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Studies on the Host Plants of Eri Silkworm and Its Effect on Haemocyte Count of Larval Stages

Nilakshi Misra Bhagawati
Research Scholar,
Deptt.of Zoology,
University of Science and Technology,
Meghalaya

Arup Nama Das
Assistant Professor,
Deptt.of Zoology,
University of Science and Technology,
Meghalaya

Rezina Ahmed
Associate Professor,
Deptt.of Zoology,
Cotton University,
Guwahati, Assam

Abstract

Ericulture is a traditional agro-based eco-friendly economic tradition in Assam, the end product of which is “Silk”. In terms of contribution to employment and income by all the four major sericulture activities of Assam Muga, mulberry & Tasar, ericulture occupies the prime position among the poor people. Moreover, because of its utility and comparative lower price than the other silk varieties; it gained much popularity among the middle and lower middle income population across the Assam. In Assam, more than 1.35 lakh families were engaged in ericulture activities during 2005 - 2006 (about 70% of the then total sericulture practising families in the state) produced about 700 MT of eri cocoon (Directorate of Sericulture, Government of Assam, 2007). It has been mostly practised by the poor tribal communities like Misings, Kacharis, Bodos, Mikirs, Rabhas, Karbis and Garos and in most cases their women folk practice the same to supplement their family income and also provide nutritional support through the delicious by-product, pupae. A large number of families were found to come out of poverty or acute poverty due to their engagement in eri rearing and weaving activities. Therefore, if undertaken properly, there is ample scope for sustainable employment and income of a wider section of rural population. Although there are some studies on the scientific aspects of ericulture till now no serious study has been made on the host plant aspects of silk-culture (sericulture) especially on ericulture of Assam. An earlier survey was conducted by the Sericulture and Weaving Department of Government of Assam during 1975 - 1976 in 598 Gaon Panchayats of the plain districts of Assam to assess the position of the silk industry in terms of production and employment. It was reported that the majority of population pursuing the silk culture as leisure time occupation. Also, it is important to identify the better breed of Host plant and its new areas of its development in Assam for the betterment of society.

Keywords: Ericulture, Tribal Community, Silk Industry, Host Plants, Rural Population.

Introduction

Sericulture is an industry concerned with the rearing of the silkworm species (Mulberry, Eri, Muga and Tassar), reeling and spinning of cocoons for production of raw silk and cultivation and maintaining food plants for yield of quality leaves. It is an important cottage industry in India. The natural silk was reported to be discovered in China (2640BC). The North – eastern states occupy a distinctive place in the global silk map for being the all four varieties of commercial silk. Assam is called the home land of Eri and Muga silkworm as their food plants are widely distributed in the entire region. In the year 1679, an English trader attempted to export several bales of Eri silk from Assam to Europe. It is an agro based industry, the end product of which is the silk (Sastry et al., 1984). Eri silkworm, *Samia ricini* (Donovan) is a multivoltine sericigenous insect. Besides the North – eastern states, the farmers of several other states viz. Andhra Pradesh, Madhya Pradesh, Tamilnadu, Karnataka, Maharastra, Uttarakhand, Uttar Pradesh, Jharkhand, Bihar, West Bengal, Orissa and Sikkim have taken up Eri culture. The quality of silk is directly related to the quality of feed which plays a remarkable role for growth and development of the silkworm.The ideal range of temperature for the growth of Eri silkworms is from 20 ^{o}C to 40 ^{o}C. The best natural food plants of eri silkworm is considered to have originated at the foot hills of the Himalayas, though according to some it may have originated both in Indian and North Africa (Chowdhury,1970 and Sarkar,1977). In eri silk worm eight eco–races are found,viz Barduar ecorace, Titabar ecorace, Dhanubhanga eco –race,Khanapara eco –race, Sile eco –

race, Nongpoh eco – race, Mendipathar eco – race and Kokrajhar eco –race. These races were evaluated during 1995-97. Among these Barduar, Tita bar and Kokrajhar eco races are exploited due to their better economic traits.

The systematic classification of Eri silkworm is

Kingdom : Animalia
Phylum : Arthropoda
Class : Insecta
Order : Lepidoptera
Family : Saturniidae
Genus : *Samia*
Species : *S. ricini* (Donovan)

Since eri silkworm are polyphagous, they feed on a variety of plants. Among these castor (*Ricinus communis*) is the primary food plant and kesseru (*Heteropanax frograns*) is considered as the major food plant. Tapioca (*Mannihot esculata*), Payam (*Evodia flaxinifolia*), Gomari, Bhotera, Bojramani, Gulancha etc. are the secondary host plants.In Assam and other North –East states, food plants grow naturally among the villages, thus rearers collect leaves from scattered castor plants or kesseru tree, which are on the other hand cultivated in regular fashion for the production of soil seeds. Castor plants are grown in the early Spring or late Autumn seasons. Ghosh (1949), Chowdhury (1960), Navaty (1964),Kapil(1967), Krishnaswami et al. (1974),Sengupta et al. (1974), Roy Chowdhury (1976 b), Bharali(1978), and Sarkar (1980) studied the rearing of eri silkworm on different food plants and reported castor as the primary food plant.

