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Research Article

Effect of Environmental Temperature on Cypovirus Multiplication and Biomolecules in Silkworm *Bombyx mori* L.

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ABSTRACT

Two strains of mulberry silkworm, namely Pure Mysore and NB_4D_2, at fifth instar first day were inoculated with 40µl of BmCPV suspension from the stock of 1.215 X 10^6 PIB/ml in 0.75% of NaCl solution by 'oral injection'. Three batches of such inoculated worms were allowed to continue larval development at 20±1°C, 24±1°C and 30±1°C in separate BOD incubators. The crude extract of midgut tissue was prepared from the survivals and used to count the total number of polyhedra per larva in each batch. Further, during virus multiplication at different rearing temperatures, the total amount of glucose, proteins and amylase as well as succinate dehydrogenase activity levels in haemolymph were studied. The experimental results clearly indicated that the atmospheric temperature has a direct influence on a number of polyhedral formations and biomolecules.

Keywords: *Silkworm, Cypovirus, Protein, Glucose, Amylase, Succinate dehydrogenase.*

INTRODUCTION

When a pathogen or a parasite enters into the biological system, it brings about an alteration in the biological reactions either directly by involving in the biochemical reaction or indirectly by modifying the essential material of the reaction (Mahesha, 1997). Silkworm *Bombyx mori* L., being a poikilothermic animal, it is highly influenced by the external atmospheric temperature on its metabolism. However, the optimum temperature of 23-24°C during the fifth instar is ideal for maintaining good physiological status. The metabolic status of an animal is evaluated in terms of the quality and quantity of biochemical constituents, which, in turn, is determined by the interaction between the nature and nurture of the animal (Mahesha, 1997). Most of the biochemical studies associated with cytoplasmic polyhedrosis in silkworm *Bombyx mori* are daily changes in the quantity of nucleic acid and protein in the blood and midgut (Kawase & Hayashi, 1965);

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activities of several enzymes responsible for carbohydrate metabolism in the midgut epithelium of *Bombyx mori* (Yaginuma et al., 1990); amino acids decreased in the haemolymph and midgut epithelium (Kawase, 1965); enhanced protein metabolism in the midgut epithelium (Watanabe, 1970); amylase and succinate dehydrogenase activity levels (Mahesha et al., 2000); and effect of cytoplasmic polyhedrosis on haemolymph proteins (Mahesha et al., 2000) and glucose (Mahesha & Thejaswini, 2013) of the silkworm. However, studies combining biomolecules like glucose, proteins, amylase and succinate dehydrogenase during cytoplasmic polyhedrosis at different temperature conditions are rather scarce. Hence, the present investigation was undertaken.

MATERIALS AND METHODS

Two mulberry silkworm breeds, namely Pure Mysore (multivoltine) and NB_4D_2 (bivoltine) and a stock of cytoplasmic polyhedrosis virus (*Bm*CPV) were used for the present study. Silkworm breeds were obtained from the Department of Studies in Sericultural Sciences, University of Mysore, Mysore and the silkworm rearing was conducted in the laboratory following the method described by Krishnaswami (Krishnaswami, 1978). The Cytoplasmic Polyhedral Inclusion Bodies obtained from the Sericulture Department, University of Agricultural Sciences, G.K.V.K. Bangalore, India, were *per orally* inoculated into the silkworm larvae (NB_4D_2) immediately after the second moult for the multiplication of the virus. Purification was carried out by following the method described by Balakrishnappa and Honnaiah (Balakrishnappa & Honnaiah, 1992). Finally, the stock suspension was prepared, which contained 6.25 X 10^6 polyhedral Inclusion Bodies (PIBs) per ml. Enumeration of PIBs was done by following Neuber's haemocytometer. Based on LC_{50} values (Mahesha, 1997, & Mahesha & Honnaiah, 2002) 1.251 X 10^6 PIB/ml in 0.75% NaCl solution was selected for treatment.

