STUDIES ON DIFFERENTIAL HAEMOCYTE AND TOTAL HAEMOCYTE COUNTS OF INSECTS OF ORDER HYMENOPTERA OF JABALPUR

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Abstract Insect immune responses are divided into humoral and cellular responses. Humoral immunity includes the production of antimicrobial peptides, reactive oxygen and nitrogen derivatives, and coagulation and melanization of hemolymph,whereas cellular immunity comprises hemocytemediated reactions such as phagocytosis, nodule formation, and encapsulation. During the present study, total haemocyte count assessed for the ants of different sample collecting sites were found to be different. On the comparison of the ants collected from five different sites the THC of ants collected from the construction area were found to be the highest as 103 cells in one drop of haemolymph while that of ants collected from garden were found to be the lowest as 31 cells in one drop of haemolymph. The THC of ants collected from play-ground were found to be 63 cells, from fish-market area 46 cells and from roadside area 47 cells were found in one drop of haemolymph.During the present relative study higher THC values were observed in the ants collected from the construction area and then in the ants collected from play-ground in comparison to ant samples collected from other sites. The density of haemocytes in ants generally depends upon the volume of the haemolymph. The feeding efficiency of the ants increases the haemocyte counts in them.

Key Words:- Insect immune responses, haemocyte, Hymenoptera.

INTRODUCTION

Insect immune responses are divided into humoral and cellular responses. Humoral immunity includes the production of antimicrobial peptides, reactive oxygen and nitrogen derivatives, and coagulation and melanization of hemolymph (Bogdan et al., 2000; Lowen berger, 2001; Buyukguzel et al. 2007), whereas cellular immunity comprises hemocyte-mediated reactions such as phagocytosis, nodule formation, and encapsulation (Lavine and Strand, 2002; Durmuş et al., 2008). Both these systems are influenced by environmental conditions such as starvation, thermal stress, and pesticides (Sharma et al., 2003; Giron-Perez, 2010; Ghasemi et al., 2014).

Immune responses of insects show great similarity to the innate immune response of mammals (Kavanagh and Reeves, 2007). Jones (1964) suggested that the morphology, embryonic origin, amoeboid movement, and phagocytic activity of insect hemocytes are comparable to those of the white blood cells of mammals.

Haemocytes are a morphologically distinct cell type (Price & Ratcliffe 1974; Mead et al. 1986), comparable to vertebrate leucocytes (Jones 1977), that constitute important and crucial components of the haemolymph in the open circulatory system of insects (hexapods) as well as in other arthropods and invertebrates. These cells were first discovered by Swammerdam in 1669, and their versatile features within a species and the duplication of similar and compatible shapes amongst different species encouraged Cuenot (1896) to be the first to classify them, dividing them into four different categories (Millara 1947). The classification has been revised several times (Yeager 1945 et al.,)

Haemocytes are blood cells found to occur in the haemolymph of insects, most of them normally resting on the surfaces of organs and some circulate freely in the body. These cells are derived from the embryonic mesoderm and later seem to appear from the pre-existing prohaemocytes.

Functionally, haemocytes are the generally accepted cellular defence units in insects and are partially responsible for their immune system (Gupta & Sutherland 1967 et al.,) Insect haemocytes respond to internal changes during development (at ecdysis) and to conditions such as starvation, wounding, parasitism, diseases andchemicals (Ratcliffe and Rowley, 1979 and Balavenkatasubbaiah *et al*., 2001).

Haemocytes help the animal to digest the dead cells and tissues during the process of metamorphosis and to cast the moult skin during the process of moulting (Kalia *et al*.,2001) and haemocytes are known to be involved in remodelling of the basement membrane which is broken down and rebuilt during metamorphosis (Nardi *et al*.,2001).

Environmental pollutants such as insecticides and heavy metals can alter the number of and/or induce structural abnormalities in hemocytes. These changes can be used to characterize the genotoxic, physiological, and biochemical effects of pollutants. Therefore, hemocytes (via changes in cell number and development of structural abnormalities) are frequently used to demonstrate the cytogenetic damage caused by toxic chemicals (yeh et al.,2005). Genotoxicity biomarkers are widely measured in eccotoxilogy as molecular toxic effects of major environmental pollutenats (wessel et al., 2007). The micronucleus is a biomarker widely used in biomonitoring studies performed to evaluate the effects of pesticide exposure.

