

# **Dyeing Effluent –Induced Alterations in the Haemogram of Dragon Fly Larvae *Diplocodes Trivialis***

D.Chitra, A.Reniprabha, G.Dhanalakshmi and K. Sivakumar

PG and Research, Dept. of Zoology, Chikkaiah Naciker College , Erode, Tamil Nadu. India.

**ABSTRACT :** The present investigation was carried out to assess the toxicity of dyeing effluent on haemogram the haemolymph of dragonfly larvae , *Diplocodes trivialis* . The experimental larvae were collected from their natural breeding sites and textile dyeing effluent was collected from the sampling site of local dyeing unit. Static bioassays were carried out to find  $LC_{50/96}$  hr value from which these sub lethal concentrations were selected and the larvae were exposed to the effluent for about 30 days . After experimental exposure , the larval haemolymph was collected to analyze the haemogram. The haemocyte picture of an insect at particular time is called haemogram and consists of measurements of THC ,DHC , AHC and haemolymph volume.

The THC pattern showed an upsurge during normal development .The prohaemocytes and spherule cells were relatively in small proportions where as plasmatocytes and granulocytes were found in increased proportions .In treated larvae, there was a significant decrement of THC , AHC and DHC. This alteration in the haemogram of the affected larvae might have inhibited the phagocytic activity and detoxification efficiency of haemocytes . The larvae would become prone to the toxicity of effluent and thus mortality resulted.

**KEY WORDS:** Haemogram , Textile dyeing effluent , *Diplocodes trivialis* .

## **I.INTRODUCTION**

The insect haemocytes include a complex of several types of mesodermal cells that circulate in the haemolymph .The insects possess three basic categories of haemocytes namely prohaemocytes , plasmatocytes and granular haemocytes .The haemocytes are nucleated , and physiologically similar to the leucocytes of vertebrates . In circulating haemolymph , an accurate count of haemocytes is difficult as large number of cells adhere to the tissues . It is also reported that the number of haemocytes vary not only in different species of insects but also in the same species under various conditions .The haemocyte number is found to be influenced by various factors such as stage of development, parasitism , bacterial infections , hemorrhage , plant extracts , endocrine extracts and various toxicants .The above literature evidenced that studies on odonate haemocytes are scanty .

Among the industrial effluents the textile dyeing effluent is found to be very toxic. There is a dearth of literature on the effect of textile dyeing effluents on Odonates , as most of the studies have contributed to the effect of pesticides , heavy metals and industrial pollutants on fishes ,crustaceans and other insects . Thus the effect of textile dyeing effluent on haemocytes is not much revealed, in particular to Odonates.

## **II.MATERIALS AND METHOD**

The dragonfly larvae were collected manually from their natural breeding places and were reared individually in separate plastic containers of two liters capacity. The larvae were fed *ad libillum* and observed daily to find whether moulting had occurred in them and to record the duration of instars.

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The dyeing effluent was collected from the discharge of local dyeing industry at Manickampalayam near Erode. The collected raw effluent was considered as 100% and was diluted with dechlorinated tap water for experimental purposes. The  $LC_{50/96}$  hr value of the effluent to each larval instar was determined by the method of Sprague (1973). From this value three different sub lethal concentrations of the effluent namely 1%, 2% and 3% were selected for chronic exposure. For experimentation four containers were used, three for the test concentrations and the fourth one as the control. The larvae were fed throughout the experimental period.

## Hemogram

The hemocyte picture of an insect at particular time is called hemogram and consists of measurements of total hemocyte count (THC), differential hemocyte count (DHC), the absolute hemocyte count (AHC) and the hemolymph volume (HV).

THC - The total haemocyte counts were made according to the method employed by Gupta and Sutherland (1968).

AHC-It was estimated by multiplying the THC by the HV.

HV - Haemolymph volume was determined by adopting the method of Moens (1975) using Amaranth dye solution.

