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Influence of bacterial infection *Serratia marcescens* septicemia on the quality of coocoons of spinned by larval instars of multivoltine mulberry silkworm *Bombyx mori* Linn

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Keywords

Septicemia bacteria, *Serratia marcescens*, *Bombyx mori*, cocoon yield parameters.

Abstract

The control of silkworm diseases at the larval stages has been emphasized so far, whereas the disease occurrence in cocoons has rather been neglected. However, silkworms which died of diseases and putrefied in larval haemolymph and cocoons contaminate the inside of the cocoons, causing deterioration of cocoon filament quality. As the occurrence of such contaminated in larval haemolymph and cocoons have increased widely, the problem of silkworms died in cocoons has suddenly come to be regarded very important. Silkworms infected with bacterial diseases at the larval stage are almost unable to produce cocoons. Therefore, dead silkworms in cocoons seem to occur at a limited condition infection when the cocooning is finished and then disease occurrence. Septicemia is regarded a disease which causes infection, death of larva and pupa, and putrefaction in cocoons within an extremely short time. A series of experiments were carried out to clarify the problem, i.e., by what route the pathogenic bacteria of septicemia can enter into haemolymph of larvae and cocoons. Septicemia caused by *Serratia marcescens* bacterial infection influenced the growth and development of silkworm larva and ultimately the economical cocoon traits like larval weight, cocoon weight, shell weight, shell percentage, filament length and denier. Significant reduction of larval weight (2.87gm), cocoon weight (0.77gm), shell weight (0.10gm), shell ratio (13.80%), filament length (505.7m), and denier (2.67D) was recorded in the experimental silkworm compared to control.

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Introduction

Diseases of silkworm are a big challenge for the rearers. Due to prolong domestication, silkworm develops less immunity and less adaptability for which they are often attacked by different agents. Although some attempt has been made to develop disease resistant silkworm variety, but only a limited success is obtained till date. In spite of taking many efforts on the rearing environment, silkworms are vulnerable for bacterial infection. Mode of transmission is after taking contaminated leaves and other pathogenic disease affected the larvae life. In the rearing bed bacterial growth occur in the gut of larva and consequent deposition of lactate, acetate etc. reduces the gut permeability and lower the pH and infection spreads all over the body of the larvae. Larvae release stool with bacterial spores and then next set of infection starts.

Entomopathogenic *Serratia marcescens* septicemia bacterial disease is acting as a parasite on multivoltine mulberry silkworm. It is causing *Serratia marcescens* septicemia bacterial disease injected into haemolymph of silkworm larvae poses a major threat to silk cocoon production during different meteorology. The rainy and winter meteorology are congenial for the spread of *Serratia marcescens* septicemia bacterial disease in larval instars of silkworm, *Bombyx mori* (Linn). It is therefore important to resort to appropriate management practices to prevent the outbreak of the disease at the field level. Obviously, the success of sericulture industry primarily depends on the successful harvest of cocoon crops. The major problem of sericulture in a tropical country like India is the high incidence of septicemia bacterial diseases. The major diseases affecting mulberry silkworm are muscardine (fungal disease), flacherie (bacterial diseases) grasserie (viral diseases) and pebrine (protozoan disease). Silkworm crop loss occurs in all the silkworm growing areas of the world, but the type of severity varies. They differ from region to region, crop to crop and even from farmer to farmer. Leaving aside minor variations, it has been found that crop loss is generally more in the tropics than in the temperate regions. The magnitude of the disease damage is on the higher side in India. It is a general observation that out of 5-6 crops per year, two are usually lost due to diseases and other meteorological reasons and even the successful once are partially lost. Thus the frequent outbreak of diseases is one of the main handicaps for the progress of sericulture industry. The reasons attributed, are poor hygienic conditions and continuous silkworm rearing all round the year, the meteorology conditions that favors faster multiplication of septicemia bacterial disease causing germs inside the haemolymph. The accumulated germs under favorable conditions become active and cause the outbreak of diseases. *Serratia marcescens* septicemia bacterial disease is one of the most destructive bacterial pathogen of silkworm *Bombyx mori* Linn causing septicemia disease, which is common in all sericulture zones of the world. Demonstrated the contagious nature of the bacterial disease and identified the cause as a *Serratia marcescens* and suggested measures to control the septicemia bacterial disease **(Mahadevappa, 2000; Sampson, 1998).** It was a microorganism to be recognized as a bacterial disease causing agent. In India 10to 50% of loss has been accounted for septicemia bacteria in total loss due to this disease **(Cai, J.1989; Lakshmi, 2013).** In this backdrop, the study was carried out to examine the *Serratia marcescens* septicemia bacterial symptological changes in *Bombyx mori* during the progress of the disease and its influence on economical characters of the cocoons and causes silk production rearers.