Synthetic pyrethroids, such as deltamethrin, have become some of the most widely used pesticides owing to their selective action, low mammalian toxicity, and low accumulative capability in vegetables and soil compared with other pesticide types such as organophosphates and Organochlorines (erstfeld, 1999). Therefore, their possible effects on living organisms must be evaluated.

The contribution of haemocytes to the prophenoloxidase system through the involvement of granular and/or plasmatocytes prompted Gupta (1985), Yokoo et al. (1995), Tojoet al. (2000) and Ling and Yu (2006) to label these two categories of haemocytes as immunocytes. However, the type of immunocytes present and their role in insects are debatable and more information on the subject is needed for clarity (Siddiqui & Khan 1979; Alfonso & Jones2002). In addition to biological agents, fungi, protozoans and other pathogenic and nonpathogenic agents are known inducers of haemocytic responses such as a variation in total haemocyte counts (THC) and differential haemocyte counts (DHC) (Shapiro1979; Christensen et al. 1989).
While considering the importance of insect haemocytes in intermediary metabolism and the role of ecdysones in regulating growth and moulting in insects, the application of exogenous ecdysones including natural (β-ecdysone) and its analogues was performed to study the haemocyte response.

Seven types of hemocytes have been frequently described in various insects: prohemocytes, plasmatocytes, granulocytes, spherulocytes, adipohemocytes, oenocytoids and coagulocytes (Jones, 1979; Gupta, 1985; Brehelin and Zachary,1986, among others).

In this review we will be observing that how the physiological parameters affects the haemocyte count of fire ants of order Hymenoptera (species: Solenopsis invicta)

Solenopsis invicta, also known as the fire ant or RIFA, is a species of ant native to South America.it was described by the swiss entomologist Felix Santschi as a variant in 1916. Its current name is invicta was given to the ant in 1972 as a separate species. The red imported fire ant is polymorphic as workers appear in different shapes and sizes. The ant's colours are red and somewhat yellowish with a brown or black gaster. However males are completely black. They live in wide variety of habitats such as rain forests, desserts, grasslands, alongside roads and buildings.

Materials and method:

Sampling sites: The ants were collected from the following five different locations of Jabalpur city-

- Garden area in Jasuja city
- Roadside area near Medical College
- Construction area from Jasuja city
- Fish market of Dhanwantri Nagar
- Play ground in Tripuri, Garha.

Sample Collection method:

Collecting can be as simple as picking up stray ants and placing them in a glass jar or as complicated as completing an exhaustive survey of all species present in an area and estimating relative abundances.To collect wide range of ants, several methods should be used. These includes hand collecting, using baits as attractants, pitfall traps.

Procedure: During the present study period ant sample (order: hymenoptera) were collected from five different places (gardens, roadside, construction area, fish-market area, play ground) of Jabalpur city to study their haemocytes and affect on their count by physiological stress. The sample collection was conduct from January to march 2019. Collected ants were kept in a plastic bottle/ jar with holes big enough to allow air to pass and small enough to not allowing the ants to escape. Before extraction of haemolyph, the ants are killed by suffocating them.Then two eppendorf tubes of different volume are taken (0.500µl and 1.5ml). on the bottom part of the tip of 0.5ml eppendorf, a small hole is made and dead ants were placed in it. After this, the small eppendorf tube is placed inside the other eppendorf tube of volume 1.5ml and is allowed to centrifuge for about 10 minutes. There is no anticoagulant used as there is no need to store the haemolymph sample for later. After centrifugation, the extracted haemolymph is deposited on the bottom of the 1.5ml eppendorf tube and further the staining procedure is done before the sample is subjected to differential count of haemocytes.**Staining procedure:** A smear is made by placing a drop of haemolymph on a slide and allowed it to dry for 10 minutes. Further the staining is done first by may-grunwalds stain and then by giemsa stain for 5minutes each. After every staining the slide is washed by water with the help of dropper and then further the sample is subjected for differential haemocyte count under light microscope (40X). Differential haemocyte count was determined as per the methods given by Jussila et al. and total haemocte counts by method given by S.R. Pugazhvendan and M. Soundararajanwith some modifications.

Results and Discussion

The present study revealed that the immunocompetence status of ants are directly guided by its haemolymph parameters which may in turn respond differently to various ecological factors.

