REVIEW OF LITERATURE

Pastuer (1870) reported that bacteria as etiological agents of silkworm flacherie. Dasgupta (1950) classified the major diseases affecting silkworm, *Bombyx mori* are flacherie, grasserie, muscardine and pebrine. Seventy per cent loss due to flacherie in sericulture industry was reported by Sidhu and Singh (1968) and Aruga and Tanada (1971) and Chitra et al. (1975) observed 30-40 per cent cocoon crop loss in silkworm is due to flacherie caused by bacteria were restricted to intestine and haemolymph. Savanurmath et al. (1992) reported that the flacherie as the most predominant (47.9 per cent) among the disease of *B. mori* followed by grasserie (42.6 per cent) and kenchu (9.5 per cent) while Selvakumar et al. (2001) investigated that *Streptococcus* and *Staphylococcus* are primarily responsible for flacherie in silkworm.

Symptomology

Chitra et al. (1975) investigated that loss of appetite, sluggishness, flaccidity of worms, rapid pulsation of dorsal vessel, vomiting of brown fluid, soft excreta, decomposition of gut contents and diarrhoea are the symptoms of flacherie diseases in *B. mori*. Poonia (1979) observed that the flacherie infected larvae are completely inactive and lethargic. The colour of the body turns brownish and blood becomes turbid. The dead larvae becomes blackish brown, liquefies and emits bad odour. He also observed that flacherie causes considerable biochemical abnormalities and result in
change of protein, aminoacid, lipid and carbohydrate contents of the insect haemolymph.

Liu Fan (1991) reported that silkworm showed the flacherie symptoms like pale body colour, shrunken skin, stunted growth and empty fore gut, while Lian (1991) revealed that the symptoms of bacterial toxicosis are sudden cessation of feeding, lifting of head, spasms and tremors, paralysis, distress, sudden collapse and death. The flacherie affected larvae become semitransparent in the anterior part of the body in early stages and digestive juice, which is initially yellow in colour and turns blackish brown colour. Aruga (1994) reported that poor growth, higher percentage of adipohaemocytes in haemolymph with lower amount of protein, calcium, magnesium chloride and decreased acidity of haemolymph occurred due to the infection of flacherie disease in *B. mori*, and Prasad (1999) suggested that flacherie affected worms shows an equalness with slightly smaller in size but the worms continue to feed.

**Biodiversity of bacterial pathogens**

A spore forming bacterium was isolated by Ishiwata (1901 and 1902) from diseased larvae of *B. mori* and he (1905) also described this bacterium as sotto disease *bacillus*. Pathogenic spore forming *Bacillus* were isolated by Toumanoff and Vago (1951) and Ohba *et al.* (1979) from silkworm larvae affected by flacherie disease and from silkworm litters.

Chitra *et al.* (1975) isolated the bacterial pathogens from cadavers of *B. mori* and divided into two classes, *viz.*, those that are restricted to
intestine and those which enter the haemolymph. Vasantharajan and Munirathnam (1978) isolated pigmented and non pigmented strains of *Serratia marcescens* and *Staphylococcus* sp., from kenchu diseased silkworm, while Enomoto *et al.* (1987) isolated *S. marcescens, Pseudomonas* sp., *Proteus* sp., *Aeromonas* sp., *Streptococcus faecalis var. liquifaciens* and *Bacillus* sp. from bacteria affected cocoons. Anitha *et al.* (1994) isolated *Bacillus, Staphylococcus* and *Serratia* from diseased silkworm. Bacterial toxicosis of silkworm caused by *Bacillus thuringiensis* infection was reported by Govindan and Devaiah (1995). Nataraju *et al.* (2002) isolated bacteria such as *S. faecalis, Streptococcus aureus* and *Streptococcus epidermis* and infectious flacherie virus from the flacherized cadavers of silkworm and found to be involved in synergistic association in causing the disease.

