STUDIES ON IDENTIFYING VARIOUS DISEAESES AFFECTING THE SILKWORM *BOMBYX MORI* UNDER REARING

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Abstract

India is the highest producer of silk in the world as well as the largest consumer. Although there is so much demand both in production and supply, the farmers are facing lots of problems because the silkworms are easily susceptible to various kinds of diseases. Hence the present study was mainly focused on assessment of diseases in silkworm *Bombyx mori* in the sericulture unit situated at Kannivadi in Dindigul. The study period was carried out from the 2nd of January to 31st, 2021. There were two kinds of diseases such as White muscardine and Flacherei have been identified and recorded in the sericulture unit from II instar to V instar stage. The date wise data of infected number of worms with such kind of disease in each day were tabulated and interpreted with relevant literature.

Introduction

In integrated farming system sericulture is an important component; which is an agro based rural industry, with tremendous potential for employment generation in rural areas. It is the biggest village industry after handloom and khadi providing full or partial employment to about *6.5* million people in India (Pankajet al., 2005).

In the world, India ranks second in raw silk production next to China. Karnataka state alone produces bulk of Indian raw silk (Govindan and Devaiah, 1995). Diseases in silkworm mulberry plants caused by pathogens reduce the quantity and quality of silk production which in turn affects national economy and farmers' income. Although there are several commercial species of silkworms, *Bombyx mori* is the most widely used and intensity studied (Goldsmith, et al., 2005). The silkworm is the larva or caterpillar of the *Bombyx mori* moth. The major diseases of silkworm are Flacherie (Bacterial), Nuclear

cytoplamic polyherosis (Viral), Pebrine (Microsporidian), Muscardine (Fungal). The prime objectives of this study are to visit the sericulture unit for examining the intensity of the diseases and the percentage of prevalence at different instar levels have to be elucidated.

Materials and Methods

Collection and Identification of Diseased silkworm

The sericulture unit situated was visited at Kuttathauavarampatti in kannivadi near Dindigul, Tamil Nadu, during the month of January from 2nd to 31st. Based on the appearance of symptoms, changes found over its body surface, sluggishness, poor feeding the infected larvae were identified from the healthy individuals and it was subjected for screening to identify the type of infection affecting the worms. Productive bivoltine silkworm is obtained and used as a study material. The silkworm, *Bombyx mori* is a native of China but has long been domesticated throughout the world largely in the temperate and sub-tropical regions for its fine cocoon filaments. This study was carried out to find out the diseases of silkworm. The mulberry silkworm is prone to infection of various pathogenic organisms.

The pathogen infected individuals were isolated. Diseased and dead silkworms, their faecal matter, contaminated mulberry leaves and rearing appliances act as sources of infection. Wide fluctuation in temperature and humidity with poor quality mulberry Leaves are the major predisposing factors for flacherie. The diseased larvae showed symptoms such as cessation of feeding, flaccidity, loss of body lustre, sluggishness and dysentery. During the initial stages of infection the larva becomes lethargic and stops eating. At an advanced stage of infection the larva exhibits retarded growth, vomits gut juices and excretes semisolid faeces. The larva becomes soft and translucent. These insects were kept frozen until analyzed. Finally the larvae ferments and the inner content turns into a black coloured liquid, which emits foul odour.

Though the rearing site was carefully maintained the occurrence of diseases were normally seen which had implied that the environmental factor also caused some detrimental effect on the rearing individuals. The pathogenicity was examined from the period between 2nd January 2021 to 31st January 2021 and the rate of susceptibility to diseases had been noted down at different instar levels under rearing. The rate of

infestation were investigated at different instar level had been reported in the present study.