During the present study, total haemocyte count assessed for the ants of different sample collecting sites were found to be different. On the comparison of the ants collected from five different sites the THC of ants collected from the construction area were found to be the highest as 103 cells in one drop of haemolymph while that of ants collected from garden were found to be the lowest as 31 cells in one drop of haemolymph. The THC of ants collected from play-ground were found to be 63 cells, from fish-market area 46 cells and from roadside area 47 cells were found in one drop of haemolymph.During the present relative study higher THC values were observed in the ants collected from the construction area and then in the ants collected from play-ground in comparison to ant samples collected from other sites. The density of haemocytes in ants generally depends upon the blood volume of the haemolymph. The feeding efficiency of the ants increases the haemocyte counts in them.

Table1: Differential haemocyte counts of ants of 5 different sample collecting sites

Types of Haemocytes	Ants collected from				
	Garden	**Road side**	**Construction area**	**Fish-market**	**Play-ground**
Granulocytes	03	01	14	06	04
Prohaemocytes	-	07	09	02	07
Plasmatocytes	11	16	42	27	39
adipohaemocytes	17	23	38	11	13

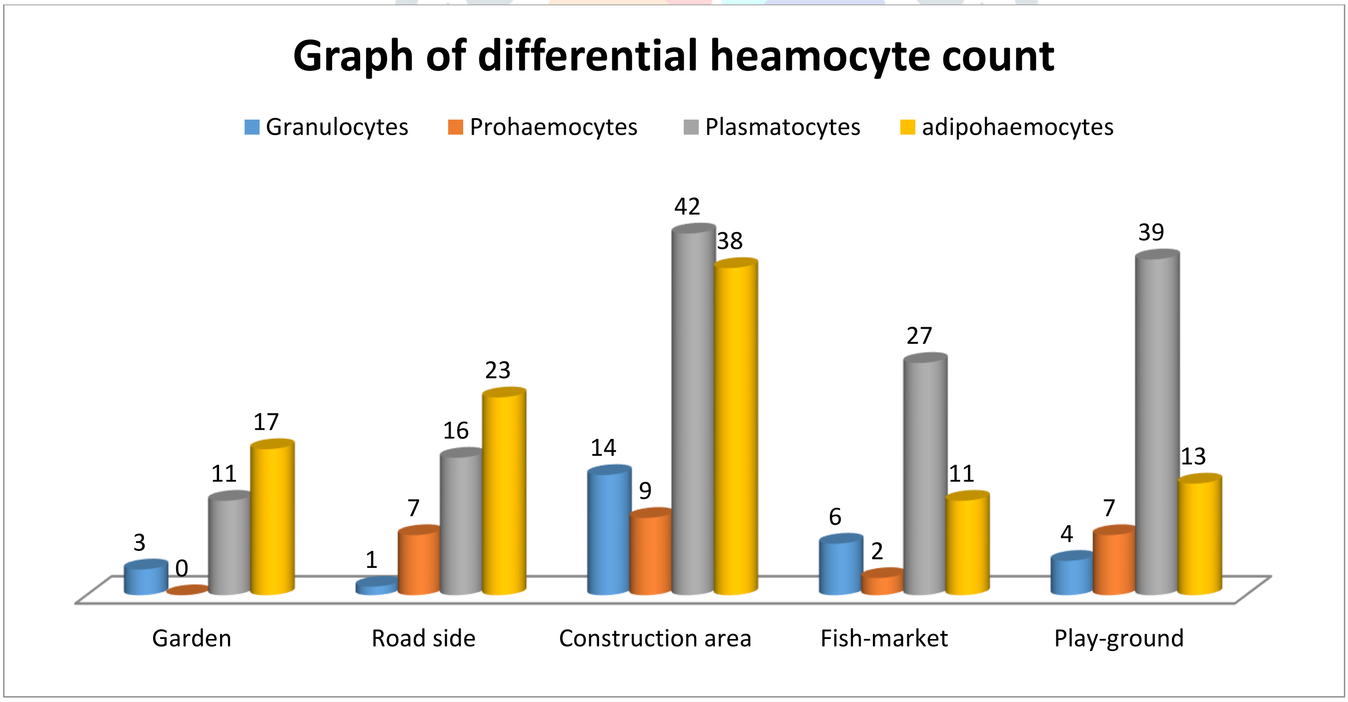

Table 2: Total haemocyte counts of ants of 5 different sample collecting si

On the comparison of the ants collected from five different sites the THC of ants were determined as follows:The THC of ants collected from the garden area is found to be 31 cells in one drop of haemolymph. Similarly, the THC of ants collected from the roadside area is found to be 47 cells and from the construction area is found to be 103 cells in one drop of haemolymph. Rest the THC of the ants collected from the fish market area is found to be 46 cells and from play ground 63 cells are found in one drop of haemolymph. The free haemocytes in the haemolymph of insects are responsible for the defense reactions against foreign agents that penetrate the haemocoel (Inoue *et al*., 2001; Russo *et al.* 2001; Watanabe, 2002 and Narayanan, 2004).The gradual rise in haemocytes may due to differentiation of prohaemocytes into plasmatocytes and also may be due to continuous division of haemocytes already in circulation. The prohaemocytes serves as stem cells in the haemolymph (Siliva *et al.*, 2002) and reduction in prohaemocytes could be correlated to the greater transformation of prohaemocytes into other type of cells which play their role in phagocytosis (Saxena and Srivastava, 2001; Bhatti, 2002 and Lavine and Strand, 2002).

Plasmatocytes and granulocytes are principal haemocyte type capable of adhering to invading pathogens and they are actively involved in phagocytosis mechanism which was investigated by Wago (1982); Lackie (1988); Pathak (1990); Ratcliffe (1993) and Strand and Pech (1995). Arnold (1974) suggested that increase in plasmocytes indicated the physiological functions of insect.

The increase in granulocyte number could be related to their activation in defense responses against invaders (Boucias and Pendland, 1998).The effective physiological mechanisms of phagocytosis, encapsulation, and other related defense mechanisms were primarily due to the availability of circulatory immune cells particularly plasmatocytes and granulocytes. As haemocytes respond very instantly against adversaries, it is expected that by using haemocyte catalogue as indicator, impact of several biotic and abiotic factors can be evaluated.

Haemocytic count varies between male and female insects of the same species (Saran *et al*., 2002 and Malikarjuna *et al.*, 2002) and the counts are associated with special conditions like parasitism, oviposition and ecdysis (Tauber and Yeager, 1936).The degree of variations in the number of haemocytes can be used as an index for diagnosis of the disease (Tepass *et al.*, 1994; Falleiros and Gregorio, 1995 and Inoue *et al.*, 2001). The number of haemocytes range from 100 to 167,000 per unit volume of haemolymph (Romosen and Stofolano, 1998). The haemocytes also change in number and morphology accompanying metamorphosis (Gardiner and Strand, 2000and Beetz *et al*., 2004). They perform phagocytosis, encapsulation of foreign bodies in the insect body cavity, wound healing (Essawy, 1990), coagulation to prevent loss of blood, nodule formation, transport of food materials, hormones and detoxification of metabolites and biological active materials (Patton, 1961).

