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STUDY ON MODULATIONS IN PROTEIN CONTENT IN THE SILKWORM BOMBYX MORI INOCULATED WITH THE FUNGAL PATHOGEN BEAUVERIA BASSIANA AND TREATED WITH PLANT POWDER

Entomology

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ABSTRACT

The silkworm Bombyx mori is a biological machine that produces mulberry silk with a lot of economic significance. Silkworms are susceptible to a variety of pathological agents that influence the qualitative and quantitative aspects of the silk cocoons. The biomolecule traits reflect the physiological condition of an organism and also may guide the potential use of biochemical markers to evaluate the health status of silkworms. The fungal pathogen Beauveria bassiana, a destructive pathogen that is highly prevalent in rainy and winter seasons, is confined to haemolymph until the death of the host. The precise understanding of the interaction between biomolecules in the ha emolymph, the host, and the pathogen in response to infection and treatment with herbal powder is essential. Therefore, the present study focused on examining changes in protein levels in the haemolymph of silkworm Bombyx mori that were infected with the fungal pathogen Beauveria bassiana, the causative organism of white muscardine disease, and treated with different herbal powders, as biomolecule-like protein traits reflect the physiological condition of an organism. Silkworms were inoculated with the fungal pathogen Beauveria bassiana in sublethal concentration immediately after the fourth moult and then treated with herbal powder in four concentrations (25%, 50%, 75%, and 100%). Gradual enhancement of protein levels was noticed in the silkworms inoculated with Beauveria bassiana and treated with nine herbal powders, namely, Phyllanthus emblica, Azadirachta indica, Ocimum tenuiflorum, Cymbopogon citratus, Psidium guajava, Murraya koenigii, Sapindus mukorossi, Manilkara zapota, and Lawsonia inermis, in combination with slaked lime powder, with reference to the fungal pathogen-inoculated silkworm. Herbal powders offer a wide array of health advantages and are eco-friendly and natural, helping prevent the attack of diseases in silkworms and boost cocoon quality.

KEYWORDS

Bombyx Mori, Beauveria Bassiana, Azadirachta Indica, Haemolymph, Proteins

INTRODUCTION

The silkworm *Bombyx mori* is an insect with a lot of economic significance and a biological machine that manufactures mulberry silk. Sericulture is a sustainable farm-based sustainable economic enterprise with a relatively low fixed capital and higher returns on investment and positively favoring the rural poor. Sericulture activities encompass a wide range of income and employment-generating vocations in both rural and urban areas. The success of the silk industry majorly relies on the successful harvest of cocoon crops.

Silkworms are prone to different diseases caused mainly by four groups of microorganisms, viz., viruses, protozoans, bacterial pathogens, and fungal pathogens. Most of the pathogens occur in the environment itself. The silkworms exposed to various pathogens lead to disease breakouts and mortality in silkworms, which leads to cocoon crop loss and, in turn, reduces profit. Among the major diseases affecting silkworms, white muscardine caused by *Beauveria bassiana* (Bals.) Vuill. is one of the destructive and infectious diseases of Bombyx *mori, which* is common in rainy and winter seasons and inflicts heavy loss on the cocoon crop every year. According to Chandrasekharan and Nataraju (2008) and Aina Bhat (2021), in India the average cocoon crop loss due to diseases is around 15–47%, while it is 10–15% in other leading sericulture countries. In the total cocoon crop loss, a 10-40% loss is accounted for by muscardine.

Chemical-based bed disinfectants are being used to manage the diseases in the silkworm *Bombyx mori*; these disinfectants leave residual toxicity in the environment. Using plant powders/extracts is an alternative to tackle the issue. Curative properties of herbs are being increasingly studied in different parts of the globe. Herbal plants have a variety of secondary metabolites and biologically active constituents that impact the physiological functions of insects. Gayathri (2007) stated that botanicals possess properties influencing the silk gland proteins in silkworms. Mathavan *et al.* (1984) reported that spirulina protein catalyzes a better conversion of proteins consumed into silk protein.