Priyadharshini (2006) isolated *Bacillus subtilis, Staphylococcus albus, Bacillus cereus, Klebsiella cloacae* and *S. aureus* from flacherie diseased cadavers. Ramesh *et al.* (2009) isolated and identified the bacterial pathogens as *Klebsiella pneumoniae* sp., *Ozaenae, Enterobacter aerogenes* and *S. odorifera, Echerichia coli* and *Yersinia enterocolitica* from *B. mori* based on the results of biochemical tests. Paramasiva and Prasad (2009) studied the morphological, histopathological and biochemical changes in silkworm *B. mori* due to incidence of bacterial flacherie caused by *B. thuringiensis var. kurstaki.*
Characterization of bacterial pathogens

Barrow and Feltham (1993) classified *Staphylococcus* strains based on biochemical tests which included coagulase production, oxidase, acetoin production, susceptibility to novobiocin and a set of sugars. Anitha *et al.* (1994) characterized *Staphylococcus* sp. as creamy, opaque, irregular colonies with spherical cells which appeared as clusters. Kloos and Bonnerman (1995) described the BBL\textsuperscript{TM} Staphyloslide TM latex test, a latex slide agglutination test, for the differentiation of *Staphylococcus* which possess clumping factor and/ or protein A, usually present with *S. aureus* from *Staphylococci* that do not possess these properties. Kunst (1997) described *Bacillus* as rod shaped gram positive, sporulating aerobes or facultative anaerobes.

Adrinae and Merie (2001) isolated spore forming, thermophilic, strictly aerobic gram positive, catalase positive, actively motile *Bacillus* sp. Takashi and Yamamoto (2001) characterized *B. thuringiensis* as producing parasporal crystal bodies which are highly toxic to silkworm. Robert Pollack *et al.* (2002) analysed the mannitol salt agar test to identify the presence of *Staphylococcus* sp., whereas growth with colour change, indicated the presence of *S. aureus*. *Staphylococcus* undergoes fermentation yielding lactic acid and also showed negative result for oxidase test. Monika *et al.* (2005) characterized *B. cereus* strains by molecular methods such as RAPD and PCR and phonetic methods *viz.*, protein profiling and biochemical assay methods. Manikandan *et al.* (2005) explained that *Staphylococcus* were Gram positive, non motile, non-sporulating and capsulated spherical
organisms and the enzyme coagulase positive and a coagulase negative pathogen. On nutrient agar medium, *Staphylococcus* species appeared to be white, circular, entire convex colonies. On blood agar, *S.aureus* showed haemolysis of the agar in the areas around the colony (http://itech.edu/fduncan/mcb 1000/stap pdf).

Schultes (1978) classified the useful antimicrobial phytochemicals into several categories like phenolics, polyphenols, quinines, flavones, flavonoids, tannins, terpenoids and essential oils. Verma (1985) reported that the plant extracts contain inhibitory substances such as protein, glycoprotein, flavanones, phenols, polysaccharides, alkaloids, glycosides, cyanogenic glucocides and saponins. Malik (1987) revealed that the plant and their constituents are less phototoxic, more systemic, easily biodegradable and induced host metabolism to resist pathogen infection.

Osato et al. (1993) found that the latex of papain to be bacteriostatic to *B. subtilis, E. clocae, S. aureus,* and *E. coli* while, Hufford et al. (1993) recorded the excellent antibacterial activity of glove against *B. subtilis* and *S.aureus* and lower activity against gram negative bacteria. Sakanaka et al. (1994) and Tsuchia et al. (1994) noticed that flavonoids present in Oolong, green lias inhibited *Streptococcus* mutants under *invitro* conditions. Ahmed et al. (1995) studied the leaf and bark extracts of *Azadirachta indica* and observed antibacterial activity against *S. epidermidis, S. aureus* and *Bacillus* sp.
Garg and Jain (1998) demonstrated the antibacterial effect of essential oil from rhizomes of *Curcuma caesia* (Roxb), rich in curcumene, ionine and turmeron. Asimachakraborty and Brantner (1999) studied the stem park of *Holarrhea pubescens* L. against *S. aureus*, *S. epidermis*, *S. faecatus*, *B. subtilis* and *P. aureginosa*. Saleh *et al.* (1999) isolated lactic acid from *Lantana camara* L. had broad spectrum antibacterial activity against *B. cereus*. Khandelwal *et al.* (1999) found that the ethanolic, aqueous and hydro alcoholic extracts of *Nyctantues arbortristis* Linn. were active against both antibiotic, resistant and non-resistant strains of *S. aureus*. Abo and Ashidi (1999) reported the antimicrobial activity of *Boerhavia diffusa* L. against gram positive bacteria.

