Total Haemocyte Count in Silkworms Bombyx Mori (Lepidoptera: Bombycidae) During Pathogenic Diseases in Akola District (Maharashtra)

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Abstract

Total haemocyte count was studied in Silkworm Bombyx mori larvae infected by pathogenic diseases Grasserie and Flacherie in Akola district. THC in silkworms infected with, Grasserie, and Flacherie recorded significant decrease at early infection (on second day with Grasserie 335.0 THCx103/mm^3 and with Flacherie was 319.0 THCx103/mm^3, as compared to control healthy non infected worm of the same developmental stage, which had 389.8 THCx103/mm^3) however, late infection i.e. on day five of the 5th instar larva, these diseases led to a more significant decrease in total hemocytes count as compare to non infected healthy silkworms of the same developmental stages (Grasserie 288.0THCx103/mm3 and Flacherie 244.5 THCx103/mm^3, as compared to control worm 465.0 THCx103/mm3). The results indicating that these cells respond quickly to the pathogenic diseases at both early and late infections and also pointed to the effective role of the hemocytes in the response of Silkworm to Grasserie, and Flacherie pathogens.

KEYWORDS: silkworms Bombyx mori, 5th instar larvae, Grasserie, Flacherie, haemolymph

Introduction:

Akola is a district in the Indian state of Maharashtra (Vidarbha) with an area of 5,431 km² lie on Latitude 20° 42' 10.59"N Longitude 76°59'57.97" E Altitude 285 m and is largely a fertile plateau. Though the district is well known as cotton district, farmers in many areas are diverted in opting rearing of mulberry silkworm, using CSR2 and Kolar gold breeds of silkworm *Bombyx mori*, and taking commercial crops round the year with small sericulture units in the farms. Continuous domestication, year after year leads to perpetuation of diseases in the silkworm, causing a great economic loss. Pathogenic diseases like Grasserie, Flacherie and Muscardine are common and are vary seasonally in Vidarbha region (Rashmi *et al.,* 2013). 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 in to the alimentary canal and penetrate midgut wall and then to the haemolymph. Haemolymph is the first line of pathogenic attack through midgut. Therefore study of Haemolymph is significant in monitoring and control of diseases at early stages. Hemocytes, in Haemolymph, carry out many essential physiological activities and are chief mediators of cellular defense response (Nahla *et al.,* 2010). Hemocytes responses during pathogenic attack in insect are good indicators of host-pathogen interaction (Da Silva *et al.,* 2000; Gillespie *et al.,* 1997). THC (total haemocyte count) in one cubic milliliter of haemolymph indicates the number of cells available for these defensive interactions. Stress conditions affect total haemocyte count as variation reported during fungal, bacterial, and viral infection (Hung *et al.,* 1993 and Nataraju, 1995). The variations in total hemocytes count in response to Grasserie and Flacherie in silkworm's haemolymph so far not studied in Akola district. Therefore the present study is carried out to

investigate the same in fifth instar larvae of Silkworm *Bombyx mori* infected with pathogenic Grasserie and Flacherie.

Material and methods:

With due consent of the farmers, worms infected with Grasserie and Flacherie were collected on the basis of gross pathology their early infection (first 2-3rd day of fifth instar) and late infection (4-6th day of fifth instar) states. Fresh haemolymph was collected from all the diseased larvae and from the healthy non infected larvae of same developmental stages, following the methods of Jalal and Rasoul (2010). Haemolymph was drawn into a the pipette up to 0.5 mark and diluted up to the 11 mark with Toisson's solution (NaCl- 1.0gm, Na_2SO_4 - 8.0gm, Neutral glycerine - 20ml, Methyl violet - 0.025 gm, Distilled water - 160ml).

Neubauer ruling of Hemocytometer was filed with diluted haemolymph and the hemocytes counted in its four corner and one central (1mm^2) squares under a microscope. The number of hemocytes per cubic millimeter (mm3) was calculated using the following formula of Jones (1967).

$$\frac{\text{Total haemocyte counted X Dilution X Depth factor of Chamber}}{\text{Number of Square counted}}$$

Where,

Dilution =20 times,

Depth factor of the chamber =10 (constant) and No. of squares counted = 5.

