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1291. Status of Turcicum Leaf Blight *(Exserohilum turcicum)* of Maize in Kashmir. **T. A. Wani, Mushtaq Ahmad.**

1297. Suppression of Dead-Heart and Folded Leaf Symptoms in Paddy by *Trichogramma japonicum* Ashmead in Seppa Area of Arunachal Pradesh, India. **Pravin Pati Tripathi, Anup Chandra, Asha Srivastava.**

1300. Evaluation of Plant Growth Promoting Traits of Mungbean Rhizobia. **Monika, Leela Wati.**

1305. Influence of Date of Sowing and Cultivars on Growth and Yield of Fodder Oats (*Avena sativa* L.) in New Alluvial Zone of West Bengal. **C. K. Kundu, A. K. Hansda, N. C. Sarkar, Pintoo Bandopadhyay, P. S. Bera.**

1309. Variability and Heritability Studies in Advanced Tomato (*Solanum lycopersicum* L.) Lines. **N. Kavyashree, Revanappa, D. Sathish, S. Gururaj.**

1314. Genetic Diversity for Yield and Its Component Traits in Fenugreek (*Trigonella foenum-graecum* L.). **Jyothi Veerayya Hosamath, R. V. Hegde, A. G. Vijayakumar, C. K. Venugopal, M. G. Hegde.**

1318. Influence of Different Sources of Potassium on Fruit Quality and Shelf-Life of Mango cv Himsagar. **Kaushik Das, Mou Roy, Debjit Roy, Pallab Dutta.**

1323. Prevalence of Emerging Disease of Macrophomina Stem Canker of Pigeonpea in North Eastern Karnataka, India. **Swamy Chikkanna, M. K. Naik, Y. S. Amaresh, N. K. Jayalakshmi.**

1327. Standardization of Seed Coating Polymer on Seed Quality of Rice (*Oryza sativa* L.). **S. Alur Rajeshwari, N. M. Shankutala, S. R. Doddagoudar, Vijaykumar Kurnallikar, R. S. Roopa Bai.**

1331. Biological Properties of Vermicompost Produced by Locally Isolated Earthworms from Temperate Kashmir Region. **Tabinda Sehar, M. Y. Zargar, G. C. Sheikh, Z. A. Baba.**

1336. Effect of Iron Sulfate Application on Yield, Nutrient Uptake and Available Nutrient Status of Soybean at Harvest (*Glycine max* L.) in *Vertisols* of Karnataka, India. **Shivanand, B. M. Radder, Sayyed Phayaz Husen, Vishwajith.**

1341. Genetic Variability for Iron Deficiency Chlorosis Resistance in Groundnut (*Arachis hypogaea* L.) under Calcareous Soil. **Ishwar H. Boodi, C. D. Soregoan, Shruti Koraddi, B. S. Patil.**

1348. *In-vitro* Screening of Plant Extract to Control Bacterial Leaf Blight of Rice Disease Caused by *Xanthomonas oryzae* pv *oryzae*. **R. K. Ranjan, Bimla Rai, P. K. Jha.**

1354. Hemolymph Glucose Dynamism in Silkworm *Bombyx mori* Infected with Grasserie and Flacherie. **Rashmi. P. Joshi, I. A. Raja.**

1357. Genetic Divergence in Chickpea (*Cicer arietinum* L.) Genotypes under Normal and Late Planting. **Akanksha Tiwadi, Anita Babbar.**

1364. Field Evaluation of Consortium of *Azospirillum*, PSB and AM Fungus on Yield Parameters of Direct Seeded Rice. **M. D. Siddaram, G. P. Santhosh, S. Shubha, R. C. Gundappagol, M. R. Umesh.**

1368. Comparative Study of Organic Matter and Humic Acid on N Mineralization in Rice– Mustard Cropping Sequence. **Niladri Paul, Debabrata Dhar, Ashim Datta, Dipankar Saha.**

1376. Genetic Analysis of Grain Yield and Phenological Traits in Bread Wheat (*Triticum aestivum* L.). **Sandeep Kumar, Pradeep Kumar, S. A. Kerkhi.**

