complex and efficient system of biological defense against pathogens and parasites. Infection in insects stimulates a complex defensive response (Gillespie et al., 1997). In cellular defense mechanism, unlike vertebrates which have red blood corpuscles and white blood corpuscles in a closed circulatory system, insects lack lymphocytes, the major source of vertebrate immunity to viral infection. But they have only free blood cells called haemocytes (Narayanan, 2004). The free haemocytes in the haemolymph of insects are responsible for the defense reactions against foreign agents that penetrate the haemocoel (Tepass et al., 1994; Falleiros and Gregario, 1995 and Inoue et al. 2001). Insects show defense response through cellular and humoral components (Dunn 1986 and Gupta, 1986). Different types of blood cells have an important role in the protection of insects against invading microorganism. Cellular responses were mediated by changes in subpopulations of haemocytes and their morphology (Gupta, 1986 and Ratcliffe et al., 1985). Hence identification and classification of various types of insect blood cells based on the structure and function is important (Gotz and Boman, 1975 and Narayanan and Jayaraj, 1976).

In the present study, six types of haemocytes were observed in *B. mori*. They are prohaematocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoids and degenerated cells. Based on Jones
classification (Jones, 1962) 5 types of haemocytes were identified in mulberry silkworm *B. mori*. They are prohaematocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs) and oenocytoids (OEs) (Plate 3.1a-c). Structure of prohaemocytes are small, round with various sizes. It was in agreement with Gupta and Sutherland (1966) and Gupta (1979). They described the structure of prohaemocytes. Plasmatocytes are rounded and larger than prohaemocytes (Gupta, 1979). In the present study the plasmocytes are observed as round shaped (Plate3.1). Granulocytes are small to large spherical or oval cells (Plate3.1b). This is in agreement with Goffinet and Gregoire (1975) and Gupta (1979). According to them the population of these cells fluctuates in different instars, being the lowest (10.5%) in the pupa, highest (37.8%) in 5th instar larvae. Spherulocytes are ovoid and round cells (Plate 3.1b). Nittono (1960) reported that these are ovoid and round cells, usually larger than granulocytes and the nucleus is generally small, rich in chromatin bodies. Akai and Sato (1973) reported that it possess an internal structure made of membranes arranged in concentric layers in *B. mori*.

Oenocytoides are small, thick, oval shaped cells Plate 3.1c. Akai and Sato (1973) observed that oenocytoides are small to large, thick, oval, spherical or elongate cells, plasmamembrane with micropapilae, cytoplasm thick, homogenous with plate, rod cells or needle like inclusions, large pleomorphic cells with homogenous cytoplasm that is devoid of cytoplasmic inclusions. In the present study there was an increase in prohaemocyte count. This result was in agreement with Beaulaton (1979); Ratcliffe
et al. (1985) and Yamashita and Iwabuchi (2001). They reported that the gradual rise in haemocytes was due to differentiation of prohaemocytes into plasmatocytes and also due to continuous division of haemocytes already in circulation.

According to Narayanan (2004) among the six major groups of insect haemocyte in recognizing the self (isografts) and non-self (allografts), plasmocytes and granulocytes are the major effector cells. Gotz and Boman (1985) reported that they react to foreign invaders either by phagocytosing like micro organism or nodulating and encapsulating objects too large to be individually engulfed by way of haemocytes attaching and forming many layers, thereby causing the death of the parasitoid through starvation and/or anoxia mechanism. Changes in total haemocyte during growth and development of healthy insects had been reported by Narayanan (2004).