Haemocytes are complex of several types of circulating cells and regularly found in the haemolymph of insects are responsible for the defence mechanism against foreign body that enter into the haemocoel. Present knowledge of insect haemocytes is limited to studies of not more than 200 insect species in about 100 genera (Arnold, 1979). The first study of THCs in insects was made by Tauber and Yeager (1934). In 1935, they studied Orthoptera, Odonata, Hemiptera and Homoptera. A year later, these same authors extended their study to include Neuroptera, Coleoptera, Lepidoptera and Hymenoptera. The insects were heat –fixed (60 ^{o}C for 5 – 10 minutes) and bled from a proleg, after which the blood sample was diluted with physiological saline. The THC (i.e, the number of circulating haemocytes per cubic millimetre) was determined by the method employed for mammalian blood counts. This work represents an outstanding contribution and has served as a model for subsequent investigations. Nittono (1960) classified the blood cells in the silkworm, *Bombyx mori* L. into six types viz., Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes, Imaginal Spherulocytes (observed only at the adult stage, but occasionally in pupa on the day before emergence) and oenocytoids. Akai and Sato (1973 and 1976) and Nakahara et al. (2009) classified silkworm haemocytes into five types based on their morphology and function viz., Granulocytes, Plasmatocytes, Oenocytoids, Prohaemocytes and Spherulocyte. Sarah et al. (2012) studied the diversity of Eri silkworm eco races and their utilization for sustainable development in north east India. They have identified the following six strains of Eri silkworm on the basis of larval colour and marking.

1. Yellow Plain (YP)
2. Yellow Spotted (YS)
3. Yellow Zebra (YZ)
4. Greenish Blue Plain (GBP)
5. Greenish Blue Spotted (GBS)
6. Greenish Blue Zebra (GBZ)

Study Area

The study was done at Khanapara, Sericulture Farm which is located in Guwahati, Assam, India. It is located in extreme south of Guwahati city. The region lies at co - ordinates between 26◦7 ′12" N and 91◦48 ′ 58 " E.

Aim of the Study

Although diversity of Eri silkworm eco races are reported by many of the Researchers, the present study is an attempt to study the different haemocyte count of two strains (GBP and GBZ) of Eri silkworm growing on castor and Kesseru plants with the following objectives:

1. Different haemocyte counting at different larval stages of the selected strains of Eri silkworm (*Samia ricini*) grown on two host plants.
2. Selection of the best strain among the selected strains of Eri silkworm.
3. Ecological Significance of Haemocyte count.

Review of Literature

Studies on Haemocytes

It is inevitable to ascertain the role of haemolymph in insects. Studies on the cellular components of haemolymph have been carried out by different researchers from time to time to understand the physiological as well as immunological aspects.

The insect body cavity (haemocoel) contains haemolymph which serves a function analogous to blood in mammals in that it transports nutrients, waste products and several micro and macro molecules. Several types of haemocytes circulate in insect haemolymph, originated from mesodermally derived stem cells that differentiate into specific lineages. Schwammerdam (1758) was the first person who studied about the insect blood cells (haemocytes) as transport globules.

Wigglesworth (1939) found the role of haemocytes in the growth and moulting of *Rhodinu sprolixus.* Further, in the year 1972, he examined haemocytes and basement membrane formation in

