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Modulations of Haemolymph Parameters in Fifth Instar Silkworm *Bombyx Mori* L. Parasitized with *Beauveria Bassiana*

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Abstract

The study focused on understanding the impact of Beauveria bassiana infection on the haemotological parameters such as pH, specific gravity and rate of pulsation of the economically significant silkworm, Bombyx mori. Silkworms, due to centuries of domestication, have developed sensitivity to environmental conditions and various micro-pathogens affecting their nutrition, metabolism, and physiological aspects of haemolymph. The researchers used the bivoltine double hybrid silkworm race (CSR2 X CSR27) X (CSR6 X CSR 26) for the investigation. The results revealed that the pH levels of the haemolymph on the first day of the fifth instar in healthy silkworms were 6.615, and on the sixth day, they were 6.596. In the experimental batch, there was a drastic reduction from the first day (6.590) to the second day (6.253), which gradually increased until the fourth day (6.426) of the instar and gradually decreased on the fifth (6.398) and sixth days (6.374). A lower level of specific gravity of the haemolymph was noticed on the first day (1.024) of the 5th instar in silkworms inoculated with Beauveria bassiana. Then a gradual increase was observed till the sixth day (1.038) of the 5th instar. Conversely, in the healthy silkworms, there was a gradual decline in the specific gravity of the haemolymph from the first day (1.025) to the sixth day (1.012) of the fifth instar. The pulse rate in silkworms parasitized by Beauveria bassiana showed a significant increase from the first day (35) to the second day (48), followed by a gradual decrease to the sixth day (32) of the instar. But in control silkworms, the heart rate increased gradually from the first day (35) to the fifth day (45) of the instar and then suddenly decreased on the sixth day (38).

Keywords: *Silkworm, Beauveria bassiana, Haemolymph, pH, Specific gravity, Pulse rate, neurotransmitter, acetyl choline, cardio acceleratory peptides, biogenic amines, abdominal ganglia, neuropeptides, neuro harmones, adrenaline*

1. Introduction

The versatile, sustainable agrarian sector known as sericulture is primarily focused on the production of silk. The final result of raising domesticated silkworms (Bombyx mori) is natural silk fiber (Singh *et al*., 2000). A number of tasks are involved in sericulture, such as growing mulberries, feeding the leaves to silkworms, obtaining cocoons, weaving, dying, and selling silk and its derivatives. Thus, it encompasses a number of industries, including textiles, art, design, research and innovation, and agriculture. In addition to giving many people job possibilities, it also contributes to raising the general economic standards of the populace and the nation at large. The most beneficial result of raising silkworms is the production of cocoons, which serve as the raw material for the silk industry. Apart from cocoons, a variety of secondary silk products are processed and utilized as animal feed, including silkworm proteins, moths, and wastes. Silkworm farming has improved the ecological, economic, and social conditions of many states' modern economies worldwide. The production of silk increases environmental conservation and silkworm feed (Gamble, 2011), creates jobs (Benchamin and Jolly, 1986), benefits society and can be a primary or secondary source of income.

An important commercial insect that supports the nation's economic growth is the mulberry silkworm. The silkworm is an extremely delicate monophagous insect that exclusively consumes mulberries. It gets all the nutrients and water it needs from the leaves in order to develop, grow, and produce silk proteins as well as spin cocoons. In addition to other requirements, the availability of nutritious mulberry leaves is critical to the success of silkworm rearing. One essential physiological component that affects the growth and development of silkworms as well as the production of silk is nutrition and assimilation. The silkworm's fifth instar is a voracious feeding stage that calls for gradual modifications to its physiology and biology. The silkworm's rate of mulberry leaf consumption and utilization is influenced by both biotic and abiotic factors. Both the nutrition and host pathogen interaction affect the physiology of the haemolymph in silkworm. Infection influences the conversion efficiency of the ingested mulberry leaf into the body biomass, silk synthesis and spinning of the cocoon.

