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HAEMOLYTIC ACTIVITY OF HOUSEFLY LARVAL EXTRACT IN VARIOUS SEASONS.

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ABSTRACT
Housefly, *Musca domestica* is distributed worldwide and considered as a pest of both farm and home. These insects are always associated with human activities, animal faeces as well as adapted to feeding on garbage. They transport disease causing organisms. The haemocytes of these animals are involved in innate immunity. Haemocytes are circulated in haemolymph of the insects. The main objective of our study is to understand how the houseflies are adapted in abrasive conditions during various seasons (summer, Rainy and winter). In the present study, an attempt has been made to understand the haemolytic activity especially from the larval maggots of domestic housefly, *Musca domestica* (Linnaeus). It was revealed that the haemolytic activity was less in Rainy season (June 2010- September 2010) from the larval extract followed by summer (February 2010-May 2010) and winter season (October 2010 - January 2011).

KEYWORDS: Haemolytic activity, Housefly, Larval extract, Maggots.

INTRODUCTION

Arthropods are major representative groups of invertebrates, and insects are characterized by containing an open type of circulatory system. The haemolymph is the fluid in the circulatory system of houseflies and is similar to the fluids and the cells making up both blood and interstitial fluid. It contains water, inorganic salts and organic compounds. Within the haemolymph, haemocytes are present which the free floating cells are and they play major role in innate immunity (Tsakas and Marmars, 2010; Wikkipedia, 2012). Most of the workers have studied the role of haemocytes present in the haemolymph in cellular responses including phagocytosis, nodulation and encapsulation (Nappi *et al.*, 2004). Various workers have studied the haemocyte from the insects circulating in the haemolymph and observed granulocytes (Lavin and Strand, 2002). Haemocytes are involved in defense mechanism namely phagocytosis, nodule formation and have a role in wound healing and transport of nutrients and hormones (Pandey and Tiwari, 2012). According to Anderson (1981) haemolymph activity can be induced by wounding. Various workers have demonstrated this haemolytic activity in haemolymph of various classes of invertebrates including Annelids (Tuckova *et al.*, (1986); Arthropods (Weinheimer *et al.*, 1969) Molluscs (Bertheussen, 1983) and Echinoderms (Canicatti, 1991). Cohen *et al.*, (1986) studied the haemolymph volume and amino acid concentrations from desert blister beetle, *Cysteodemus armatus.* They have stated that changes in the concentration of amino acid appeared to be important to osmotic balance during desiccation. Wyatt and Kalf (1957) studied the chemical nature of insect haemolymph. They have noticed small amount of glucose and apparent glycogen in plasma of *Bombyx mori* insects.

Sapcsaliu (2009) has studied the haemocytes from haemolymph samples in honey bees, *Apis mellifera carpatica* from Romania. According to Jalal *et al.*, (2010) while studying the haemolymph of citrus spiders from Pakistan stated that the haemolymph from these animals contained a blend of components having biological and toxicological prospective. They have further stated that the species abundance is mainly dependent on various seasons and thereafter haemolymph quantity and quality were also changed with the course of time significantly. Mallik and Reddy (2009) have studied mulberry nutrition on regulation of haemolymph in low sodium high potassium, magnesium and moderate calcium concentration from the haemolymph and stated that larger quantity of leaf ingestion have a stronger impact on haemolymph cationic composition. Velide and Rao (2011) carried out studies on cold stress in fifth instar of *Tussar* silk worm and analyzed biochemical components from the haemolymph and noticed increased amino acid content and decrease in fat bodies from the various treatment groups of Andhra local larvae (low temperature). Williams *et al.*,(2011) while studying the effect of freezing and dehydration on haemolymph volume in the golden fly *Urosta solidahinis* observed the lower levels of magnesium when the larvae is subjected to freezing. The main purpose of present study was to determine the haemolytic activity in the larval maggots of housefly *Musca domestica* , collected from native sites such as animal excreta. This will give an idea of the synchronization of various activities in different seasons as the houseflies survive virtually in the habitat of number of animal pathogens and it will give some direction on the immune defense system of these insects.

MATERIAL AND METHODS

Housefly maggots, *Musca domestica* (L) were collected from local dairy farm from the dumping site of cow dung at Rupabhawani temple site Solapur, Maharashtra. The present work was carried out from February 2010 to January 2011.