Rhodinus. He found during the period between feeding and ecdysis in the fourth instar larvae of *Rhodinus* the basement membrane below the epidermis of the abdomen increases about threefold in thickness. This increase takes place during the time, about 5 to 7 days after feeding, when the plasmatocytes settle and flatten in great number on the inner surface of the membrane. Insect haemocytes respond to internal changes during development (atecdysis) and to conditions such as starvation, wounding, parasitism, diseases, chemicals including insecticides. Hollande (1911) attempted to classify haemocytes and categorised the haemocytes. Nittono (1960) studied the blood cells in the *B. mori.*Bahadur and Pathak (1971), studied the changes in total haemocytes counts (THC) in relation to sex, development, ecdysis and hot water fixation in *Halysdenta* (Hemiptera). They found there is no difference in the THC of the two sexes; the THC differs in the immature stages and adults. There is a gradual increase in counts during metamorphosis. The fixation of insects in hot water before withdrawal of haemolymph abruptly affects the THC shows a significant increase in the numbers of haemocytes. Their studies made in relation to ecdysis showed that the THC slightly decreases 24 hour before ecdysis, whereas after ecdysis it abruptly falls and then rises again during the middle part of the instar. Arnold (1970) found that the size, form and character of haemocytes were found to change according to the changes in the physiological condition of the insect. Plasmotocytes and Granulocytes are described as the main cell types involved in all defence mechanisms (Beaulaton and Monpeyssin, 1977; Ratclifffe et al. 1985 ; Ratcliffe and Rowley 1987 ; Wiesner and Götz, 1993). Phagocytosis is considered the first barrier against pathogens and it has been described in the haemolymph of many insect species against biological (Ratcliffe and Rowley,1979; Ratcliffe et al. 1985 ;Götz and Boman, 1985 ; Ratcliffe, 1986) and non – biological agents (Wiesner, 1991, 1992 ; Slovak et al., 1991). Saxena and Saxena (1985) studied the cytopathological and numerical changes in haemocytes of *Periplanata americana* after treatment with Malathion. The possible involvements of insect haemocytes in curbing the biological and chemical control efforts of pests are also apparent due to their implication in the detoxification of insecticides (Lackie, 1988). A number of toxicological studies have examined the effects of insecticides on the chemical changes in insect haemolymph (Nath et al., 1997 ; Serebrov et al. 2001). Haemocytes in the haemolymph of insects are responsible for the defence mechanism against foreign body that enter into the haemocoel (Tepass et al. 2001). The degree of variations in the number of haemocytes can be used as an index for diagnosis of the disease. They investigated the recognition of pathogens may be accomplished by plasma or haemocyte proteins that bind specifically to bacterial or fungal polysaccharides. Several morphologically distinct haemocyte cell types cooperate in the immune response. Begum et al. (1998) investigated the effect of sub lethal concentration of deltramethrin (DELM) on the haemocytes of larva, pupa and adult *P. ricini* on the basis of total haemocyte count, differential haemocyte count (DHC) and cytological changes after an interval of 24, 48 and 72 hour after treatment. They found that SLC of DELM caused a decrease in THC and some cytological changes of haemocytes such as cytoplasmic extension, increased vacuolization, cytoplasmic and nuclear distortion, cellular aggregation and degeneration. Spherulocytes and Plasmatocytes were actively involved in the detoxification process. In the silkworm, Plasmatocytes are normally larger than Granulocytes and Plasmatocytes are the major haemocyte type that takes part in encapsulation (Strand et al.,2008). Oenocytids have been considered as the only source of prophenoloxidase (PPO) which is an important immunity protein in insects. In insects, haemocytes have been considered as the only source of ppo (Ashida and Brey, 1998). However, recent studies have shown that there is also PPO in the hind gut and wing discs of the silkworm (Diao et al., 2012; Shao et al., 2012). Higher larval weight in Greenish Blue Zebra (GBZ) and YZ were reported by Singh et al., (2012). N. Bhagawati and R. Mahanta (2014) reported on the changes in haemocyte count in haemolymph of different larval stages of eri silkworm on application of Malathion. Nisar A. Ganie,Afifa S. Kamili, Baqual, M. F., Sharma, R.K., Dar, K.A. & Masarat Bashir (2015) reported that the haemolymph of the silkworm, *Bombyx* mori L. contained five types of haemocytes namely Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes and Oenocytoids. Prohaemocytes were found to be round in shape and Plasmatocytes were amongst the most polymorphic and prominent types. Their shapes ranged from oval, elliptical to spindle with very pointed ends (fusiform) and have a large centrally placed nucleus.

Studies on Effect of Nutrition on The Silkworm

The silkworm nutrition is considered as a major area of research in sericulture (Legay, 1958).The effect of providing foods of different variety in *Bombyx mori* was reported by Takahasi (1961), Hassanein and Shaarawy (1962), Narayanan et al. (1966), Das and Sikdar (1970) and Krishnaswami et al. (1970).Chowdhury (1965) conducted experiments on nutrition with som, soalu and dighlati food plants and obtained the highest percentage of silk with som plants though certain combinations of som, soalu and dighlati also gave the encouraging results. Chowdhury (1970) reported two mutant varieties of yellow and blue coloured larvae in Muga in addition to the existing green larval forms. Koul et al. (1979) worked on the growth and silk production in *Bombyx mori* by feeding three varieties of *Morus alba* and observed maximum larval weight on *Morus alba* local (B). Nataraj et al.(1980) made a feeding trial with different varieties of mulberry in relation to the yield of cocoon crop in different seasons and found significant influence of

season and mulberry varieties in respect of larval duration and E.R.R.

Pillai et al.(1980) in *Bombyx mori* observed higher larval weight and heavier cocoon weight on two mulberry varieties (S54 and Kanava 2). Radha and Sridhar studied the productivity of silkworm as influenced by three varieties of mulberry. Sarkar et al. (1983) performed rearing of *Bombyx mori* on diploid, triploid and tetraploid mulberry varieties and obtained the highest values for all the cocoon characters by feeding mulberry leaves from triploid variety. Raja Sekharagouda (1991) reported the growth promoting effect of plant products. Genetic analysis of scatters population of the Indian Eri silkworm has been studied by R. P. Appukuttanair, A. K. Awasthi. Rearing performance of Eri silkworm fed with different Castor genotypes (E. Getu and Kedir Shifa, 2013).

