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Research Article

Assessment and Management of Silkworm Diseases and their Effect on Silkworm Growth and Cocoon Production

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Abstract | The present study was carried out to assess and manage silkworm diseases and to study their effect on silkworm growth and cocoon production during silkworm rearing seasons at Sericulture Laboratories of Pakistan Forest Institute, Peshawar. Silkworm is an important primary productive insect species reared to boost the sericulture industry. Silkworm eggs were incubated under laboratory conditions at 25±2 °C temperature and 70±10% RH up to 5th larval instar which is optimal level for successful development of silkworms. Most common diseases that affect silkworms are pebrine, grasserie, flacherie and muscardine which can severely impact silkworm growth and cocoon production leading to significant economic losses. The infected larvae are smaller in size, had lower body weights and showed delayed developmental stages results in lower quality silk and cocoon production. Healthy and infected silkworm larvae, pupae were assessed for visual inspection of diseases. The quality of the cocoons were assessed based on their size, shape, color and texture. Any irregular or abnormal features were recorded. The study comprised of 15 number of larvae and cocoons of Chinese C-21 strain. The parameters that were studied in the present experiment and recorded were larval weight of full grown larvae of Batch I (1.9-3.6 gm) and Batch II were (1.6-2.6 gm), cocoon weight of Batch I (1.4-2.5 gm) and Batch II were (1.3-1.7 gm). Thirty larvae of silkworm was examined and it was observed that 10 larvae out of 30 were infected with some kind of pathogens. The percentage of healthy silkworms larvae were 67% and the infected were of 33%. The overall pupal mortality were calculated as 4% in which 3.1% mortality was caused by bacterial disease, 0.5% mortality was caused by viral disease and 0.4% pupal mortality was due to fungal disease. Infected larvae negatively affects cocoon production. So the effect of diseases on cocoon yield and quality were determined by calculating the percentage of good and bad cocoon production. In sampling of 30 cocoons, 67% were good cocoons and 33% were bad. The study shows that carefully controlling the temperature and humidity levels and managing the feeding of silkworms ensure the optimal growth and development resulting in healthy and productive silkworms which minimize the chances of disease spreading.

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Introduction

Silkworms (*Bombyx mori)* are insects that belong to the order Lepidoptera is an important primary productive insect species reared to boost the sericulture industry. Sericulture process involves rearing silkworms on mulberry tree leaves and harvesting the silk fibers produced by the silkworms to create silk fabric usually in controlled environments (Krishnaswami *et al*., 1973). The silkworms spin cocoons which are carefully collected and processed to extract the silk fibers. This process requires careful management of the silkworm lifecycle including temperature, humidity and feeding schedules (Shen *et al*., 2011). It requires specific knowledge and techniques to ensure the well-being of the silkworms and the quality of the silk.

Silkworms are the larvae of silk moths and have a cylindrical body shape with chewing mouthparts. Female silkworms are generally larger than males. The female lays eggs which hatch into silkworm larvae. Silkworms typically have a creamy white in color. The size of silkworms depends on their age and developmental stage. Optimal temperature (25°C) and humidity (75%-95%) is required for successful development of silkworms (Wang *et al*., 2015). Silkworms are highly valued for their silk production and are commonly reared in many parts of the world for this purpose. However like any other living organism silkworms are susceptible to various diseases caused by bacterial, viral, fungal and protozoan pathogens that can significantly impact their growth and cocoon production (Sathe *et al*., 2018).

One of the most common disease that affect silkworms is called pebrine which is caused by the microsporidian parasite *Nosema bombycis*. Pebrine can severely impact silkworm growth and cocoon production leading to significant economic losses in sericulture industries (Patil *et al*., 2014). The infected larvae are smaller in size, had lower body weights and showed delayed developmental stages (Shetty and Chadda, 2006). Pebrine-infected silkworms also produced fewer and smaller cocoons compared to healthy silkworms. The infected silkworms showed a reduction in silk production and lower quality silk fibers (Nagmani, 2013). Infected silkworms exhibited higher mortality rates compared to healthy silkworms. The disease weakens the immune system of the silkworms making them more susceptible to other infections and complications which can further impact their growth and cocoon production (Rajendran and Sathiah, 2007).