Preparation of RBC- The haemolytic activity of larva of housefly, *Musca domestica* was determined by using adult Wistar rat erythrocytes.The known quantity of rat blood (2-3 ml) was drawn into 12 ml of heparinised 5mM Hepes buffer (pH 7.4) containing 150 mM NaCl. This homogenate was centrifuged at 4000 rpm for 5 minutes. The cell pellets were thoroughly washed with Hepes buffer. The working stock of RBC were made by diluting the RBCs (0.5-0.8ml) to about 15 ml with the buffer. Different volumes (5-40 µl) of the diluted extract were drawn into 1ml of water containing 0.1% of Triton ×100 and buffer with RBCs was used as blank. The solution was centrifuged and the haemolymph was measured spectrophotometrically (Perkin Elmer model lambda 35 uv vis spectrometer. The volume of RBC was standardized so as the absorbance was about 0.2 O. D. (about 2×10^6cells/ml range). Similar methodology was adapted for various extract samples collected during various seasons. The absorbance of O.D was considered as the haemolytic activity. For the conformation of the experimentation three sets were run simultaneously.

RESULTS AND DISCUSSION

The haemolytic activity of housefly extract of various seasons was determined by measuring O.D.at 540 nm of the released haem, against 100% lysis using Melatin as a positive standard. It was observed that during summer from all the sets of larval extracts (500µL) the O.D. was 0.2201(Feburary 2010 to May 2010). During Rainy seasons (June 2010 to September 2010) the larval extract from all the sets showed the mean OD value as 0.1825 whereas in the Winter season (October 2010 to January 2011) the larval extract from all the sets revealed the OD at 540 nm as 0.2365. (Table 1; Figure1).

Table: 1 Effect of housefly larval extract on haemolytic activity in various seasons

Seasons	Control O.D	Experimental O.D	Percentage of Lysis
Summer (February 2010-May 2010)	0.0100 ±0.001	0.2201* ± 0.010	48.9
Rainy (June 2010- September 2010)	0.0100 ±0.001	0.1825 *± 0.006	40.0
Winter (October 2010 - January 2011)	0.0100 ±0.001	0.2365* ± 0.006	52.5

* indicates value significant at P‹0.05

The haemolytic activity was determined on the basis of absorbance. Hence, from the present result it is evident that the haemolytic activity was maximum in winter seasons which were followed by summer and rainy seasons. Total lysis showed 0.4500 O.D. whereas the extract of rainy season (June 2010 to September 2010) showed 0.1825 O.D. (40%). Hence, data indicates that the extract shows about 40% minimum haemolysis in rainy season which indicates differential haemolytic activity in different season (Feburary 2010 to January2011), it may be due to self-defense mechanism in insects.

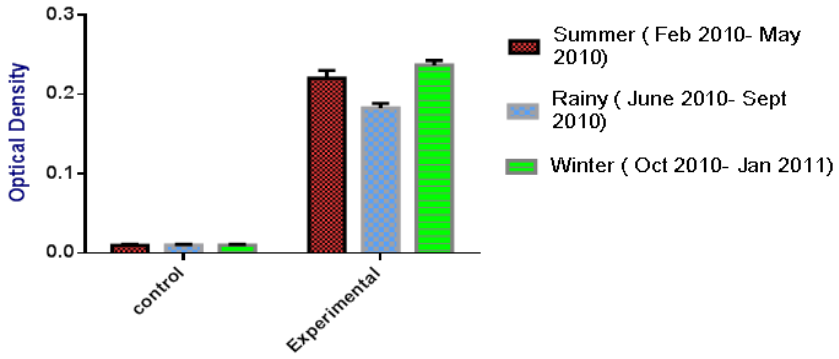

Figure 1. Effect of house fly larval extract on hemolysis activity during different seasons

Our results after comparing with different seasons revealed that in rainy season the percent lysis of RBC of rat was less (40.0%) as compared to summer (48.9%) and winter (52.5%). On the basis of our result it can be suggested that, rainy season being favourable for breeding and reproductive activity. The haemolysis is less in rainy season and hence, can be used for the therapeutic use for varying wound healing process. Houseflies are abundant throughout the year but they are dominant at particular season under prevailing condition and food availability. Therefore, their number tends to increase or decrease with respect to seasons. A particular humidity and temperature was considered as favorable season for the growth of *Musca domestica* . Their number sharply increases during mid-rainy seasons and continued for some time and as their number falls off progressively as the winter season approaches (Mohamed Humayun *et al* ., 2002, Hausmaun 1996). Haemolytic activity is directly related with the innate immunity system in insects' defense response (Lavin *et al.*, 2002). As the houseflies live in pathogenic environment the innate immune response of houseflies has been elaborated in different seasons. During various seasons the housefly confronted variable amount of bacteria under unhygienic conditions, however due to their innate immune response, these flies survive and transmit various diseases (Zaidman -Remy *et al*., 2006). Mallik and Reddy (2008) studied effect of high temperature on

haemolymph sugar level in three selected silkworm races of *Bombyx mori* . They have noticed decrease in the activity and the levels of haemolymph threolase with the pupae of CSR-2 treated with high temperature. The decrease was more at 36°C than at 32°C. Lavin and Strand (2002) studied insect haemocytes and their role in immunity. They have stated that the insect haemocytes uprise from the mesodermal tissue. They have quoted that these hemocytes identify a variety of foreign targets and haemocytes mediated defense responses are regulated by various signaling factors which control cell adhesion and cytotoxicity. Pandey and Tiwari (2012) while overviewing the insect haemocytes stated that these haemocytes respond very quickly against various changes. From the present study it can be stated that the houseflies can be used as therapeutic agent for wound healing. It is considered that less haemolytic activity will be useful for wound healing. A variation in different seasons shows that there was less haemolytic activity in rainy season which is safer for therapeutic use. From our results it can be highlighted that low haemolysis in rainy season (June 2010 to September 2010), the secreted proteins in this season may be convenient for therapeutic use, hence larval extract may require extensive investigations for drawing further inferences.