Haemolymph is the life-saving extracellular circulatory fluid in insects, with diverse functions similar to blood and lymph in vertebrates. It supplies nutrients, hormones, and immune cells throughout the open body cavity. The degree of variations in biomolecules like carbohydrates, proteins, lipids etc., in the haemolymph can act as an index to assess the health status of silkworms, as haemolymph is a physiological pool for all the biomolecules required for all the metabolic activities.

Biomolecules, viz., carbohydrates, proteins, lipids, etc., are the basic chemical constituents of living systems and enable all physiological, structural, and metabolic functions. Proteins are essential and abundant macromolecules with diverse functions in living systems, involved in every biological activity and indispensable for life. Cozzone (2010) stated that the structural arrangement enables proteins to perform a wide range of functions, from catalyzing biochemical reactions to providing structural support.

With this backdrop, the study examined the impact of nine herbal powders, namely, Phyllanthus *emblica, Azadirachta indica, Ocimum tenuiflorum, Cymbopogon citratus, Psidium guajava, Murraya koenigii, Sapindus mukorossi, Manilkara zapota*, and *Lawsonia inermis*, on the changes in protein molecules in *Beauveria bassiana*-infected silkworms and in inoculated worms treated with the herbal powders mixed with slaked lime in four ratios. in comparison to untreated silkworms (control).

Methodology

For the study, bivoltine double hybrid (CSR2 X CSR27) X (CSR6 X CSR26) was selected, and chawki worms were procured from Chawki Rearing Centre (CRC), Vedurukuppam, Chittoor (Dist.). Silkworm stock was maintained according to Dandin *et al.* (2003) for the experiment with meticulous synchronisation of several factors required for the healthy growth of silkworms. The silkworms were taken to experiment with the stock at the beginning of the 5th instar.

Beauveria bassiana culture was maintained on potato dextrose agar media. After achieving the required growth, conidia from a single colony were harvested from the *Beauveria bassiana* culture and transmitted to PDA slants, and the pure culture was maintained through repeated transfers every 15 days. The culture of *Beauveria bassiana* was maintained under absolutely sterile conditions.

Fresh and matured leaves of *Phyllanthus emblica* (Usiri), *Azadirachta indica* (neem), *Ocimum tenuiflorum* (Tulasi), *Cymbopogon citratus* (lemongrass), *Psidium guajava* (guava), *Murraya koenigii* (curry leaf), *Sapandus mukorossi* (Indian soap berry), *Manilkara zapota* (sapota), and *Lawsonia inermis* (Henna) were collected in fresh condition and taken to the laboratory of the Department of Biosciences and Sericulture, SPMVV, Tirupati. The leaves were thoroughly cleaned with distilled water, and then the leaves were shade-dried. The dried leaves were ground into a fine powder and kept in airtight containers for the experimentation.

The fine herbal powders of the nine medicinal plants have been mixed

with slaked lime in four ratios: 25% (25 grams of herbal powder + 75 grams of slaked lime), 50% (50 grams of herbal powder + 50 grams of slaked lime), 75% (75 grams of herbal powder + 25 grams of slaked lime), and 100% (100 grams of herbal powder) for the experiment.

Silkworms were inoculated with *Beauveria bassiana* inoculum in sublethal concentration (2.15×10^4 conidia/ml) per treatment. After 2 hours of inoculation, the silkworms were treated with herbal powders and slaked lime mixtures in four concentrations, i.e., 25%, 50%, 75%, and 100% @ 5 grams/sq. ft. Every day before the first feeding, silkworms were dusted with an herbal mixture. from the 1st to the 6th day of the 5th instar silkworm. Three replications were maintained @ 100 silkworms/replication. Two controls were maintained, i.e., silkworms inoculated with a fungal pathogen and another normal control without any treatment.

Haemolymph was collected daily from the first to the sixth day from silkworms inoculated with the fungal pathogen and treated with 9 herbal powders mixed with slaked lime in 4 different ratios, along with both control groups. The silkworms were selected randomly for the collection of haemolymph, then the worms were cleaned and kept in ice for 2-10 minutes. Haemolymph was collected by cutting the 3-5 abdominal legs in pre-chilled centrifuge tubes. Then the haemolyph has been taken for the assessment of total proteins in the experimental and control batches. Total proteins were estimated by the method of Lowry et al. (1951). The data have been recorded and statistically analyzed by using three-way ANOVA, and the total protein levels were expressed as mg/ml of haemolymph.