Other diseases of silkworms are Grasserie, caused by the *Borrelina virus*, also known as the *Bombyx mori* nuclear polyhedrosis virus (BmNPV), is a significant disease in silkworms that infects the larvae and leads to high mortality rates and reduced cocoon quality. Infected silkworms often display stunted growth and decreased weight gain compared to healthy individuals (Devi and Pandiarajan, 2019). Flacherie, another major disease, is primarily caused by the bacterium *Pseudomonas aeruginosa*. This bacterial infection significantly impacts silkworm growth and cocoon production, resulting in reduced growth rates and high mortality among infected individuals, often linked to poor rearing conditions and inadequate sanitation (Gholap *et al*., 2018; Ramachandra *et al*., 2015). Additionally, muscardine, caused by the fungal pathogen *Beauveria bassiana*, negatively affects silkworm growth and cocoon production. Infected larvae exhibit white, powdery fungal growth on their bodies, leading to reduced larval weight and higher mortality rates compared to healthy ones (Liu *et al*., 2014). These diseases collectively pose substantial challenges to silkworm rearing, significantly affecting productivity and cocoon quality.

Assessing and effectively managing silkworm diseases is crucial for maintaining healthy populations and maximizing silk production. This study aims to identify and characterize various types of diseases affecting silkworms, including viral, bacterial, fungal, and protozoan infections. By evaluating the impact of these diseases on silkworm growth, larval survival rates, cocoon quality, and silk production, the research study will contribute to the development of effective management strategies. Once the diseases are assessed, effective management strategies can be implemented to control and prevent further infections (Fan *et al*., 2015).

Materials and Methods

The present experiment was conducted to assess and manage silkworm diseases and to study their effect on silkworm growth and cocoon production at Sericulture laboratories of Pakistan Forest Institute, Peshawar during rearing seasons of 2023. The following procedures were adopted for conducting the research work.

Materials

Healthy and infected silkworm larvae and pupae were visually inspected for diseases. Pathogen features, such as fungal spores or bacterial cells, were examined using a compound microscope with suitable magnification. A magnifying glass was employed to observe small lesions, discolorations, spores, or other signs of disease on the silkworms. A suitable light source, such as a desk lamp, was used to illuminate the silkworms during inspection. Plastic trays of appropriate size and design housed the silkworm larvae throughout the experimental period. Tools like forceps or a brush were used to handle individual silkworms during inspection.

Methods

Visual inspection was performed on the silkworm larvae, pupae, and cocoons to identify and assess the presence of diseases. The assessment involved determining external symptoms and signs indicating various silkworm diseases with careful observation and examination of the silkworms, rearing environment and exhibited symptoms. This process aids in the identification, diagnosis and monitoring of diseases, as well as the implementation of appropriate control measures.

First, a detailed visual examination and assessment of silkworm larvae were conducted. The larvae were taken out from their rearing container and placed on a clean tray for better visibility. The external appearance of the silkworms was carefully observed for any abnormalities or signs of disease including changes in color, size, shape, and texture of the body as well as the presence of lesions, deformities or unusual behaviors. The body color and general appearance of the silkworm larvae were noted and any unusual discoloration, spots or abnormal growth were recorded. The locomotion and behavior of the larvae were observed with unusual sluggishness, excessive crawling or any abnormal movement being noted. The external structures of the silkworm larvae including the legs, head, prolegs, and mouthparts, were examined for any signs of abnormalities, deformities or damage. The quality of the cocoons was assessed based on their size, shape, color, and texture with any irregular or abnormal features being recorded. Infected silkworm larvae or tissues showing signs of disease such as abnormal behavior, discoloration or lesions were also recorded.