On the First Day, the fifth instar larvae were inoculated with 40µl of PIBs suspension from the stock of 1.251 X 10^6 PIB/ml in 0.75% of NaCl solution by 'oral injection'. Three batches of such inoculated worms were allowed to continue larval development at 20±1°C, 24±1°C and 30±1°C in separate BOD incubators. Each batch consisted of 60 worms in triplicate. Of the survivals (on the 6^{th} Day in NB_4D_2 and 8^{th} Day in Pure Mysore), the whole midgut tissue from twenty five silkworm larvae was pooled separately from all the three batches. Such midgut tissue was homogenized with a small quantity of distilled water, finally made up to 50 ml with distilled water, and filtered through a double-layered muslin cloth. The crude extract was used to count the total number of PIBs per larva in each batch, and fifty PIBs bodies from each batch were screened at 600 x magnification for irregular (an abnormal shape) outline.

About 2-4 larvae were collected daily at regular intervals of 24 hours from the time of inoculation from each batch until the onset of spinning. The abdominal legs were punctured, and the haemolymph was collected in pre-chilled microcentrifuge tubes containing 1mM thiourea to prevent oxidation (Mahesha, 1997). The total proteins were determined by following the method of (Lowry et al., 1951). Bovine serum albumin was used as standard protein. The results were expressed as µg of protein/µl of haemolymph. Blood glucose level was estimated by following the method of Folin-Wu as described by (Oser, 1976). The blood glucose level was expressed as µg glucose per µl haemolymph. Quantitative analysis of amylase activity was done in haemolymph following the method of (Noelting & Bernfeld, 1984), as modified by (Ishaaya & Swirski, 1976). The specific activity of the enzyme was expressed as µg of glucose generated/mg protein/min at 37°C. Succinate dehydrogenase activity levels were estimated by the method of (Nachlas et al., 1960) as modified by (Mahesha & Honnaiah, 2002). The specific activity levels were expressed in µmoles of formazan formed/mg protein/min at 37°C. The data obtained from

the biochemical experiments were statistically analyzed through SPSS by two way ANOVA (Fisher & Yates, 1953) and DMRT (Duncan Multiple Range Test) wherever they were applicable.

RESULTS AND DISCUSSION

In the Pure Mysore race, the role of temperature on the rate of polyhedral Inclusion Bodies formation was more when compared to NB_4D_2 race. In the Pure Mysore breed, the mean number of PIBs per worm was 0.982 x 10^6, 1.021 x 10^6 and 2.714 x 10^6 at 20±1°C, 24±1°C and 30±1°C, respectively. The variation among the experimental sets is found to be statistically significant at 0.0113 level. Further, the results of DMRT revealed that the silkworm batches reared at 20±1°C showed significant ($P<0.05$) variation from the remaining two experimental sets. In the case of NB_4D_2 race, the average number of PIBs per worm was 0.571 x 10^6, 0.75 x 10^6 and 0.833 x 10^6 at 20±1°C, 24±1°C and 30±1°C, respectively. The variation among the experimental sets is found to be statistically significant at 0.0113 level. Further, the results of DMRT revealed that the silkworm batches reared at 20±1°C showed significant ($P<0.05$) variation from the remaining two experimental sets. Apart from this, the temperature did not have any influence on the shape of the PIBs, *i.e*., only regular types, namely tetragonal and hexagonal polyhedral bodies, were observed in all the experimental sets.

