Bacterial Diseases of Silkworm

Introduction

Silkworm rearing is a highly enterprising vocation for a large number of sericulturists across the country. The success of silkworm crop depends on many factors. The good quality mulberry leaf, proper temperature and humidity conditions, larval spacing, aeration, protection from diseases and pests, *etc*., are some of the factors that contribute to the success of silkworm crops. Many silkworm diseases are caused due to the infection of various bacteria, virus, fungus, microsporidia, *etc*., in the silkworm colonies and this is a major limiting factor in the successful cocoon production. Diseases such as grasserie, flacherie, muscardine and pebrine are caused due to these pathogens. Flacherie diseases are caused by microbial and amicrobial agents and microbial flacherie is caused by both bacteria and viruses. Bacteria are involved in causing flacherie as individual pathogen or in combination with viruses thereby contributing for disease epizootics. In China and Japan the silkworm crop loss due to diseases are less, but in India it is more and severely affects the economic returns of the rearers. Of the total disease occurrence in Karnataka the bacterial flacherie is to the extent of 57.22% as assessed by microscopic examination of haemolymph and midgut smears (Samson *et al.*, 1990). Infection with several bacteria causes severe mortality and majority of the deaths occur during moult and when the worms are about to spin. Mortality becomes maximum among fifth instar silkworm larvae.

Flacherie in silkworms is a syndrome characterized by flaccidity of larval body. The flaccidity caused by bacteria is referred as bacterial flacherie. Louis Pasteure reported bacteria as etiological agent of flacherie in silkworms as early as in 1870. The diseases in silkworms caused by bacteria are generally classified as bacteremia, septicemia and toxemia. For better appreciation, the topic is being explained into different sub-headings and they are:

1. BACTERIAL DISEASES IN SILKWORMS
2. PREDISPOSING FACTORS
3. LARVAL INSTAR AND HOST SUSCEPTIBILITY
4. NUTRITION AND HOST SUSCEPTIBILITY
5. ENVIRONMENT AND HOST SUSCEPTIBILITY
6. MIXED INFECTION OF BACTERIA WITH OTHER PATHOGENS
7. MANAGEMENT OF BACTERIAL DISEASES

1. Bacterial Diseases in Silkworms

In 1870, Pasteur had reported that a *coccus* and *bacillus* bacteria caused the flacherie in silkworms. Later, many researchers have reported that different bacteria are causing bacteriosis in silkworms. Cuboni and Garibini (1890) thought that *Bacillus cubonianus* was the cause of flacherie. In Japan, the causal agent of Sotto disease was identified as *Bacillus sotto* (Iwashita, 1902), which presently is

known as *Bacillus thuringiensis* var. *sotto*. Sawamura in 1960 isolated *Bacillus megaterium* and *Bacillus ellenbachi* from the silkworms. *Serratia marcescens* (Metalnikov and Chorine, 1928), *Bacillus bombysepticus* (Hartman, 1931), *Bacillus bombycoides* (Paillot, 1942), *Streptococcus bombycis* (Steinhaus, 1949), *Bacillus mucoides* and *Bacillus latrosporus* (Steinhause, 1949), *Bacillus cereus* var. *alesti* (Tourmanoff and Vago, 1950), *Aerobacter cloaceae* and *Achromabacter superficialis* (Nakasuji and Kodama, 1969), *Streptococcus faecalis*, *Serratia piscatorium*, *Proteus vulgaris*, *Proteus inconstina*, *Proteus morganii*, *Proteus aerobacter*, *Proteus aerogenes*, *Micrococcus flavus* (Kodama and Nakasuji 1969; Nakasuji and Kodama, 1969; 1970; Vago, 1963) were also reported to be pathogenic to silkworm causing flacherie. Chitra *et al*., (1973) isolated *Aerobacter cloacae*, *Achromobacter delmarvae* and *A. superficalis*, *Achromobacter delmarvae*, *Pseudomonas boreopolis*, *P. ovalis*, *Escherichia freundii* and *Staphylococcus albus*.