Results

The sericulture farm has been maintained with supplying the disinfectant vejitha in a scheduled manner. The temperature and humidity had also been maintained properly. Though the farm is maintained with proper physiological parameters the silkworms are commonly susceptible to various kinds of pathogens. The pathogenicity was examined from the period between 2nd January 2021 to 31st January 2021 and the rate of susceptibility to diseases had been noted down at different instar levels under rearing. Two types of diseases were identified in the sericulture unit during the study period. 1. Bacterial disease – Flacherie, 2. Fungal disease – White Muscardine. Bacterial flacherie were caused primarily by *Serretia marcesens. Streptococcus sp, and Staphylococcus sp* of bacteria.

Table-1 shows the percentage of prevalence of fungal disease ***White muscardine*** in silkworm larvae infected from II instar to V Instar larval Stage.

S. No	Date	Stages of Instar	No.of Sikworms Infected	Range of Silkorm (Infected in Numbers)	No.of Silk worm infected	% of disease prevalence
1	10.01.2021 11.01.2021 12.01.2021 13.01.2021	II	30 36 42 53	30 to53	161	7.4%
2	14.01.2021 15.01.2021 16.01.2021 17.01.2021 18.01.2021 19.01.2021	III	60 63 69 75 80 90	60 to 90	427	19.7%
3	20.01.2021 21.01.2021 22.01.2021 23.01.2021 24.01.2021 25.01.2021	IV	103 111 116 120 126 129	103-129	705	32.6%
4	26.01.2021 27.01.2021 28.01.2021 29.01.2021 30.01.2021 31.01.2021	V	132 137 140 145 149 154	132-154	857	39.6%

Table – 1 shows the Comparative analysis between the rate of prevalence and number of worms affected from II instar to V instar stage. The study report revealed that from 10th to 13th January the number of infected worms increased every day from 30 to 53 at the stage of II instar and 161 silkworms were totally affected by white muscardine diseases. The estimated rate of percentage of disease prevalence was 7.4%. The rate of infestation gradually increased to 19.7%, 32.6% and 39.6% in the III, IV and V instar stage respectively. The report portrayed that the number of infected silkworms found 30 on the first day of study later it raised to 90, 129 and 154 from II to V instar and the total number increased in each instar was 161, 427, 705 and 857 respectively. It clearly stated that the adult worms were easily susceptible to the pathogenicity rather than that of young worms. The young worms were able to sustain against the disease.

Table-2 Shows the percentage of prevalence of diseases in silkworm infected by Flacherie Disease in the IV th Instar.

Date	Instar	Flacherie Infected silkworm	% of prevalence
1 Jan 2021	IV Instar	7	70%
24 Jan 2021	IV Instar	3	30%

Table – 2 portrayed that the number of silkworms infected by another disease known as Flacherie caused by bacteria such as *Serretia marcesens, Streptococcus sp, and Staphylococcus sp* during IV instar stage. Compared with the white muscardine disease the rate of infestation caused by the flacherie was much less. When the larva entered into IV instar stage a least number of worms infected with flacherie. The investigated report revealed that 7 worms were infected with the rate of infestation was estimated about 70% on 21st January. But 2 days later the infestation rate was reduced only to 30% on 24th of January. This was the contradictory report against white muscardine which showed increasing trend of infestation from earlier period to adult stage.

Table 3 shows the larval weight, cocoon weight, Shell weight and Shell ratio in control as well as infected silkworm.

Parameters Group	Matured larval weight(g)	Cocoon weight (g)	Shell weight (g)	Shell ratio%
Control	2.55	1.77	0.35	17.04
Infected silkworm	2.07	0.75	0.1	13.01

In the sericulture farm the overall parameters such as matured larval weight (g) ,cocoon weight (g), shell weight(%) and shell ratio between controlled and disease silkworms had been taken and portrayed in table-3.

In the control group the weight of matured larvae was 2.55 g , the cocoon weight was 1.77 g and shell weight was 0.35g, and the overall shell ratio is 17.04%, but the diseased silkworm group the larval weight reduced to 2.07g cocoon weight 0.75g, shell weight 0.1 and the shell ratio reduced as much as to 13.01%. Thus the parameter clearly showed that if any larval forms would have severely affected by various kinds of infection and diseases that would cause prompt effect on the individual worm. Moreover the quality and weight of the cocoon, shell and shell ratio also reduced.