RESULTS AND DISCUSSION

Biochemical analysis is crucial for understanding the physiological and biological processes related to health status. Consequently, knowledge of biochemical changes aids researchers in comprehending the underlying mechanisms of diseases, monitoring these conditions, and developing prophylactic strategies. Proteins are vital biomolecules, widely associated with all biological functions. Proteins are essential molecules, especially in *Bombyx mori*, primarily for the development of silk glands and structural growth. Understanding biological relationships between proteins and pathogens and the dynamics of biomolecules of silkworms during the progress of the disease and treatment with herbal powder is critical for improving silk yield and combating destructive diseases.

The changes in protein levels in the haemolymph of silkworms inoculated with *Beauveria bassiana* and treated with herbal powders at four ratios are presented in Table -1. A significant elevation in total protein levels was observed in worms treated with almost all herbal powders compared with infected worms. The protein content in the healthy control haemolymph was 43.24±0.1 mg/ml, and in inoculated worms, it was 32.19±0.26 mg/ml.

Dusting of herbal powders (9 plants) in combination with slaked lime in four ratios (25%, 50%, 75%, and 100%) showed significant restoration of protein content in the silkworms inoculated with fungal pathogen *Beauveria bassiana* with reference to inoculated worms and nearly on par with protein levels of normal control silkworms. The most significant restoration of protein content occurred in silkworms treated with *Phyllanthus emblica. This was followed by treatments with Azadirachta indica, Ocimum tenuiflorum, Cymbopogon citratus (lemon), Psidium guajava, Murraya koenigii, Sapindus mukoross, Manilkara zapota,* and *Lawsonia inermis.*

Gradual elevation of protein levels was recorded, viz., 36.62 ± 0.31, 38.53 ± 0.46, 41.37 ± 0.30, and 43.17 ± 0.14 at 25%, 50%, 75%, and 100% ratios of *Phyllanthus emblica* powder + slaked lime. Similar trend was noticed in *Azadirachta indica* and slack lime-treated silkworms in four ratios. The values recorded in four ratios are 36.29 ± 0.12, 38.29 ± 0.16, 41.20 ± 0.22, and 43.08 ± 0.71 at 25%, 50%, 75%, and 100% ratios, respectively. *Ocimum tenuiflorum* also demonstrated substantial recovery of protein levels, viz., 35.90 ± 0.14, 37.63 ± 0.36, 39.81 ± 0.17, and 41.31 ± 0.56 at 25%, 50%, 75%, and 100% ratios, correspondingly. Treatment with *Cymbopogon citratus* produced a similar pattern of improvement of protein levels in different concentrations, viz., 34.82 ± 0.17, 36.66 ± 0.27, 38.85 ± 0.15, and 40.87 ± 0.10 at 25%, 50%, 75%, and 100% concentrations, respectively. The *silkworms dusted with Psidium Guajava* powder *in combination with slaked lime* in four ratios significantly recovered protein levels in a concentration-dependent manner. The protein levels recorded in the treatment were 33.54 ± 0.50, 35.60 ± 0.55, 37.03 ± 0.98, and 40.20 ± 0.07 at 25%, 50%, 75%, and 100% concentrations, respectively. Treatment with *Murraya koenigii* powder in disease-inoculated silkworms significantly restored the protein levels compared to disease-inoculated silkworms. The protein levels recorded in four treatments are viz., 32.87 ± 0.16, 34.73 ± 0.32, 36.67 ± 0.44, and 39.85 ± 0.28 at 25%, 50%, 75%, and 100% concentrations, respectively.

Moderate to low recovery of protein content was observed in silkworms infested with the fungal pathogen *Beauveria bassiana* and treated with herbal powders from *Sapindus mukorossi, Manilkara zapota*, and *Lawsonia inermis*. The protein content in silkworms treated with *Sapindus mukorossi* was recorded as 31.47 ± 0.33, 34.02 ± 0.03, 35.46 ± 0.46, and 39.07 ± 0.57 at concentrations of 25%, 50%, 75%, and 100%, respectively. *Manilkara zapota* treatment resulted in protein levels of 30.46 ± 0.48, 33.67 ± 0.19, 34.24 ± 0.28, and 38.77 ± 0.58, while *Lawsonia inermis* treatment produced values of 30.31 ± 0.23, 33.49 ± 0.25, 34.62 ± 0.39, and 38.46 ± 0.24 at 25%, 50%, 75%, and 100% concentrations, respectively.

