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Dynamics of pH and Specific Gravity of Haemolymph in different breeds of Silkworm Bombyx Mori L. Infested with Beauveria Bassiana(Bals) Vuill

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Abstract
The present study concentrated on understanding the influence of Beauveria bassiana infection on the dynamics of pH and the specific gravity of haemolymph in three hybrids of the silkworm Bombyx mori. After being domesticated for millennia, silkworms are now sensitive to a variety of micro-pathogens and environmental factors that might impact the nutrition, metabolism, and physiological characteristics of haemolymph. For the investigation, the researchers employed bivoltine double hybrids (CSR2 X CSR27) X (CSR6 X CSR 26), bivoltine single hybrid (CSR2 X CSR4), and crossbreed (PM × CSR2) silkworm hybrids.
The bivoltine double hybrid, crossbreed, and bivoltine single hybrid showed an elevation of pH level up to the fourth, third, and second days, respectively. In the remaining days of the instar, all three breeds showed a progressive decline in pH. All of the breeds that were chosen for the study had a lower pH level than the control. Lower specific gravity was recorded, and it stayed constant from the first to the fourth day, then significant enhancement of specific gravity was recorded in a bivoltine double hybrid. With reference to the crossbreed and bivoltine single hybrid, it was constant until the third and second days, respectively. The remaining days of the instar in the experimental silkworms were marked by a significant increase in specific gravity.

Keywords: Silkworm Hybrids, Bombyx mori, haemolymph, pH and Specific Gravity, Beauveria bassiana..

I. INTRODUCTION

Sericulture is a labour-intensive, agro-based industry that helps to boost the rural economy by giving abundant self-employment opportunities to people not only in rural but also in semi-urban and urban areas, particularly for women. The promotion of industrial and agricultural interfaces was a strong suit for sericulture, bolstering the rural economy.

Silkworm is a sensitive, tiny insect with very good commercial importance that produces silk, which is very much valued by high-end fashion designers. One of the major constraints of the sericulture industry is the menace of diseases. The loss generally occurs in the last stages of the culturing of silkworms after a significant amount of time and money has been invested. According to Selvakumar *et al.* (2002), the cocoon crop loss in India as a result of silkworm diseases is estimated to be between 27 to 35%, while the cocoon crop loss varies by 11 to 15 kg/100 DFLs depending on the season. According to Chandrasekharan and Nataraju (2008), the cocoon crop loss due to muscarine has been estimated at 10–40% in the total crop loss. The metabolic disorders caused by pathological agents like bacteria, viruses, and fungi show a variety of physiological abnormalities that lead to the death of the silkworm.

Silkworm hybrids play a significant role in determining the quantity and quality of silk produced. Despite being qualitatively superior hybrids, these cultivars are easily damaged by mishandling, which can result in repeated crop failures from illness. Hybrid vigour is an essential tool for evaluating, sustaining, and increasing cocoon production in inbred strains. Additionally, it aids in locating potential hybrids for use in commerce (Nagarajuet al 1996, and Singh et al 2002). Therefore, three popular hybrids viz., bivoltine double hybrid (CSR 2 x CSR 27) x (CSR 6 x CSR 26), crossbreed (PM × CSR2) and bivoltine single hybrid (CSR2 X CSR4)were selected for the investigation.

Haemolymph respond very instantly against adversaries and it is life saving fluid and it performs a variety of functions and acts as a physiological reservoir for all the biomolecules needed for every metabolic process. Additionally, haemocytes in the blood of silkworms plays a significant role in the immune system. When bacteria, fungi, or any other foreign body enters the silkworm body, the innate immune response is set off, and haemolymph plays a crucial part in this process. Numerous roles for haemolymph have been documented, including storage, nutrient transfer, excretion, defence, moulting, and metamorphosis (Mullins 1985).pH indicates the alkalinity and acidity of a solution on a logarithm scale. In insects' the blood pH level is in between 6.4 to 8.0. A well-balanced pH is very much important in living organism for healthy growth and development. Any fluctuation in the pH level of the blood disturbance the level of sodium and chloride ions that leads to respiratory problem consequently it leads to death of the organism. The specific gravity of the haemolymph of insects reflects many metabolic

changes. Abnormalities in the physiology of haemolymph due to stress induced by pathological agents might lead to distinct alterations in the blood. The degree of variation of pH and specific gravity in the haemolymph can act as index to assess the health of any organism. Hence, the present study focused on understanding the kinetics of pH and specific gravity in the blood of the silkworm Bombyx mori in different hybrids treated with Beauvoir bassiana fungal spores.