Flacherie in silkworms is a syndrome characterized by flaccidity of larval body. The flaccidity caused by bacteria is referred as bacterial flacherie. The symptom caused by different bacteria is atypical and are of general type. The larvae lose appetite, become lethargic and vomit gut juice. The growth becomes stunted and the larvae become flaccid. The dead larvae develop different colours, depending on the species of bacteria involved in causing the infection, become rotten and foul smelling. However, based on the site of infection and pathogenicity caused by the bacteria in silkworms, the bacterial diseases are categorized into three types. They are:

A. Bacteremia
B. Septicemia and
C. Toxicosis

A. Bacteremia

Bacteremia or bacterial disease of digestive tract is caused due to the multiplication of bacteria in the gut. The bacteria infect silkworm primarily through mouth and digestive tract and results in the development of a chronic type of flacherie. The cephalothoracic region becomes translucent and in the advanced stage even the posterior portion becomes translucent. The larvae show loss of appetite, sluggishness, retarded growth and develops diarrhea. *Streptococcus faecalis* and *Streptococcus faecium* are the most common bacteria associated with bacterial disease of digestive organ in the silkworm *Bombyx mori*. These are gram positive bacteria measuring 0.7 - 0.9 μm in diameter. The *Streptococcus faecalis* multiplies for 2-3 days and form large colonies attached to the larval peritrophic membrane. After 6 days, the peritrophic membrane dissolves and the goblet and cylindrical cells become vacuolated. The larva dies as the fluid in digestive tract and the bacteria passes through the vacuolated midgut epithelium into the hemolymph. In the digestive tract, the bacteria produce enzymes such as chitinase, lecithinase and proteinase which act on the midgut epithelium and enable the bacteria to enter hemocoel. Bacterial exotoxins and endotoxins also play a major role in invasion of the digestive tract by different bacteria. The toxins damage the gut wall and enable the bacteria to enter the hemocoel. Infection of *S. faecium* in fifth instar larvae result in

lowering of gut pH from the range of 8.0 and 9.5 to 7.5 and 7.6. The lowering of gut pH is conducive for most septicemic bacteria.

B. Septicemia

Septicemia in silkworms is caused by the septic bacterial invasion into the silkworm body and their multiplication, which results in the mortality of silkworms. The majority of silkworms that die from this disease vomit the gut fluid. The body of the diseased silkworm becomes soft, shrunken and the thoracic region swollen. A large number of bacteria are observed in the Haemolymph. The body wall ruptures easily liberating foul smelling fluid containing large number of bacterial cells. The larvae die in short span of infection. Several bacteria *viz*., *Serratia marcescens*, *Pseudomonas* sp., *Bacillus proteus*, *Bacillus aergenes*, *Bacillus prodigiousus*, *Bacillus pyocyanes*, *Streptococcus* sp. have been isolated from silkworms affected with septicemia. But most common are *Serratia marcescens* and *Bacillus* sp. In case of septicemia caused by *Bacillus* sp., the silkworm shortly after death develop dark greenish tinge on the dorso-thoracic region which later extends to the whole body. In case of septicemia caused by *Serratia marcescens*, the larvae after death develop dark brown spots and reddish tinge, which extends to whole body. Injection of 6-8 x 10^2 bacteria causes death within 18 hours. The pathogenicity of orally inoculated Serratia causes a high mortality at high humidity, although the mortality varies with the amount of bacteria added to the feed. Bacterial involvement in septicemia of pre-pupal or pupal stages leads to the spoilage of cocoon quality and reduction in grainage productivity.

