

# **Changes Observed in the Coagulation Process of Haemocytes in the Larvae of Dragon Fly *Diplocodes Trivialis* under Effluent Stress**

D.Chitra ,A.Reniprabha , G.Dhanalakshmi and S.M.Thoulah basha

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

**ABSTRACT:** In insects , haemolymph coagulation is considered to be initial step in wound healing which involves coagulocytes and granulocytes .In the present study , it has been aimed to observe the changes in the process of coagulation in the haemolymph of dragonfly larva *Diplocodes trivialis* when exposed to the textile dyeing effluent .The antepenultimate larvae of *Diplocodes trivialis* and effluent collected from a local dyeing unit were used for the present study .The larvae were exposed to the effluent for the period of 30 days .After experimental duration , the haemolymph was collected directly on a clean slide by cutting the legs of the larvae and haemolymph was allowed to ooze out .After necessary time , the coagulation was observed. It was found that, the textile dye effluent impaired the normal coagulative process by blocking the precipitation of cytoplasmic strands and this could also be due to reduced number of haemocytes.

**KEYWORDS:** Textile dyeing effluent, coagulation process, *Diplocodes trivialis*.

## **I.INTRODUCTION**

The haematology is growing field of research in animal biology and importance of hematological research has considerably increased due to the impact of environmental pollution to indicate the level of harmful effect on animals. Haemocytic immunity in insects depend on phagocytes , encapsulation , coagulation and poison detoxification process .Extensive literature are available to reveal the phagocytic action of haemocytes (Pech and strand , 1995 ; Carlos *et al .* , 1996 ; Griesch and Vilcinskas , 1998 ; Tojo-sumio *et al .* , 2000).The role of haemocytes in the process of coagulation of blood through coagulocytes has been established by Arnold (1974) and Gregoire and Coffinet (1979) in various insects .The role of haemocytes in the formation of connection tissue has been envisaged by Messner (1976) and Crossley (1979).But works related to the effect of textile dyeing effluent on the coagulative process of odonate haemocytes are scanty .Therefore the present study has been aimed to investigate the effect of textile dyeing effluent on coagulative process of haemocytes in the dragon fly larvae *Diplocodes trivialis*.

## **II.METARIALS AND METHODS**

The larval instars of *Diplocodes trivialis* were collected from their natural breeding places and textile dyeing effluent was collected from local industry and brought to laboratory. Static bio assays were carried to find out LC50/96 hr value and it was found to be 30 % .In the sub-lethal concentrations of the effluent the larvae exhibited no moulting and died on day 35. After the experimentation, the haemolymph was collected directly by cutting the legs as rapidly as possible at the edge of the cover glass lying on a slide and was allowed to spread into a thin film .Then the slide was immediately observed under the microscope at specific intervals of 10 minutes up to 60 minutes to study the coagulative process .The preparations were microphotographed.

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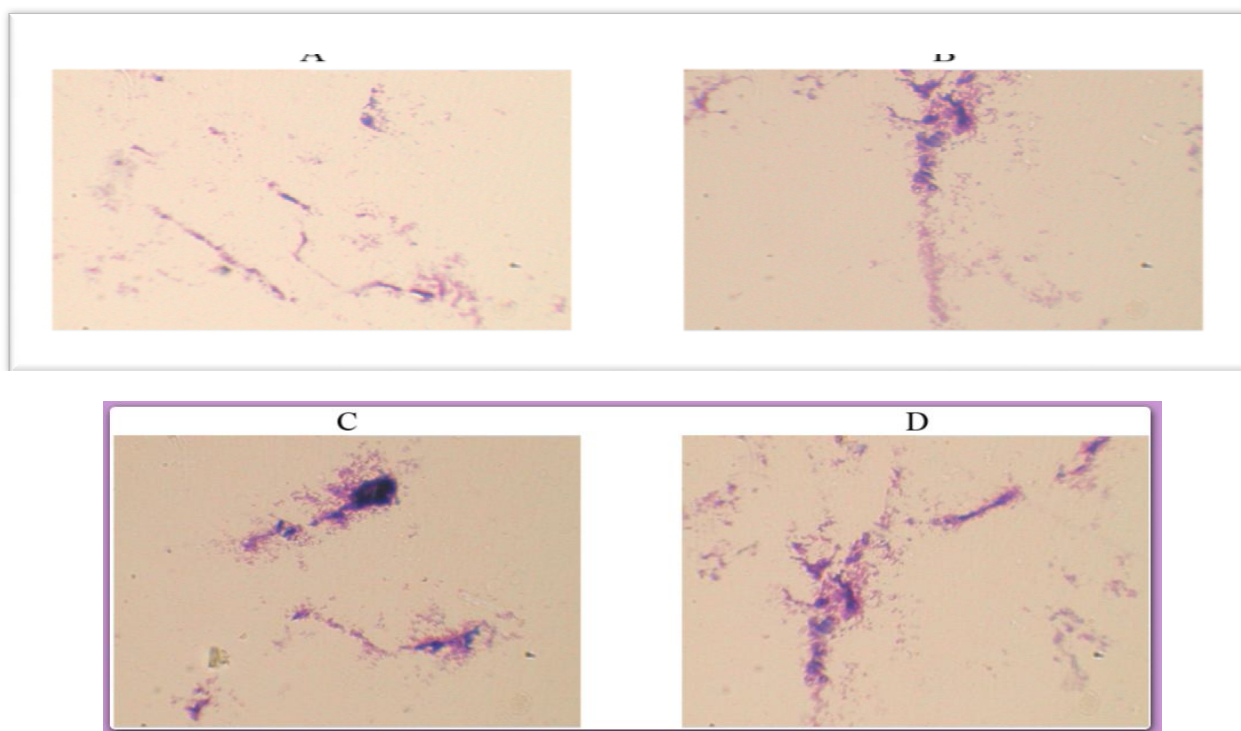
### III.RESULTS AND DISCUSSION