Discussion

There are several silk worm species are cultured for the production of silk. Among which the *Bombyx mori* is the most widely and intensively used for studying. The mulberry silkworm is easily susceptible to various diseases and is affected by parasites and pests. Various types of silkworm diseases are identified from the larval forms to the adult moth stage are caused by virus, bacteria, fungus and protozoa. When the larvae are infected with such a kinds of pathogens, the mortality rate is increased which ultimately reduce the quality and quantity of cocoon that would cause a great economic loss. In view of this the silkworm diseases are to be treated seriously otherwise it can create epidemic.

Many researchers worked on the control and prevention of silkworm diseases caused by microbes is flacherie, grasserie, muscardine and pebrine during rearing helps to increase the silk productivity by preventing the mortality to a great extent. The report was stated by Das and Shamsuddin, (2006) that temperature played a vital role on the growth of the silkworms. As silkworms are cold-blooded animals, temperature have a direct effect on various physiological activities. The temperature had a direct correlation with the growth of

silkworm; wide fluctuation of temperature was harmful to the development of silkworm.

Rahmathulla, (2014) stated that water is an essential requirement for metabolic activity and optimum growth. At higher temperature probably evapo-transpiration at body surfaces and respiratory epithelium of tracheal system significantly increases. The problem of water balance in silkworm at ambient temperature is further complicated by poor moisture content of the leaf, which finally affects the growth and productivity of Silk yield. Silkworms are greatly affected by White muscardine disease due to *B. bassiana* causesa which cause the cocoon yield loss upto 30 percent almost throughout the year (Lakshmi *et al.*, 2013).

In the present study the silkworm was heavily infected by white muscardine disease. This report was similar to the report stated by Chandrasekaran and Nataraju, (2008) who revealed that silkworm showed symptoms of white muscardine caused by fungus *Beauveria bassiana* the most common one in India.

In the present study the bacterial disease flacherie was infecting the IV and V instar in their later part of their life cycle. The result of the present study was similar to the study of Nataraju *et al*., (2005) who stated that the major fact responsible for bacterial flacherie was the rearing conditions. The rise in temperature and humidity in rearing place leads to dysfunction of alimentary canal which encourages flacherie.

The mummified stage of fungal disease was seen in the present study which was considered as highly contagious and dreadful. The whole body covered with white powdery mycelium and produces millions of conidia except the chitinous parts of the head region. It was proved by the report of Ishikawa & Miyajima, (1964). They portrayed that mummified larva remains hard, do not decay, spoil or smell.

The present study of fungal diseases was evidenced from the report of Bulmer & Formtling, (1983). Their report showed that infected survival larvae were spun the cocoons and unable to emerge as silk moth due to secondary infection was found in pupal stage. The Muscardine infection was due to body contamination by fungus and direct penetration by germ tube. This disease was acute with young worms and chronic with adult worms.

Many studies were carried out in India and other sericulture countries on white muscardine infecting the silkworms. Among different types of muscardine, white muscardine was the most common, caused by *Beauveria bassiana* (Bals.)

In the present study reported that muscardine was common and highly infected the

silkworm under rearing condition. According to the report of Samson et al.,(1990) and Anon, (1992), the muscardine of *B. bassiana* was a well known entomopathogen of worldwide distribution (Low temperature and relatively high humidity) played a great role for spread and development of muscardine disease in the rearing bed. Highest rate of infection and mortality was found during rearing, similarly there was a possibility for larvae to get infected either through food or other sources of contamination. Sometimes few worms are infected, it spreads within the host and affected worms release pathogens either through excreta or by direct contact leading to the secondary infection. This may ultimately lead to the spread of diseases in the rearing bed.

The incidence of muscardine disease was caused by high humidity and low temperature. The present study report was proved from the study of Samson et al., (1990). His investigation report stated that fungus infect primarily the third and fourth instars silkworm but the disease symptoms appear at late stage of infection and affected all stages of life cycle of silkworm. In Several reports from farmer of different Sericultural areas in India exhibit the cocoon crop loss due to silkworm diseases.