According to Eloff (2000), acetone extracts of the *Celastraceae* had antibacterial against *E. faecalis*, *P. aeruginosa* and *E. coli*. Mandel *et al.* (2000) evaluated antibacterial activity of *Asparagus recemosus* (wild root) against *B. subtilis*, *S. aureus* and *Pseudomonas* sp. Choudhury *et al.* (2002) reported that the fresh garlic extracts was most effective against silkworm pathogen *A. assama* under *in vitro* condition. Chattopadadhyay *et al.* (2004) have done an exclusive work to establish the biological activities and pharmalogical actions of turmeric and its extracts. Garlic preparation showed a wide spectrum antibacterial activity against gram negative and gram positive bacteria including species of *Escherichia*, *Staphylococcus*, *Streptococcus*, *Proteus* and *Bacillus* (www. Health reports.com. 2006 and Tattleman, 2005). Manimegalai and Chandramohan (2005) reported the efficiency of *Thuja orientalis* L. at 10,000 ppm and *Curcuma domestica* V. at
40,000 ppm against B. *thuringiensis*. Latha and Kannabiran (2006) investigated the antibacterial activity and phytochemicals of *Solanum trilobatum*. They found the presence of tannins, saponins, flavonoides, phenolic compounds and carbohydrates indicate *S. trilobatum* is one of the potential medicinal plant for therapeutic use.


Panzaru *et al.* (2009) studied the aqueous extracts of garlic the most potent plant material against bacterial and fungal pathogens. They reported that garlic contains organosulphur groups, that can act as metal chelators, powerful nucleophiles or electrophiles and hence confer antimicrobial properties on this compound. Chaithradhyuthi *et al.* (2009) evaluated the antioxidant and antimicrobial properties of four different members of *Allium*. They found that, among the members of *Allium* tested for reducing capacity, *Allium cepa* L. showed maximum reducing property and *Allium ascalonicum* L. showed lowest reducing property. Ravikumar *et al.* (2009) screened forty-five crude methonolic extracts from twenty three marine halophytes against five botanical and two fungal saprophytic pathogens of diseased silkworm *B. mori*. They found that among thirty four mangrove samples screened for
antibacterial activity, the leaf extract of *Rhizophora mucronata* Lamk. showed maximum inhibitory activity against *S. aureus*.

Wigglesworth (1935) found the role of haemocytes in the growth and moulting of a *Rhodius prolixus*. Bahadur and Pathak (1971) studied the changes in total haemocyte counts (THC) in relation to sex, development, ecdysis and hot water fixation in *Halys denta* (Hemiptera). They found there is no difference in the THC of the two sexes, the THC differs in the immature stages and adults. There is a gradual increase in counts during metamorphosis. The fixation of insects in hot water before withdrawal of haemolymph abruptly affects the THC shows a significant increase in the number of haemocytes. Their studies made in relation to ecdysis showed that the THC slightly decreases 24 hour before ecdysis, whereas after ecdysis it abruptly falls and then rises again during the middle part of the instar. Arnold (1972) found that the size, form and character of haemocytes were found to change according to the changes in the physiological condition of the insect.

Wigglesworth (1973) examined haemocytes and basement membrane formation in *Rhodinus*. He found during the period between feeding and ecdysis in the fourth instar larva of *Rhodinus* the basement membrane below the epidermis of the abdomen increases about threefold in thickness. This increase takes place during the time, about 5 to 7 days after feeding, when the plasmatocytes settle and flatten in great numbers on the inner surface of the membrane. Chain and Anderson (1983) revealed the inflammation in insects by the release of a plasmatocyte depletion factor.
following interaction between bacteria and haemocytes. Saxena and Saxena (1985) studied the cytopathological and numerical changes in haemocytes of *Periplanata americana* after treatment with malathion. Begum and Gohain (1990) observed the differential counting of haemocytes in different larval instars of eri silkworm *P.ricini*. Chain *et al.* (1992) studied the haemocyte heterogeneity in the cockroach *P. americana* using monoclonal antibodies. Klunner *et al.* (1994) characterized the binding factor for LPS in haemocyte membranes of *Galleria mellonella* and *Hyalophora cercopia*. Gillespie *et al.* (1997) investigated the biological mediators of insect immunity. They investigated that recognition of pathogens maybe accomplished by plasma or haemocyte proteins that bind specifically to bacterial or fungal polysaccharides. Several morphologically distinct haemocyte cell types cooperate in the immune response. Begum *et al.* (1998) investigated the effect of sublethal concentrations of deltamethrin (DELM) on the haemocytes of larva, pupa and adult *P. ricini* on the basis of total haemocyte count differential haemocyte count and cytological changes after an interval of 24, 48 and 72 hour after treatment. They found that SLC of DELM caused a decrease in THC and some cytological changes of haemocytes such as cytoplasmic extension, increased vascuorization, cytoplasmic and nuclear distortion, cellular aggregation, and degeneration. Spherulocytes and plasmatocytes were actively involved in the detoxication process.