Silva et al., 2001 worked on haemocyte type, in addition to total and differential counts were studied in parasitized and unparasitized Anastrepha oblique larvae at the beginning and at the end of the third instar. In both developmental phases, in parasitized and unparasitized larvae, six well-defined haemocyte types were distinguished in the hemolymph: prohemocytes, plasmatocytes, granulocytes, adipohemocytes, oenocytoids and spheurulocytes. The THC of parasitized larvae was not significantly different from the unparasitized larvae at thr beginning of the 3rd instar, but was significantly higher at the end of the instar. By means of the DHC it was verified that at the beginning of the 3rd instar 97% and 94% of the total circulating haemocyte were comprised of prohemocytes and plasmatocytes in both Parasitized and unparasitized larvae, respectively. The remaining hemocyte types are present in the circulation, but their proportion was never greater than 1.0%. Despite the general similarities in the average number and percentage of hemocyte in the two analysed phases of the third 3rd larval instar, some distict alteration were noted. At the end of the instar, the proportion of prohemocytes and plasmatocytes in parasitized and unparasitized larvae diminishes to 87.8% and 80.5% respectively. In addition,granulocyte proportion increased from 11% in parasitized larvae to 18.1% in unparasitized. The proportion of the other cell types remained unaltered, not comprising more than 1%.Dr. Parnashree Mukherjee et al., (2017) worked on the Investigation of the physiological stress indicators and immunocompetence status of road side insects in comparison to the insects of domestic gardens of Jabalpur city.The basic aim of the study was to investigate the physiological stress indicators in haemolymph of insects of domestic gardens and road sides and relative analysis of the immunocompetence status of insects of roadside and domestic gardens of Jabalpur city to keep a check on their biodiversity loss and to assess their role in maintaining ecological balance. During the study period insect samples were collected from 2 busy roadsides habitat and 10 domestic gardens of Jabalpur city to investigate the immunocompetence status of the insects of road sides and domestic gardens of Jabalpur city. Haemolymph of insects were collected in ice cooled 1.5 ml microcentrifuge tubes prefilled with 300µl sodium cacodylate based anticoagulant followed by relative analysis of various commonly used physiological stress indicators in insect haemolymph such as total and differential haemocyte counts, total protein composition (in terms of refractive index),clotting capacity of hemolymph and protein profile to portray the immunocompetence status of the insects of road side in comparison to those found in the domestic gardens. During the estimation of Heamolymph proteins of insects of all the 05 orders of road side insects by SDS-PAGE followed by Commassic blue and silver staining revealed the presence of the heat shock proteins in the insects which help to increase their resistance against adverse conditions of the roadsides. The study indicated that the high values of THC of the adult insects of both roadsides and domestic garden correlated with less coagulation time which serve as indicator of good immunocompetence status. The present comparative study of insects of both the habitats indicates that the haematological parameters like DHC%, THC, serum protein content and clotting time of insects of domestic gardens were found to be lesser than that of the road side insects which indicates poor immunocompetence status of insects of domestic garden in comparison to the insects of roadsides of Jabalpur city.