II. METHODOLOGY :

For the investigation, three silkworm hybrids, viz., Bivoltine Double Hybrid (CSR2 X CSR27) X (CSR6 X CSR 26), Pure Mysore x CSR2 (crossbreed), and CSR2 × CSR4 (bivoltinesingle hybrid), were chosen. The silkworm stock was maintained in the silkworm rearing laboratory under the optimum required environmental conditions, as suggested by Dandin et al. (2003), to carry out the experiment. In the study, pH and specific gravity of blood were analysed in the fifth-stage silkworms treated with the fungal pathogen Beauveria bassiana in the breeds chosen.
For the study, chawki worms were procured from the Chawki Rearing Centre (CRC), Jakkadona, Vedurukuppam (Mandal), Tirupati Division, Chittoor District of Andhra Pradesh, in the cooler hours of the day. Prior to getting the chawki worms, the silkworm rearing house, its surroundings, and rearing appliances had been thoroughly disinfected by using the recommended disinfectants.

Mummified Beauvoir bassiana-infected silkworms were cut into small pieces with a sterile blade, transferred into a Petri plate that contains 20 ml of Potato Dextrose Agar (PDA) media, and incubated for 7 to 9 days at 25°C. Then, an individual colony from the culture was transferred to sterile PDA slants, and maintained the pure cure with repeated transfers every 15 days. From a three-week-old culture, fungal spores were collected into a beaker consisting of 50 millilitres of sterile distilled water and added with a drop of tween-20 to the inoculums.

The LD50 value was calculated by the method of Reed and Muench (1938) for the three breeds chosen for the study of the 5th instar silkworms (2.15 x 106 conidia/ml in crossbreed and bivoltine single hybrid and 2.15 x 105 conidia/ml in bivoltine double hybrid) by using probit analysis (Leora Software, 1987). Accordingly, Beauvoir bassianal spore suspension was prepared from the culture. All the above-mentioned operations were carried out under strict aseptic conditions.

For the experiment, 100 silkworms were selected randomly from the stock per replication and treated with the fungal spore suspension, and silkworms treated with double-distilled water were used as a control. For the study, four replications were maintained. After a day of induction with Beauvoir bassiana spores, the silkworms were taken for day-to day analysis, i.e., from the first day to the sixth day of the 5th stage silkworms. Every day, haemolymph was collected from the experimental and control batches by cutting the third pair of prolegs and adding a pinch of phenylthiourea. Then the blood of the silkworm was taken for analysis of pH and specific gravity.

The pH and the specific gravity of the haemolymph were determined by using a digital pH meter (ELlCO) and following Van Slyke's copper sulphate method by Pilmer (1944).

III. RESULTS AND DISCUSSION

pH

In comparison to healthy controls, the pH values of haemolymph in Beauveria bassiana-infected silkworms were examined during the fifth instar (Graph 1). In untreated worms, elevation pH was recorded from the first day to the fourth day of the 5th instar of bivoltine single hybrid (6.61 to 6.72), bivoltine double hybrid (6.64 to 6.87), and a reduction in pH was noticed in both breeds, i.e., bivoltine single hybrid (6.44 and 6.31) and bivoltine double hybrid (6.74 and 6.76) on the 5th and 6th days of the instar. The elevation of pH was observed up to the 3rd day of the instar in crossbreed (6.62 to 6.73), and on other days, drops in pH levels were observed (6.7 to 6.47).