Krishnan *et al.* (2000) investigated the relationship between nodulation response to bacterial infection and exogenous application of 3,4-dl-dihydroxy phenyl alanine in the silkworm *B. mori*. 
Balavenkatasubbaiah *et al.* (2001) investigated the difference in response in terms of THC and DHC in susceptible and in tolerant breeds. They observed that in tolerant breeds of *B. mori* there was increase in THC, DHC and period of survival of haemocytes with regard to granulocytes, plasmatocytes and spherulocytes under normal condition and during progressive BmNPV infection. Nittono (1960) studied the blood cells in the *B. mori*. Krishnan *et al.* (2002) analysed the haemocytes and haemolymph of the silkworm *B. mori* against superoxide dimutase (SOD) activity following *B. subtilis* incubation using a phytochemical assay system. Narayanan (2004) provided the various aspects of insect defense mechanisms through their innate, ie. non-specific reactions, and how the various microbial control agents *viz.*, entomopathogenic fungi, bacteria, virus and nematodes try to overcome the insect host defense.

Ottaviani (2005) studied the mechanism of the innate immunity in the insects and also reported the cellular component like phagocytosis, encapsulation, melanization, nodule formation, wound healing, haemolymph clotting and transplantation and the humoral component *viz.*, lectins, cytokine-like molecules and anti microbial peptides of the haemolymph. Jalali and Salehi (2008) studied the types of haemocytes of *Papilio demoleus* L. they found six types of haemocytes *viz.*, prohaemocytes, plasmatocytes, granulocytes, spherulocytes, adipohaemocytes, and oenocytoides. They found that the THC steadily decreases during the developmental stages, attains its peak in the late 5th instar and steeply declines in the pupa. Singh *et al.* (2008) studied the antiviral activity of phytoextract against cytoplasmic
polyhedrosis virus and its effect on total and differential haemocyte count in tasar silkworm, *A. mylitta*. They observed that total haemocyte count increased in haemolymph up to sixth days post inoculation of phytoextract treated batches. While in the inoculated control the increase was within three days, indicating positive haemocyte mediated response in silkworm treated with phytoextract.

Thomas and Nair (2010) studied the ultrastructure of haemocytes of *Rhynchophorus ferrugineus Oliver*. They observed the presence of six types of haemocytes in the haemolymph of *R. ferrugineus* namely the prohaemocytes, plasmatocytes, granulocytes, adipohaemocytes, oenocytoids and spherulocytes. Comparision of cell size and nuclear size of haemocytes in the different stages of red palm weevil indicated that prohaemocytes are the smallest of all the haemocyte types, and adipohaemocytes are the biggest of all.

Rajasekharagouda (1991) reported the growth promoting effect of plant products. Application of petroleum ether extracts of *P. corylifolia* and *T. terrestris* increased the larval weight, cocoon weight and shell ratio of *B. mori*. Sivaprakasam and Rabindra (1996) concluded that the disinfection of bamboo mountages followed by application of Resham Keet Oushadh (RKO) after each moult and feeding of mulberry leaves treated with aqueous extract of *P. corylifolia* at 800 ppm once during third, fourth and fifth instar of silkworm reduced the grasserie disease besides enhancing the larval and cocoon parameters. Patil *et al.* (1997) tested the efficacy of leaf extracts of seven plants, viz., *P. corylifolia*, *T. terrestris*, *C. nucifera*, *A. indicum*,
B. diffusa, P. niruri and S. vulgare pers. He also observed that the botanicals did not show any toxic impact. On the contrary marginal increase in the larval, pupal and shell weights, shell ratio and silk filament length were also noticed.

