Biochemical Changes In Haemolymph of The Mulberry Silkworm, Bombyx Mori L. In Response to The Injection of Bacillus Sp. And Staphylococcus Sp.

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Abstract- *Pathogens induce several biochemical and physiological alterations in insects. Major biochemical changes in haemolymph of the fifth instar larvae of the silkworm in response to the injection of Bacillus sp and Staphylococcus sp were studied. In the haemolymph of control larvae protein, carbohydrates, lipids and trehalose were found to be increasing day by day. About 70% and 72.5% mortality were observed in Bacillus and Staphylococcus injected larvae respectively. In both Bacillus injected larvae and Staphylococcus injected larvae, the following biochemical changes in their haemolymph were observed. i) The protein level was found to be increasing on the first three days and then decreasing after wards till the last day. When compared to control larvae about 10% and 11% of increase in protein were observed in Bacillus injected and Staphylococcus injected larvae respectively on the third day. But about 28 % and 31% of reduction in protein were observed in Bacillus and Staphylococcus injected larvae respectively on the last day. ii) Gradual increase in the carbohydrates from the first day to the fifth day and a decrease on the last day were observed in both cases.iii) Day to day increase in trehalose level was observed in both the cases. Likewise everyday the trehalose level was greater in the pathogen injected larvae than that of the control larvae. iv) Gradual decrease in the lipid content was observed in both the cases. The lipid level was lesser in pathogen injected larvae than that of the control larvae. Decrease in the level of protein on the later days and decrease in the level of lipid could be attributed to reduced feeding, Degenerative effects caused by the bacteria and utilization of lipid to compensate the reduced feeding. The increase in carbohydrate might be due to the gluconeogenesis of the conversion of non- carbohydrate substance into carbohydrates to meet out the energy decrease arised by the injected bacteria.*

Keywords- haemolymph, protein, carbohydrates, lipids and trehalose.

I. INTRODUCTION

Insects are one of the most successful animal groups that are living in every habitat on the Earth. Hence, they have developed effective defense strategies against the invading microorganisms. Insects rely solely on the innate defense mechanism against the invading microbes since the adaptive immunity is totally absent (Hoffman, 1995). Innate immune response of insects has many apparent similarities to those of mammals (Gillespie et al., 2000). Particularly, cell mediated innate immunity through haemocytes and biochemical based humoral innate immunity through cell free haemolymph proteins (Lavine and Strand, 2002). Haemolymph is the only extracellular fluid of insects with diverse functions and reservoir for the products required for every physiological activity of the insect body. Thus changes in the composition of haemolymph reflect the physiological and biochemical transformations taking place in the insect tissues. Pathogenic infections are reported to induce biochemical and physiological alterations in insect tissues. The progress of infection by a pathogen in the host tissue can be monitored by studying the degree of variation in metabolic constituents (Rajitha and Savithri, 2014). Silkworm larvae are used as model organism in pathogenicity study due to its body size (5 cm), generation time (40-60 days), easy rearing, comfortable handling, low cost rearing, minor space required, outcome of results within a day, high throughput results and non-ethical issue (Evelina et al., 2013). Human pathogenic bacteria cause the disease to the silkworm similar to mammals. For example Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli and Vibrio cholerae cause the diseases to silkworm also (Kaito and Sekimizu, 2007). In our research Bacillus sp. (Gram negative short rods) and Staphylococcus sp. (Gram positive cocci) were used to infect the fifth instar larvae to study the mortality rate and their effect on major biochemical components of haemolymph. Despite several studies made on bacterial disease of silkworm, information regarding the exact biochemical and physiological changes occurring inside the body of silkworm throughout the progress of disease is scanty. Hence we have carried out a study to find out the biochemical changes in the haemolymph of the mulberry silkworm, Bombyx mori L. infected with Bacillus sp. and Staphylococcus sp.

II. MATERIALS AND METHODS

Bacterial injection

Silkworm Double hybrid of (CSR6 X CSR26)×(CSR2 X CSR27) was selected for the study. The silkworm larvae were reared in the laboratory under optimum conditions according to Dandin et al., (2003). Immediately after fourth moult i.e. on the first day of the fifth instar, the larvae were injected with 50 µl of suspension of Bacillus sp. (1.1 x 106 cells/ml) and 50 µl of Staphylococcus sp. (1 x 108 cells/ml).