The level of blood glucose in the control set of Pure Mysore larvae showed a significant drop during the 5th instar except on the 6th Day (Table 1). The *Bm*CPV inoculated worms also followed the same pattern but with a decreased amount of glucose. When the average blood glucose level during the 5th instar was taken, control worms showed 2.909 µg/µl, followed by T_1 of 2.402 µg/µl, T_2 of 2.271 µg/µl and T_3 of 2.224 µg/µl. In the case of control as well as *Bm*CPV infected NB_4D_2 larvae (Table 2), the glucose level is gradually increased from the beginning to the end of the fifth instar. A well defined change in the quantity of glucose could be observed between control and diseased worms as well as among the infected batches. Of all the batches tested, the average blood glucose level during the fifth instar was found to be the highest in control with 3.014 µg/µl followed by T_1 set with 2.602 µg/µl, T_2 set with 2.521 µg/µl and T_3 set with 2.413 µg/µl. In control worms of the Pure Mysore breed, haemolymph proteins showed a significant increase in their levels every Day up to the 6th Day and a gradual reduction till the end of the fifth instar. This pattern of haemolymph proteins was observed even in the *Bm*CPV inoculated worms except on the first Day when a low concentration of protein was noticed on the third Day when compared to the previous Day (Table 3). When the mean protein concentration during the fifth instar was considered, the highest concentration of protein was observed in the control set (63.29 µg/µl) followed by T_1 set (58.96 µg/µl), T_2 set (55.72 µg/µl) and T_3 set (51.17 µg/µl). In case of NB_4D_2 breed, the haemolymph protein levels of control set showed significant increase in their levels every Day till the end of fifth instar. The *Bm*CPV inoculated batches are also followed the same pattern in protein levels as in the case of control larvae, except 3rd Day when a significant reduction in protein level was observed (Table 4). The average protein concentration at fifth instar stage, was high (47.74 µg/µl) in the control set followed by T_1 worms of 43.64 µg/µl, T_2 worms of 40.26 µg/µl and T_3 worms of µg/µl. The amylase activity levels in haemolymph of control worms of Pure Mysore was increased with the increase in the age during the 5th instar and reached its peak on the 5th Day (Table 5). But, gradual reduction in the activity was observed from 6th Day onwards. The *Bm*CPV inoculated silkworms of all sets also showed the same pattern of enzyme activity as that of control silkworms; but the infected sets showed a reduced rate of enzyme activity. The average activity in the control set during fifth instar was highest with 0.114µg glucose generated /mg protein /min at 37°C followed by T_1 worms with 0.097 µg glucose generated /mg protein/min at 37°C, T_2 worms with 0.092 µg

glucose generated /mg protein/min at 37°C and T_3 worms 0.089 µg glucose generated /mg protein/min at 37°C. In the case of NB_4D_2 larvae, the amylase activity levels in the haemolymph of control silkworms showed its peak activity on 3rd Day of the 5th instar (Table 6). From 4th Day onwards, a gradual reduction was noticed. The *Bm*CPV inoculated batches, are all showed their peak activity levels on 2nd Day only. Of all the examined batches, the average enzyme activity of control worms during the fifth instar was 0.149 µg glucose generated /mg protein/min at 37°C, followed by T_1 worms of 0.138 µg glucose generated /mg protein/min at 37°C, T_2 worms of 0.137 µg glucose generated /mg protein/min at 37°C and T_3 worms of 0.131 µg glucose generated /mg protein/min at 37°C.

The succinate dehydrogenase (SDH) activity in the haemolymph of control larvae of Pure Mysore showed a gradual increment from 2nd to 4th Day (Table 7). Again, from 6th Day, it showed gradual decrease till the end of 5th instar. The *Bm*CPV inoculated batches are also showed the same pattern with reduced enzyme activity. And this reduction of activity was directly proportional to the temperature. When average SDH activity was considered, the silkworms of control set exhibited more enzyme activity of 2.60 µmoles of formazan formed/mg protein/hour at 37°C, followed by T_1 worms of 1.964 µmoles of formazan formed/mg protein/hour at 37°C, T_2 worms of µmoles of formazan formed/mg protein/hour at 37°C and T_3 worms of 1.744 µmoles of formazan formed/mg protein/hour at 37°C. The SDH activity levels in the haemolymph of control larvae of NB_4D_2 showed its maximum enzyme activity on 3rd Day of fifth instar (Table 8). The batches of all experimental sets are also followed the same pattern with a reduced rate of enzyme activity. When average enzyme activity during the fifth instar was observed, the silkworms of control set exhibited more activity of 2.704 µmoles of formazan formed/mg protein/hour at 37°C followed by T_1 worms of 2.268 µmoles of formazan formed/mg protein/hour at 37°C, T_2 worms of 2.154 µmoles of formazan formed/mg protein/hour at 37°C and T_3 worms of 1.842 µmoles of formazan formed/mg protein/hour at 37°C.