The frequency and occurrence of blood cell types (haemocytes) in both sexes of adult *Z. variegatus* fed on four different food plants were studied by Idowu A. B. and Sonde O.A. (2003). Only 6 types were recognised, namely Prohaemocytes (PRS), Plasmohaemocytes (PLS), Granulocytes (GRS), Spherulocytes (SPS), Adipohaemocytes (ADS) and Oenocytes (OES) (Chapman, 1982). There was a siginificant decrease in the number of haemocytes as adult age Increases except for Adipohaemocytes.

D.K. Gogoi et al. (2012) reported that Kesseru plants are less susceptible to diseases and pests in comparision to castor. Kesseru feed silkworms produce small cocoon but they are compact with strong fibre. Singh et al. (2012) reported higher larval weight in Yellow Zebra (YZ) and Greenish Blue Zebra (GBZ) when reared on castor food plants during November –December under north India climatic condition. Priyanki Sarmah and Jogesh Chandra Kalita (2013) also reported highest larval body weight in YZ under climatic condition of Assam.

Materials and Method

Equipments

Materials include compound light microscope, glass slides, specimen, cover slip, Borosil beaker, Thermometer, Warm water, Forcep, Blotting paper, Eosin - Methylene Blue for staining and sharp blade.

Collection of The Experimental Seed

Eggs were collected from Eri Seed Grainage, Khanapara in the month of February, 2016. Eri eggs are of medium size compound to Muga or mulberry and oval shaped. The egg has hard chitinous shell and is candid white with colourless glue which helps the egg to adhere on the surface. The eggs of first two days are kept for rearing.

Rearing of Silkworm

The eggs of *Samia ricini* were reared at the Government Basis Muga Farm, Khanapara in the month of February and March, 2016. By following the method of Chowdhury (1982). The cluster of eggs are wrapped in a piece of cloth and kept in a safe place for hatching. After about 13 days i.e. on 14th February larvae hatched out of the eggs.

After hatching the larvae were divided into two groups and transferred them gently to two different rearing trays. They were fed with leaves of Castor (*Ricinus communis*) and Kesseru (*Heteropanax fragrans*) separately. Sterile conditions were maintained so that they could be well protected from unfavourable conditions as well as attack of parasites, predators and diseases.

Separation of Strains and Collection of Larvae

After attaining the third larval stage, larvae of two strains are collected from the two trays separately. Two strains have been found –

1. Greenish Blue Plain (GBP)
2. Greenish Blue Zebra (GBZ)

Collection of Haemolymph

For smear preparation, at first the larvae were kept at 50 ^{o}C water bath for 2 minutes to fix the haemolyph. A small amount of haemolymph was collected by cutting the proleg on the 7th abdominal segment and a smear was prepared on the slide. The dried smear was then stained with Eosin – methylene blue stain and kept for 15 minutes and after rinsing in distilled water, mounted by DPX.For differential haemocyte count, cell categories were counted in 100 cells in each smear using a compound light microscope based on morphological features as described by Nittono (1960). Cell morphology is the basic method which is performed using a variety of microscopy formats, including light microscopy (Gupta, 1985; Brehelen and Zachary, 1986). The relative numbers of different haemocytes were compared between two strains of 3rd, 4th and 5th larval stages on feeding with castor and kesseru leaves separately. The values were statistically analysed following standard statistical procedure.

Results and Discussions

The present study was undertaken for differential haemocyte count of Eri silkworm. Accordingly an investigation was carried out during the month February – March, 2016. Two different food plants were provided and 5 different haemocyte types were identified in the third, fourth and fifth instar larval haemolymph of Eri Silkworm (*Samia ricini*) as reported by Akai and Sato (1973 and 1976) and Nakahara et al., (2009).

Plasmatocytes (PL)

Plasmatocytes were observed as round, fusiform and spindle shaped with a relatively smaller nucleus.The irregular shape of the cells is due to cytoplasmic extensions.

Granulocytes (GL)

These were rounded and ovoid in shape. The centrally located nucleus was found to be relatively small, round, elongate and surrounded by abundance of cytoplasmic granules. The nucleus occupies the central position. Cells with intermediate features between PLs and GRs were also observed.

Adipohaemocytes (AD)

Adipohaemocytes were spherical and oval cells. Compared with that of the plasmatocytes, the nucleus was relatively small rounded and

eccentrically located. The cytoplasm contains small refrigent fat droplets and vacuoles.

Prohaemocytes (PR)

These were found to be small, round, oval and elliptical cells with variable sizes. The nucleus was larger compared with haemocyte types and centrally located.

Spherulocytes (SP)

Cells were observed as oval with a small nucleus. The cytoplasm was thick and homogeneous with a number of spherules present around the nucleus.