DISCUSSION

The present study demonstrates that infection caused a significant reduction in total protein content in silkworms, decreasing from 43.24 ± 0.10 in the control group to 30.19 ± 0.94 in the infected group. Protein is one of the most important biochemical constituents in silkworms, playing a crucial role in growth, metabolism, immune defense, tissue development, and silk protein synthesis. The observed reduction in protein levels following infection suggests severe physiological stress and metabolic disruption in the infected silkworms.

In silkworms, pathogenic infections are known to alter nutrient metabolism by increasing protein catabolism and suppressing protein biosynthesis. During infection, proteins are extensively utilized for the production of immune-related molecules, detoxification processes, and repair of damaged tissues. Additionally, pathogens may consume host nutrients and interfere with digestive and absorptive functions, leading to reduced protein accumulation. The decrease observed in the infected group therefore reflects the adverse effects of infection on the nutritional and physiological status of the silkworm.

Treatment with all plant extracts significantly improved protein levels in a concentration-dependent manner, indicating their ability to alleviate infection-induced biochemical stress and restore normal metabolic activities. The gradual increase in protein content with increasing extract concentration suggests that the active phytochemicals present in these medicinal plants exert protective effects against pathogen-induced damage.

Among all treatments, *Phyllanthus emblica* showed the highest recovery of protein content, reaching **43.17 ± 0.14** at 100% concentration, which was almost identical to the control value. This near-complete restoration suggests that the herbal powders effectively protected silkworm tissues from infection-associated damage. The efficacy of *P. emblica* may be attributed to its abundance of ascorbic acid, phenolic compounds, tannins, and flavonoids, which possess strong antioxidant and antimicrobial properties. These bioactive compounds may reduce oxidative stress, improve nutrient utilization, and promote protein synthesis in infected silkworms(Krishnaveni *et al.,* 2010 and Baliga *et al.,* 2012).

Similarly, *Azadirachta indica* demonstrated excellent recovery, increasing protein levels to 43.08 ± 0.71 at 100% concentration. *Azadirachta indica* is well known for its antimicrobial, antifungal, antibacterial, and immunomodulatory activities. The phytoconstituents present in neem, including azadirachtin, nimbin, and quercetin, may inhibit pathogen proliferation and enhance the silkworm's innate defense mechanisms. Consequently, protein degradation is minimized and normal metabolic activities are restored (Biswas *et al.,* 2002 and Subapriya *et al.,* 2005).

Ocimum tenuiflorum also produced substantial recovery of protein content, reaching 41.31 ± 0.56 at the highest concentration. *Ocimum tenuiflorum* contains eugenol, rosmarinic acid, and various phenolic compounds that possess antioxidant and antimicrobial activities. These compounds may reduce cellular damage caused by infection and support tissue repair processes, thereby improving protein metabolism in silkworms (Pattanayak *et al.,* 2010).

Treatment with *Cymbopogon citratus* resulted in a notable increase in protein levels from 34.82 ± 0.17 at 25% concentration to 40.87 ± 0.10 at 100% concentration. The recovery observed may be associated with the presence of citral and other bioactive constituents that possess antimicrobial and antioxidant properties. These compounds likely help maintain cellular integrity and improve physiological functions in infected larvae (Shah *et al.,* 2011).

The extracts of *Psidium guajava* and *Murraya koenigii* also significantly enhanced protein levels. *Psidium guajava* leaves contain flavonoids, tannins, and phenolic compounds, whereas *Murraya koenigii* are rich in carbazole alkaloids and antioxidants. These phytochemicals may improve digestion, nutrient assimilation, and immune function, thereby contributing to the restoration of protein content in infected silkworms (Ningappa *et al.,* 2007 and Joseph *et al.,* 2011).