Table 1: Amount of Glucose in haemolymph of Pure Mysore larvae treated with *Bm*CPV (Values expressed as µg/µl)

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	Average concentration
Control (24˚C±1˚C)	4.890	3.460	2.665	1.974	2.240	2.885	2.810	2.909
T_1 (20˚C±1˚C)	4.510	3.260	2.040	1.440	1.720	2.010	1.839	2.402
T_2 (24˚C±1˚C)	4.410	3.010	1.910	1.310	1.640	1.910	1.712	2.271
T_3 (30˚C±1˚C)	4.340	3.180	1.810	1.230	1.580	1.810	1.621	2.224

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 2: Amount of Glucose in haemolymph of NB_4D_2 larvae treated with *Bm*CPV (Values expressed as µg/µl)

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	Average concentration
Control (24˚C±1˚C)	1.025	2.230	2.489	3.067	6.257	3.014
T_1 (20˚C±1˚C)	1.071	2.073	2.117	2.67	5.080	2.602
T_2 (24˚C±1˚C)	1.092	1.96	2.181	2.42	4.89	2.521
T_3 (30˚C±1˚C)	1.008	1.930	2.010	2.250	4.870	2.413

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 3: Amount of protein in haemolymph of Pure Mysore larvae treated with *Bm*CPV (Values expressed as µg/µl)

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	Average concentration
Control (24˚C±1˚C)	20.548	23.730	44.500	72.690	101.120	98.644	98.10	63.29
T_1 (20˚C±1˚C)	26.180	22.110	41.50	67.740	94.050	82.600	79.010	58.96
T_2 (24˚C±1˚C)	25.16	21.95	39.32	56.47	92.77	79.83	74.56	55.72
T_3 (30˚C±1˚C)	23.910	21.71	35.15	46.17	89.92	75.33	66.02	51.17

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 4: Amount of Protein in haemolymph of NB_4D_2 larvae treated with *Bm*CPV (Values expressed as µg/µl)

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	Average concentration
Control (24˚C±1˚C)	28.31	32.65	40.39	58.08	79.26	47.74
T_1 (20˚C±1˚C)	36.66	29.79	39.56	49.61	62.58	43.64
T_2 (24˚C±1˚C)	34.87	28.54	36.87	46.38	54.67	40.26
T_3 (30˚C±1˚C)	33.44	29.46	33.50	44.43	50.89	38.34

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 5: Amylase activity levels (µ moles of glucose generated/mg protein/min at 37°C) in haemolymph of Pure Mysore larvae treated with *Bm*CPV

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	Average concentration
Control (24˚C±1˚C)	0.114	0.116	0.119	0.121	0.115	0.111	0.102	0.114
T_1 (20˚C±1˚C)	0.097	0.098	0.105	0.108	0.092	0.094	0.086	0.097
T_2 (24˚C±1˚C)	0.092	0.093	0.096	0.098	0.093	0.090	0.083	0.092
T_3 (30˚C±1˚C)	0.089	0.090	0.092	0.094	0.089	0.087	0.088	0.089

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 6: Amylase activity levels (µ moles of glucose generated/mg protein/min at 37°C) in haemolymph of NB_4D_2 larvae treated with *Bm*CPV

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	Average concentration
Control (24˚C±1˚C)	0.192	0.177	0.141	0.117	0.112	0.149
T_1 (20˚C±1˚C)	0.174	0.162	0.128	0.107	0.102	0.138
T_2 (24˚C±1˚C)	0.178	0.165	0.131	0.109	0.104	0.137
T_3 (30˚C±1˚C)	0.170	0.160	0.120	0.106	0.101	0.131