The insect blood, also known as haemolymph, is the intracellular circulating fluid that fills the haemocoel and is in direct contact with tissues and organs while bound and non-vascular. Within the body of the insect, the haemolymph freely flows, soaking various tissues (Jones, 1979). In addition to cellular components, the haemolymph of insects contains substances similar to vertebrate blood, such as carbohydrates, proteins, lipids, salts, water, and hormones. According to Kerenhap *et al*., (2005), the two main components of hemolymph are corpuscles, or hemolymphocytes, and plasma. The innate immunological response that is set off when bacteria, fungi, or any other foreign body enters the silkworm body is greatly aided by hemolymph. Haemolymph is known to perform a wide range of tasks, including storage, nutrient transfer, excretion, defense, moulting, and metamorphosis (Mullins, 1985). The only extracellular fluid found in insects is the hemolymph which serves a variety of purposes and serves as a physiological reservoir for all the biomolecules needed for every metabolic process. Additionally, hemoglobin plays a significant role in the immune system of insects. Throughout an insect's development and survival phase, hemolymph's physical characteristics, including pH, specific gravity and osmotic pressure are constantly kept at their ideal levels (Buck 1953; Florkin and Jeuniaux 1974). Insect cell media requires a precise relative concentration of sodium, potassium, calcium, and magnesium ions (Baldwin, 1962). Haemolymph contains a variety of biomolecules, including urea, uric acid, ammonia, proteins, carbohydrates, trehalose, and inorganic components. According to Robinson (1966), tolerance is the host's capacity to prevent the occurrence of disease even when it is being attacked by the pathogen, whereas resistance is the host's capacity to impede a pathogen or agent that causes disease. Schafer (1971) asserts that in a resistant variety, the pathogen's own proliferation is hindered, but in a tolerant variety, the pathogen's or parasite's proliferation is not always hampered.

Understanding the composition and functions of silkworm hemolymph is essential for researchers and sericulturists working to enhance silk production and study the physiology of these economically important insects. It provides insights into the intricate mechanisms that govern the life cycle and silk production process in silkworms.

When assessing and forecasting the pathogenic effect on the insects, the alterations in the haemolymph's physical parameters are helpful. In addition, knowing how pathogenic fungi may affect the physical and biochemical environments of insects and their hemolymph is crucial for managing diseases. Modifications in the hemolymph caused by the pathogenic agent can also aid in determining the best course of action against the intended insect. A further deficiency in a thorough and organized comprehension of the hematological and hemochemical changes in Bombyx mori infected with Beauveria bassiana prompted an investigation into the function of hemolymph in different metabolic profiles, defense mechanisms and changes in hemolymph during the development of fungal pathogen in the chosen hybrid. The study's findings will open the door to plan and manage the diseases in large populations, which will shield sericulture farmers from financial loss.

2. Materials And Methods

The current investigation has been carried out on the commercially popular bivoltine double hybrid (CSR2 X CSR27) X (CSR6 X CSR26). The stock of silkworms has been maintained by following the standard protocol as suggested by Dandin *et al.*, (2003). Silkworms were reared by careful synchronization of several factors such as maintenance of a mulberry garden to provide suitable mulberry leaves for the silkworms, strict maintenance of hygiene in the rearing house and its surroundings, manipulation of required environmental conditions, procurement of good quality chawki worms etc. in the silkworm rearing laboratory of Department of Biosciences and Sericulture, Sri Padmavati Mahila Visvavidyalayam, Tirupati.

Simultaneously pure *Beauveria bassiana* culture was maintained by using Potato Dextrose agar medium. Conidia were collected from the 3-week-old *Beauveria bassiana* pure culture and prepared sterile inoculum in a beaker that contained 50 ml of sterile double distilled water and a drop of tween-20. LD50 value was calculated for the 5^{th} instar silkworms by following the probit analysis (Leora software, 1987). Then the silkworms were inoculated immediately after passing the fourth moult, with sub-lethal concentration (2.15 X 10^4 conidia/ml) and healthy silkworms were treated with double distilled water and used as control. Four replications were kept for the experiment with hundred silkworms in each replication.

After induction of the myco-pathogen, *Beauveria bassiana*, the larvae were taken for experimentation to scan the dynamic daily changes occurring in the pH, specific gravity and pulse rate in the infected silkworm.

The pH of the Haemolymph was determined immediately after the collection of body fluid of healthy and Beauveria bassiana infected silkworm by using (ELlCO) digital pH meter equipped with a combined glass electrode.

The specific gravity of the haemolymph of silkworms was determined by Van Slyke's copper sulphate method by Pilmer (1944). A stock of copper sulphate solution (saturated) was prepared with distilled water at room temperature (25-30°C). With the use of a hygrometer, serial-specific gravities of 1.01, 1.02, 1.03, 1.04, 1.05, 1.06, 1.07, 1.08, 1.09, and 1.10 were prepared from this solution, and the solutions were placed in small beakers in a serial order. The haemolymph was collected using a micropipette, and the droplets of haemolymph were carefully placed into beakers containing a known specific gravity copper sulphate solution. The specific gravity of the haemolymph was determined by the specific gravity of the solution in which the droplets of the haemolymph remained suspended, i.e., did not sink to the bottom or float to the surface.

