J. Soils and Crops 33 (2) 289-294, December 2024

ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

HAEMOCYTIC PROFILE OF THE TROPICAL TASAR SILKWORM, *Antheraea mylitta* (DRURY, 1773) (LEPIDOPTERA : SATURNIIDAE)

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ABSTRACT

The haemocytes plays a significant role in determining the overall physiological condition of the insect. The present study was carried out on the tasar silkworm, *Antheraea mylitta* (D.) during the year 2017-2022. The main aim of the present investigation was to study the haemocyte profile of the fifth instar larvae, pupae and adult of the tasar silkworm *Antheraea mylitta* (Drury, 1773). The healthy fully grown fifth instar larva, pupa and adults of the tasar silkworm were collected from the different rearing fields of the Bhandara and Gondia District. Collected insects were acclimatized in the laboratory. After acclimatization the haemolymph was collected and the total and differential haemocyte count was undertaken by following the standard procedure. The total haemocyte count (THC) was found to be highest in the fifth instar larvae followed by pupal and adult stages. During the diapausing generation of the pupa, the THC was significantly decreased as compared to the non-diapausing generation. It significantly increased in the female fifth instar larvae than in the pupal and adult stage. The light microscopic study of the differential haemocyte count revealed that, there were five types of haemocytes i.e. Prohaemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytes (OEs) which were identified in the haemolymph of tasar silkworm. The per cent population of haemocytes showed that the granulocytes have the highest population followed by PLs, SPs, OEs and PRs in both diapausing and non-diapausing generation. All the five hameocytes studied tasar silkworm, *Antheraea mylitta* (D.) was found to be significantly increased in the female as compared to the male.

(Key words: Tasar silkworm, *Antheraea mylitta,* Haemolymph, Total haemocyte count (THC), Differential haemocyte count (DHC))

INTRODUCTION

Haemolymph is the fluid present in the general body cavity of the insect haemocoel. It is composed of plasma and numerous haemocytes. The haemocytes comprise a complex of several types of mesodermal cells which are nucleated, comparable to the leucocytes of other vertebrates (Levine and Strand, 2002).

Jones (1962) developed the system for classifying Prohaemocytes, insect haemocytes. The plasmatocytes and granulocytes are the three well-defined types of haemocytes found in most of the insects. Apart from these, one or more of four other types of haemocytes like coagulocytes, spherulocytes, adipocytes and granulocytes are present in some other insects.

The various physiological functions in the body of insects like coagulation, connective tissue synthesis, self-recognition, immune response and opsonization, phagocytosis and encapsulation, melanization and discharging elements of the phenoloxidase system and synthesis and storage of the respiratory pigments were performed by the haemocytes (Xylander, 2009 and Hillyer, 2016). The haemocytic profile of the insect may depend on the environment, the health of the insect and the soil health of the rearing field (Yadav and Verma, 2019).

The role of haemocytes was significantly found in the physiological condition of the insect as a whole. The little work has been carried out on the tasar silkworm, *Antheraea mylitta* (Drury, 1773). The present investigation was carried out in the haemolymph of fifth instar larvae, pupae and adults of the tasar silkworm, *A. mylitta* to assess the haemocytic profile of the diapausing and nondiapausing generations of tasar silkworm, *Antheraea mylitta* (D.)

MATERIALS AND METHODS

The present investigation was carried out on the tasar silkworm, *A. mylitta* (Lepidoptera : Saturniidae) during the year 2017 to 2022.

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Healthy fully grown fifth instar larvae, pupae and adults of the tasar silkworm were collected from the different rearing fields of the Bhandara and Gondia districts. The collected animals were acclimatized in the laboratory. After acclimatization the haemolymph was collected and the total and differential haemocyte count was undertaken by following the standard procedure.