Material was collected from different stages of the silkworm lifecycle including larvae, pupae, and adults. Before examination, the collected material was typically fixed in a suitable fixative compound such as formalin or alcohol to preserve cellular structures and prevent degradation. After fixation, the material was processed for sectioning, staining, and mounting on a microscope slide. Microscopic examination can reveal the presence of pathogenic organisms such as bacteria, viruses, fungi or parasites within the tissues. It also allows for the identification of specific morphological or cellular abnormalities associated with the disease such as necrosis, inflammation or tumors.

In addition to observing the silkworm larvae, the examination also included inspection of the rearing environment which was assessed periodically. This involved checking the humidity, temperature, cleanliness and ventilation of the rearing trays or boxes. Efforts were made to identify and eliminate any potential sources of infection or stress for the silkworms such as contaminated food, overcrowding or inadequate hygiene. Different management strategies were implemented to control diseases affecting silkworm growth and cocoon production. These strategies included regular cleaning of rearing equipment, trays and tools to minimize the spread and contamination of diseases. The rearing environment was optimized by maintaining appropriate temperature and humidity levels, regularly removing waste and providing adequate ventilation. Beneficial microorganisms such as Bacillus thuringiensis were used as biocontrol agents to control disease-causing pathogens. This organism can outcompete or produce toxins against pathogens protecting silkworms from infections. High-quality mulberry leaves were provided ensuring that the feed was free from contaminants and of good nutritional value which helps in preventing diseases and is essential for the overall health and immunity of silkworms. Regular monitoring and surveillance of the silkworms for any signs of diseases facilitated early detection and prompt action to prevent the spread of diseases among the silkworm population. Disinfectants like potassium permanganate or hydrogen peroxide in diluted form were used to clean the rearing trays avoiding any harm to the silkworms. Any silkworm larvae exhibiting symptoms of disease were isolated immediately to prevent the spread of infection to healthy silkworms.

Results and Discussion

The present experiment was conducted to assess and manage different diseases of silkworm affecting the silkworm growth and cocoon production at Sericulture laboratories of Pakistan Forest Institute, Peshawar. The data were recorded on the following parameters discussed below:

Incubation of Bombyx mori eggs

The 4 gram of silkworm eggs were kept in an incubator under the required optimum conditions including humidity (75-85%), temperature (25-26°C) and light for successful hatching for the rearing season as shown in Table 1.

Table 1: *Incubation of Bombyx mori eggs.*

Days	Humidity (%)	Light (Lux)	Temperature (°C)
1-3	70-75	30-50	20
4	76-80	30-50	20
5-6	80-85	30-50	23-25
Till Hatching	80-85	30-50	25-26

Larval weight and cocoon weight of Batch I

After 10-12 days, the eggs were hatched into larvae and were transferred into rearing trays where they actively feed on mulberry leaves and go for their further development. After 5 mounts, they were ready to spin cocoon. Examination at this stage involve the observation of larval and cocoon weight in grams as shown in Table 2. The maximum and minimum larval weight were recorded 3.6g and 1.9g while the maximum and minimum cocoon weight were recorded 2.5g and 1.4g respectively as shown in Table 2 with the average larval weight 2.83g and average cocoon weight 1.98g.

Table 2: *Larval weight and cocoon weight of Batch I.*

Range	Larval weight (g)	Cocoon weight (g)
Mean	2.83	1.98
Maximum	3.6	2.5
Minimum	1.9	1.4

Larval weight and cocoon weight of Batch II

The eggs that were not hatched were kept further in incubator for few more days under the required range of humidity, light and temperature. After 3 more days of incubation, the eggs hatched into larvae and were considered as Batch II. Similarly batch I the larvae were taken out from incubator and transferred to rearing trays to feed on mulberry leaves. It was observed that the larvae consume less amount of mulberry leaves as compare to the earlier ones. Other abnormal behavior were also observed including stunted growth and slow cocoon formation. The cocoon filament formed by these larvae were also weak. Before their microscopic examination, the weight of cocoon and larvae were recorded in grams for better results as shown in Table 3. The maximum and minimum range of larval weight that recorded were 2.6g and 1.6g, respectively. The maximum and minimum range of cocoon weight recorded were 1.7g and 1.3g, respectively as shown in Table 3 with the average larval weight 2.05g and average cocoon weight 1.52g.