Krijgsman *et al.,* (1950) described the isolated technique of the heart, in which the heartbeat can be observed by dissecting the worm. Submerging the dissected worm in a physiological solution for a few minutes rendered it inert. The worms were then placed on a wax plate, dorsal side down. In a shallow petri dish, this plate was submerged in physiological solution. An incision was made in the back end of the worm and this continued until the worm's thoracic region was reached. To expose the heart, the digestive tract and fatty tissue were meticulously removed. After that, it was left for 15-20 minutes without any disturbance to recover from the shock and return to a normal heartbeat. Every day the frequency of heartbeat was measured. The measurements are in beats per minute. The physiological solution was prepared by following the protocol suggested by Naidu (1955). The solution was prepared at room temperature and comprised 9.62 g NaCl, 0.77 g KCl, 0.5 g CaCl2, 0.18 g NaHCO3, 0.01 g/l NaH2PO4, dextrose/g + 2 drops of 0.1 NH4Cl2 per litre of distilled water with a pH of 0.0 pH.

Statistical Analysis

The data were collected from the four replications of the experiment and control batches and analyzed the data by using Analysis of Variance (ANOVA) and presented in the tables.

3. Results and Discussion

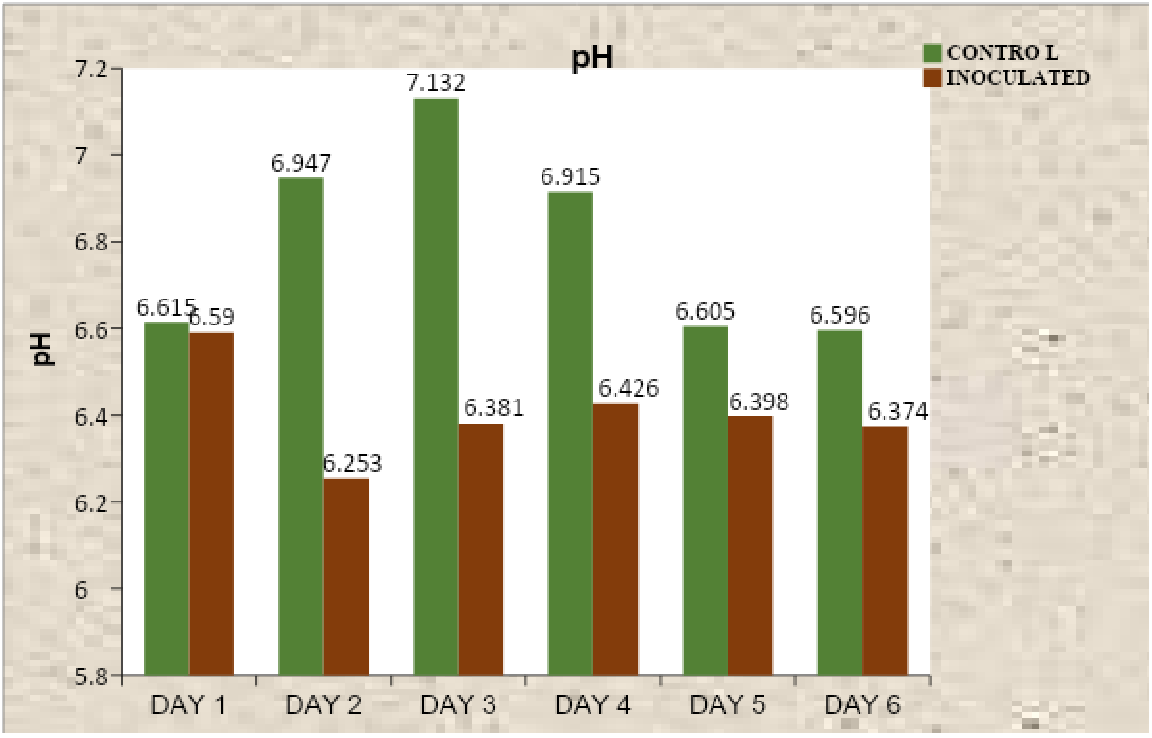
One of the most dangerous and contagious fungi that affects silkworms, Bombyx mori, and causes white muscardine disease is Beauveria bassiana (Bals.) Vuill. Before dying, the fungal pathogen Beauveria bassiana attaches itself to the silkworm Bombyx mori's body cavity and causes damage and transformation to the fat and hemolymph body cells. Haemocytes are a crucial part of the insect immune system, and hemolymph is a vibrant tissue fluid that has a close metabolic relationship with other tissues and organs. It also serves as a reservoir for the products needed for every physiological activity. In insects, infection triggers a sophisticated defense mechanism. Insect hemolymph frequently contains complexes of various circulating cell types called hemoglobins. The defense mechanism against foreign bodies that infiltrate the hemoglobin is their responsibility (Tepass *et al*., 1994; Falleiros and Gregorio, 1995; Inoue *et al.,* 2001). The presence of immune cells in the hemolymph was primarily responsible for the biological defense mechanisms such as phagocytosis, encapsulation, nodule formation, and others that were found to be effective. Studies on hematology are crucial for assessing the health of the host insect and comprehending the physical conditions and disorders that arise as Beauveria bassiana progresses through it. Changes in the enzymatic, biochemical, and haematological parameters of infected larvae would predict the metabolic stress that the insect would encounter as the pathogen developed. With this background, daily variations in a haemotological parameters like pH, specific gravity and pulse rate were examined in bivoltine double hybrid (CSR 2 × CSR 27) × (CSR 6 ×CSR 26). The results of the investigations are presented in the following tables and graphs.