Total haemocyte count

With the help of a clean graduated WBC pipette, the haemolymph was sucked up to the 0.5 mark. The excess solution sticking to the tip of the pipette was wiped out. It was followed by sucking the Tauber-Yeager's fluid (Tauber and Yeager, 1935) up to 11 mark. A few drops were discarded after a thorough gentle shaking . Some drops were transferred to the platform of the Naubauer's counting chamber. A cover slip was applied over the bar, before transferring the fluid mixture from the pipette. Wait for a few minutes for the settlement of the haemocytes and counted by standard method.

Differential haemocyte count

The air-dried smear was made by taking a drop of haemolymph on the glass slide. For staining, the stock solution of Giemsa stain prepared by the method of Yeager (1945) was diluted 10 times with distilled water. The smear was stained with the diluted Giemsa solution for about 20 minutes after complete air drying. To differentiate red-staining structures the stained smear was immersed briefly in distilled water mixed with a few drops of lithium carbonate. To differentiate blue-staining structures the stained smear was immersed briefly in distilled water mixed with a few drops of dilute hydrochloric acid.

Using the Yeager (1945) classification system as modified by Jones (1959), the Prohemocytes (PRs), Plasmatocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs) and Oenocytoids (OEs) were counted.

Statistical analysis

Each assay was replicated three times. Statistical analysis of the data was done using online Graph Pad Quick calcs Software (San Diego, California USA, www.graphpad.com). Values were expressed as Mean±SEM of replication. Student's t-test was applied to locate the significant difference between different groups at a 0.05 significance level.

RESULTS AND DISCUSSION

A haemogram of insect includes the total haemocyte count (THC) and differential haemocyte count (DHC). The amount of haemolymph, sex, stage of development, and physiological state affect the THC in insects (Siddiqui and Al-Khalifa, 2013).

In the present study, the fifth instar larvae showed a maximum value of THC than the pupal and adult stages. NDD showed a significant increase in the number of THC than the DD (Table 1). Present findings are supported by the study of Ghasemi *et al*. (2013), who studied the haemocytes in different life stages of the Mediterranean flour moth, *Ephestia kuhniella.* Zeller (1879) found that the THC increased at a relatively constant rate during larval life and reached a peak at the pupal stage. Apparently, the haemocytes of *A. mylitta* are most active during larval stages. Probably they are required during pupation for removal of hystolysed tissues. Less value of THC in adults implied a lesser degree of activity (Kaur *et al.,* 2017).

In male and female of tasar silkworm, *A. mylitta* the THC was found to be significantly increased in the female than the male fifth instar larvae (Table 2). This seems to be associated with the oviposition and other reproductive function of the female insects. Present findings are supported by the study of Pandey *et al.* (2010) and Lokesh *et al.* (2012), who reported that the THC in response to temperature stress increased with the rise in temperature in Daba and Laria ecorace of *A. mylitta* and it is also associated with the sex of an insect.

In the present study, differential haemocytes were studied in the fifth instar, pupal and adult stages of the tasar silkworm, *A. mylitta.* Based on Jones classification for haemocytes (Jones, 1962), five types of haemocytes i.e. prohaemocytes, plasmatocytes, granulocytes, spherulocytes and oenocytoid were identified (Table 3). The per cent population of haemocytes showed that the GRs have the highest population followed by PLs, SPs, OEs and PRs in DD and NDD generations of fifth instar, pupal and adult stage. Morphologically the haemocytes differed in shape, size and position of the nucleus.

The PRs showed highest population in all the stages followed by PLs, SPs, OEs and PRs. In holometabolous insects, the PLs count increases during larval growth and granular haemocytes seem to account for the peak in number prior to pupation (Amaral *et al.*, 2010).

The PRs have been described as rounded, oval or spindle in shape and small in size compared to other haemocytes (Figure 1 A and C). In the tasar silkworm, *A. mylitta,* the PRs remain high in number throughout the larval and pupal stages and the population declined in adult stage. As their population declines in the adult stages the per cent of PLs increases, it may be taken to indicate the conversion of PRs into the PLs. Due to its surface projections, the PRs are similar in size to GRs entitling them to be called PRs-GRs intermediates (Jalali and Salehi, 2008).