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 7: Succinate dehydrogenase activity levels ((µ moles of formazan formed/mg protein/hour at 37°C) in haemolymph of Pure Mysore larvae treated with *Bm*CPV

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	Average concentration
Control (24˚C±1˚C)	2.43	2.51	2.63	2.54	2.48	2.74	2.88	2.60
T_1 (20˚C±1˚C)	1.80	1.86	1.94	1.88	1.84	2.03	2.13	1.925
T_2 (24˚C±1˚C)	1.89	1.88	1.98	1.89	1.87	2.14	2.10	1.964
T_3 (30˚C±1˚C)	1.63	1.68	1.76	1.71	1.66	1.84	1.93	1.744

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

Table 8: Succinate dehydrogenase activity levels ((µ moles of formazan formed/mg protein/hour at 37°C) in haemolymph of NB_4D_2 larvae treated with *Bm*CPV

Dose of *Bm*CPV	2nd Day	3rd Day	4th Day	5th Day	6th Day	Average concentration
Control (24˚C±1˚C)	2.76	2.94	2.72	2.44	2.66	2.704
T_1 (20˚C±1˚C)	2.31	2.47	2.28	2.05	2.23	2.268
T_2 (24˚C±1˚C)	2.19	2.33	2.16	1.96	2.13	2.154
T_3 (30˚C±1˚C)	1.88	2.01	1.85	1.66	1.81	1.842

The variation between experimental sets, between age groups and the interaction effect between experimental sets and age groups are all found to be statistically significant at 0.000 level.

The influence of temperature on the rate of polyhedra formation was found to be highly significant. In Pure Mysore breed it was 0.982 x 106, 1.021 x 106 and 2.714 x 106 PIBs/worm at 20±1°C, 24±1°C and 30±1°C respectively. In case of NB_4D2 breed it was 0.571 x 106, 0.75 x 106 and 0.833 x 106 PIBs/ worm at 20±1°C, 24±1°C and 30±1°C. Pure Mysore strain had more number of polyhedral than NB_4D_2 strain. As temperature is considered to be one of the important factors in determining the rate of metabolism, it might have directly influenced the rate of PIBs formation also. It is believed that as the temperature is increased up to a certain limit, the rate of metabolism in silkworm also increases, which in turn might have helped to increase in the number of viruses. Therefore, the rate of polyhedral formation might have become higher at high temperature. At the same time, temperature did not play any role on the shape of polyhedral bodies; only regular types like hexagonal and tetragonal were observed. The shape of cytoplasmic polyhedral bodies is controlled by either virus particle (Aruga et al., 1961) or by the change of cell environment (Bergold, 1963) or by the change of polyhedral protein. Since the temperature does not modify these factors, it does not control the shape of the polyhedral. Hence the regular shape of the polyhedral was maintained.

Glucose is continuously released into the bloodstream from the midgut cells. Such additions are increasingly high in the fifth instar since the feeding activity is relatively high, that is of the total food consumed in the entire larval stage, as much as 83.83% of it utilized in the fifth age itself. As shown in the table 1 and 2, the average quantity of glucose during 5th age was found to be high in the control. But, the haemolymph of the worms *Bm*CPV inoculated worms showed a lower concentration of blood glucose level. Among *Bm*CPV inoculated batched, the decrease in quantity of glucose is directly correlated with the increase in rearing temperature.

The protein concentration in the haemolymph of both the strains infected with *Bm*CPV showed significant reduction. The

decrease in blood protein was found to be high as the rearing temperature increased. The decrease in blood protein could be due to gluconeogenesis induced by the lower inputs of glucose in to the haemolymph since the infected worms ate a low quantity of mulberry leaves in addition to utilization by the pathogen for synthesis of viral and polyhedral proteins. (Kawase & Hayashi, 1965) results also support our observations.