DHC- The differential count was made according to the method recommended by Yeager (1938).

## III. RESULTS AND DISCUSSION

Hematological indices are very important parameters for the evaluation of physiological status in organisms under toxic stress. The changes in the haematological indices (THC, AHC and DHC) depend on the concentration of the effluent and duration of exposure of larvae to the effluent.

In the current investigation, an increased THC pattern was observed in the control larvae of *Diplocodes trivialis* (Table.1). Same trend in THC pattern was observed by Kali *et al.* (2000) in American bollworm, *Helicoverpa armigera*.

The higher total haemocyte counts in the last larval instar of the present species could be attributed as an indication of higher activity of immune system to infections. (11).

On the contrary to the controls, textile dyeing effluent in treated larvae brings out a significant decrement of THC in the haemolymph of odonate larvae. These alterations observed in the present study in congruence with the findings of Ayyangar and Rao (1). Peter and Anantha Krishnan (10) also reported reduced haemocytes in the haemolymph of insects during stress caused by starvation, neem extract, poisonous chemicals, foreign substances and so on.

In most insect larvae, the prohaemocytes and the spherule cells are relatively a small proportion of haemocyte population in the haemolymph while granulocytes and plasmatocytes are at a higher proportion (13;14). A similar trend is also applicable in the odonate larvae which is revealed from the occurrence of higher proportion of plasmatocytes and granulocytes during metamorphosis of the control larvae of *Diplocodes trivialis*. Hinks have noticed that the plasmatocytes are involved in immune response in wax moth larvae, *Galleria mellonella*. The plasmatocytes of insects are shown to be capable of phagocytosis as investigated by many researchers like (8;12;2;13 and 14).

Thus the present study revealed that the larvae of *Diplocodes trivialis* tried to resist the toxic stress at lower concentration (1%) of the effluent with the increase in the number of plasmatocytes. But the immune response of the larvae might have failed at higher concentration by showing reduction in the number of plasmatocytes leading to mortality of the larvae.

Increased granular haemocytes was due to detoxification of haemocytes. Reduction in granular haemocytes at certain effluent concentration was due to the transformation of granular haemocyte into cystocyte (6.9). Therefore, the changes in number of plasmatocytes and granulocytes would block the phagocytic activity so that the larvae would become more susceptible to the toxicity of effluent as observed in the present study.

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Table .1.Total haemocytes count in the haemolymph of *D.trivialis* larvae when exposed to TDE II (Each value is the mean  $\pm$  SD of 5 observations.

Experimental samples	Total haemocyte (cells /cu.mm)Exposure period (Days)		
	10	20	30
control treated	18416.80 $\pm$ 201.83	17934.20 $\pm$ 165.75	18204.00 $\pm$ 129.10
1%	18513.60 $\pm$ 209.47** (0.53)r =0.694 Y=0.720x+5251.706	17107.80 $\pm$ 110.68** (-4.61)r= 0.824 Y=0.550x+7243.148	13346.00 $\pm$ 38.47*# (-26.69)r = 0.918 Y=0.273x+8368.365
2%	1.6710.20 $\pm$ 109.46** (-9.27)r =0.895 Y=0.485x+7771.383	14037.40 $\pm$ 103.82** (-21.73)r= 0.937 Y=0.587x+3516.630	12309.60 $\pm$ 29.17*# (-32.38)r=0.928 Y=0.210x+8492.598
3%	14436.20 $\pm$ 36.38 (-21.61)r=0.959 Y=0.173x+11251.969	13427.60 $\pm$ 28.78 (-25.13)r=0.728 Y=0.126x+11161.215	11208.00 $\pm$ 107.10*# (-38.43)r =0.992 Y=0.822x-3765.862
ANOVA(F) 5118.11* ,3555.68 $\Delta$			

\*Significant at  $t^{0.05}$  NS –Not significant(t),# -Not significant ( $x^2$ )  $\Delta$ Significant at F-0.05; $\Delta^{NS}$  –Not significant (F) ANOVA(F)-Two factor with repetition represents decrease from control values in parameters1;r-correlation coefficient

Table .2.Absolute haemocyte count in the haemolymph of *D.trivialis* larvae when exposed to TDE II (Each value is the mean  $\pm$  SD of 5 observations.