Table - 1 and Graph – 1: Everyday changes in pH of silkworm ***Bombyx mori*** L. inoculated with fungal pathogen ***Beauveria bassiana*** (Bals.) Vuill. with reference to control during 5th instar.

DAYS	CONTROL		INOCULATED		p value	sig	Days
	Mean	Std. Deviation	Mean	Std. Deviation			
DAY 1	6.615	0.036	6.590	0.034	0.247	NS	0.000***
DAY 2	6.947	0.093	6.253	0.043	0.000	**	
DAY 3	7.132	0.115	6.381	0.040	0.000	***	
DAY 4	6.915	0.058	6.426	0.072	0.000	***	
DAY 5	6.605	0.265	6.398	0.081	0.097	NS	
DAY 6	6.596	0.266	6.374	0.067	0.075	NS	
BATCHES	p value = 0.000			***			
NS = Not Significant: * = p< 0.05 : **=p<0.01 : *** = p< 0.001							

ANOVA: pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BATCHES	2.851	1	2.851	181.813	0.000
DAYS	0.628	5	0.126	8.012	0.000
BATCHES * DAYS	1.281	5	0.256	16.340	0.000
Error	0.941	60	0.016		
Corrected Total	5.702	71			



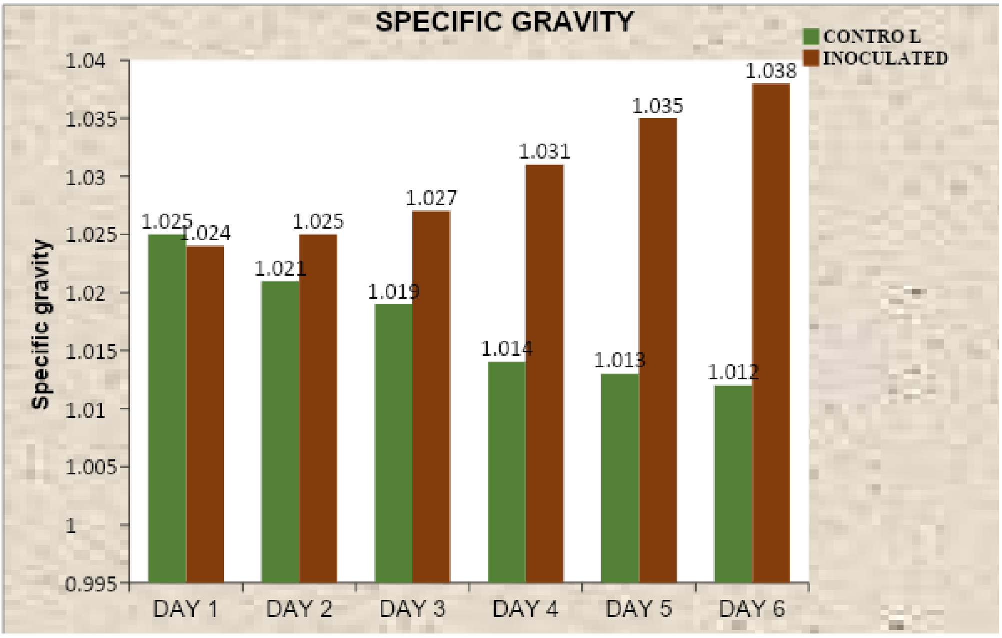
pH levels (Table-1 and Graph-1) were examined in the blood of silkworm Bombyx mori inoculated with Beauveria bassiana fungal pathogen during the 5th instar in comparison with control. In healthy silkworms, pH of the haemolymph on the first day was recorded as 6.615 and on the sixth day it was recorded as 6.596. A gradual raise of pH was noticed from 1st to 3rd day (6.615 to 7.132) and gradual reduction of pH was recorded till sixth day (6.596). In experimental batch there was a drastic reduction from first day (6.590) to second day (6.253) and gradually increased till fourth day (6.426) of the instar and gradually decreased on the fifth (6.398) and sixth day (6.374). Fluctuations in the pH of haemolymph in the inoculated silkworms were observed. Significant decrease in the haemolymph pH of diseased silkworms with reference to control was observed on second, third and fourth day of instar. The pH of the inoculated worms was less compared to control on all the days. In inoculated silkworms' maximum pH (6.590) was observed on the first day and least pH (6.253) was noticed on the second day of the instar. Haemolymph is an insect's circulating fluid, comparable to mammalian blood, that travels via the open circulatory system and bathes the organs and tissues directly. Insect haemolymph is devoid of erythrocytes and contains a high quantity of free amino acids. Plasma, haemocytes, and dissolved inorganic and organic compounds make up haemolymph. In insects, the haemolymph serum is the only extracellular fluid. Haemolymph also transports nutrients, nitrogenous waste products, hormones, and a range of other chemicals to other tissues. The amount and types of haemocytes differ according to the insect species, developmental stage, and physiological state. The negative logarithm of hydrogen ion concentration, or the quantification of acidity or alkalinity, is the pH. Insect haemolymph's pH is usually between 6.4 and 6.8. The pH of the haemolymph in the silkworm system is significant because the silkworm has an open circulatory system, and haemolymph travels from the posterior to the anterior end of the body through the dorsal blood vessel for appropriate brain coordination with various