The α -amylases (α -1, 4-glucan-4-glucanohydrolases; EC 3.2.1.1) are the hydrolytic enzymes and are one of the key enzymes involved in digestion and carbohydrate metabolism in insects (Baker, 1983, & Baker, 1987). And digests the starch contained in mulberry leaves and releases as simple sugars. On the other hand, the role of haemolymph amylase is not yet known. The *Bm*CPV inoculated silkworms exhibited significant reduction in amylase activity. This reduction is a clear indication of decreased rate of feeding, digestion and assimilation. In haemolymph, the enzymatic proteins might undergo gluconeogenesis as it is observed in connection with the blood protein. Such a metabolic diversion becomes very much necessary as the drop in blood glucose levels might lead to serious consequences like cell death. Even in higher animals like man the blood glucose levels are maintained within a certain range and hypoglycemia leads to incapacitation and instant death.

The succinate dehydrogenases (succinate: acceptor oxidoreductase; EC 1.3.99.1) is an enzyme complex, bound to the inner mitochondrial membrane of mammalian mitochondria, insects and many bacterial cells (Farshid & Mahesha, 2012). It is the only enzyme that participates in both the citric acid cycle and the electron transport chain and the activity levels may be correlated with the level of oxidation in a particular cell/tissue. The *Bm*CPV infection affects on succinate dehydrogenase activity levels in both the breeds. The enzyme activity is significantly lowered, and this might be due to scarcity of essential material for the synthesis of enzyme, as these materials might have been deviated for the synthesis of viral and polyhedral proteins.

CONCLUSION

The atmospheric temperature during cypovirus infection in silkworm *Bombyx mor*i L., increases the number of PIBs formation, reduces the total amount of glucose, proteins and; amylase as well as succinate dehydrogenase activity levels. The increment in a number of PIBs and reduction in the amount and activity levels of biomolecules is gradual; and correlates with increment in the rearing temperature as it influences almost all metabolic reactions including viral multiplication within the host cell. In addition, due to the diversion of digested food materials towards the metabolic activity of the host, multiplication of viral proteins, formation polyhedral proteins and defense mechanism of the host; more number of PIBs and low level of biomolecules appeared. Such a basic knowledge about these aspects during polyhedrosis might offer an important tool to manage insect pathogens in general and cypovirus in particular. Also, the information gathered in this research work contributes to the basic virology in general.

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REFERENCES

Aruga, H., Hukuhara, T., Yoshitake, N., & Israngkul, N. A. (1961). Interference and latent infection in the cytoplasmic

polyhedrosis of the silkworm *Bombyx mori* (Linnaeus). *J. Insect Pathol*., *3*, 81-92.

Balakrishnappa, Y. K., & Honnaiah S. (1992). Isolation and purification of cytoplasmic polyhedrosis virus from *Bombyx mori* L. *J. Mysore University, sect. B, XXXII*, 331- 333.

Baker, J. E. (1983). Properties of amylases from midguts of larvae of *Sitophilus zeamais* and *Sitophilus granarius*. *Insect Biochem*., *13*, 421-428.

Baker, J. E. (1987). Purification of isoamylases from the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) by high-performance liquid chromatography and their interaction with partially purified amylase inhibitors from wheat. *Insect Biochem*., *17*, 37-44.

Bergold, G. H. (1963). *The nature of nuclear polyhedrosis viruses*. In Insect Pathology, an Advanced Treatise (E. A. Steinhaus, ed.), *1*, 413-456.

Farshid, G. K., & Mahesha, H. B. (2012). Studies on succinate dehydrogenase and its relationship with economic characters of silkworm *Bombyx mori* L. *Annals Bio. Res*., *3*(7), 3638-3651.

Fisher, A. R., & Yates, F. (1953). *Statistical tables for biological, agricultural and medical research*, 6th Ed. Pub. by Longman Group Ltd., England.