The differential haemocyte count profile during the larval stages (3rd, 4th and 5th instars) of two strains of eri silkworm grown on two different food plants showed some variations as shown in the

Table 1: Haemocyte counting in the castor feeding 3rd instar larvae of two strains

Haemocytes 3rd Instar	Mean % ± SD	
	GBP	GBZ
Plasmatocytes	38.5 ± 0.91	40.5 ± 2.27
Prohaemocytes	20.6 ± 0.24	18.5 ± 2.86
Granulocytes	22.3 ± 1.35	24.5 ± 0.27
Spherulocytes	08.5 ± 0.95	09.8 ± 0.75
Adipohaemocytes	5.85 ± 2.25	4.85 ± 0.92

Table 2 : Haemocyte counting in the Kesseru feeding 3rd instar larvae of two strains

Haemocytes 3rd Instar	Mean % ± SD	
	GBP	GBZ
Plasmatocytes	35.05 ± 1.15	39.5 ± 2.76
Prohaemocytes	20.08 ± 1.40	19.5 ± 0.80
Granulocytes	38.20 ± 2.35	40.8 ± 0.57
Spherulocytes	08.40 ± 0.85	08.0 ± 0.75
Adipohaemocytes	04.88 ± 1.25	04.85 ± 0.92

Table 3 : Haemocyte counting in the castor feeding 4th instar larvae

Haemocytes 4th Instar	Mean % ± SD	
	GBP	GBZ
Plasmatocytes	38.05 ± 1.02	42.5 ± 0.56
Prohaemocytes	20.08 ± 2.64	19.8 ± 1.80
Granulocytes	28.20 ± 2.35	23.8 ± 4.56
Spherulocytes	08.40 ± 0.80	09.0 ± 1.70
Adipohaemocytes	06.20 ± 1.25	05.6 ± 0.25

Table 4 : Haemocyte counting in the Kesseru feeding 4th instar larvae of two strains

Haemocytes 4th Instar	Mean % ± SD	
	GBP	GBZ
Plasmatocytes	38.05 ± 2.02	40.5 ± 0.86
Prohaemocytes	21.08 ± 1.64	19.8 ± 2.80
Granulocytes	39.20 ± 2.35	40.8 ± 1.56
Spherulocytes	08.80 ± 0.65	08.0 ± 1.50
Adipohaemocytes	04.60 ± 0.55	04.8 ± 0.45

Table 5 : Haemocyte counting in the castor feeding 4th instar larvae of two strains

Haemocytes 5th Instar	Mean % ± SD	
	GBP	GBZ
Plasmatocytes	39.05 ± 1.02	41.5 ± 0.80
Prohaemocytes	20.08 ± 1.64	19.5 ± 2.80
Granulocytes	27.30 ± 0.35	26.8 ± 0.56
Spherulocytes	08.02 ± 0.65	07.5 ± 0.50
Adipohaemocytes	05.80 ± 0.65	05.1 ± 0.45

Table 6: Haemocyte counting in the Kesseru feeding 5th instar larvae of two strains

Haemocytes 5th Instar	Mean % ± SD	
	GBP	GBZ
Plasmatocytes	39.01 ± 1.80	40.1 ± 0.56
Prohaemocytes	20.20 ± 1.97	19.0 ± 0.55
Granulocytes	41.62 ± 1.30	41.6 ± 1.45
Spherulocytes	08.69 ± 1.61	7.98 ± 0.45
Adipohaemocytes	04.16 ± 0.12	4.01 ± 0.46

The effect of castor feeding on 5tn larval haemocytes were shown in the (table 5, fugure 5). The Plasmatocytes of GBP were (39.05 ± 1.02) and GBZ were (41.5 ± 0.80). The Granulocytes of GBP were (27.30 ± 0.35) and GBZ were (26.8 ± 0.56). The Prohaemocytes of GBP were (20.08 ± 1.64) and GBZ were (19.5 ± 2.80). The Spherulocytes of GBP were (08.02 ± 0.65) and GBZ were (07.5 ± 0.50). The Adipohaemocytes of GBP were (05.80 ± 0.65) and GBZ were (05.1 ± 0.45). After castor feeding it was observed that the percentage of Plasmatocytes was more followed by the Granulocytes, Prohaemocytes, Spherulocytes and Adipohaemocytes.The percentage of Plasmatocytes were more in the GBZ strain, while that of other haemocytes were found less in the GBZ strain in comparison to the GBP strain.

Comparative Account of Effects of Different Host Plants on Differential Haemocyte Count (DHC)

From those observations comparing the effects of different host plants it was obtained that at the 3rd instar GBP strain the percentage of all haemocytes were slightly more on the castor feeding [PL (39.05%±0.92)PR(21.8% 2.43) GR (24.37%± 1.63) SP (8.67% ±0.94) AD (5.87%±2.10)] than that of Kesseru feeding [PL(38.03%±1.20)PR (20.01 %±1.80) GR (40.2%±2.88) SP(8.2%±0.82) AD (4.82%±1.23)] except the Granulocytes which were obtained more on Kesseru feeding (40.2%±2.88) in comparison to the castor feeding (24.37% ± 1.63).