The bivoltine double hybrid showed an elevation of pH until the fourth day (6.51 to 6.64), after which the pH level began to decline (6.42 and 6.37) on the fifth and sixth days. In contrast, the crossbreed (6.52 to 6.62) showed an increase in pH up to the third day, after which the pH level gradually decreased (6.49 to 6.34). The first and second days of the instar in bivoltine single hybrids (6.6 and 6.63) showed a little increase in pH level; however, from the third day onwards, larvae treated with Beauveria bassiana showed a gradual decline in pH level (6.5 to 6.3). However, lower pH levels were seen in all experimental larval breeds throughout the instar compared to control.

The measure of the degree of acidity or alkalinity, or pH, is the negative logarithm of hydrogen ion concentration. Insect hemolymph typically has a pH of 6.4 to 6.8.Because silkworms have an open circulatory system, the pH of their haemolymph is crucial for the brain's efficient coordination with other organs and body parts.

Pristavko (1967) observed changes in pH levels in the haemolymph of Leptinotarsa decemlineata infected with the fungal pathogen Beauveria bassiana. Any change in the pH concentration disrupts the worm's metabolism. The presence of acidic metabolites in the haemolymph could explain the pH's tendency towards the acidic side (Wiggleworth, 1972).The release of certain chemicals into the haemolymph of diseased worms during their growth phase may be the cause of the pH drop. These compounds are naturally acidic. Secondly, it can be the result of the pathogen breaking down carbohydrates and compounds that are released from the metabolism of carbohydrates.

Finally, the lack of buffer inability, which results in "acidemia," can also be linked to increased metabolites in the haemolymph. It is evident that infectious pathogens possess the capacity to secrete and release certain chemical substances or enzymes. Furthermore, invading agents do take the necessary nutrients from the haemolymph to thrive, which uses the resources needed for host upkeep. Acidity is caused by Beauveria bassiana infection, which also modifies the haemolymph's concentrations of different organic acids.

The pH of 5th instar worms was found to drop abruptly from 6.6 to 6.2 on the first day of infection, followed by an elevation of pH (6.4) on the fourth day. This progressive increase in pH was noted by Kusunoki and Watanabe (1982). However, even though there was a lot of variation, the initial pH level of 6.6 was noted on the fifth day of the fifth instar in healthy worms. In 1990, Ambika noted a decrease in the pH of the haemolymph in all three silkworm races: Pure Mysore, KA, and Pure Mysore x KA. By the fifth day of the fifth instar, the pH of the haemolymph reached an acidic state, showing that infection causes acidemia. The pH drop was greatest in the KA race, lowest in the Pure Mysore, and moderate in the PM x KA hybrid. However, even though there was a lot of variation, the initial pH level of 6.6 was noted on the fifth day of the fifth instar in healthy worms. The experiment's findings suggest that, in comparison to healthy worms, the fungus may be generating some molecules that have an acidic character, which causes acidemia in silkworms infected with Beauveria bassiana. The high production of organic acids and the deposition of ammonium oxalate crystals by Beauveria bassiana during its growth within the host body contribute to the acidic character of the hemolymph.

Specific gravity:
In comparison to the control, the specific gravity (Graph 2) of the haemolymph of silkworm Bombyx mori inoculated with the fungal pathogen Beauveria bassiana during the fifth instar was measured. The bivoltine double hybrid and crossbreed showed a greater specific gravity level (1.04) in the first two days of the fifth instar, followed by a modest decrease in specific gravity (1.03) in the remaining days of the instar in both races of healthy silkworms. In bivoltine single hybrids, the first three days of the instar showed an elevation of specific gravity (1.04), while the following days of the instar showed a little drop of specific gravity (1.03).