Haemolymph sample collection

After 24 hours post injection, mortality of control and infected larvae were recorded. Live silkworm larvae were used for collection of haemolymph samples. First abdominal leg of larva was cut by using a forceps and free flowing haemolymph were collected into pre-chilled eppendorf tube containing phenylthiourea crystals. Immediately these tubes were used for obtaining cell-free haemolymph.

Estimation of Protein, Carbohydrate and Amino acid

Total haemolymph protein was estimated according to Lowry et al., (1951) using Bovine Serum Albumin (BSA) as standard. The quantitative estimation of total carbohydrates in haemolymph was done by anthrone method (Dubois et al., 1956) using glucose as standard. Total haemolymph lipids was estimated by the method of Zoellner and Kirsch (1962) using cholesterol as standard. Total haemolymph trehalose was estimated by Anthrone method (Roe, 1995) using trehalose as standard. The results were statistically analyzed and discussed.

III. RESULTS AND DISCUSSION

About 70% of mortality due to injection of Bacillus sp. and 72.5% mortality due to injection of Staphylococcus sp. (Table 1) were observed, whereas non-infected larvae remained healthy with many biochemical changes. This might be due to the total upset of immune system and inhibition of feeding as suggested by Dettloff et al., (2001).

The biochemical parameters of haemolymph of an insect such as, proteins, carbohydrates, lipids, trehalose, nucleic acids etc., vary significantly when they are infected by pathogens. The results on the investigation of biochemical changes in protein, lipid, carbohydrate and trehalose content in haemolymph of Bacillus sp. and Staphylococcus sp. injected silkworm larvae were presented in table 2 and 3 respectively. The levels of all the major biochemicals of the haemolymph studied in this work were to be increasing from the first day till last day in the control larvae. Significant differences in the concentration of major biochemical contents were noticed in the bacteria injected larvae when compared to the control larvae.

Protein:

The total protein content consists of structural and soluble proteins involved in the architecture and metabolites of cell, respectively. Nagata and Yashitake (1989) explained that the quantitative variation in proteins in the body of insects depends upon the nutritional status of the food and its utilization during the growth, metamorphosis and infection of microorganisms. In this study, the difference in protein concentration between control and pathogen injected larvae was more pronounced as the disease progressed. The changes in the protein level of haemolymph in Bacillus injected larvae and Staphylococcus injected larvae were almost same. In both of these larvae, the protein level was found to be increasing on the first three days and it was decreasing on later days. The highest increases were of 11.65% and 12.46% over the previous day were observed on the third day in Bacillus injected and Staphylococcus injected larvae respectively, (Table 2 & 3). Compared to control, protein level was higher in the bacteria injected larvae on the first three days whereas it was lower on the next three days. Such a significant hyperproteinemia was also observed in Bacillus thuringiensis injected larvae of Plodia maculipennis (Narayan and Jayaraj, 1974) at 24 hr post injection. The decreased protein content during infection therefore could have inhibited the process of larval development, which was evident from the retarded growth of the larvae during infection.

About 28-31% lower protein level was observed on the 6th day in bacteria injected larvae than the control larvae. It was interesting to note that the level of protein on the 6th day was found to be lower than that of the 1st day in both cases. The decrease in protein on the later days could be attributed to the harmful effects of the bacteria, less consumption of mulberry leaves, poor assimilation, less digestive capacity, less conversion efficiency, activated proteolysis or impaired protein synthesis in the tissues due to infection (Manohar Reddy, 2004) or due to coagulation of protein substance with the increased moisture requirement of the pathogen, as suggested by Kodaira (1961).

The reduction of protein content in these advanced stages of infection might be attributed to the consequences of changes in the metabolism of proteins and amino acids of haemolymph by the developing pathogen and cessation of feeding by the host organism. The results of this work were in corroboration with that of Thirupathamma and Savithri (2014) who reported similar reduction in the protein level in the fifth instar larvae of B.mori infected with Beaveria bassiana. Rajitha et al., (2013) also observed the reduction of protein content in the advanced stage of bacterial infection in B.mori and attributed the same reason. The infection of fungal pathogen, B.bassiana also resulted in gradual reduction of protein content (Thirupathamma and Savithri, 2014). However, no significant difference was observed between the impact of Bacillus and Staphylococcus with reference to protein level of haemolymph.