Rajashekhargouda et al. (1997) investigated the effect of crude seed extracts of P. corylifolia on economic characters of silkworm, B. mori. Murugan et al. (1998) reported the growth promoting effect of aqueous extracts of T. terrestris, P. niruri, B. diffusa, P. corylifolia, C. coriaria and Parthenium hysterophorous, while Ramesha and Govindan (1999) observed the influence of some plant growth regulators on larval growth and cocoon traits of silkworm, B. mori. They observed that the fifth instar larval duration was reduced and maximum larval weight, spinning percentage, cocoon weight, shell weight, shell percentage and silk productivity were enhanced due to topical application of plant growth regulators.

Mahesha et al. (1999) studied the extrafoliation of aqueous extract of botanicals on larval, cocoon and egg parameters of B. mori. Jyothi et al. (1999) studied the effect of aqueous neem seed kernel extract and neem oil on development and survival of silkworm, B. mori. Raman et al. (1999) observed that the relative effects of methoprene on the improvement of fecundity in different races of mulberry silkworm, B. mori. Krishnaprasad et al. (2001) investigated the effect of Solanaceous leaf extracts on growth, development and cocoon traits of PM×NB4D2 silkworm.

Rajeshwari and Isaiarasu (2004) observed the influence of the leaf, flower and pod extracts of Moringa oleifera on the growth and reproductive
parameters of *B. mori*. The mean larval weight and the weight and size of cocoon increased significantly as a result of this supplementation. Sampoorani *et al.* (2004) revealed the effect of Phytoecdysteroids (*Tribulus prosterata* and *Achyranthus japonica*) on mulberry leaves to accelerating spinning and this effect on the cocoon and reeling parameters of silkworm and to assess its influence on larval development of silkworm. Sridevi *et al.* (2004) investigated the effect of extracts from *Tagetes erecta, Withania somnifera, Tinopora cordifolia, Leptadenia retioulata, Terminalia arjuna* and *Adhathoda vasica* on cocoon reeling parameters of *B. mori*, wherein they reported improvement in silk reelability percentage.

Patil *et al.* (2005) reported that *Parthenium* root extract induced silkworms resulting in higher cocoon and pupal weights and better survival. Murugesh and Mahalingam (2005), studied the spraying of aqueous leaf extract of *T. terrestris* on silkworm during third, fourth and fifth instars resulted in higher cocoon and shell weights, silk productivity and shell ratio. Kamalakanaan *et al.* (2005) reported that the application of petroleum ether extract of *C. longa* stems on 24, 48, 72, 90 and 120h old fifth instar multivoltine larva resulted in increased cocoon and shell weight, shell ratio and silk filament length. Pratheesh kumar *et al.* (2007) have reported that feed supplementation of 5% concentration (v/v) of *T. terrestris* and *W. somnifera* has contributed for improvement of reproductive parameters viz., higher fecundity and egg recovery in silkworms. Murugesh and Bhaskar (2008) studied the silk productivity of silkworm, *B. mori* through the use of
botanicals. They found higher larval weight and effective rate of rearing when the larvae were topically applied with aqueous extracts of plants, than control.

Manimuthu and Isaiarasu (2010) recorded the influence of herbal tonic *Aloe* on the overall performance of the mulberry silkworm, *B. mori*. They found the mean larval weight, relative growth rate, effective rearing rate, larval consumption index of the final instar larvae of *B. mori* increased with this supplementation of *A. vera*. Maribashetty *et al.* (2010) studied botanicals that influence the silkworms by increasing either food consumption, thereby enhancing cocoon yield. Shubha *et al.* (2010) revealed the effect of medicinal botanical on the grainage parameters. Manimegalai *et al.* (2010) found the administration of botanicals like *P. corylifolia* and *P. amboinicus* improved the economic parameters viz., larval weight, cocoon weight shell weight and shell ratio in the treatments compared to treated control.