Moderate recovery was observed in silkworms treated with *Sapindus mukorossi*, *Manilkara zapota*, and *Lawsonia inermis*. Although these extracts significantly increased protein levels compared with the infected group, their effectiveness was comparatively lower than that of *P. emblica* and *A. indica*. Nevertheless, the observed improvements indicate that these plants also possess bioactive compounds capable of reducing infection-induced metabolic disturbances and enhancing protein retention(Upadhya *et al.,* 2012 and Chetan joshn *et al.,* 2025).

An important observation of the study is the clear concentration-dependent response exhibited by all plant extracts. As the concentration increased from 25% to 100%, protein levels consistently improved. This trend suggests that higher concentrations provide greater amounts of bioactive phytochemicals, resulting in stronger antimicrobial activity, improved antioxidant protection, and enhanced recovery of metabolic functions. Such dose-dependent effects further support the therapeutic potential of these medicinal plants in silkworm disease management.

From a sericultural perspective, restoration of protein content is particularly significant because proteins are essential for larval growth, silk gland development, and fibroin and sericin synthesis. Maintenance of adequate protein levels contributes directly to larval health, cocoon quality, and silk productivity. Therefore, the improved protein content observed in herbal powder-treated silkworms indicates not only recovery from infection but also the potential for better growth performance and silk yield.

Beranhaum (1988) reported that allelochemicals have a deleterious effect on disease causing pathogen of insects and the insects ingesting such allelochemicals could survive infections better. Mallikarjuna *et al.* (2002) observed the enhancement of protein content in *Beauveria bassiana*-infected silkworms treated with systemic fungicide, which is in accordance with the present results. Kiran Kumar *et al.*, (2012) observed an elevation of proteins in the haemolymph of Antheraea mylitta during the 5th instar of the silkworm infested with CPV and treated with *A. barbadensis*, *P. corylifolia*, and *Bougainvillea spectabilis* aqueous extracts. Chavan, J. A. and Bhawane, G. P. (2013) observed that Bacillus thuringiensis-infected silkworms had decreased protein levels compared to the normal control, while silkworms treated with plant extracts exhibited elevated protein levels compared to the infected silkworms and a drop in the protein content compared to the normal control. They stated that the growth of infected silkworms was hindered by the physiological damage, but treatment with plant extracts reduced disease symptoms and improved biomolecule levels in the inoculated silkworms.

CONCLUSION

Overall, the findings suggest that medicinal plant extracts can effectively mitigate infection-induced protein depletion in silkworms. Among the tested plants, *Phyllanthus emblica* and *Azadirachta indica* exhibited the strongest restorative effects that can be considered promising botanical agents for enhancing silkworm health and protecting larvae against pathogen-associated biochemical alterations. A decrease in protein content was observed in silkworms infested with fungi; the drop may be attributed to alterations in protein metabolism caused by the developing fungal pathogen, as well as the cessation of food intake. The herbal treatment promoted growth and silk yield, altering biochemical constituents like total protein levels in the haemolymph. The biochemical study may track the response of herbal powders to the silkworms infested with *Beauveria bassiana*, which is useful to evolve diagnostic markers and prophylactic strategies. The biochemical studies in silkworms inoculated with *Beauveria bassiana* and treated with herbal powders in combination with slaked lime revealed significant improvements, mitigating the fungal infection and restoring physiological balance. The study indicated that treatment with selected plant powders is useful in silkworm disease management. The results support the potential application of these eco-friendly and cost-effective plant-based treatments in sustainable sericulture practices. Therefore, plant powders can be exploited to reduce the occurrence of diseases and to improve cocoon yield.

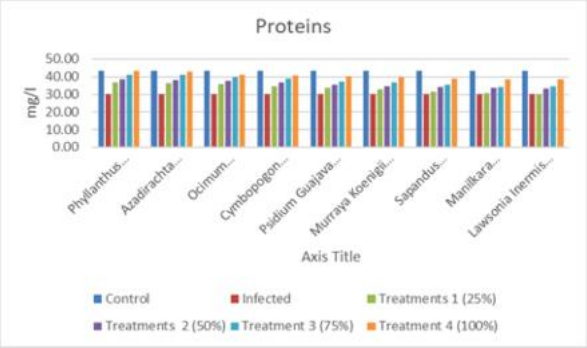

Fig: 1
*all are Significant at 0.01 level

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