Lipid:

Lipids always represent the major component of the fat body and the main source of metabolic fuel in insect. Though an initial insignificant increase was observed in both the bacteria injected larvae, the level of lipid was found to be decreasing gradually from the second day till the last day. When compared to control, highest percentage of reduction in lipid level was observed on the 6th day in both cases, (Table 2 & 3). The similar report of initial elevation of lipid level in response to infection was also made by, (Mullen L and Goldsworthy, 2003) and Cheon et al., (2006) and attributed this to mobilization of lipids to the haemolymph in response to immune challenge for membrane biogenesis in the sites of infection. Increased lipid content in infected silkworm larvae with NPV was reported by Govindan et al. (1998). Monir Siraj et al. (2007) reported that there was a significant increase in total lipid content in both haemolymph and midgut tissues after inoculation of BmDNV1 in susceptible breeds and concluded that lipids serve as a source of metabolic energy as well as essential for structural components of cells.

Significant reduction of lipid content was observed in the present study during the course of bacterial infection. The decrease in lipid level might be due to blocked food ingestion as suggested by Benett and Shotwell (1972) and the rapid utilization of fat reserves for the metamorphosis as reported by Tripathi and Singh (2002). It might be due to the utilization of lipids in the metabolic activity of the host to combat against the infection of the pathogen. Increased lipase activity in infected haemolymph might also be cited as a reason for decreased content of lipid in the haemolymph. Mallikarjuna et al., (2002) also noticed the reduction of lipid content in the haemolymph of B. bassiana inoculated larvae and suggested that the lipids are used as a source of energy required for the growth and development of fungus. In contrast to the present study, two-fold increase in lipid content was recorded by Aboul-ela et al., (1991) in Plodia interpunctella larvae after treatment with Bacillus thuringiensis and suggested that it might be due to the conversion of some proteins to fats during the course of starvation. Almost same trend of changes were observed in both Bacillus injected and Staphylococcus injected larvae with reference to lipid level. The increase in trehalose level might have led to the reduction of lipid level as reported by Arrese et al., (1996) implying that an inverse relationship exists between the haemolymph concentration of lipid and trehalose.

Carbohydrate:

Carbohydrate level in the haemolymph is maintained at a steady state in insects through homeostatic regulation at all stages of the life cycle (Wyatt 1967). Simex and Kodrik (1986) have reported that the free carbohydrates in the haemolymph changed significantly during last larval instar and metamorphosis in silk worms. The late age silkworm larvae accumulate higher carbohydrates compared to young age worms. Day to day observation of carbohydrate level of haemolymph revealed that the level was increasing from the first day to the last day in both Bacillus injected and Staphylococcus injected larvae. Highest increase was noticed on the 4th day in both bacteria injected larvae like the control larvae. When compared to control, level of carbohydrate was insignificantly higher in the bacteria injected larvae for the first four days and it was lower in both cases on the next two days (Table 2 & 3).

Carbohydrates are the major components in the food of all the living organisms which either directly or indirectly used as the source of energy for all vital activities. Carbohydrates serve as main source of energy in a number of insect species. As energy is a vital force in the biological system, a breakdown of organic constituents mainly carbohydrates is required to meet the energy under stress condition. The decreased carbohydrates levels in haemolymph might be attributed to excessive utilization of carbohydrates to meet the demand of energy for the multiplication and development of pathogen. In the inoculated B.mori, the carbohydrate content reduced when compared to control. The present work was in agreement with Rajitha et al., (2013) who reported that inoculation of bacteria resulted in gradual reduction of protein, lipid and carbohydrate contents in the haemolymph during the progress of B. bassiana because of the utilization of carbohydrates by the pathogen and low food intake.