The PLs were rounded, fusiforms or spindle in shape, and large in size with a relatively smaller nucleus (Figure 1 A and G). The PLs increased from the fifth instar larval to the adult stage. The population of these cells was found to be higher in the males in NDD generation but in the DD generation, it was higher in female larvae, pupae and adults. The present results are also in agreement with that of Andrade *et al.* (2003) in *Anticarsia gemmetalis* in Embrapa Soja, Londrina-PR, Brazil.

The PRs have been considered as young PLs and therefore, the latter have been regarded as stem cells that give rise to the other cell types (Gupta and Suderland, 1966) (Table 5). The level of PLs decreased in DD generation and in the adult stage essentially following the pattern of oxygen

consumption during diapause and development (Raina and Bell, 1974).

The GRs are considered as pleiomorphic haemocytes. These are the only haemocyte type that has been reported in all major Arthropod groups and Onychophora (Gupta, 1985). The relative percentage of GRs decreased in the late fifth instar onwards. The GRs are involved in a fast defense response (Andrade *et al.,* 2010).

Light microscopic observations of the DLC in the tasar silkworm, *A. mylitta* revealed that the haemocytes were arranged in five classes on the basis of morphological and cytological features. They were the PRs, PLs, GRs, SPs and OEs (Figure 1 A-G). The PRs showed the highest population in all the stages followed by PLs, SPs, OEs, and PRs.

The amount of haemolymph, sex, stage of development, and physiological state affect the THC in insects. The quality of the leaf will also affect the overall physiological state of the insect (Raut *et al.,* 2020). In Lepidoptera, the granular cells and plasmatocytes are the only haemocyte types reported to be phagocytic (Lavine and Strand, 2001, 2002). Similar roles may perhaps have been played by granulocytes and plasmatocytes in the tasar silkworm, *A. mylitta.*

Table 1. Total haemocyte count (THC) (Cells/mm^3) in diapausing and non-diapausing tasar silkworm, *A. mylitta*

Source	NDD	DD	t- stat
Fifth Instar	66214.20±1145.04	64164.95±876.07	1.42^{NS}
Pupa	61751.85±2601.04	34709.78±3225.09	4.6605***
Adult	7955.05±169.57	8105.65±161.49	0.6431^{NS}

Values are mean SE(m)±, n=20 for each group, NDD- Non-Diapause Destined, DD- Diapause Destined

Table 2. Total haemocyte count (THC) (Cells/mm^3) in male and female tasar silkworm, *A. mylitta*

Source	Male	Female	t- stat
Fifth Instar	63370.85±872.43	67008.30±1039.13	2.681*
Pupa	41161.18±3908.37	41779.43±4123.34	0.1088^{NS}
Adult	7823.90±123.25	8236.80±189.08	1.8294^{NS}

Values are mean±SEM, n=20 for each group

Table 3. Morphological characteristics of the haemocytes of tasar silkworm, *A. mylitta*

Type of haemocyte	Shape	Size (µm)	Position of nucleus	
		Width	Length	
Prohaemocytes (PRs)	Round or spherical	5.6-11.8	8-13.2	Central
Plasmatocytes (PLs)	Elliptical	9.0-15.4	17-28.5	Central
Granulocytes (GRs)	Spherical or oval	11.5-21	13.5-25	Central or eccentric
Spherulocytes (SPs)	Round or oval	7.5-11	14-23.5	Generally eccentric
Oenocytes (OEs)	Rounded	13.6-21	13.6-21	Eccentric

Table 4. Per cent differential haemocytes (DHC) in diapausing and non-diapausing generation of fifth instar tasar silkworm, *A. mylitta*