Experimental samples	Absolute haemocyte count (cells)Exposure period (Days)		
	10	20	30
control treated	1535249.90 $\pm$ 44389.01	1570536.20 $\pm$ 25054117	1865957.68 $\pm$ 19767.13
1%	1417398.23 $\pm$ 209.47** (-7.68)r =0.911 Y=0.931x-12653.509	1428135.77 $\pm$ 42001.78** (-9.07)r= 0.986 Y=1.653x1168887.368	1032616.66 $\pm$ 33387.24*# (-44.66)r = 0.991 Y=1.673x-2089474.526
2%	1255125.03 $\pm$ 19670.70** (-18.25)r =0.854 Y=0.378x+674339.691	112566.90 $\pm$ 14569.04*# (-28.33)r= 0.908 Y=0.718x-140390.689	911873.67 $\pm$ 9409.32*# (-51.13)r=0.980 Y=0.466x+41789.078
3%	1026393.25 $\pm$ 25361.72*# (-33.15)r=0.897 Y=0.512x+239729.802	987532.72 $\pm$ 19812.28*# (-37.12)r=0.908 Y=0.718x-140390.689	732166.02 $\pm$ 11886.36*# (-60.76)r =0.853 Y=0.513x-224588.883
ANOVA(F) 1853.74 $\Delta$ 20864 $\Delta$			

\*Significant at  $t^{0.05}$  NS –Not significant(t),# -Not significant ( $x^2$ )  $\Delta$ Significant at F-0.05; $\Delta^{NS}$  –Not significant (F) ANOVA(F)-Two factor with repetition represents decrease from control values in parameters1;r-correlation coefficient .

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Table .3 Prohaemocyte count in the haemolymph of *D.trivialis* larvae when exposed to TDE II (Each value is the mean  $\pm$  SD of 5 observations.

Experimental samples	Prohaemocyte (%)Exposure period (Days)		
	10	20	30
control treated	16.27 $\pm$ 1.58	17.33 $\pm$ 1.47	17.20 $\pm$ 1.86
1%	15.41 $\pm$ 0.71 <sup>NS#</sup> (-5.20)r =0.765 Y=0.343x+0.827	14.14 $\pm$ 1.13 <sup>*#</sup> (-16.87)r= 0.878 Y=0.678x+2.660	11.35 $\pm$ 1.04 <sup>*#</sup> (-34.02)r = 0.738 Y=0.413x+4.28
2%	14.16 $\pm$ 0.82 <sup>*#</sup> (-12.94)r =0.772 Y=0.403x+7.607	14.49 $\pm$ 1.57 <sup>**</sup> (-16.37)r= 0.0847 Y=0.906x-1.213	9.88 $\pm$ 1.15 <sup>*#</sup> (-42.56)r=0.926 Y=0.572x+0.046
3%	12.51 $\pm$ 1.19 <sup>*#</sup> (-23.12)r=0.888 Y=0.699x+1.643	10.93 $\pm$ 1.33 <sup>*#</sup> (-36.92)r=0.843 Y=0.762x-2.276	8.49 $\pm$ 1.25 <sup>*#</sup> (-50.64)r =0.711 Y=0.480x+0.24
ANOVA(F) 60.80 <sup>A</sup> , 29.39 <sup>A</sup>			

\*Significant at t<sup>0.05</sup> NS –Not significant(t),# -Not significant (x<sup>2</sup>) <sup>A</sup>Significant at F-0.05; $\Delta$ <sup>NS</sup> –Not significant (F) ANOVA(F)-Two factor with repheccitation represents decrease from control values in parameters1;r-correlation coefficient .

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