The *Bacillus* species of bacteria causing septicemia are rod shaped ranging in size from 1-1.5 x 3 μm with sub-terminal spore and peritrichate flagella. *Serratia marcescens* appear as small rods measuring 0.6-1.0 x 0.5 μm. These are non sporulating bacteria with peritrichate flagella. *Serratia marcescens* in the digestive tract of silkworm are non-pathogenic. But when they enter the hemocoel they multiply rapidly and become pathogenic leading to acute disease and death in 1-3 days. Apart from the damaged gut wall the septic bacteria invade silkworms through wounds caused by injury and multiply rapidly in the hemolymph. The invasion by *Serratia pisatorium* into haemocoel in silkworm is observed to enhance by the presence of *Streptococcus faecalis/Streptococcus faecium* in the larval midgut. The lowering of pH by these bacteria favours the *Serratia piscotorium* to multiply and invade the hemocoel and produce fatal septicemia.

C. Toxicosis

The cause of bacterial toxicosis is *Bacillus thuringiensis.* The common *Bacillus* involved is *B.t.* var. *sotto*. The larvae suffering from bacterial toxicosis will lose appetite and become sluggish. The dorsal vessel pulsates at rapid rate and the larvae wriggle as though they are in severe pain. Three exotoxins, *viz*., α-exotoxin, β-exotoxin, γ-exotoxin and an endotoxin, δ-endotoxin have been isolated from B. thuringiensis. The poison ingested by the larva is first dissolved by the digestive fluid and partly

absorbed by the body, which causes toxicity to the silkworms. This affects the nerves causing convulsions or paralysis. There are two types of bacterial toxicosis.

i. ***Acute bacterial toxicosis:*** This is a poisoning caused by the ingestion of parasporal crystal bodies called δ-endotoxin produced by *Bacillus thuringiensis.* The major symptoms of bacterial toxicosis caused by *Bacillus thuringiensis* are – sudden cessation of feeding, lifting of heads, spasms and tremors, paralysis, distress, sudden collapse and death. Death may occur within 10 minutes to few hours. Shortly after the death, the corpse appears hard for touch with the head retracted so as to assume a hook-shaped appearance. Gradually the corpse become black and rotten, exuding a foul smelling dark brownish fluid.

ii. ***Chronic bacterial toxicosis:*** This is caused by ingestion of small quantity of Bt crystal toxin. Mulberry leaf intake is reduced, faeces become irregular shaped and occasional vomiting is observed and develops muscle paralysis. The thorax and abdominal tip become transparent and the worms become motionless. After 10-12 h of bacterial infection sluggishness is observed and loss of clasping power of the legs, flaccidity and death are observed.

Now let us study, second aspect.

2. Predisposing Factors

A healthy silkworm is generally more resistant to infection than stressed one. Stress brought about by the malnutrition, metabolic imbalance, physical and other factors results in weakened larvae and increased susceptibility to bacterial infection. In order to check the outbreak of the bacterial disease in silkworm, it is essential to eliminate the stress factors by feeding with nutritious mulberry and rearing them under congenial and hygienic environment. The nutritional stress lowers the resistance of silkworms by rendering them inefficient in production of antibacterial and antiviral factors in the gut and haemolymph. The ability of the larvae to produce such factors in the gut is dependent on the quality of leaves. The mulberry of poor nutritive value will not be able to provide sufficient quantity of essential requirement to the larvae to produce anti-bacterial factor. It results in high rate of multiplication of infectious bacteria and the development of flacherie disease. Weakness due to physiological starvation due to feeding of poor quality and insufficient mulberry leaf should be avoided. These stress factors in early instars predisposes the outbreak of flacherie during later instar silkworms. Feeding wet leaves under unhygienic and humid conditions may also lead to physiological disturbance thereby predisposing the disease development. The improper incubation of eggs weaken the growing embryo, resulting in weak larvae susceptible to infection by bacteria causing flacherie.

Now let us look into the third aspect.