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REFERENCES

Anderson R. S. (1981). Inducible haemolytic activity in Mercenaria mercenaria haemolymph. Devl. camp. *Immunol*. 5: 575-585.

Bertheussen K. (1983). Complement-like activity in seaurchin coelomic fluid. *Devl. camp. Immunol.* 7: 21-31.

Canicatti C. (1991). Binding properties of ***Paracenwotus lividus*** (Echinoidea) haemolysin. *Comp. Biochem. Physiol.* 98: 463-468.

Cohen C., Allen C., Ralph B., March and Jhon D Pinto (1986). Effects of Water Stress and Rehydration On Haemolymph Volume and Amino Acid Content in the Blister Beetle, *Cysteodemus armatus*. *Comp. Biochem. Physiol.* 85(4): 743-746.

Hausmaun K. and Hulssmann N. (1996). Protozoology 2nd Edition New York Thieme Medical Publishers. 67-68.

Jalal F., Shakila M., Shahnaz A.R., Anjum S., Tanveer M. and Ibrahim R. (2010). Comparative Profile of free ions in venom and haemolymph in citrus spiders from Pakistan *Pak. J. Zool.* 42 (6): 687-691.

Lavin M.D. and Strand M.R. (2002). Insect haemocytes and their role in immunity. *Insect Biochem. Mol. Biol.* 32: 1295-1309.

Mohammad H., Raheela H., Saeed A. K., Naseer A. C. and Mohammad T. (2002). Seasonal Variation of parasites in Houseflies from different areas of Lahore. *Pak. PG. Med. J.* **13** (2):64-66.

Malik F.A. and Reddy S.Y. (2008). Effect of high temperature on haemolymph sugar levels in three selected silkworm races *Acta Entomol. Sinica.* 51(11):1113-1120.

Nappi A.J., Kohler L. and Mastore M. (2004). Signalling pathways implicated in the cellular innate immunne response of Drosophila. *ISJ.* 5-33.

Pandey J.P. and Tiwari R.K. (2012).An overview of Insect Haemocyte. *Am. J. Biochem. Mol. Biol.* 2 (3): 82-105.

Sapcsaliu I., Radoi., Crenguta P., N. Tudor., Eliza C., Siceanu A. and Meiu F. (2009). Research regarding Haemocyte Profile *Apis mellifera* carpatica bee haemolymph originate in the South Romania **.** *Lucrari Sti.*42 (2): 393-397.

Tuckova L., Rejnek J., Sima P. and Onderjova R. (1986). Lytic activities in coelomic fluid of *Eisenia foetida* and *Lumbricus terrestris. Devl. camp. Immunol.* 10**:** *181-189.*

Tsakas V. J. and Marmaras .(2010). Insect Immunity and its signaling: an overview Department of Biology, University of Patras, Greece. *ISJ*. 7:228-238.

Velide L and Rao .P (2011) Effect of temperature Alterations on Biochemical Component of the Fat Body and Haemolymph and excretory products of Tropical Tasar Silkworm A. Mylitta Drury. *Asian J .Exp. Biol. Sci.* 2(4) *: 583-588.*

Wikkipedia (2012). http://www.org/wiki/haemolymph.

Weinheimer P. F., Evans E. E., Stroud R. M., Acton R. T. and Painter B. (1969). Comparative immunology: natural haemolytic system of the spind lobster, *Panulirus argus. Proc. Sot. Exp. Biol. Med.* ***13:*** *322-326.*

Wyatt G.R and Kalf G.F. (1957). The Chemistry of Insect Haemolymph. Trehalose and other Carbohydrates. *J. Gen. Physiol.* (4):6.

Williams J.B., Richard E. and Lee. Jr (2011). Effect of freezing and dehydration on ion and cryoprotectant distribution and haemolymph volume in golden rod gall fly,*Eurosta solidaginis J. Insect physiol* -1163-1169.

Zaidman-Remy A., M. Herve M. Poidevin, S. Pili-Floury, M-S. Kim, D. Blanot, B-H. Oh, R. Ueda, D. Mengin-Lecreulx, and Lemaitre B. (2006). The Drosophila amidase PGRPLB modulates the immune response to bacterial infection. *Immunity*. 463-473.