In comparison to the control, the inoculated silkworm exhibited a lower specific gravity until day four (1.02), then there was a little elevation of specific gravity (1.03 and 1.04) on the fifth and sixth days of the instar in the bivoltine double hybrid. In the cross breed, decreased specific gravity levels were recorded up to the third day (1.02) on the fourth and fifth (1.03) days, identical specific gravity levels in both healthy and inoculated silkworms. However, on the sixth day (1.04), the inoculated worms showed higher specific gravity levels than the control group. The bivoltine single hybrid exhibited a gradual elevation specific gravity level during the instar i.e., first day to end of the instar (1.02 to 1.04). However, the specific gravity of the three breeds included for the study showed no discernible change. In the case of fifth instar silkworms inoculated with the fungal pathogen Beauveria bassiana in a bivoltine double hybrid, specific gravity was constant from the first to the fourth day (1.02), and then increased in the remaining days (1.03 and 1.04). In contrast, in the case of crossbreeds, specific gravity was constant up to the third day (1.02) and increased in the remaining days of the instar (1.03 to 1.04).A progressive increase in specific gravity was noticed in the bivoltine single from the first day to the sixth day (1.02 to 1.04) of the fifth instar silkworm infected with Beauveria bassiana treated silkworms. In healthy worms, Kusunoki and Watanabe (1984) reported a specific gravity of 1.025 at the start of the fifth instar. The specific gravity in treated silkworms with Beauveria bassiana was increased significantly overtime, reaching 1.035 on the fourth day, while the specific gravity of the healthy larvae increased very gradually and slightly, measuring 1.03.Since the fungus has begun to reproduce inside the body, the haemolymph fills with free cells, or hyphal bodies. As a result, the hemolymph's consistency thickens, leading to an elevation of specific gravity. According to Raghavaiah et al., (1988), infected 5th instar silkworms with Beauveria bassiana showed a progressive decrease in the osmotic pressure of their haemolymph as the infection progressed. Conversely, throughout the entire length of the fifth instar of silkworms, the osmotic pressure of healthy larvae stayed constant at 0.7% NaCl. As the osmotic pressure drops, the insect perishes. The diseased silkworm has a higher specific gravity than the healthy one. Kodaira (1961) determined the haemolymph density of silkworms. When a silkworm becomes infected with Beauveria bassiana, symptoms like dehydration and fasting towards the conclusion of the infection cause the body's water content to drop and its fluid composition to become more concentrated, which raises its specific gravity. Syeda Fakrunnisa Begum and G Savithri (2023) observed a lot of fluctuations in the pH levels of the silkworm blood, and a lower level of specific gravity was recorded in the initial stage (1.024) of the instar. Gradual elevation of the specific gravity was observed till the end of the instar (1.038) in the treated silkworms, and there was a gradual drop in the specific gravity in healthy silkworms from the first day (1.025) of the instar.

The investigation of the pH levels in the haemolymph of Beauveria bassiana-infected silkworms showed significant changes in the acidic-alkaline balance. The pH fluctuation suggests a dynamic host-pathogen interaction, which suggests that the silkworm's immune response is influencing its internal milieu and the invaded fungal pathogen may utilise the haemolymph as a source of nutrition for growth and development, as this depletes the host's resources needed for host maintenance, which may cause a drop in pH level.

The study of specific gravity revealed significant variations in the density of haemolymph in fungal-infected silkworms. These differences may be due to modifications in metabolic processes, the proliferating fungal mycelia in the haemocoel, and drawing nourishment from the haemolymph. As the haemolymph is filled with hyphal bodies and free cells, body fluid may gradually become viscous in consistency, resulting in increased specific gravity. The variations in the pH and specific gravity of diseased silkworms draw attention to the need for a more thorough understanding of how fungal infections affect the overall health of the silkworm.

In conclusion, the work adds to the growing body of knowledge on silkworm pathology and opens up new avenues for research. It is necessary to understand the nuances of pH control and the specific gravity changes in infected silkworms in order to develop targeted therapies aimed at enhancing the resilience of silkworm populations and, consequently, the silk industry. This work provides an invaluable basis for further research in the field. The investigation concentrated on the intricate dynamics of host-pathogen interactions in silkworms; this work offers a vital foundation for future research in the field.