organs and portions of the body. The silkworm's physiology is disrupted by any changes in pH values. Wigglesworth (1972) stated that the acidic pH in the haemolymph could be due to the availability of metabolic products in the form of acids. The infected fungus may secrete various acidic compounds into the haemolymph of diseased worms during its growth phase, resulting in a decrease in pH in the haemolymph of diseased worms. These acidic compounds favour the growth and development of Beauveria bassiana which is acidophilic in nature. Secondly, it could be related to the pathogen's breakdown of carbohydrates and the compounds released during carbohydrate metabolism. Finally, elevated metabolites in the haemolymph can be linked to a lack of buffering capacity of the haemolymph; which when lost accumulates the carbonic acid resulting in "acidemia." Infectious pathogens clearly have the ability to secrete certain chemical compounds and release them into the haemolymph; in other words, pollutants are introduced, resulting in "haemolymph pollution." Furthermore, invaded pathogens use the haemolymph's essential nutrients for their growth, depleting the resources meant for the host's upkeep. Beauveria bassiana infection causes acidity in the haemolymph and changes the amount of several organic acids. Pristavko (1967) noted the fluctuations in the haemolymph pH of Leptinotarsa decemlineata infected with Beauveria bassiana. Ambika (1990) observed a decrease in the pH of silkworm haemolymph in all three silkworm races viz., Pure Mysore, KA and Pure Mysore x KA due to Beauveria bassiana infection. By the fifth day of the fifth instar, the pH of the haemolymph had reached an acidic level, showing that infection causes acidemia. In 5th instar worms, Kusunoki and Watanabe (1984) reported a drastic drop in pH from 6.6 to 6.2 on the second day of infection, then a gradual increase in pH until it reached 6.4 on the 4th day of infection. However, in healthy worms, the original pH level of 6.6 was reported on the 5th day of the 5th instar, despite significant variation. The results of the experiment suggest that the fungus may be creating acidic chemicals, resulting in acidemia in Beauveria bassiana infected silkworms when compared to healthy worms. The researchers also noted the pH of gut juice in healthy fifth instar silkworm to be 10.5 to 10.7. But in silkworms severely infected by CPV, the alkalinity decreased considerably recording a pH of 9.5 or less indicating its inefficiency to destroy the disease-causing germs entering through food. Many researchers reported that Beauveria bassiana produces a considerable number of organic acids and deposits ammonium oxalate crystals in the host body, resulting in the acidic character of the haemolymph. Similar to the pH of the haemolymph, different parts of the gut record different pH which may also be influenced by the disease. Terra and Ferreira (1994) reported the pH of foregut in most lepidopteran larvae as 7.0, anterior ventriculus having a pH of 9.8, middle ventriculus has a pH of 10 and posterior ventriculus had a pH of 9.5.