Conclusion:

According to this review of haemocyte count of ants (order: hymenoptera) on the comparison of the ants collected from five different sites the THC of ants collected from the construction area were found to be the highest as 103 cells in one drop of haemolymph while that of ants collected from garden were found to be the lowest as 31 cells in one drop of haemolymph. The THC of ants collected from play-ground were found to be 63 cells, from fish-market area 46 cells and from roadside area 47 cells were found in one drop of haemolymph.It is reported that hemocytes are biochemically very active and very sensitive cellular components of insects and hence a catalogue on them will be very helpful in identifying and quantifying the stresses. It will be useful to study the hemocyte immune responses of insects in benefits of human kind in the field of applied and biomedical sciences. Proper understanding of insect hemocytes will lead to help the betterment of applied sciences of insects and related industry. For example in recent past, due to global warming, day time temperature (May-June) shoots up and it goes up to 35 to 45°C or even more. It causes adverse impact on the survival of tasar silkworm pupae and invites heavy loss to the tasar silk industry. If hemocyte based indicator will be standardised, the suitable temperature in reference to proper storage can be evaluated.Pest control: Synthetic chemical insecticides have played important and beneficial roles in the control of insect pests and the reduction of insect borne diseases for nearly 50 years. Their use will remain necessary for many more years. Nonetheless, it also poses real hazards. Some leave unwanted residues in food, water and the environment. Low doses of several insecticides are toxic to humans and other animals and some are suspected to be carcinogens. Hence, many researchers, farmers and homeowners are seeking less hazardous alternatives to conventional synthetic insecticides. Presently it focuses on microbial insecticides; botanical insecticides and insecticidal soaps; attractants and traps; and beneficial insects and mites (natural enemies of pests). Proper understanding of hemocyte in the form of catalogue may lead the effective control of pests in assuring healthy crops and enhancing agricultural productivity.

Use in medical and pharmaceuticals sciences: A detailed understanding of insect immune system presumes significance for scheming novel disease control strategies against insect-vector borne diseases. Towards this goal, several large-scale genome and transcriptome projects have been initiated in insects in the past decade. Noduler was found to be evolutionarily conserved among insects and it would be worthwhile to analyze its role in other insects. Recently numerous analogues have been observed between insect and mammalian innate immune pathways. However, nodulation like response is not reminiscent of any of the vertebrate innate immune mechanisms. It is reported that several mammalian extra cellular matrix proteins of unidentified function show resemblance with Noduler. Thus, it will be interesting to explore their role in vertebrate immune pathways in view of the insect hemocytes. Insects possess a complex and efficient system of biological defence against pathogens and parasites. An essential component of immunity is a mechanism for surveillance by which an organism can detect foreigners or the presence of nonself molecules. The recognition of nonself stimulates defence responses with the recruitment of hemocytes and with humoral response. The hemocytes of insects play a very important role in general metabolism and in defending against the foreign bodies as these are the main immune competent cells of insect cellular immunity. Present catalogue will help studying the role of hemocyte in drugs and vaccines.

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