Type of haemocyte	Stage	NDD	DD	t stat
Prohaemocytes	V instar larva	1.94±0.23	1.18±0.226	0.73^{NS}
	Pupa	1.62±0.14	2.14±0.16	2.45*
	Adult	0.81±0.11	0.93±0.09	0.89^{NS}
Plasmatocytes	V instar larva	23.94±1.31	24.34±0.70	0.27^{NS}
	Pupa	27.99±1.70	23.53±1.52	1.95^{NS}
	Adult	42.09±2.49	39.82±1.07	0.83^{NS}
Granulocytes	V instar larva	51.84±1.29	52.07±1.18	1.26^{NS}
	Pupa	48.21±1.47	55.82±1.45	3.69***
	Adult	45.43±1.00	46.61±0.81	0.91^{NS}
Spherulocytes	V instar larva	19.44±1.19	20.00±1.87	1.11^{NS}
	Pupa	16.89±0.64	16.93±0.57	1.22^{NS}
	Adult	9.91±0.27	10.04±0.41	2.31*
Oenocytoid	V instar larva	2.71±0.25	1.65±0.10	0.24^{**}
	Pupa	2.40±0.25	2.72±0.24	0.91^{NS}
	Adult	1.29±0.12	1.86±0.09	3.62**

Values are mean SE(m)±, n=15 for each group**, NDD-** Non-Diapause Destined, **DD-** Diapause Destined

Table 5. Per cent differential haemocytes (DHC) in male and female of fifth instar tasar silkworm, *A. mylitta*

Type of haemocyte	Stage	NDD		t stat	DD		t stat
		Male	Female		Male	Female	
Prohaemocytes	V instar larva	1.70±0.22	2.17±0.41	0.99^{NS}	1.968±0.26	2.39±0.369	0.93^{NS}
	Pupa	1.30±0.11	1.94±0.15	3.46***	1.81±0.18	2.48±0.17	2.68*
	Adult	0.56±0.06	1.05±0.12	3.65**	0.73±0.08	1.13±0.09	3.28*
Plasmatocytes	V instar larva	24.04±1.47	19.84±0.87	3.62***	18.22±0.85	21.46±0.94	1.76^{NS}
	Pupa	29.16±2.32	26.82±2.63	0.66^{NS}	22.58±2.09	24.48±2.35	0.60^{NS}
	Adult	47.82±2.77	36.36±1.98	3.36**	37.34±0.82	42.31±1.18	3.43**
Granulocytes	V instar larva	49.68±1.50	54.01±1.71	1.89^{NS}	55.04±1.58	50.09±1.81	0.80^{NS}
	Pupa	45.21±2.31	47.22±1.95	0.66^{NS}	50.49±1.59	48.16±2.35	1.17^{NS}
	Adult	43.37±0.97	50.48±1.21	2.64*	47.89±1.05	42.35±1.01	1.76^{NS}
Spherulocytes	V instar larva	19.34±2.10	19.53±1.41	0.07^{NS}	20.86±3.05	22.95±2.45	0.53^{NS}
	Pupa	19.84±0.83	19.94±1.06	0.08^{NS}	20.51±0.71	21.32±0.93	0.71^{NS}
	Adult	6.33±0.28	9.50±0.27	3.02*	11.26±0.45	12.83±0.48	2.38*
Oenocytoid	V instar larva	2.93±0.32	2.49±0.39	0.85^{NS}	2.59±0.11	2.70±0.18	0.54^{NS}
	Pupa	2.72±0.40	2.08±0.25	1.36^{NS}	2.89±0.24	2.55±0.44	0.69^{NS}
	Adult	0.98±0.09	1.60±0.11	4.19**	1.66±0.12	2.07±0.09	2.78*

Values are mean SE(m)±, n=15 for each group**, NDD-** Non-Diapause Destined, **DD-** Diapause Destined