Results and Discussion:

Table 1 and Fig.1, envisaged significant change in total haemocyte count of silkworms infected with pathogenic diseases, Grasserie and Flacherie which is along the lines of Witting, (1962) ; Shapiro, (1969) ; Rabindra, (1974) ; Chiang, (1988) and Abd-El-Aziz and Awad (2010). Witting (1962) quoted that higher circulating haemocytes is the immediate immune response in some insects at cellular level which depends on the type and dose of the pathogen. On contrary to this we reported reduction in THC, as on second day with Grasserie THC was (335.0 THCx103/mm^3) and with Flacherie was (THC 319.0 THCx103/mm^3), as compared to control healthy non infected worm of the same developmental stage, which had THC (389.8 THCx103/mm^3). Gunnarsson (1988) and Manachini *et al.,* (2011), too reported that total hemocytes levels in *Schistocerca gregaria* (Forskal) and *Rhynchophorus ferrugineus* respectively decreased with attack of pathogenic infections, and supported the results obtained in the present study.

The reduction in THC was highly significant on late infection (day 5) with Grasserie (288.0THCx103/mm3) and Flacherie (244.5 THCx103/mm^3), as compared to control healthy non infected worm (465.0 THCx103/mm3) of the same developmental stage and are in accordance with Ericsson *et al.,* (2009) who reported significant reduction in the haemocyte count in *Trichoplusiani* after pathogenic infection of *E. coli.* Abir *et al.,* (2013) too observed reduction in the THC due to entomopathogenic bacteria infection with (G+) *Bacillus thuringeinsis* on *Bombyx mori* (Lepidoptera: Bombycidae) after 48 h post-infection. Morton *et al.,* (1987) and Rivers *et al.,* (2002), suggested that the existence of infectious pathogens would cause a decrease in the number of circulating haemocytes which is to make the infection booming. This decrease is associated with the

nodule formation and encapsulation around the invading pathogen, as well as the de-granulation of some cell types.

The decrease in THC reported in the present investigation during infectious diseases, according to Edwards (1999) and Russo *et al.,* (2001) might be due to the presence of diseases pathogens in the haemocoel inhibiting the haemopoiesis, and decreasing the number of the cells in the haemolymph. These results may be indicatives of the pathogenic attack on the defense mechanisms of the silkworm against the pathogen and may help in the understanding the role of the haemocytes during Grasserie and Flacherie diseases in Silkworm *Bombyx mori* in small sericulture units in the area.

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Table 1: ***Total haemocyte count in silkworms Bombyx mori (Lepidoptera: Bombycidae) during pathogenic diseases, in Akola District.***

Duration of infection	**Control Larva**	**Grasserie**	**Flacherie**
Early infection (2^{nd} day of 5^{th} instar)	**389.8 ±18.44**	**335.0 ±18.07**	**319.0 ± 25.44**
Late infection (5^{th} day of 5^{th} instar	**465.0 ± 16.26**	**288.0 ±21.16***	**244. 5 ± 19.55***

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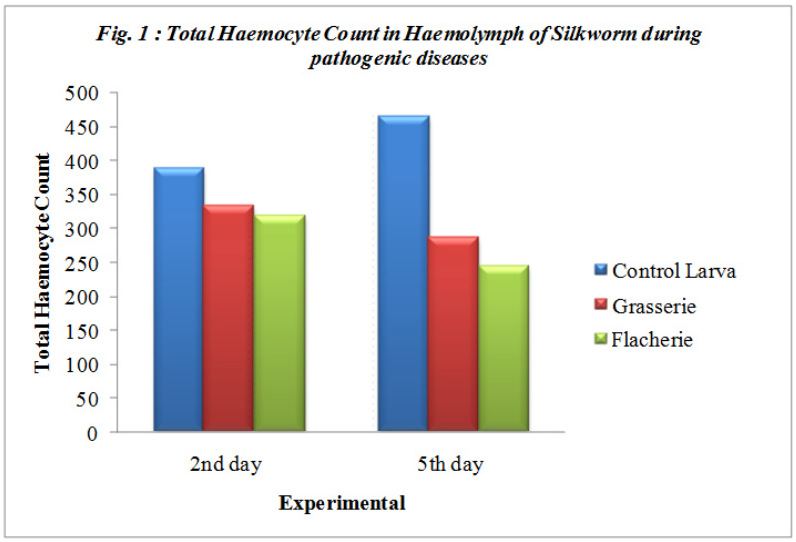
Fig. 1 : Total Haemocyte Count in Haemolymph of Silkworm during pathogenic diseases
Total Haemocyte Count
500
450
400
350
300
250
200
150
100
50
0
2nd day
5th day
Experimental
Control Larva
Grasserie
Flacherie