Ishaaya, I., & Swirski E. (1976). Trehalase, invertase and amylase activities in the black scale, *Saissetia oleae* and their relation to host adaptability. *J. Insect Physiol*., *22*, 1025-1029.

Kawase, S., & Hayashi, Y. (1965). Nucleic acid and protein changes in blood and midgut of the silkworm *Bombyx mori* (Linnaeus), during the course of cytoplasmic polyhedrosis. *J. Invertebrate Pathol*., *7*, 49-54.

Kawase, S., & Hayashi, Y. (1965). Nucleic acid and protein changes in blood and midgut of the silkworm, *Bombyx mori* (Linnaeus), during the course of cytoplasmic polyhedrosis. *J. Invertebrate Pathol*., *7*, 49-54.

Kawase, S. (1965). Free amino acids in the haemolymph and midgut of the silkworm, *Bombyx mori* (Linnaeus), infected with cytoplasmic polyhedrosis virus. *J. Invertebrate Pathol*., *7*, 113-116.

Krishnaswami, S. (1978). *New technology of silkworm rearing*. Central Sericultural Research and Training Institute, Bulletin no. 2, Central Silk Board, India, pp. 1-23.

Lowery, O. H., Rosebrough, N. J., Favor, A. L., & Remdall, R. J. (1951). Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem*., *193*, 265-275.

Mahesha, H. B. (1997). Cytogenetic and biochemical studies in a few races of *Bombyx mori* L., treated with cytoplasmic polyhedrosis virus and a mutagen. *Ph. D*. Thesis, University of Mysore, Mysore, India.

Mahesha, H. B., Thejaswini, P. H., & Honnaiah, S. (2000). Effect of cytoplasmic polyhedrosis on amylase and succinate dehydrogenase activity levels in silkworm *Bombyxmori* L. *Entomon., 27*(3), 269-279.

Mahesha, H. B., Thejaswini, P. H., & Honnaiah S. (2000). Effect of cytoplasmic polyhedrosis on haemolymph proteins of silkworm *Bombyx mori* L. *Ann. Entomol, 18*(1), 27- 32.

Mahesha, H. B., & Thejaswini P. H. (2013). studies on the haemolymph glucose level in silkworm *Bombyx mori* L. during cytoplasmic polyhedrosis., *Int. J. Pure App Biosci*., *1*(3), 11-16.

Mahesha, H. B., & Honnaiah S. (2002). Effects of cytoplasmic polyhedrosis on silkworm (*Bombyx mori* L) and its transmission from generation to generation. *J. Seric*., *8-10* (1&2), 38-43.

Mahesha, H. B., & Honnaiah, S. (2002). Amylase and succinate dehydrogenase activities in F1 progeny raised from

ethyl methanesulfonate treated silkworm *Bombyx mori* L. *Indian J. Seric.*, *41*(1), 24-28.

Nachlas, M. M., Marguleis, S. I., & Seligman, A. M. (1960). Sites of electron transfer to tetrazolium salts in the succinoxidase system. *J. Biol. Chem*., *235*(9), 2737-2743.

Noelting, G., & Bernfeld, P. (1984). Sur les enzymes Amylolytiques III. La. B-amylase Dosage. D. activite'et controle de L' absence d'α-amylase. *Helv. Chim. Acta*., *31*, 286-290.

Oser B. L. (1976). *Hawk's physiological chemistry*. 14th Edn. Tata McGraw Hill publishing company Ltd, New Delhi, India.

Watanabe, H. (1970). Enhancement of protein metabolism in the midgut epithelium of the silkworm, *Bombyx mori*, by infection with a cytoplasmic polyhedrosis virus. *J. Invertebrate Pathol*., *15*, 247-252.

Yaginuma, T., Kobayashi, M., & Kawase, S. (1990). Changes in activities of several enzymes responsible for carbohydrate metabolism in midgut epithelium of the silkworm *Bombyx mori*, infected with cytoplasmic polyhedrosis virus. *J. Seric. Sci*. Japan., *59*(1), 64-70.