Materials and Methods

The silkworm multivoltine Pure Mysore race was selected for the study and the silkworms were reared under optimum conditions with meticulous coordination of many activities such as maintenance of mulberry garden, preparation and disinfection of the rearing room and appliances, procurement and handling of silkworm eggs and incubation, young and late age silkworm rearing, general hygiene, moulting care, mounting, spinning, harvesting of cocoons etc **(Ono et. al. 1965).** The larvae were processed further as per the guidelines enlisted by **(Krishanaswami et al. 1978; Rath, 2005; Rao, 2006; Iwano, 1985)**. On the 1st day of the fifth instar, the larvae were inoculated by dipping in sub lethal concentration of *Serratia marcescens* septicemia bacteria (2.15x 106 haemolymph/ml @ 50ml/100 worms for 45Sec) and larvae treated with double distilled water were used as control. From the day of inoculation to the end of the 5th instar silkworm larvae were kept under continuous surveillance to examine the symptom logical changes during the development of bacterial pathogen *Serratia marcescen*s septicemia bacterial disease and taken observations. By the end of the fifth instar the silkworm showed the symptoms of spinning i.e., cessation of feed, body shrinkage, translucent light black colour skin etc. These larvae are known as matured worms, the larval weight of the silkworms was recorded. These worms were transferred to mountages to spin the cocoons. After completion of the spinning process, the cocoons were harvested on the 6th day of mounting and then these cocoons were taken for the assessment of various economic parameters viz., cocoon weight, shell weight, shell ratio, filament length, and denier. The qualitative and quantitative parameters of cocoons were determined by following the methods as given by.

Larval weight: The Quantitative profile analysis of fifth instar larval silk worms was studied by weighing and measuring the worms during one to six days of fifth instar larva. For this purpose, ten larvae from every replication were randomly selected and their individual weight was recorded. This character indicates the healthy and robust disposition of the larvae.

Cocoon weight: The silk cocoon weight which is important trait in sericulture because cocoon weight has moderate heritability and is actually easy to quantify researchers select original populations based on this trait. Cocoon weight indicates the approximate quantity of raw silk that could be reeled from the cocoons. In the present experiment a sample is drawn from each replication comprising around ten cocoons. The sample drawn represents the entire quality of each replication. Individual cocoon weight was taken from each sample of ten cocoons and mean cocoon weight was calculated. The weights were taken in gram units.

Shell weight: Cocoon shell weight is economic trait represents the total quantity of silk in a cocoon. Average single shell weight was calculated from ten cocoon shells used for the assessment of cocoon weight.

Shell ratio: Cocoon shell denotes the total amount of silk available in a single cocoon and is expressed in percentage. It is calculated by dividing the shell weight reading by cocoon weight reading. Quotient thus obtained was multiplied by hundred to obtain the shell ratio or shell percentage in entire cocoon.

Filament length: Silk filament is the total length of silk filament, unwound from a single cocoon measured in meters. In the present experiment a sample is drawn from each replication comprising around ten cocoons shell. Ten cocoons were cooked and reeled on an eprouvette (such as a mortar for testing) with a circumference of 1.125m and the mean value of filament length in meters was calculated as per the standard formula 1.125 **(Sonwalkar, 1993)**.

Filament length (m) =

Number of rotations X Circumference of the wheel.

Denier: Denier scale is the weight in grams of 9,000 meters of the filament. Denier represents the size of the yarn. It was calculated using the formula as given by **(Sonwalkar, 1993)**.

Denier Scale= Weight of filament (g) ÷ Length of filament (m) X 9000

Statistical analysis: For the consistency in the results, all the attempts were revised three times. The data was collected. All the experimental data recorded from the six replicates have been subjected to statistical analysis by following **T-test**.