Table - 2 and Graph – 2: Everyday changes in specific gravity of silkworm ***Bombyx mori*** L. inoculated with fungal pathogen ***Beauveria bassiana*** (Bals.) Vuill. with reference to control during 5th instar.

DAYS	CONTROL		INOCULATED		p value	sig	Days
	Mean	Std. Deviation	Mean	Std. Deviation			
DAY 1	1.025	0.002	1.024	0.003	0.503	NS	0.653
DAY 2	1.021	0.002	1.025	0.003	0.022	*	
DAY 3	1.019	0.002	1.027	0.003	0.000	***	
DAY 4	1.014	0.004	1.031	0.005	0.000	***	
DAY 5	1.013	0.004	1.035	0.007	0.000	***	
DAY 6	1.012	0.004	1.038	0.007	0.000	***	
BATCHES	p value = 0.000			***			
NS = Not Significant: * = p< 0.05: **=p<0.01 : *** = p< 0.001							

ANOVA: SPECIFIC GRAVITY

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BATCHES	0.003	1	0.003	165.029	0.000
DAYS	0.000	5	0.000	0.663	0.653
BATCHES * DAYS	0.002	5	0.000	19.451	0.000
Error	0.001	60	0.000		
Corrected Total	0.006	71			



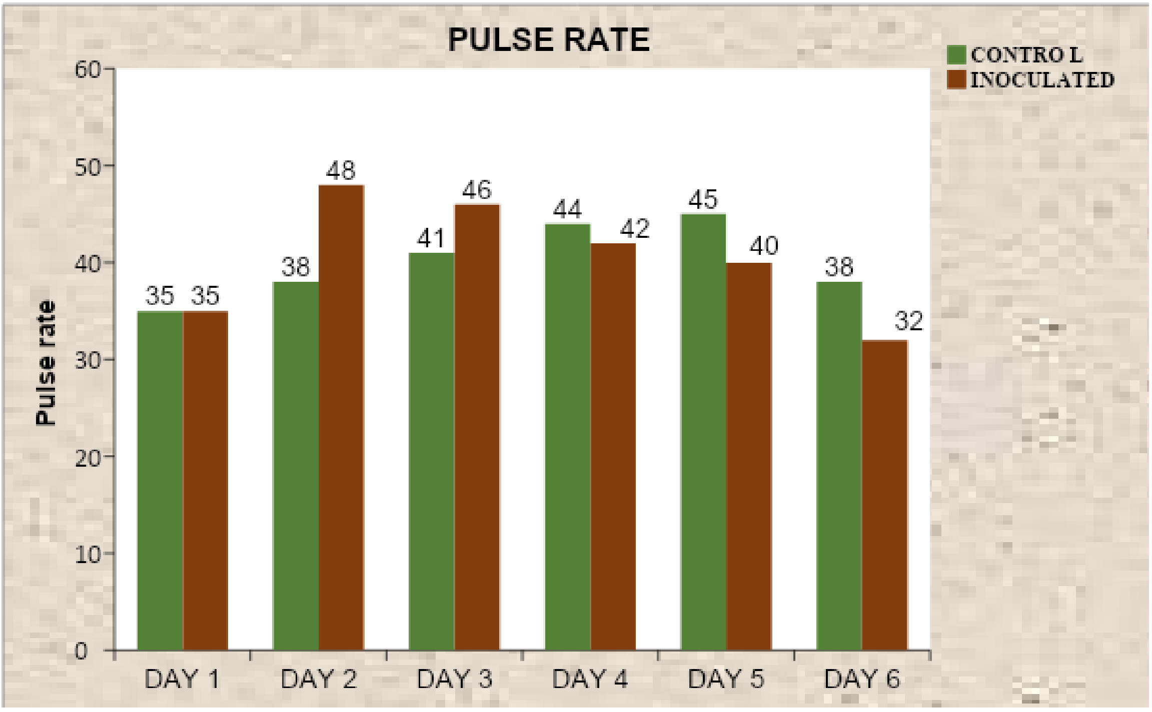
Specific gravity (Table-2 and Graph-2) was examined in the haemolymph of silkworm Bombyx mori inoculated with fungal pathogen Beauveria bassiana during 5th instar in comparison with control. Lower level of specific gravity of the haemolymph was noticed on the first day (1.024) of the 5th instar in silkworms inoculated with Beauveria bassiana. Then a gradual increase was observed till the sixth day (1.038) of the 5th instar. Conversely, in the healthy silkworms there was a gradual decline in the specific gravity of the haemolymph from the first day (1.025) to the sixth day (1.012) of the fifth instar. On the first day the specific gravity of the haemolymph of the inoculated silkworms was less compared to control. From second day onwards a significant difference between inoculated with respect to the healthy silkworms was noticed. In healthy worms, Kusunoki and Watanabe (1984) found the specific gravity of 1.025 at the beginning of the fifth instar. Infected larvae had a significant increase in specific gravity over time, reaching 1.035 on the fourth day after infection, whereas healthy larvae had a very gradual and modest increase, reaching 1.03 on the fourth day after infection. The haemolymph becomes filled with hyphal bodies as the fungus reproduces inside the system, causing the haemolymph to become viscous in consistency, resulting in an increase in specific gravity. In Beauveria bassiana infected 5th instar silkworms, Raghavaiah *et al*., (1988) observed a higher specific gravity in diseased silkworms than the healthy silkworms. The density of body fluid in silkworms was measured by Kodaira (1961). In the case of diseased silkworms infected with Beauveria bassiana, symptoms such as dehydration and fasting near the end of infection cause a decrease in water content and a more concentrated body fluid composition, resulting in an increase in specific gravity.

