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Quantitative changes of total haemocytes count in insect *Lethocerus indicus* (Hemiptera : Bellostomatidae)

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Abstract : Present experiment was conducted to study the Total Haemocytes Count (T.H.C) of *L. indicus.* The quantitative analysis of haemocytes have shown that the total number of haemocytes increase continuously throughout the development stages (from1st nymphalinstar to adult), and reach a peak level in the fifth nymphal instar in both the sexes. The increasing number in THC was most probably correlated with an increasing demand for nutrient supply, cellular defense and production of immunologic factor. It was also marked that the level of THC in adult individual of both the sexes were found to be lower than that of previous stage (fifth nymphal instar). During experiment it was observed that the total haemocyte count in adult female was higher than adult male. This higher haemocytes concentration in adult female seems to be associated with the reproductive function of female insects.

Key Words: *Lethocerus indicus*, Insect, Total Haemocyte Count (THC) .

INTRODUCTION

By virtue of the blood, in insect is a colorless fluid largely present in the body cavity called haemocoel and hence it is known as "**Hemolymph"** or "**Haemolymph".**Hemocytes are found in the hemolymph of invertebrates **(Lavine and Strand, 2002; Williams,2007, Arnold, 1974)[1-3].**These cells originate from mesodermal stem cells and are either circulating cells or associated with various tissues as sessile cells. They function as cellular immune surveillance cells and are part of a primitive immune system that recognizes a variety of foreign targets as well as alterations to self. The cells play a major role in the elimination of potentially harmful structures by phagocytosis and encapsulation and the secretion of antimicrobial peptides. Small targets are cleared by phagocytosis, whereas larger objects are eliminated by encapsulation (Ratcliffe and Rowley, 1985)[4]. Hemocytes are also important for embryonic tissue formation and organ remodelling during metamorphosis because they have the ability to remove apoptotic cells by phagocytosis. Insect haemocytes are studied also as model system for vertebrate hematopoiesis **(Crozatier and Meister , 2007)[5]**
Despite several studies made on haemocytes of different insects by various scientists as mentioned above. Yet, there are no studies on the haemocytes of *L. indicus* . So, here we present the investigatory report on the total haemocyte counts in the haemolymph of *L. indicus.*

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MATERIALS AND METHODS

For the total haemocytes counts (THC), the haemolymph was drawn into a Thoma white blood cell pippete up to the 0.5 mark and diluted up to the 11 mark with Tauber-Yeager fluid (Tauber and Yeager, 1935)[6]. The pipette was shaken for several minutes and the first three drops were discarded. A double line with improved Neubaur ruling haemocytometer was filled with diluted haemolymph and the haemocytes were counted in their four corners and one control (1 mm2) sqares. The number of circulating haemocytes per cubic millimeter (mm3) was calculated using the following formula of Jones (1962):

Haemocytes in five 1 mm2 × Dilution × Depth factor of chamber
No. of squares counted
Where dilution = 20
Depth factor of the chamber = 10 (constant) and No. of squared counted = 5

RESULTS AND DISCUSSIONS

Haematological investigations provide diagnostic keys to various diseases and valuable indicators of the physiological status of the organisms. It is hypothesized that variation in hemocytes profile/catalogue and other histological/enzymatic changes consequent to stress conditions can be used as indicator for health and it can be utilized in biomedical field.

In present experiment the total haemocytes showed progressively increasing value throughout the nymphal development (From 1st to 5thnymphal instars) in all the three seasons (Autumn, Rainy and Winter) in both of the working years (2009 – 2010) in both the sexes , however **Andrade et al. (2003)**[7] reported decreasing tendency in the number of hemocytes in *A. gemmastalis* during larval period.

The lowest value was recorded in first nymphal instars , 4305 /mm^3, 4394 / mm^3 and 4320/ mm^3, in male and 4574/mm^3 , 4596/mm^3 , and 4562/mm^3 in female ,the peak value in the fifth nymphal instars (5210/mm^3 , 6086/mm^3 ,and 5306/mm^{3}in male and6354/mm^3 **,** 6638/ mm^3 **,** 6112/mm^{3}in female individualsin Winter, Autumn and Rainy season of the year 2009 – 2010 respectively**(Table :1- 3)** . Likewise, in adults individuals of both the sexes these values were decreased to 4974/ mm^3 , 5588/mm^3 , and 5003/mm^3 in male and 5023/mm^3 , 6015/mm^3 , 6102/mm^3 in female in Winter, Autumn and Rainy season of the year 2009 – 2010 respectively. These result of THC were agreed with the result of earlier investigator such as **Tauber and Yeager (1935)**[6] **and Nardi *et al.* (2001)**[8] ,they reported the differences in the amount of total haemocytes in male and female of same species was found to be high in female as compare to male . **Sanjayanet. al.; (1996)**[9] also reported such significant increase in female than male in the haemocytes of *S. hopes* . This sexual differences was mainly due to an increase in number of PLs, which is more prominent in female, **(Beetz et. al. 2008)**[10]. The increasing number in T.H.C in all nymphal instars in present case was most probably correlated with an increasing demand for nutrient supply for growth , cellular defense and production of immunologicfactor in both the sexes as reported by **Pugazhvendam&Soundararajan (2012)**[11]. The increasing number of THC in female individuals were seems to be associated with the reproductive function of female insects. In present investigation the lowest number of THC in winter season of both the sexes were supposed to be due to the clumping of the cells by low temperature and thus rendering the haemocytes unavailable from circulating haemolymph for counting, as also observed by **Pandey *et al.* (2004)**[12] **and Pandey&Tiwari (2012)**[13] , Or some of the haemocytes got adhered with the organs bathed by haemolymph.