Results and Discussion

Pathological Symptom changes: No conspicuous symptoms were noticed immediately after inoculation of *Serratia marcescens* septicemia bacterial disease. But gradually an infected silkworm became inactive, sluggish, stopped the feed and remained underneath the mulberry leaves. After 48 hrs, the infected worms started to vomit digestive juice, haemolymph leaked from the body and later, the worms gradually became stiff and the movement of the worms was very much restricted and colour of the body changed to brown/ black colour with oily specks. Initially the oily specks are in small in size with the advancement of the age, the size and number of the oil specks was enhanced**.** Then the silkworm body became soft, pliable and later stiff and hard. Nearly on 7th or 8th day of the infection white efflorescence noticed near intersegment region, spiracles, and then complete body was covered with septicemia bacterial disease and finally haemolymph black colour developed on the body **(Fig: 1)**. The mummified cadaver became brittle and breaks into pieces when dropped from a certain height. Infected worms fail to spin the cocoons, but those which spin form flimsy cocoons**.** Cocoons formed by these infected worms were smaller and lighter in weight and the worms not emerged as moths.

Economical cocoon parameters: The overt changes observed in the economical traits of cocoon in *Serratia marcescens* septicemia bacterial disease infected silkworm with reference to control are shown in **(Table: 1)**. The results of the present study clearly indicated that *Serratia marcescens* septicemia bacterial disease infestation influences the growth and development of silkworm and the economical cocoon parameters. Significant reduction of matured larval weight (2.87gm), cocoon weight (0.77gm), shell weight (0.10gm), shell ratio (13.80%), filament length (505.7m), and higher denier (2.67D) was recorded in the experimental silkworm larvae. In contrast, to the experimental control larvae significantly higher values were recorded in the majority of the economical characters such as 5^{th} instar larval weight (2.99gm), cocoon weight (1.23gm), shell weight (0.21gm), shell ratio (16.33%), filament length (652m) and textile denier value (2.88D) in control experiment. Appropriate matured larval weight is an indicator to measure the health of silkworm and in turn to obtain good quality of cocoon.

Table: 1: Influence in economical cocoon parameters of multivoltine silkworm *Bombyx mori* Linn inoculated with bacterial infection *Serratia marcescens* septicemia to control.

Parameter	Larval weight	Cocoon Weight	Shell Weight	Shell ratio	Filament Length	Denier
Control	2.99gm ±0.01	1.23gm ±0.05	0.21gm ±0.02	16.33% ±0.11	652.0m ±17.88	2.88D ±0.13
Infected with septicemia	2.87gm ±0.06	0.77gm ±0.16	0.10gm ±0.01	13.80% ±0.09	505.7m ±14.89	2.67D ±0.06