In control larvae, immediately after withdrawal of haemolymph there were individual haemocytes which slowly aggregated to form the coagulum by the entrapment of haemocytes. When the duration was extended, the coagulocytes developed cytoplasmic extensions. After 60 minutes from the withdrawal period, coagulation process ended with the formation of cytoplasmic strands, which is exemplified in plate I. On treated larvae, the coagulation process was disrupted because of the prevention of precipitation of plasma proteins which was involved in coagulation process, thus affecting the formation of cytoplasmic strands. This could also be correlated with the reduced number of haemocytes in the haemolymph of the treated larvae as shown in plates II and III. It is also evident that the concentration of effluent and duration of exposure affects the process of coagulation in *D. trivialis*.

In insects, the haemolymph coagulation is considered to be an initial step in wound healing which involves the coagulocytes (Rowley and Ratcliffe, 1976; Rowley, 1977; Ratcliffe and Rowley, 1979). Francois (1975) Beaulaton and Monpeysson (1976) have investigated that the haemocytes synthesize and secrete glycol and lipo proteins which form matrices for haemolymph coagulum.

In the present study, the textile dyeing effluent blocks the precipitation of plasma proteins around coagulocytes and the development of cytoplasmic strands, thus impairing the process of coagulation in the haemolymph of *D. trivialis* larvae. This observation of the present study falls in line with the findings of Chindah and Sikoki (2004). Because of coagulative disruption, the immune response of the larvae was inhibited resulting in mortality of larvae in the effluent treatment.

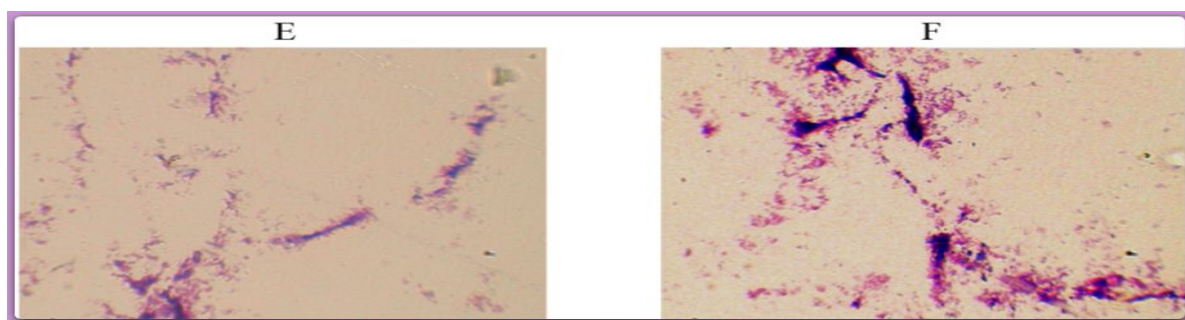
#### **Giemsa-stained smear of haemolymph after 60 minutes of withdrawal showing the completion of coagulative process by the formation of cytoplasmic strands (400X).**



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