In the present study the fall in total haemocyte count (8816 ± 105 to 3066 ±48) was observed in inoculated group. This result was in agreement with Narayanan and Jayaraj (1973) and Hung et al. (1993). They reported that drastic reduction in the number of haemocytes during various microbial infections like fungal, bacterial, viral and parasitic infection. Difference in THC in indigenous silkworm breeds has earlier been investigated by Nataraju (1995). According to Wittig (1962) increase in circulating haemocytes is the instant cellular immune response in some insects and the response also depends on the type and dose of the pathogen. Non pathogenic bacteria or heat killed cells of pathogenic bacteria resulted in increase in THC in armyworm Pseudoletia unipuncta and in wax moth
G. mellonella was reported by Shapiro (1967). Begum et al. (1998) studied the effect of sub lethal (SL) concentration of deltamethrin (DELM) on the haemocytes of larva, pupa and adult P. ricini on the basis of total haemocyte count and differential count. SLs of DELM caused a decrease in THC that SPs and PLs were actively involved in the detoxification process. Balavenkatasubbaiah et al. (2001), Balavenkatasubbaiah and Nataraju (2005) and Krenhap et al. (2005) reported that there was a possibility of differential response by haemocytes to microbial infection in different breeds of B. mori. There was increase in THC, DHC and period of survival of haemocytes with regard to granulocytes, plasmatocyte and spherulocytes under normal condition and during infective BmNPV infection. The present study has also conformed the dominance of granulocytes in B. mori treated with A. cepa.

Koundinya et al. (2007) reported the increase in THC in the bacteria infected larvae of selected bivoltine breeds. Singh et al. (2008) reported that haemocytes involved in defense mechanism against virus and phytoextract prevent the multiplication of virus by enhancing the haemocyte mediated defense response. In control, the total haemocyte count was significantly low (13375 / mm³) as compared to the treated (15,677 / mm³) groups because the increase may represent the defense response of silkworm, B. mori against the invading pathogen. The observed data agreed with Vey and Gotz (1986); Butt et al. (1988) and Butt and Humber (1989). As they investigated that once entomophagous fungi have penetrated in the host integument and gained access to nutrient rich haemocoel, they are
conformed with host humoral and/or cellular defenses. Gupta (1986) reported that higher number of THC may also be due to mitosis of large number of circulating haemocytes in the robust larvae as THC was correlated to blood volume.

Begum et al. (1998) had reported that the response of haemocytes of *P. ricini* to deltamethrin progressed through morphological and pathological changes resulting in cell breakdown and decreases in total haemocyte counts. Munson and Yeager (1994) have reported the appearance of fat droplets in the haemocytes as glycogen disappeared from the haemocytes. Jalali and Salehi (2008) reported the pattern of the THC changes during postembryonic development is largely similar to holometabola i.e., it increases during the larval stages attains its peak by end of 5th instar (prepupa) and declines in the pupa. The reason that seems more plausible could be an elevated rate of mitosis that characterizes all other tissues during the period of active growth and it might also increase the number of haemocytes as observed.

Wigglesworth (1959) and Crossley (1979) had reported that the haemocytes are known to be involved in intermediary metabolism such as protein synthesis, transport of nutrients, phenol metabolism and growth stimulation. Hoffman (1970), Hinks and Arnold (1977) and Prasadara Rao et al. (1984) had reported that due to active growth during larval stage, intermediary metabolism process should be higher and therefore needs the services of a large number of haemocytes. Hinks and Arnold (1977) had demonstrated that ecdysone enhances the rate of mitosis in haemocytes of
lepidopterans. Since the ecdysone titre is high towards the latter part of each instar. The cellular responses to infections have been worked out in many insects by Chain and Anderson (1982); Dunn and Drake (1983) and Horohove and Dunn (1983). Similar observations have also been made by Mallikarjuna et al. (2002). He studied the effect of systemic fungicide on the total haemocyte count in *B. bassiana* infected silk worm, *B. mori*. Salt (1970) reported that haemocytes are extremely efficient in removing pathogens by accomplishing a series of reactions designated as phagocytosis, nodule formation as encapsulation.