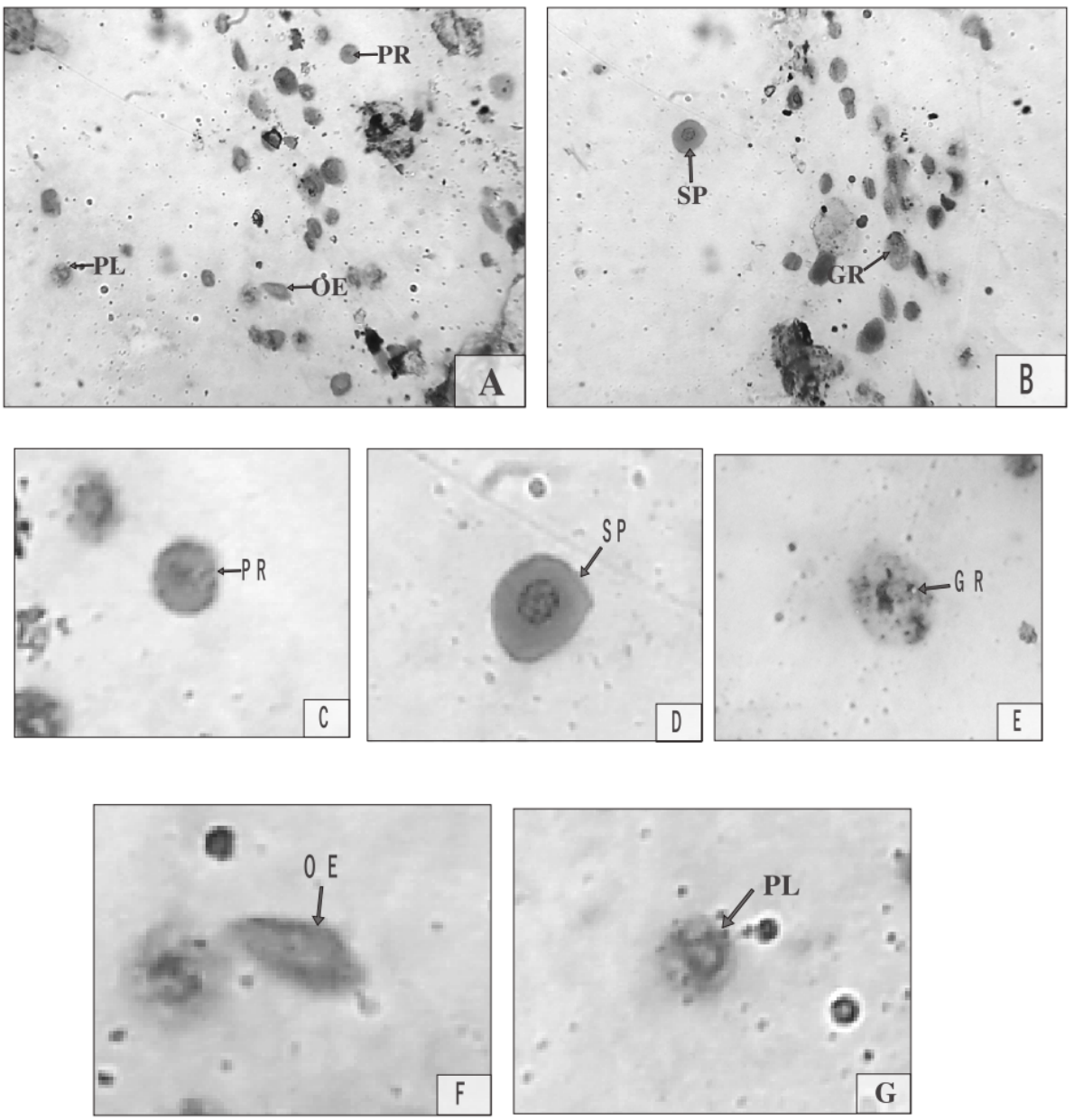

Figure 1 (A-G): Type of haemocytes of tasar silkworm, *A. mylitta*.

A: Smear of haemolymph showing PR, OE and PL, **B:** Smear of haemolymph showing SP and GR, **C:**Prohaemocyte (PR), **D:**Spherulocyte (SP), **E:** Granulocyte (GR), **F:**Oenocytoid (OE) and **G:**Plasmaticyte (PL). **Abbreviations**: PR- Prohaemocyte, SP- Spherulocyte, PL- Plasmatocyte, GR- Granulocyte and OE- Oenocytoid

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Rec. on 23.04.2024 & Acc. on 09.05.2024