Pugazhvendam&Soundararajan (2012)[11]were conducted to study on the Total Haemocytes Count (T.H.C) of *Chrysocorispurpureus.*, during metamorphosis and reproduction and found the quantitative analysis of haemocytes have shown thatthe total number of haemocytes increase continuously throughout the post embryonic development stages(from the third nympal instars to adult) reach a peak level in the adult stage.The increasing number in T.H.Cis correlated with an increasing demand for nutrient supply, cellular defense and production of immunologicfactor**Jalal and Salehi (2008)**[14]. This higher haemocytes concentration seems to be associated with the reproductive function of female insects. In present result of THC in the haemolymph of *L. indicus* was agreed with the work of **Pugazhvendam&Soundararajan (2012)**[11]except the peak level was found in the fifth instar nymph instead of adult. But in contrast to present result the value of THC in male was greater than that of female individual as reported by **Luckhart, et. al.; (1982)**[15]**.**

Table 1: Total haemocytes counts in the haemolymph of *L indicus* in Rainy season with respect to sex (2010 – 2011)

S.N.	HAEMOCYTES COUNTS IN DIFFERENT DEVELOPMENTAL STAGES IN RAINY SEASON											
	MALE						FEMALE					
	INS I	INS II	INS III	INS IV	INS V	ADULT	INS I	INS II	INS III	INS IV	INS V	ADULT
1	4459	4612	4780	5095	5895	5253	4699	4920	5475	6196	6550	6095
2	4456	4608	4784	5099	5898	5252	4710	4925	5472	6199	6545	6098
3	4490	4620	4790	5086	5922	5265	4725	4939	5485	6225	6570	6092
4	4475	4635	4785	5110	5890	5245	4696	4921	5498	6192	6555	6125
5	4500	4615	4801	5120	5905	5280	4705	4950	5500	6218	6590	6130
MEAN	4476	4618	4788	5102	5902	5259	4707	4931	5486	6206	6562	6108
S.D	17.0	9.3	7.2	11.8	11.1	12.3	10.2	11.6	11.4	13.0	16.3	16.1

Table 2: Total haemocytes counts in the haemolymph of *L indicus* in Winter season with respect to sex (2010 – 2011)

S.N.	HAEMOCYTES COUNTS IN DIFFERENT DEVELOPMENTAL STAGES IN WINTER SEASON											
	MALE						FEMALE					
	INS I	INS II	INS III	INS IV	INS V	ADULT	INS I	INS II	INS III	INS IV	INS V	ADULT
1	4420	4599	4725	4990	5231	4925	4695	4850	5300	6170	6395	6068
2	4450	4595	4722	4995	5329	4928	4672	4859	5310	6178	6390	6065
3	4470	4585	4750	5005	5345	4937	4683	4875	5320	6175	6385	6075
4	4415	4625	4765	5020	5360	4950	4695	4847	5300	6187	6399	6081
5	4480	4641	4728	5000	5335	4920	4710	4899	5345	6165	6406	6062
MEAN	4447	4609	4738	5002	5320	4932	4691	4866	5315	6175	6395	6070
S.D	26.0	20.7	16.7	10.2	45.7	10.5	12.7	19.1	16.7	7.4	7.2	6.6

Table 3 : Total haemocytes counts in the haemolymph of *L indicus* in Autumn season with respect to sex (2010 – 2011).

S.N.	HAEMOCYTES COUNTS IN DIFFERENT DEVELOPMENTAL STAGES IN AUTUMN SEASON											
	MALE						FEMALE					
	INS I	INS II	INS III	INS IV	INS V	ADULT	INS I	INS II	INS III	INS IV	INS V	ADULT
1	4490	4699	4811	5210	6085	5822	4722	4895	5615	6225	6655	6145
2	4486	4690	4815	5216	6089	5818	4728	4896	5612	6230	6659	6142
3	4499	4701	4839	5209	6081	5835	4750	4899	5630	6225	6675	6158
4	4535	4710	4865	5245	6100	5825	4715	4925	5645	6245	6690	6170
5	4550	4695	4870	5260	6115	5840	4755	4920	5618	6250	6651	6140
MEAN	4512	4699	4840	5228	6094	5828	4734	4907	5624	6235	6666	6151
S.D	25.6	6.66	24.4	20.6	12.2	8.22	15.7	12.8	12.1	10.4	14.5	11.3

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