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Hemolymph Glucose Dynamism in Silkworm *Bombyx mori* Infected with Grasserie and Flacherie

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Abstract The effects of infectious diseases Grasserie, viral and bacterial Flacherie on hemolymph glucose were examined in the hemolymph of fifth instar larvae at early and late experimental stage. The hemolymph collected from the control non infected healthy worms and that from the diseased worms. We reported that relative to controls, early infection with Grasserie slightly altered the hemolymph content of the glucose, in all the experimental worms. However a marked decrease in glucose level with the advancement of the infection on 5th day of the fifth- instar silkworms infected with Grasserie, viral Flacherie and bacterial Flacheric was noticed. This decrease in glucose contents was much higher in silkworm infected with bacterial Flacherie. The decreased reported, could be due to the utilization of less food material, reduced rate of conversion and metabolism.

Keywords Hemolymph, Silkworm *Bombyx mori*, Grasserie, Flacherie, Glucose.

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Introduction

Silkworm, *Bombyx mori* is a purely domesticated insect since 4,500 years, but like other domesticated animals it become quite delicate venture and might be easily susceptible to a number of diseases, causing great economic loss. Pathogenic diseases like Grasserie and Flacherie are common and vary seasonally in Vidarbha region of Maharashtra [1], high temperature and the dry climatic conditions of the district are conductive to the incidence of these diseases. The pathogens causing Grasserie and Flacherie, usually enter per orally through the contaminated food into the alimentary canal and penetrate midgut wall and then to the hemolymph. Hemolymph is the first line of pathogenic attack through midgut. Therefore study of hemolymph is significant in monitoring and control of diseases at early stages, most of which develops seasonally [2, 3]. All animals, have their own specific seasonal diseases, which usually emerge at the time a species becomes so abundant in a particular phase of year, that it menace the affluence of the coming generations [4].

Though major bimolecular such as carbohydrates, proteins and lipids take active part in physiological process underlying growth and development, they also help to resist the stress and disease. Alterations in the carbohydrates of a silkworm plays vital role in the interface between the host and disease pathogens as a part of survival approach including physical defends.

Table 1. Glucose (mg %) in hemolymph of 5th instar silkworms during Grasserie, viral Flacherie and bacterial Flacherie.

Control		Grasserie		Viral Flacherie		Bacterial Flacherie	
Early Day 1	Late Day 6	Early Day 1	Late Day 6	Early Day 1	Late Day 6	Early Day 1	Late Day 6
10.1 ± 0.70	10.58 ± 1.37	8.44 ± 0.61	6.18 ± 0.57	5.7 ± 0.45	4.76* ± 0.27	7.1 ± 0.71	4.18* ± 0.37

Martignoni [5] and Shigematsu and Noguchi [6] reported that pathogenic infections induce biochemical and physiological alterations in insect Mahesha et al. [7] and Mahesha et al. [8] reported the same in silkworms. Chino and Gilbert [9] pointed out that biomolecule like glucose are the major components in living organisms food that either directly or indirectly used as energy source for all vital activities. It serves as main source of energy to a number of insect species and in other animal species. Changes in glucose either quantitative or qualitative, acts as sensitive alarm in disease monitoring mechanism. Therefore alteration in the glucose contents of the body may be used as indicator parameters and may provide a specific fingerprint in disease monitoring and control. Therefore, we have studied, hemolymph glucose dynamism, as a possible sensitive indicator of stress caused by infectious diseases like Grasserie and Flacherie (bacterial and viral) in silkworm *Bombyx mori.*

Materials and Methods

Mildly diseased larvae are identified at early larval stages, collected from various sericulture units in the district and were continued to rear on mulberry leaves at 25° C. The measurements of glucose were made in the hemolymph of fifth instar larvae, beginning from the newly molted stage (day one) and continued till the 6th day of the instar. The fifth instar larval period was divided into two chronologically identified states as early experimental stage on day one and late experimental stage on day five. The hemolymph was collected from the control non infected healthy worms of the same state and that from the diseased worms by cut through the proleg and used for measurement of glucose. To avoid the activity of prophenol oxidase followed by melanisation of hemolymph, 1 mg of phenylthiourea was added to the samplex and preserved at -20 degree. An ELICO clinical chemistry analyzer Cl 162 and prescribed assay kits were used for the estimation of the glucose.