Table 7: Haemocyte counting in different feeding of 3rd larval stage of GBP strain		
Haemocytes	**Castor Feeding**	**Kesseru Feeding**
Plasmatocytes	38.5 ± 0.91	35.05 ± 1.15
Prohaemocytes	20.6 ± 0.24	20.08 ± 1.40
Granulocytes	22.3 ± 1.35	38.20 ± 2.35
Spherulocytes	8.5 ± 0.95	08.40 ± 0.85
Adipohaemocytes	5.85 ± 2.25	04.88 ± 1.25

Fig 7: Graphical Representation of Haemocyte Count in Different Feeding in 3rd Larval Stage]

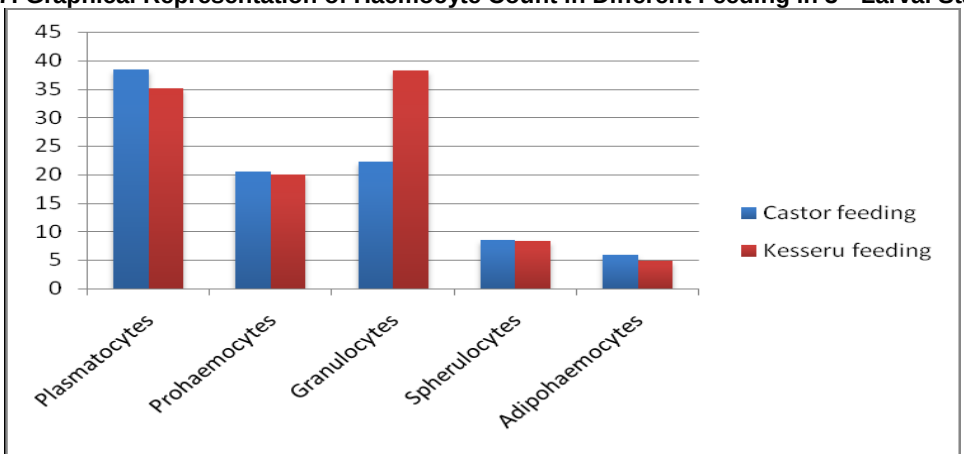


Table 8 : Haemocyte counting in Different Feeding of 3rd Larval Stage of GBZ Strain		
Haemocytes	**Castor Feeding**	**Kesseru Feeding**
Plasmatocytes	40.5 ± 2.27	39.5 ± 2.76
Prohaemocytes	18.5 ± 2.86	19.5 ± 0.80
Granulocytes	24.5 ± 0.27	40.8 ± 0.57
Spherulocytes	9.8 ± 0.75	08.0 ± 0.75
Adipohaemocytes	4.85 ± 0.92	04.85 ± 0.92

Table 9 : Haemocyte Counting in Different Feeding of 4th Larval Stage pf GBP Strain		
Haemocytes	**Castor Feeding**	**Kesseru Feeding**
Plasmatocytes	38.05 ± 1.02	38.05 ± 2.02
Prohaemocytes	20.08 ± 2.64	21.08 ± 1.64
Granulocytes	28.20 ± 2.35	39.20 ± 2.35
Adipohaemocytes	06.20 ± 1.25	04.60 ± 0.55

Fig 8: Graphical Representation of Haemocyte Count in Different Feeding In 3rd Larval Stage of GBZ Strain

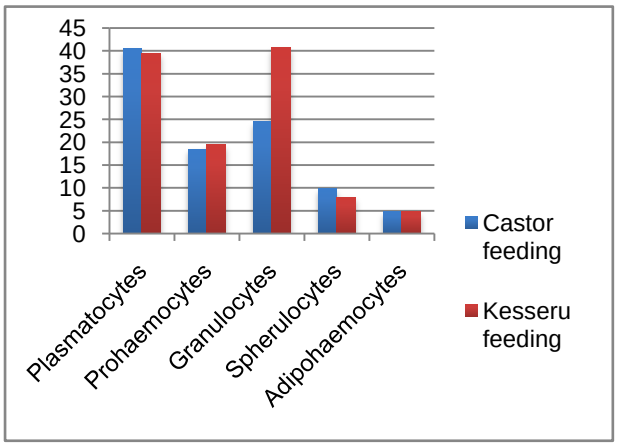


Fig 9: Graphical Representation of Haemocyte Count in Different feeding in 4th Larval Satge of GBP Strain