Table - 3 and Graph - 3: Everyday changes in pulse rate of silkworm ***Bombyx mori*** L. inoculated with fungal pathogen ***Beauveria bassiana*** (Bals.) Vuill. with reference to control during 5th instar.

DAYS	CONTROL		INOCULATED		p value	sig	Days
	Mean	Std. Deviation	Mean	Std. Deviation			
DAY 1	35	3.464	35	6.603	1.000	NS	0.000***
DAY 2	38	3.162	48	3.633	0.000	**	
DAY 3	41	2.828	46	5.727	0.084	NS	
DAY 4	44	2.608	42	5.329	0.428	NS	
DAY 5	45	2.966	40	5.586	0.272	NS	
DAY 6	38	3.162	32	5.55	0.044	*	
BATCHES	p value = 0.207			NS			
NS = Not Significant: * = p< 0.05: **=p<0.01 : *** = p< 0.001							

ANOVA: PULSE RATE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
BATCHES	32.000	1	32.000	1.630	0.207
DAYS	1092.000	5	218.400	11.124	0.000
BATCHES * DAYS	490.000	5	98.000	4.992	0.001
Error	1178.000	60	19.633		
Corrected Total	2792.000	71			



The experimental data of the pulse rate of silkworm, Bombyx mori was collected every day and presented in Table-3 and Graph-3. From the data analysed, it was found that pulse rate in the silkworms inoculated by Beauveria bassiana increased significantly from first day (35) to second day (48) followed by a gradual decrease to the sixth day (32) of the instar. But in control silkworms, the heart rate increased gradually from first day (35) to fifth day (45) of the instar and drastically decreased on the sixth day (38). A significant change was observed on the second and sixth day of the instar in the diseased worms compared to control. On the second day, the inoculated worms showed a significant increase (48) in the pulse rate over the control (38) and a significant decrease on the sixth day of the instar. The second day and the third day recorded an increase in diseased (48,46) than in control (38,41) which may be due to the neurosecretions which regulate the heartbeat indicating the neural reaction to parasitization.