Results and Discussion

In the present study we explored that the infection with pathogens of Grasseric and Flacheric affected glucose content in the hemolymph of silkworm *Bombyx mori.* Table 1 and Fig. 1 releative to controls early infection with Grasserie non significantly affected the hemolymph content of the glucose, 8.44% mg as compared to the control healthy non infected silkworm at the same age 10.1% mg. However a slight decrease in glucose level in the hemolymph with the advancement of the infection on 5th day of the fifth-instar silkworms was noticed, which was 7.1 % mg as compared to 10.38% mg in control healthy worms.

The results also showed that early infection with viral Flacheric altered the hemolymph content of the glucose, 5.7% mg as compared to the control healthy non infected silkworm at the same age, 10.1% mg. However a highly significant decreases in glucose level in the hemolymph with the advancement of the viral Flacherie infection on 5th day of the fifth-instar silkworms was noticed, which was 4.76% mg as compared to 10.385 mg in control healthy worms.

Similarly, on early infection with bacterial Flacherie the hemolymph content for glucose, was not significantly affected, 7.1% mg as compared to the control healthy non infected silkworm at the same stage, 10.1% mg. However a marked decrease in glucose level in the hemolymph with the advancement of the disease on 5th day of the fifth-instar silkworms was noticed, which was 4.18% mg as compared to 10.38% mg in control healthy worms. Michitsch and Steele [10] observed that the content of carbohydrates in insect hemolymph is regulated by the adipokinetic hormone secreted by corpus cardiacum and this

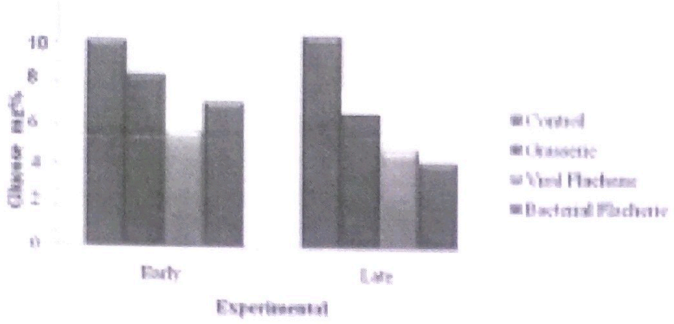

Fig. 1. Glucose (mg %) in hemolymph of silkworms during Grasserie, viral Flacherie and bacterial Flacherie.

hormone could increase the level of trehalose and reduce the level of lipid in hemolymph. In the present study the decrease of glucose at the end of larval instar may also be due to the alteration in activity of adipokinetic hormone in hemolymph Michitsch and Steele [10] further accepted that, whether microbial infection affects the content of the carbohydrates and lipids by interfering with hormone balance is unknown. According to Etebari et al. [11] the decrease of glucose due to late infection reported in the present study can first could be attributed to an outside stress or a response to subsides the sress. In the present study viral infection could prevent the feeding of larvae by unfavorable effects on midgut tissues and as a result in the early days after infection, decrease of some compounds like glucose could be due to starvation stress. Higher glucose level observed in control healthy batches, according to Mahesha and Thejaswini Manohar [12] might reflect the higher rate of food ingestion and digestion. [12] documented that the drop in the hemolymph glucose level in diseased worms represents sustained depletion of the same since the infection alters the normal function of the mid gut. A breakdown of organic constituents which is according to Manohar Reddy [13], is mainly essential to meet the energy under stress condition, suggesting its mobilization to meet the higher demands under pathogenic stress. Our study concluded, the glucose, in the viral and bacterial infected silkworms suffers negative variation in hemolymph, due to the utilization of less food material, reduced rate of conversion and low metabolism leading to less production by the surviving silkworms [11]. Further study at molecular level may pave the way for better understanding of the metabolic responses to infection.

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