In the present study the THC was 9695±103/ mm³ by the 1st day and increased as 13621± 317/ mm³ by the 6th day in *A. cepa* treated groups. This was in agreement with Singh et al. (2008). He reported that THC increased in haemolymph upto 6th day post inoculation in phytoextract treated batches while in the inoculated group the increase was within three days indicating the positive haemocyte mediated response in silkworm treated with phytoextract. Divergence in haemocyte profile of lepidopteran larvae was widely reported by Lackie (1988) and Ratcliffe (1993). According to them the plasmatocytes and granulocytes accounted for more than 50 percent of the haemocytes in circulation in the larval stage of lepidopteran. Beaulaton (1979) suggested that prohaemocytes in *B. mori* differentiated into plasmatocytes which were interturn differentiated into granulocytes and spherulocytes. In the present study range for prohaemocytes was 18-22, 26-33, and 24-27 viz.,1%, 5% and 10% respectively in *A. cepa* treated groups and 32-10 in inoculated and that in control were 20-35.
The spurt in granulocytes and plasmatocytes is explicit in silkworm breeds upon immunization. It is an established fact that the main cellular defense of insects is mediated by haemocytes. Granulocytes and plasmatocytes are principal haemocyte type capable of adhering to invading pathogens and they are actively involved in phagocytosis mechanism which was investigated by Lackie (1988) and Strand and Pech (1995). The granulocytes were ranged from 43-50, 48-51 and 38-43 in A. cepa treated groups (1%, 5%, and 10%) respectively and 29-11 in inoculated groups and 35-43 in control group was observed in this study. The granulocytes which are primarily involved in defensive mechanisms. In this study the gradual decrease of granulocytes are observed in inoculated groups. This was in agreement with Balavenkatasubbaiah *et al.* (2001). He reported that during the progressive infection, there was gradual decrease in the prohaemocyte. Shapiro (1969) reported that increase in granulocyte number upon NPV infection in *G. mellonella* whereas plasmatocytes were found decreasing with the progression of disease. The gradual decrease in prohaemocyte count may be due to the conversion of prohaemocytes to other types of haemocytes that is required for defensive mechanism. Similar observations have been made in silk worm against fungi infection by Kawakami (1965) in arthropods by Gupta and Han (1988) in insect *Periplaneta americana* by Ennesser and Nappi (1984).

However some reports suggested that there indeed was a decrease in THC and DHC upon infection by bacterial pathogen in some insects. Wittig (1962) had reported the efficiency of blood cells in defending against
septicemia depends on the type and dosage of pathogen. Light doses of *Bacilli* (1.7 to 2.8 \( \times 10^{-5} \) / larva) were phagocytosed within 30 mts. after injection in *P. unipuncta* while higher doses resulted in decrease of THC. Elrod-Erickson *et al.* (2000) reported that a decrease in number of granulocytes and plasmatocytes was noticed in immunized insects after 24-48 hr. This may be due to the involvement of these haemocytes in phagocytosis. Granulocytes and plasmatocytes are the only haemocytes involved in phagocytosis reaction in lepidoptera. They are also involved in nodulation *i.e.*, multiple haemocytes binding to aggregation with bacteria which was proved by Schmid *et al.* (2001) and Narayanan (2004) and Krishnan *et al.* (2002).

There are some Pattern-Recognition Receptors (PRRs) in the plasma that enhance phagocytosis or nodulation. PRPs include LPs binding protein, gram negative bacteria, recognition protein, peptidoglycon recognition protein lecithin, haemolin etc., Koizumi *et al.* (1999) reported the isolation of LPs binding protein from *B. mori*. A number of molecular elicitors of cellular defense have been isolated and purified from *B. mori* by Lavine and Strand (2002). Jaydeb *et al.* (2000) attributed the survival of the silkworm breed p5 against diseases to their cellular defense. Patil and Jamuna (2000) categorized several multi and bivoltine breeds based on THC. Balavenkatasubbaiah *et al.* (2001) reported 50 percent increase in plasmatocytes and nearly 100 percent rise in granulocytes in tolerant silkworm breeds like PM and Nistari when challenged with NPV. Sivaprasad *et al.* (2003) developed a haemocyte based index to distinguish the
resistant and susceptible silkworm breeds. Rowley and Ratcliffe (1976) had reported that drop in granulocytes population is due to the loss of haemocytes through degranulation in the course of immune reaction.

In the present study the increase in the THC was largely due to the increase of spherulocytes and plasmocytes. It was in agreement with Arnold (1974). Who suggested that increase in variety of PLs indicated the physiological functions in the insect at that period.