Trehalose:

Trehalose level also was found to be increasing from the first day till last day in the haemolymph of both Bacillus injected and Staphylococcus injected larvae like control larvae. However, percentage of increase over the previous day in the control was considerably different from that of the bacteria injected larvae. About 48% shoot up in the trehalose level was observed on the third day in the control larvae whereas the shoot up in the bacteria injected larvae was only about 35% (Table 2 & 3). Trehalose is a multifunctional molecule, and diverse functions like structural support, transport role, signaling and protection of membranes and proteins against heat or cold. The increase in trehalose level of the haemolymph could be directly related to the glycogen content of the fat body which is influenced by a number of endogenous organic and inorganic factors (Kochi and Kaliwal 2006). The major source of trehalose in the haemolymph appears to be from the breakdown of glycogen in the fat body, Unni et al., (1997) explained that the higher concentration of trehalose in the haemolymph may be due to the release of trehalose as a result of histolysis of various tissues or release from the fat body. The present work was supported by Rajitha et al., (2013) who reported that the significant elevation of trehalose content in infected haemolymph might be due to the conversion of glycogen into trehalose and its subsequent release into the haemolymph.

Significant elevation in trehalose content in infected haemolymph might be due to the conversion of glycogen into trehalose and its subsequent release into the haemolymph by the fat body during the starvation due to infection. And the another reason for accumulation of trehalose at the end of the fifth instar may be the efficiency of infected larvae to utilize the available haemolymph trehalose for deriving energy to put forth growth with the progress of the age in fifth instar might have been reduced. Same results were observed by Ambika (1990) during fungal infection by Beauveria bassiana and she also observed the decreased glycogen content in fat body and elevation of trehalose during the infection. Elevation in trehalose may also be due to defense mechanism in host haemolymph by the formation of protective membranes for structural support.

IV. CONCLUSION

As a conclusion, the present investigation revealed the following: Mortality was observed in all the experimental larvae injected with pathogens. This might be due to the total upset of immune system and inhibition of feeding. Protein level increased on the first three days after injection of pathogen whereas it decreased on later days. Carbohydrate level increased from the first day of injection till fifth day and it decreased on the last day. Trehalose level was greater in the pathogen injected larvae than in the control larvae. Gradual decrease in lipid was observed on all days in pathogen injected larvae.

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Table 1. Percentage of mortality of fifth instar larvae of the mulberry silkworm Bombyx mori L. in response to the injection of Bacillus sp. and Staphylococcus sp.

Bacteria Injected	Percentage (%) of Mortality					
	I day	II day	III day	IV day	V day	VI day
Bacillus sp.	24%	32.5%	40%	52.5%	60%	70%
Staphylococcus sp.	30%	40%	47.5%	54%	64%	72.5%

Table 2. Biochemical changes in the haemolymph of the fifth instar larvae of the mulberry silkworm Bombyx mori L. injected with Bacillus sp.

Biochemical content	Larvae	I day	II day	III day	IV day	V day	VI day
Protein (mg/ml)	Control	162.37±1.59	170.42±0.68 [4.95%]	174.18±0.11 [2.20%]	181.21±0.94 [4.03%]	199.07±0.49 [9.85%]	204.94±0.26 [2.94%]
	Injected	159.11±0.51 (-2.00%) t = -4.33*	173.14±0.43 (1.59%) [8.81%] t = 7.51*	193.32±0.34 (10.98%) [11.65%] t = -116.66*	178.34±0.32 (-1.58%) [-7.74%] t = 6.41*	162.73±0.85 (-18.25%) [-8.75%] t = 81.82*	146.32±0.68 (-28.60%) [-10.08%] t = 79.58*
Lipid (mg/ml)	Control	30.04±0.06	31.18±0.56 [3.79%]	32.09±0.73 [2.91%]	32.42±0.94 [1.02%]	33.29±0.57 [2.68%]	33.61±0.39 [0.96%]
	Injected	30.55±0.47 (1.69%) t = -2.35*	30.67±0.94 (-1.63%) [0.39%] t = 1.04**	29.53±0.51 (-7.97%) [-3.71%] t = 6.37*	28.46±0.32 (-12.21%) [-3.62%] t = 8.84*	27.54±0.94 (-17.27%) [-3.23%] t = 11.71*	26.45±0.53 (-21.30%) [-3.95s%] t=24.13*
Carbohydrate (mg/ml)	Control	3.41 ± 0.32	3.95± 0.07 [15.83%]	4.18 ± 0.33 [5.82%]	5.44 ± 0.32 [30.14%]	6.54 ± 0.23 [20.22%]	7.01± 0.07 [7.18%]
	Injected	3.62 ± 0.38 (6.15%) t = -0.93**	4.16± 0.27 (5.31%) [14.91%] t = -1.61**	4.35 ± 0.46 (4.06%) [4.56%] t = 0.67**	5.79 ± 0.12 (6.43%) [33.10%] t = -3.38*	6.45 ± 0.44 (-1.37%) [11.39%] t = 0.40**	6.21 ± 0.38 (-11.41%) [-3.72%] t = 4.64*
Trehalose (mg/ml)	Control	2.96 ± 0.13	3.22± 0.31 [8.78%]	4.78± 0.18 [48.44%]	5.14 ± 0.27 [7.53%]	5.85 ± 0.08 [13.81%]	6.45 ± 0.10 [10.25%]
	Injected	3.18 ± 0.05 (7.43%) t = -3.34*	3.88 ± 0.15 (20.49%) [22.01%] t = -3.52*	5.27 ± 0.15 (10.25%) [35.82%] t = -4.71*	5.98 ± 0.10 (16.34%) [13.47%] t = -6.44*	6.17 ± 0.14 (5.47%) [3.17%] t = -3.98*	7.09 ± 0.47 (9.92%) [14.91%] t = -2.94*