Table 3: *Larval weight and cocoon weight of Batch II.*

Range	Larval weight (g)	Cocoon weight (g)
Mean	2.05	1.52
Maximum	2.6	1.7
Minimum	1.6	1.3

Percentage of larval mortality of silkworms in Batch I and Batch II

Microscopic examination was done for both the larvae of Batach I and Batch II. Both the larvae were of Chinese variety C-102 strain of silkworm. It was observed that 10 larvae out of 30 were infected with some kind of pathogens.

Detail examination of 10 larvae were carried out to determine the pathogen that infect the silkworm larvae. It was observed that the 4 larvae out of 10 were attacked by the bacteria "*Nosema bombycis*" and showed the symptoms of pebrine disease that including the loss of appetite, delay moulting, retarded growth, pale colour and the shiny oval shaped spores appear under microscope as shown in Table 4. Similarly other 3 larvae out of 10 were attacked by "*Borrelina virus*" and showed the symptoms of viral diseases as shown in Table 4 that is grasserie and flacherie diseases of silkworm while remaining 3 larvae were attacked by fungus "*Beauveria bassiana*" and showed the symptoms of fungal disease as shown in Table 4 which is a muscardine disease of silkworm. The percentage of healthy and infected silkworms were also calculated as shown in Table 4. The percentage of healthy silkworms were 67% and the infected were of 33%.

Table 4: *Percentage of larval mortality of silkworms in Batch I and Batch II.*

Variety	No. of specimens examined	Infected specimens			% of healthy specimens	% of infected specimens
		By bacterial disease	By viral disease	By fungal disease		
Chinese C-102	30	04	03	03	67	33

Percentage of pupal mortality of silkworms in Batch I and Batch II

Percentage of pupal mortality due to bacterial, viral and fungal diseases were also calculated. The overall pupal mortality were calculated as 4%. Out of 4% the 3.1% mortality was caused by bacterial disease, 0.5% mortality was caused by viral disease and 0.4% pupal mortality was due to fungal disease as shown in Table 5.

Table 5: *Percentage of pupal mortality of silkworms in Batch I and Batch II.*

Variety	% of pupal mortality due to			% of Total mortality
	Bacterial disease	Viral disease	Fungal disease	
Chinese C-102	3.1	0.5	0.4	4

Cocoon grading of silkworms of Batch I and Batch II

Infected larvae negatively affects cocoon production. So the effect of diseases on cocoon yield and quality were determined by calculating the percentage of good and bad cocoon production. In sampling of 30 cocoons, 67% were good cocoons and 33% were bad as shown in Table 6.

Table 6: *Cocoon grading of silkworms of Batch I and Batch II.*

Variety	Total cocoon examined	% of good cocoons	% of bad cocoons
Chinese C-102	30	67	33