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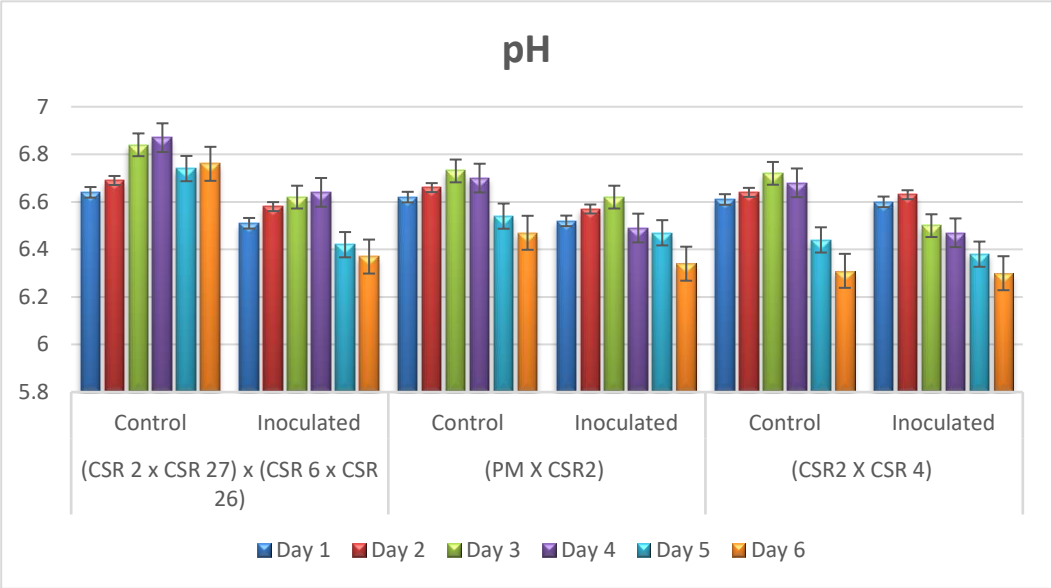
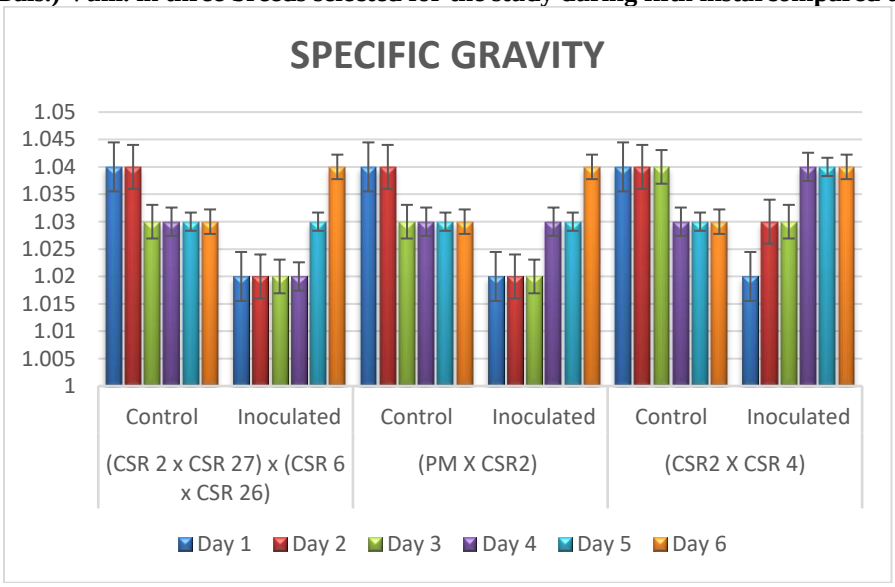
Graph 1: pH in the haemolymph of silkworm Bombyx mori L. treated with fungal pathogen Beauveria bassiana(Bals.) Vuill. in three breeds selected for the study during fifth instarcompared to control.



Graph 2:Dynamics of Specific gravity in the haemolymph of silkworm *Bombyx mori* L. treated with fungal pathogen *Beauveria bassiana*(Bals.) Vuill. in three breeds selected for the study during fifth instarcompared to control.



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