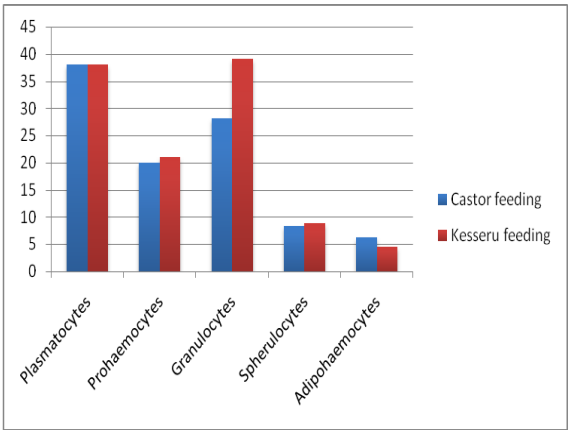


Table 10 : Haemocyte Counting in Different Feeding of 4th Larval Stage of GBZ Strain		
Haemocytes	**Castor Feeding**	**Kesseru Feeding**
Plasmatocytes	42.5 ± 0.56	40.5 ± 0.86
Prohaemocytes	19.8 ± 1.80	19.8 ± 2.80
Granulocytes	23.8 ± 4.56	40.8 ± 1.56
Spherulocytes	09.0 ± 1.70	08.0 ± 1.50
Adipohaemocytes	05.6 ± 0.25	04.8 ± 0.45

Fig 10: Graphical Representation of Haemocyte Count Indifferent Feeding in 4th Larval Stage of GBZ Strain.

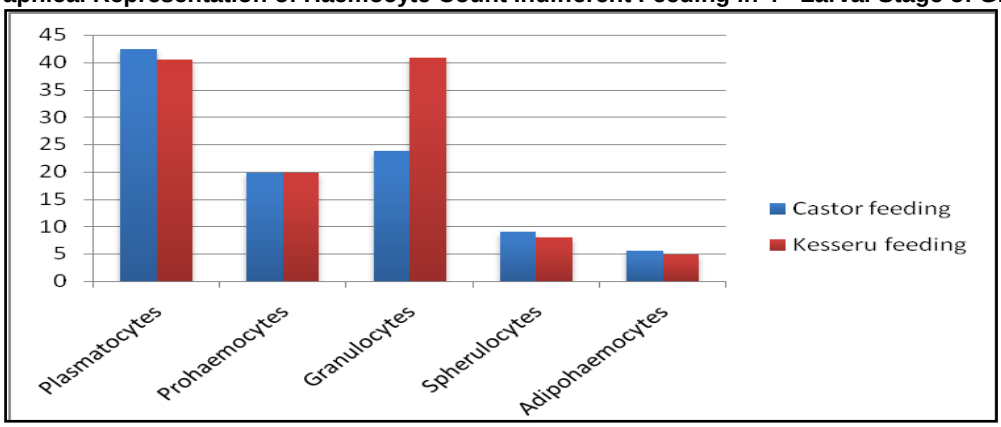


Table 11: Haemocyte Counting in Different Feeding of 5th Larval Stage of GBP Strain		
Haemocytes	**Castor Feeding**	**Kesseru Feeding**
Plasmatocytes	39.05 ± 1.02	39.01 ± 1.80
Prohaemocytes	20.08 ± 1.64	20.20 ± 1.97
Granulocytes	27.30 ± 0.35	41.62 ± 1.30
Spherulocytes	08.02 ± 0.65	08.69 ± 1.61
Adipohaemocytes	05.80 ± 0.65	04.16 ± 0.12

Fig 11: Graphical Representation of Haemocyte Count in Different Feeding in 5th Larval Stage of GBP Strain

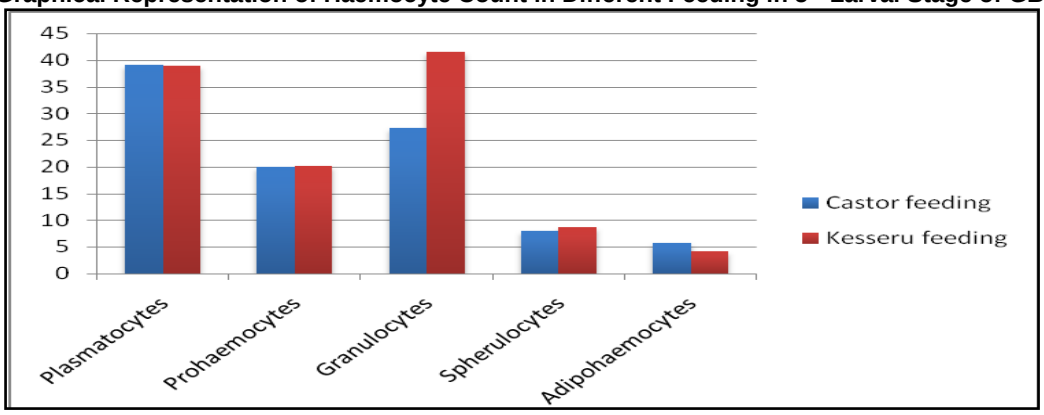
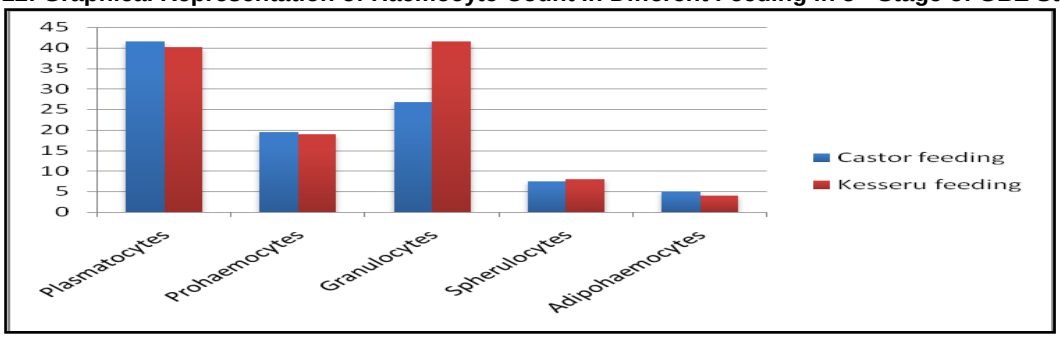