Silk production is an important industry that heavily relies on the cultivation of silkworm. A major problem for the silk production is the diseases of silkworm larvae. The different pathogens including bacteria, virus, fungus and other protozoans can attack and leads to the chronic diseases such as pebrine, grasserie, muscardine and flacherie which causes severe economic loss in sericulture by affecting the growth and cocoon production of these insects. The study determines the number of healthy and infected silkworms and differences in their cocoon production and growth parameters. For this purpose, the eggs of silkworm were incubated under the required climatic conditions. Silkworm eggs were incubated under laboratory conditions at 25±2 °C temperature and 70±10% RH up to 5th larval instar in a 15 x 14 ft rearing room. The range of temperature, humidity and light that provided to silkworm eggs were almost similar to (Shah *et al*., 2007). Two days before the expected hatching date, care was taken with the eggs laid on cards. Each egg was wrapped in wax paper to ensure optimal protection. Once the moment of hatching arrived, a feather was used to softly brush away any remnants and the newly hatched offspring were gently transferred onto a tray. Prior to this process, a layer of moist newspaper was thoughtfully placed at the bottom of each rearing tray creating an ideal environment. To preserve the critical humidity levels, a protective plastic sheet was carefully draped over the trays. To monitor the crucial element of temperature a thermometer recorded every fluctuation. The mean larval weight and cocoon weight of infected larvae were 2.05g and 1.52g respectively. The recorded weight range is low as compared to the study done by Suraporn and Terenius (2021) in which the larval weight obtained were 2.70g and cocoon weight obtained were 1.33g. The *Lactobacillus casei* supplementation had positive impact on the growth characters. Silkworm infections significantly lowered the cocooning ratio, but *L. casei* treatment made the ratio remain on an intermediate level (Suraporn and Terenius, 2021; Sudo and Watanabe, 2020). The cocoon productions by infected larvae were 33% and pupation ratio were calculated as 96%. The cocoon production and pupation ratio is also smaller as compared to the study done by (Suraporn and Terenius, 2021; Rahmathulla, 2012). The pupation ratio was 100% in the groups of healthy silkworms. The *L. casei* treatment resulted in a significantly higher pupation ratio in infected larvae which was calculated 95% with and cocoon ratio which was also 95% calculated (Suraporn and Terenius, 2021). The reason behind the variation in both the results can be attributed to the findings of the study conducted by Suraporn and Terenius (2021). In their study the infected larvae were supplemented with *Lactobacillus casei* which resulted in larvae developing resistance to various diseases. Consequently this led to lower rates of larval and pupal mortality and a significant increase in cocoon production. The inclusion of the

'*Lactobacillus casei*' supplement played a crucial role in enhancing the outcomes of the study. It is important to acknowledge that this report has its limitations. The scope of this study is limited to the assessment and management of silkworm diseases affecting growth and cocoon production. Other factors such as environmental conditions genetic factors and rearing practices may influence disease occurrence but they are not specifically addressed in this report. Additionally the discussion on management strategies and control measures is subject to variations in regional conditions, availability of resources and the economic feasibility of different approaches.

Conclusions and Recommendations

Silk production is a crucial industry reliant on the cultivation of silkworms (*Bombyx mori*) but various diseases can significantly hinder their growth and cocoon production causing substantial losses. This study aimed to comprehensively assess and manage silkworm diseases through visual inspection and microscopic examination, identifying factors that impede growth and production. Observations during the rearing season revealed that diseases notably affect larval and pupal stages with bacterial infections causing higher mortality rates compared to viral and fungal diseases. The study underscores the urgent need for proactive disease management to ensure silkworm health and productivity. Future research should explore integrating molecular techniques, such as PCR, for rapid pathogen detection and developing disease-resistant silkworm strains through selective breeding or genetic engineering. These advancements will enhance disease control, improve silkworm productivity and support the sustainability of the silk industry.

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Novelty Statement

By controlling environmental conditions and feeding practices this study demonstrates that effective management of silkworm diseases leads to improved growth and cocoon production. This approach results in healthier, more productive silkworms and reduces economic losses associated with disease outbreaks.

Author's Contribution

Muhammad Salman: Conceived the research idea and designed the study, analyzed the data and wrote the paper.
Muhammad Atif Majeed: Managed the literature review, provided critical insights and helped with the final editing and proofreading of the manuscript.
Naveed Ahmed: Provided overall guidance throughout the research study.
Mir Manzar Ud Din: Reviewed for finalizing and correction.
Arsalan Ali: Data analysis and reviewed experimental progress.
Hina Gul: Performed detailed corrections and refined the manuscript.
Uzair Hussain Shah: Data Collection and supervision.
Umer Saeed Khan: Data Collection.

Generative AI and AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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