3. LARVAL INSTAR AND HOST SUSCEPTIBILITY

Different bacterial isolates are known to vary in their pathogenicity to silkworms and the stage of growth of larvae affects their virulence (Chitra et al., 1974). Infection with some bacterial pathogens

causes severe mortality and majority of the deaths occur during moulting and just before spinning. Also, irrespective of the age of infection, mortality is maximum in the final instar. The susceptibility of fifth instar larvae to infection by different bacteria is more or less the same. The younger larvae show varying degree of susceptibility. *Aerobactor cloacae* and *Staphylococcus albus* are more lethal to older larvae than to first instar larvae and the reverse is true with *Escherichia freundii*. The most susceptible instar is third instar as revealed by maximum mortality due to infection at this instar.

Now let us study, fourth aspect.

4. NUTRITION AND HOST SUSCEPTIBILITY

The susceptibility of silkworm to bacterial infection is governed by the nutritive factors. The poor quality mulberry with low protein, sucrose or high cellulose makes silkworms comparatively more susceptible to infection by pathogens. The silkworms also derive precursors of anti-bacterial and anti-viral substances from the mulberry. The caffeic acid derived from chlorogenic acid from mulberry leaves is converted in the silkworm's digestive tract into anti-bacterial caffeoquinone. It is presumed that two antibacterial phonolic acids – the procatechuic acid and p-hydroxybenzoic acid found in the faeces of silkworms must have the origin from the mulberry.

The fifth aspect of our study is,

5. ENVIRONMENT AND HOST SUSCEPTIBILITY

The rise in silkworm rearing temperature accelerates the development of bacterial diseases. Silkworms exposed to small doses of *B.t.* var. *alesti* leads to chronic infection, but on exposure to higher temperature of around 30°C rapidly develops into fatal bacteriosis. High temperature and humidity leads to dysfunction of alimentary canal, which in turn leads to *Enterococcus* multiplication.

Now let us study the sixth aspect of our study.

6. Mixed Infection of Bacteria with other Pathogens

Since the domesticated silkworms are susceptible to many infectious agents, mixed infections are very common. Mixed infection is the association of more than one pathogen causing diseases in silkworms. Synergistic interference of different pathogens influences the disease development in silkworms. The pathogen or its infection acts as a biological stressor and increase the susceptibility of the insect to another pathogen. The mixed infection play a major role in reducing the cocoon crop yields. Mixed infections may occur simultaneously or one after the other and usually the interaction between the two or more pathogens involved results in synergism thereby affecting the cocoon productivity both quantitatively and qualitatively. The silkworms very less susceptible to *Serratia marcescens* becomes highly susceptible when already being inoculated per orally with *Staphylococcus* sp. The reason being the lowering of gut pH by the *Staphylococci* thereby enhancing the multiplication rate of *Serratia marcescenes*. Similarly if the digestive tract of silkworms is attached with viral infection, the *Bacillus*

bombycis otherwise non infective attains an infective proposition, causing damage to the silkworm crops. The symptoms of gattine are manifested only when the combination of *Streptococcus* and virus occurs in the susceptible silkworm.

`*Thatte roga*' a disease reported in Karnataka was found to be caused due to the synergistic association of bacteria such as *Streptococcus faecalis* var. *liquifacians, Staphylococcus aureus, Staphylococcus epidermidis and Bacillus* sp. and a picarnavirus namely *Bombyx mori* infectious flacherie virus (BmIFV). The disease was reported to begin in a localized area in the colony and spreads quickly at an alarming rate to the rest of the population. Due to this infection the larvae were observed to be feeble, lethargic and dull with translucent cephalothoracic region. They vomit gut juice and extrude soft faeces with high water content. The disease affects the later instar silkworms and the loss due to the disease was significant.