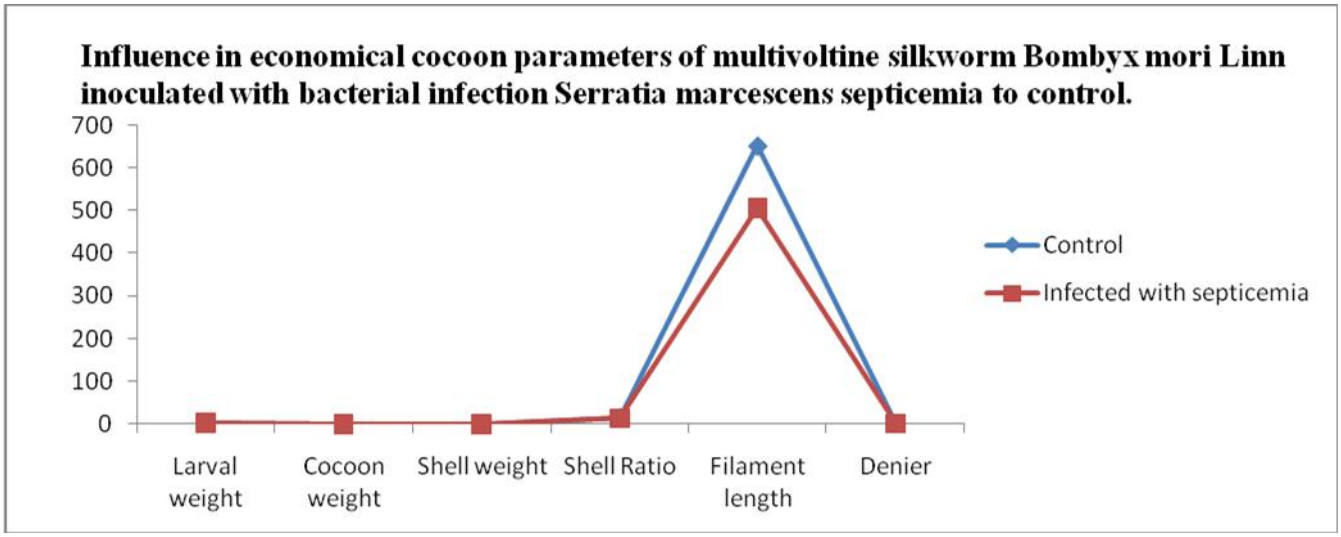

Fig: 1: Influence in economical cocoon parameters of multivoltine silkworm *Bombyx mori* Linn inoculated with bacterial infection *Serratia marcescens* septicemia to control.

Cocoon weight is an important commercial character used to determine the amount of raw silk that can be obtained. The cocoon price is determined based on the weight of the cocoon. The shell weight is more important than the cocoon weight since it is the shell that yields the silk for reeling. Thus, higher the weight of the shell, greater will be the silk yield. Shell percentage fairly indicates the quantity of raw silk that can be reeled from the cocoons and also helps in estimating and there by fixing a proper price for the cocoons. The silk filament length indicates the reel able length of silk filament from a cocoon. Reeling performance is better with cocoons having a larger filament length, which reduces the number of feeding ends. This results in increase of production, quality and also raw silk percentage of the cocoon. Generally, a longer filament with less number of breaks, higher is the reel ability. This indicates that higher the reliability percentage higher is the raw silk quality. The fineness of cocoon filament is expressed by size i.e. denier **(Bassi, 1835; Ainsworth, 1956; Vanitha, 2017).** Reduction in the matured larval weight may be due to the consequence of *Serratia marcescens* septicemia bacterial disease that leads to the decrease in food consumption, digestion, relative consumption rate, efficiency of conversion of ingested food in fifth instar of *Bombyx mori* Linn infected with *Serratia marcescens* septicemia bacterial disease **(Janakiraman,1961; Ayuzawa,1972; Li. et. al. 2001)** analyzed the *Serratia marcescens* septicemia bacterial disease and proliferation in silkworm body after 12 to 24h post- inoculation of the *Serratia marcescens* septicemia bacterial disease. The healthy growth and development of the silkworm is directly related to economical cocoon traits. It is well supported by **(Chandrase, et. al. 2008; Dandin, et. al. 2003).** She noticed poor cocoon characters from pebrine infected multivoltine Pure Mysore silkworm. The reduction in economical cocoon characters could be attributed to loss of appetite, lethargic conditions and physiological stress induced by the *Serratia marcescens* septicemia bacterial disease in 5^{th} instar silkworm larvae **(Seema, et. al. 2018; Entomo, 1985)** observed the reduction of economical parameters due to *Serratia marcescens* septicemia bacterial disease and attributed that it may be due to the decline in the synthesis of silk proteins and the direct or indirect effect of septicemia bacterial disease on the growth and development of silk gland of the silkworm *Bombyx mori* Linn. More number of breakages and higher denier were noticed in experimental cocoons with reference to healthy ones. It may be assumed that the physiological and biochemical stress induced by a fungal pathogen caused to exude uneven amounts of silk fluid in Haemolymph **(Sonwalkar, 1993; Jhanshi, 2003; Kukan, B., 1999;**

Iwanami, 1985). Have reported the decrease in shell weight in Pure Mysore larvae infected **(Mahadevappa, 1993; Rath, et. al, 2003; Jiang, 2012)** studied on the parasitization of fifth instar larvae of *Bombyx mori* by septicemia bacterial disease have reported the decrease in cocoon weight and shell weight in the infected larvae. Silkworm *Bombyx mori* is completely domesticated economic insect and *Serratia marcescens* septicemia bacterial disease is an aggressive parasite which affect the larval body haemolymph. Therefore, the disease in silkworm results in a drastic reduction in the growth and development and qualitative and quantitative cocoon yield parameters. This ultimately has a direct effect on the sericulture community of the country due to reduced returns, in turn affecting the economy of the country, since silk as the end product of sericulture industry is a good source of foreign exchange for the country. Therefore, it is the prime responsibility of sericulture scientists and sericulture farmers to explore many more strategies to prevent/control the occurrence of the *Serratia marcescens* septicemia bacterial disease to enhance the qualitative and quantitative parameters of cocoon crops.

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