Note: Values inside the parentheses indicate the percentage of change over the control.
Note: Values inside the square brackets indicate the percentage of change in biochemical content over the previous day.
Note: *Significant at the level of $p < 0.05$, **Not significant at the level of $p < 0.05$.

Table 3. Biochemical changes in the haemolymph of the fifth instar larvae of the mulberry silkworm, Bombyx mori L. injected with Staphylococcus sp.

Biochemical content	Larvae	I day	II day	III day	IV day	V day	VI day
Protein (mg/ml)	Control	151.17±0.61	159.33±0.66 [5.39%]	164.08±0.14 [2.98%]	173.18±0.66 [5.54%]	187.05±0.69 [8.00%]	195.42±0.44 [4.47%]
	Injected	148.09±1.10 (-2.03%) t = 5.47*	162.07±0.96 (1.71%) [9.44%] t = -5.20*	182.28±0.92 (11.09%) [12.46%] t = -43.35*	168.35±0.69 (-2.78%) [-7.64%] t = 11.25*	150.53±0.53 (-19.52%) [-10.58%] t = 92.69*	133.28±0.70 (-31.71%) [-11.45%] t = 167.45*
Lipid (mg/ml)	Control	29.24±0.40	30.57±0.38 [4.54%]	31.09±0.68 [1.70%]	31.24±0.56 [0.48%]	32.19±0.45 [3.04%]	32.57±0.55 [1.18%]
	Injected	29.53±0.09 (0.99%) t = -1.85**	29.76±0.16 (-2.64%) [0.77%] t = -4.06*	28.03±0.93 (-9.84%) [-5.81%] t = 6.33*	27.33±0.46 (-12.51%) [-2.49%] t = 11.75*	26.14±0.07 (-18.79%) [-4.35%] t = 29.23*	25.54±0.53 (-21.58%) [-2.29%] t = 20.19*
Carbohydrate (mg/ml)	Control	3.24 ± 0.16	3.83± 0.14 [18.20%]	4.10 ± 0.15 [7.04%]	5.46 ± 0.60 [33.17%]	6.70 ± 0.28 [22.71%]	7.00± 0.07 [4.47%]
	Injected	3.28 ± 0.17 (1.23%) t = -0.37**	3.96± 0.05 (3.39%) [20.73%] t = -1.85**	4.18 ± 0.17 (1.95%) [5.55%] t = 1.42**	5.67 ± 0.36 (3.84%) [35.64%] t = -0.65**	6.24 ± 0.17 (-6.86%) [10.05%] t = 3.04*	6.03 ± 0.16 (-13.85%) [-3.36%] t = 11.97*
Trehalose (mg/ml)	Control	2.55 ± 0.20	2.82± 0.04 [10.58%]	4.15± 0.59 [47.16%]	4.92 ± 0.17 [18.55%]	5.14 ± 0.07 [4.47%]	6.02 ± 0.37 [17.12%]
	Injected	3.04 ± 0.28 (19.21%) t = -4.02*	3.58 ± 0.13 (26.95%) [17.76%] t = -12.63*	5.00 ± 0.44 (20.48%) [39.66%] t = -2.84*	5.12 ± 0.54 (4.06%) [2.4%] t = -5.72*	6.11 ± 0.20 (18.87%) [19.33%] t = -13.87*	6.95 ± 0.24 (15.44%) [13.74%] t = -5.65*

Note: Values inside the parentheses indicate the percentage of change over the normal control.
Note: Values inside the square brackets indicate the percentage of change in biochemical content over the previous day.
Note: *Significant at the level of $p < 0.05$, **Not significant at the level of $p < 0.05$

REFERENCES

[1] JA. Hoffman, “Innate immunity of insects”, Curr. Opin. Immunol, 7, 4-10, 1995.