Bacillus bombycis helps for the infection of nuclear polyhedrosis virus by making the condition of epithelial cells for virus infection and vice versa. The highest mortality of silkworms in simultaneous mixed infection is due to flacherie virus and bacteria. The susceptibility of BmIFV infected larvae increases in the presence of *S. faecalis* and *Serratia marcescens*. Similarly, simultaneous per oral infection of silkworm with BmDNV2 and *Staphylococcus aureus* results in higher mortality as well as deterioration in cocoon quantitative traits. It is well established that synergism between BmIFV and *Streptococcus* sp./ *Staphylococcus* sp./ *Serratia* sp. of bacteria and BmDNV and *Streptococcus* sp. bacteria results in the flacherie disease in silkworms. The synergism between different pathogens generally lowers the incubation period by 5-6 days and cause comparatively increased level of mortality. The bacteria such as *Pseudomonas* sp., *Serratia* sp. and *Proteus* sp. along with microsporidian spores in the larvae cause primary septicemia. The larvae generally die within 5 days day without developing microsporidiosis due to mixed infection.

7. MANAGEMENT OF BACTERIAL DISEASES

Thorough disinfection of rearing room and rearing equipments with recommended general disinfectant is essential to ensure absence of pathogen in the rearing environment before and after each rearing programme. Maintenance of rearing and personal hygiene during the course of rearing ensures that the pathogen are kept away from the rearing environment. Use surface disinfected quality eggs and rear the early instar silkworms under optimum conditions.

In order to prevent the outbreak of bacterial flacherie, the silkworm larvae should be reared on nutritive mulberry leaf under hygienic and congenial rearing conditions. The mulberry leaves fed to silkworm should be of good quality and rich in nutritive value to silkworms, especially to early instar worms. The mulberry grown under shade or with excessive nitrogen fertilizer is not suitable for silkworm rearing. Application of silkworm body and rearing seat disinfectants as per recommended schedule and quantity prevent the spread of diseases.

In addition, it is essential to provide required bed space and ventilation. There is need to avoid accumulation of silkworm waste, feeding wet leaves, overcrowding and injury to larvae. The management of temperature and humidity to suite the requirement of silkworm is essential. High temperature (>28°C) or low temperature (<20°C) and high humidity during later instar (>75%) form the stress factor. The incubation of silkworm eggs at 26°C and relative humidity of 80-85% ensure robust growth of embryo. It is also important to pick the diseased silkworm if any in the rearing bed and dispose them by burning or using disinfectant.

In order to prevent flacherie and *Thatte roga* in silkworm rearing, the rearing trays and equipments or tools used for silkworm rearing are to be dipped in an effective disinfectant solution for 10 min. Further the practice of smearing the bamboo trays used for rearing the silkworms should not be smeared with cow dung, which harbours abundance of pathogens and acts as a source of contamination. Further, the accumulation and fermentation of silkworm litter should be avoided. Ensuring good cross ventilation helps in reducing humidity between the rearing beds. Dusting of dry slaked lime is also helpful in keeping the humidity low and reduces the pathogen buildup in the rearing environment.

Use of antibiotics to suppress bacterial flacherie, especially the bacterial disease of digestive organ is found to be effective. Antibiotics such as erythromycin, kanamycin, streptomycin, terramycin, chloramphenicol, aureomycin, neomycin and tetracycline have been reported to be suppressive against bacterial disease of digestive organ. Enriching of mulberry leaves with 0.1% gentamycin was found to reduce flacherie. As a prophylactic measure, ampicillin @ 500 ppm can be used to treat mulberry leaves and fed to silkworms during the first feeding of 3^{rd} and 5^{th} instars to manage bacterial flacherie in order to harvest better cocoon crops.

Thus the topic can be summarized that, different bacteria cause diseases in silkworms alone or in combination with other bacteria, virus or microsporidians. Bacterial disease of digestive organ, Septicemia and Toxicosis are the major bacterial diseases in silkworms. There are many predisposing factors, which contribute to the causation of bacterial diseases. The crop loss due to bacterial infection can be kept at minimum by following various management strategies during the course of silkworm rearing.