The present study was carried out between the 10th January and 31st January. Since it was the winter season, the incidence rate of infection was high. It was evidenced from the report of Dasgupta,(1961). He reported that major silkworm diseases caused by Grasserie (virus), Flacherie (bacteria), Muscardine (fungi) and Pebrine (protozoan /microspordian). Among the fungal diseases of silkworm, white muscardine and green muscardine possess a major threat to silk cocoon production during rainy and winter seasons as these two seasons were congenial for the spread of these diseases (Sengupta *et al.*, 1990).

Mummified larva looks like white chalky piece, different stages of diseased, healthy and dead larvae have been seen. The mummified stage considered as highly contagious and dreadful. The whole body covered with white powdery mycelium and produces millions of conidia except the chitinous parts of the head region. Mummified larva remains hard, do not decay, spoil or smell, unlike other diseased larvae of grasserie, flacherie and pebrine. Infected survival larvae were spun the cocoons and unable to emerge as silk moth due to secondary infection was found in pupal stage. These infectious microbes cause secondary infection and spread diseases stated by (Ishikawa & Miyajima, 1964).

According to the report of Ishikawa and Miyajima, (1964) no conspicuous

symptoms were noticed immediately after inoculation of fungal pathogen *Beauveria bassiana*. This has been proved in the present study which revealed that the infected silkworm became inactive, sluggish, stopped the feed underneath the mulberry leaves. After 48hours, the infected worms started to vomit digestive and remained juice 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 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 the white mycelia and finally conidia developed on the body. 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 produces flimsy cocoons. Cocoons formed by these infected worms were smaller and lighter in weight and the worms not emerged as moths, (Seema et al., 2019). The matured larval, cocoon and shell weight significantly reduced in the heavily infected silkworms. It was proved from the report of Seema et al.,(2018**)** who stated that the overt changes observed in the economical traits of cocoon in *Beauveria bassiana* infected silkworm. Appropriate matured larval weights are an indicator to measure the health of silkworm and in turn to obtain good quality of cocoon. Cocoon weight is an important commercial character used to determine the amount of raw silk that can be obtained, (Seema et al., 2018).

The fineness of cocoon filament is expressed by size i.e. denier. Reduction in the matured larval weight may be due to the consequence of fungal infection that leads to the decrease in food consumption, digestion, relative consumption rate, efficiency of conversion of ingested food in fifth instar of *Bombyx mori*, (Raj *et al* (2002). The shell weight is more important than the cocoon weight since the shell yields the silk for reeling. Thus, higher the weight of the shell, greater will be the silk yield with the references cited in the discussion, it was concluded that the silkworm larvae might have been affected by physical parameters such as temperature and humidity. It might have severely been affected by the pathogens such as protozoan, bacteria and fungi. Utmost care must be given to rearing silkworm and their various levels of instar from II to V stage in the sericulture farm that would prevent the culturist from economic loss.

Conclusion

When periodically the sericulture farm was visited to observe incidence of pathogenecity of silkworm *Bombyx mori* in the sericulture unit, various kinds of silkworm diseases such as White muscardine and Flacherie were identified. The form of white sporulation, vomiting and colour changes also noticed in at the rearing unit. The infected silkworms were collected from random sampling method. The percentage of prevalence rate of infection was identified at different instars were recorded.

It was concluded that the number infected silkworm was measured totally 2160 among which the larvae infected with White muscardine during II Instar were161, III Instar 427, IV instar 705 and V instar 857. The silkworm infected with flacherie disease was seen in the IV instar, totally 10 silkworms were reported to have flacherie. White muscardine was found from 2^{nd} to 5^{th} instar stage and it affected the total number of 2160 silkworm. Flacherie diseases were found only in 4^{th} instar laeval stage and it affected the total number of 10 silkworms. The results achieved were discussed with the relevant literature.

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