[2] JP. Gillespie, AM. Bailey, B. Cobb and A. Vilcinskas, “Fungi as elicitors of insect immune responses, Archives of Insect Biochemistry and Physiology”, 44, 49–68, 2000a.

[3] MD. Lavine and MR. Strand, “Insect haemocytes and their role in immunity”, Insect Biochem. Mol. Biol, 32, 1295- 1309, 2002.

[4] K. Rajitha, G. Savithri, “Amino acid profiles in the haemolymph of silkworm, Bombyx mori L. infected with fungal pathogen, Beauveria bassiana (Bals) vuill”, International Journal of Applied Biology and Pharmaceutical Technology, 5(1), 163-166, 2014.

[5] P. Evelina, D.U. Putri and J.Q. Wim, “Choosing an Appropriate Infection Model to Study Quorum Sensing Inhibition in Pseudomonas Infections”, Int. J. Mol. Sci, 14, 19309-19340, 2013.

[6] C. Kaito, K. Sekimizu, “A silkworm model of pathogenic bacterial infection”, Drug Discov. Ther, 1, 89-93, 2007.

[7] S. Krishnaswami, “Improved Method of Rearing Young Age (Chawki) Silkworms”, Central Sericulture Research and Training Institute, Mysore, Reprint of Bulletin, 3, 1990.

[8] S.B. Dandin, Jayant Jayaswal & K. Giridhar, “Hand book of sericulture technologies”, 2003.

[9] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, “Protein measurement with folin-phenol reagent”, Journal of biological chemistry, 193, pp 265-275, 1951.

[10] M. Dubois, KA. Gilles, JK. Hamilton, PA. Rebers, and F. Smith, “Colorimetric method for determination of sugars and related substances”, Analytical Chemistry, Vol 28, no3, pp 350-356, 1956.

[11] N. Zoellner, and K. Kirsch, “Colorimetric method for determination of total Lipids”, Journal of Experimental Medicine, 135, 545-550, 1962.

[12] JH. Roe, “The determination of sugar in blood and spinal fluid with anthrone reagent”, J. Biol. Chem, 242, 424-428, 1955.

[13] M. Dettloff, D. Wittwer, C. Weise, A. Wiesner, “Lipophrin of lower density is formed during immune responses in the lepidopteran insect, Gallerie mellonella”, Cell Tissue Res, 306, 449-458, 2001.

[14] N. Yoshitake, & M. Nagata, “Protein metabolism in the larval development of the silkworm. Bombyx mori L. Protein reutilization at the IV moult”, J. Seric. Sci. Japan, 58(3), 221-233, 1989.

[15] K. Narayan and S. Jayaraj, “Mode of action of Bacillus thuringiensis Berliner in citrus leaf caterpillar Papilio demoleus”, Indian J. Exp. Biol, 12, 89-91, 1974.

[16] B. Manohar Reddy, “Metabolic modulations in various tissues of pre spinning silkworm Bombyx mori (L) Larvae during grassarie (BmNPV) disease”, Ph.D. Thesis, Sri Venkateswara University, Tirupathi, India. 2004.

[17] Y. Kodaira, “Biochemical studies on the muscardine fungi in silkworms, Bombyx mori. L”, In: J. Fac. (Ed.), Text. Sci. Tech. 5, Sinshu. University Sericulture, 1-68, 1961.

[18] D. Thirupathamma, G. Savithri, “Day to day changes in protein levels of silkworm, Bombyx mori L. infected with Beauveria bassiana (Bals)Vuill”, World Journal of Pharmaceutical Research, 3 (4), 1473-1482, 2014.