Table 12: Haemocyte Counting in Different Feeding of 5th Larval Stage of GBZ Strain		
Haemocytes	**Castor Feeding**	**Kesseru Feeding**
Plasmatocytes	41.5 ± 0.80	40.1 ± 0.56
Prohaemocytes	19.5 ± 2.80	19.0 ± 0.55
Granulocytes	26.8 ± 0.56	41.6 ± 1.45
Spherulocytes	07.5 ± 0.50	7.98 ± 0.45
Adipohaemocytes	05.1 ± 0.45	4.01 ± 0.46

Fig 12: Graphical Representation of Haemocyte Count in Different Feeding in 5th Stage of GBZ Strain



Overall Comparison

The overall results showed that the percentage of granulocytes was increasing from 3rd to 5thinstar at both strains of Samia *ricini* on both castor and kesseru feeding. The percentage of Adipohaemocytes were decreasing from 3rd to 5th instar of GBZ strain on both feedings, while it was almost same in GBP strain from 3rd to 5th stage on both feedings.The percentage of Prohaemocytes was decreasing in both strains from 3rd to 5th instar larvae feeding on both the plants.The percentage of Plasmatocytes were found more among all haemocytes in the GBZ strain on castor feeding which was almost same at all the 3 stages of larvae ; while it was increasing from 3rd to 5thinstar in GBZ strain on kesseru feeding. Thus it was observed that the percentage of haemocytes were almost unchanged on feeding of different food plants, also in both the strains except the Granulocytes and Plasmatocytes which were found more in the GBZ strain of *Samia ricini* feeding on kesseru leaves. Marked variation in temperature influences the physiological level and is expressed through cellular and immunological changes, (Neven, 2000; Contreras and Bradley, 2010; Lalouette et al., 2011; Catalan et al., 2011). After prolonged starvation, Jones and Tauber (1952) in last instar *Tenebrio* larvae reported increase in the number of GRs but a decrease in the number of PLs. Reduction in the number of fusiform haemocytes was observed in starved *Bombyx* larvae (Ovanesyan, 1951) from which my result was deviated by increasing the percentage of Plasmatocytes in older stages of eri silkworm larvae. Pandey (2004) found that in repeated withdrawals of blood. Plasmatocytes and granular cells are described as the main cell types involved in all defence mechanisms (Beaulaton and Monpeyssin 1977; Ratcliffeet al. 1985 ; Ratcliffe and Rowley 1987, Wiesner and Götz 1993). Phagocytes is considered the barrier against biological (Ratcliffe and Rowley 1979; Ratcliffe et al. 1985; Götz and Boman 1985; Ratcliffe 1986) and non–biological agents (Wiesner 1991,1992; Slovák et al., 1991). Singh et al., (2012) also reported higher larval weight in Greenish Blue Zebra (GBZ) and YZ.

Thus the GBZ strain is a disease resistant strain of Eri silkworm with higher percentage of Granulocytes and Plasmatocytes which are actively involved in the process of encapsulation, nodule formation and phagocytosis a part of the cellular defence mechanism of the insect. Also it has high larval weight as mentioned earlier. Hence it may be profitable in the economy of Eri culture.

Conclusion

The present study reveals that the Greenish Blue Zebra (GBZ) strain fed on Kesseru plants (*Heteropanax fragrans)* is commercially more beneficial than the other strain of *Samia ricini*. It was established on the basis of the presence of a significantly higher number of Plasmatocytes and Granulocytes followed by less number of Prohaemocytes. The decrease in the number of PR and PL with simultaneous increase of PL and GR.respectively, suggested that a transformation of PR in PL and PL in GR took place.These haemocytes are very essential to diagnose various diseases. The GBZ strain feeding on kesseru will be more resistant against any pathogens due to the presence of higher percentage of these cells.Therefore proper selection of strains as well as their food plants may help the common farmers and also the commercial rearers to gain a profitable quantity of quality eri silk yarn with less utilization of labour.

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