In lepidopterans, two neuropeptides called cardio acceleratory peptides (CAPS) are released from abdominal ganglia which stimulate the heart to increase the frequency. So, the physiological stress due to pathogenesis might have shown an impact on cardio acceleratory peptides (CAPS) in silkworm. Many other compounds like neuropeptides and biogenic amines also influence the heartbeat. Octopamine is an example of biogenic amine. Open circulatory system is seen in silkworms and the neurohormones may be secreted which may stimulate the heart. Krijgsman (1952) reported that acetyl choline influences the heartbeat of cockroach, grasshopper, cricket and honeybees (Hamilton, 1939; Davenport, 1941). Smitha and Vijaya Bhaskara Rao (2010) stated that Selenium might have caused an indiscriminate release of neurohormones which ultimately lead to metabolic imbalance in lethal dose of Selenium. She also observed a decrease in the rate of heartbeat at its lethal dose. She attributed it to the suppression of acetyl cholinesterase activity altering the function of pacemaker ganglia under selenium stress. According to Colhoun (1963) the coordination and activity of the nervous system and active state of an organism warrants a high concentration of AChE. Ye *et al.*, (2017) stated that acetylcholinesterase which hydrolyses the neurotransmitter acetylcholine into acetate and choline to terminate synaptic transmission is encoded by two ace genes (ace1 and ace2). Chen (2009) stated that the AChE2 in Bombyx mori is the major enzyme in synaptic transmission. The pulse rate of the dorsal vessel varies depending upon the stages of metamorphism. It is slower in pupa stage where the animal is inactive and increases in imago stage. It also increases with the temperature. Heartbeat is under the nervous control by various nerves. The heart receives sensory and motor nerve fibres from the segmental ganglia of the ventral chain. The rhythmic activity of the heart is greatly influenced by the pathological tension exerted upon the heart by the alary muscles. The pace-maker of the heart is neurogenic and is affected by acetylcholine which causes acceleration. Adrenaline accelerates the activity of the post ganglionic fibres. Various muscles, enzymes and hormones involved in heartbeat might have been affected by the invading pathogen. Hormones and enzymes are basically proteins. Decrease in the total protein content of the silkworm due to infection of Beauveria bassiana might have affected the production of hormones and enzymes and lowered the pulse rate in infected silkworms. Shortage of acetylcholine interrupts the conduction of nerve impulse. In Bombyx mori, nervous impulses arising from the frontal ganglion are conducted through the anterior branches of the visceral nerve to the myocardium where they evoke epsps in the heart cells. Individual epsps or summated epsps at greater than 2 Hz trigger antidromic peristalsis from an orthodromically beating dorsal vessel. The soma of a motor neuron was located in the frontal ganglion, whose action potential was correlated with myocardial epsps. Ai and Kuwasawa (1991, 1995) noticed that the neuron in the frontal ganglion was considered a "trigger" motor neuron for antidromic heartbeat. Uchimura (2006) examined the excitatory neural control of heartbeat by the frontal ganglion in the fifth instar of Bombyx mori and noticed that the innervation of the anterior region of the dorsal vessel by the motor neurons, through the anterior cardiac nerves is responsible for the control of heartbeat in silkworm. For the smooth nervous coordination and proper functioning of the heart, synaptic transmission should be conducted systematically and needs the secretion of neurotransmitters stimulating the synthesis of acetylcholine as a behavioural adaptation by the infested larva.

4. Conclusion

It The comprehensive study on the pH, specific gravity and pulse rate of silkworms infected with fungi Beauveria bassiana has provided valuable insights into the physiological responses of silkworm to fungal infections. The research findings shed light on the intricate interplay between the silkworm's internal environment and the presence of pathogenic fungi, emphasizing the importance of understanding these factors in sericulture. The statistical analysis of pH levels of the haemolymph revealed significant alterations in the acidic-alkaline balance within the silkworms' bodies upon fungal infection. This fluctuation in pH is indicative of a dynamic host-pathogen interaction, suggesting that the silkworm's immune response may be influencing its internal environment. The investigation into specific gravity demonstrated notable changes in the density of silkworms affected by fungal infections. These variations could be attributed to physiological changes, alterations in metabolic processes, or the presence of fungal structures within the silkworms' tissues. The observed fluctuations underscore the need for a more nuanced understanding of the impact of fungal infections on the silkworm's overall health. Furthermore, the assessment of pulse rate provided valuable data on the physiological stress experienced by silkworms during fungal infections. The recorded variations in pulse rate serve as a potential indicator of the severity of the infection and its impact on the cardiovascular system of the silkworm. Such insights are crucial for developing effective strategies to mitigate the effects of fungal infections in sericulture.

To summarize, this research contributes to the growing body of knowledge on silkworm pathology and opens avenues for further exploration. Understanding the nuances of pH regulation, specific gravity changes, and pulse rate fluctuations in infected silkworms is essential for devising targeted interventions to enhance the resilience of silkworm populations and consequently, the silk industry. As we continue to delve into the intricate dynamics of host-pathogen interactions in sericulture, this study serves as a valuable foundation for future research endeavors in the field.

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