[19] K. Rajitha, G. Savithri, P. Sujathamma, “Metabolic modulations in the haemolymph of silkworm, Bombyx mori L. during the progress of fungal pathogen Beauveria bassiana”, IJETCAS, 2279-0047, 2013.

[20] L. Mullen and G. Goldsworthy, “Changes in lipophorins are related to the activation of phenoloxidase in the haemolymph of Locusta migratoria in resonse to injection of immunogens”, Insect Biochem Mol Biol 33, 661-670, 20, 2003.

[21] HM. Cheon, SW. Shin, G. Bian, JH. Park and AS. Raikhel, “Regulation of lipid metabolism genes, lipid carrier protein lipophorin, and its receptor during immune challenge in the mosquito Aedes aegypti”, J Biol Chem 281, 8426-8435, 21, 2006.

[22] R. Govindan, TK. Narayanaswamy and MC. Devaiah, “Text book of Principles of Silkworm Pathology” Seri Scientific Publishers, Bangalore, 18, 1998.

[23] Monir Siraj, Murikinati Balavenkatasubbaiah, Kuniyil

Chandrasekharan, Bhyrappa Nataraju, Sudendra Dutta Sharma, Thangasami Selvkumar and Parimi Sudhakara Rao, “Effect of BmDNV1 infection on the biochemical parameters in Densonucleosis type 1 resistant and susceptible breeds of silkworm, Bombyx mori L”, Acta Entomologica Sinica 50 (1), 74- 78, 2007.

[24] GA. Bennet and OL. Shotwell, “Haemolymph lipids of healthy and diseased Japanese beetle larvae”, J Insect Physiol 18, 53-62, 1972.

[25] R. Tripathi and N.P. Singh, “Effect of Bacillus thuringiensis var. kurstaki on biochemical changes in haemolymph of Helicoverpa armigera (Hubner) larvae”, Asian J. Exptl. Sci, 14 (1 and 2), 31-34, 2000.

[26] M. Mallikarjuna, M. Balavenkatasubbaiah, B. Nataraju, V. Thiagrajan, “Effect of systematic fungicide on total haemocyte count and haemolymph biochemical changes in silkworm, Bombyx mori L. infected with Beauveria bassiana”, Int. J. Indust. Entomol, 5, 189-194, 2002.

[27] R. Aboul-Ela, M.Y. Kamel, H.S Salama, A. El-Moursy and A. Abdel-Razek, “Changes in the biochemistry of the hemolymph of Plodia interpunctella after treatment with Bacillus thuringiensis”, Journal of Islamic Academy of Sciences, 4(1), 29-35, 1991.

[28] E.L. Arrese, B.I. Rojas-Rivas, M.A. Wells, “The use of decapitated insects to study lipid mobilization in adult Manduca sexta: effects of adipokinetic hormone and trehalose on fat body lipase activity”, Insect Biochem. Mol. Biol, 26, 775–782, 1996b.

[29] G.R. Wyatt, “The Biochemistry of sugars and polysaccharides in insects”, Advanced Insect Physiology, 4, 287-360, 1967.

[30] V. Simex, and D. Kodrik, “Changes in the tissue glycogen and free carbohydrates of haemolymph during the last larval instar and metamorphosis of silkworm, Bombyx mori L”, Acta, Entomol. Bohemaslov, 83(2), pp 92-100, 1986.

[31] BG. Unni, A. Choudhury, D. Khanikor, AC. Ghosh, “Studies on trehalase and trehalose in the fat body, haemolymph, silkgland and head region of the muga silkworm, Antherea assama during the spinning period”, Sericologia, 37 (1), 31-35,1997.

[32] G. R. Wyatt, and G. F. Kalf, “The chemistry of haemolymph, I. Trehalose and other carbohydrates”, Journal of general physiology, 40, pp 833-847, 1957,

[33] T. Ambika, “Biochemical changes in silkworm. Bombyx mori L. due to infection by Beauveria bassiana (Bols) M.Sc., Dissertation”, university of Agricultural Sciences, Bangalore, pp, 25-86, 1990.

[34] S. C. Kochi & B. B. Kaliwal, “The effect of potassium bromide on biochemical contents of fat body and haemolymph of cross breed races of the silkworm bombyx mori L.”, J. Env- Sci 4(1), 17-24, 2006.