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Short Communication

Effect of thiourea on tissue damage in the larvae of *Sarcophaga* Sp.

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Abstract

The objective of the study was to identify the tissue damage due to chemical (Thiourea) stress in the larvae of Sarcophaga ruficornis by trypan blue staining. Trypan Blue dye exclusion method is used for the assessment of cell viability. In all treatments the tissue shows differential staining pattern as compared to control. In Sarcophaga ruficornis larvae the gut tissues viz. gastric caeca, midgut, hindgut and malpighian tubules are more susceptible regions to stress as compared to non gut tissues i.e. brain ganglia and salivary gland.

Keywords: Thiourea, Trypan blue, *Sarcophaga*, tissue damage, chemical stress.

Introduction

Stress is defined as "an environmental factor causing a change in a biological system, which is potentially injurious[1]. The varieties of chemical stress which affect the tissue damage has been studied only in few dipteran families viz. Calliphoridae[2], Drosophilidae[3-8], Muscidae[9,10] and Sarcophagidae[11]. The chemicals causing tissue damage in these dipterans used are insecticides, pesticides, heavy metals, toxic chemicals etc. However, there is only a single reporting with the toxic chemical namely sodium azide on one species of the family Sarcophagidae[11]. In the present study the stress effect of a toxic chemical, thiourea was seen on larval tissues of the flesh fly, *Sarcophaga ruficornis* and also assessed the mortality rate of larvae after chemical treatment. For evaluate the level of tissue damage trypan blue dye exclusion assay was used. The method is based on the principle that live or viable cells do not take up trypan blue while it readily enters dead or non viable cells.

Materials and methods

Present investigations was carried out on laboratory stocks of *Sarcophaga ruficornis* Fab. (Sarcophagidae: Diptera). Chemical stress on larval tissue of flesh fly was examined by staining with trypan blue, a dye which is a marker of tissue damage.

Thiourea treatment and Trypan Blue staining: For control 10 healthy third instar larvae were placed in test tube, incubated and covered with muslin cloth and kept at room temperature (26± 2°C). For chemical treatment 5 sets of 10 healthy third instar larvae were placed in separate test tubes and incubated with different concentration of thiourea. The concentrations and time of exposure for chemical stress are presented in Table-1.

Table-1: Concentration and time of exposure for Thiourea.

S.No.	Concentration	Time (Minutes)
1	20 mM	30,60,90
2	30 mM	30,60,90
3	40 mM	30,60,90

After each treatment the control and treated larvae were dissected in insect saline and tissues were stained in trypan blue (0.2 mg/ml in phosphate buffer saline, pH 7.4) for 30 minutes at room temperature. Tissues were rotated intermittently to bring the entire tissue in contact with the dye. After staining tissues were rinsed thrice in phosphate buffer saline and left for washing in phosphate buffer saline for 30 minutes, immediately scored for trypan blue staining following the method of Krebs and Feder[12].

Mortality of larvae after Thiourea treatment: To identify the mortality of larvae after chemical treatment, larvae were thawed at room temperature for 1 hour and assessed the mortality rate. Moving larvae were assigned as survivors and others were assigned as dead. Mortality rate was observed after chemical treatment for different time intervals i.e.15 min, 30 min., 45 min and 60 min. In all the treatment the rate of mortality increased with treatment time.

Results and discussion

The staining patterns of different larval tissues after chemical treatment with Thiourea are summarized in Table-2.

As compare to control the brain ganglia and salivary gland show moderate staining in all treatments except 20mM/30 min. The Gastric caeca region of larvae show darker staining after 30 min. exposure of 30mM thiourea. The Mid gut and hind gut were darkly stained by trypan blue in all experiments except 20 mM/30 min. All larvae exposed to thiourea show darkest staining in malpighian tubules.

Table-2 shows that non-gut tissues (Bg and Sg) are less sensitive as these show moderate staining than gut tissues (Gc, Mg and Hg) including malpighian tubules (Mt) which shows darkest staining.

Mortality rate after Thiourea treatment: The rate of mortality increased with treatment time and concentration. It was lowest at 15 min. exposure of 20mM concentration of chemical treatment and after 60 min. exposure of 40mM concentration of thiourea treatment the mortality is approximately 100%. The mortality rate of *S. ruficornis* larvae after chemical stress is represented in Figure-1.

Table-2: Staining pattern of control and stressed larval tissues of *Sarcophaga ruficornis.*

	Bg	Sg	Gc	Mg	Hg	Mt
Control	+	-	+	-	+	+
(20mM/30 min.)	+	+	++	++	++	++++
(20mM/60 min.)	++	++	++	+++	+++	++++
(20mM/90 min.)	++	++	++	+++	+++	++++
(30mM/30 min.)	++	++	++	+++	+++	++++
(30mM/60 min.)	++	++	+++	+++	+++	++++
(30mM/90 min.)	++	++	+++	+++	+++	++++
(40mM/30 min.)	++	++	+++	++++	++++	++++
(40mM/60 min.)	++	++	+++	++++	++++	++++
(40mM/90 min.)	++	++	+++	++++	++++	++++

Bg-Brain ganglia, Sg - Salivary gland, Gc - Gastric caeca, Mg - Mid gut, Hg –Hind gut , Mt - Malpighian tubule. (-)= No staining,(+) = Pale blue staining,(++) =Moderate staining,(+++)=Darker staining,(++++) = Darkest staining.

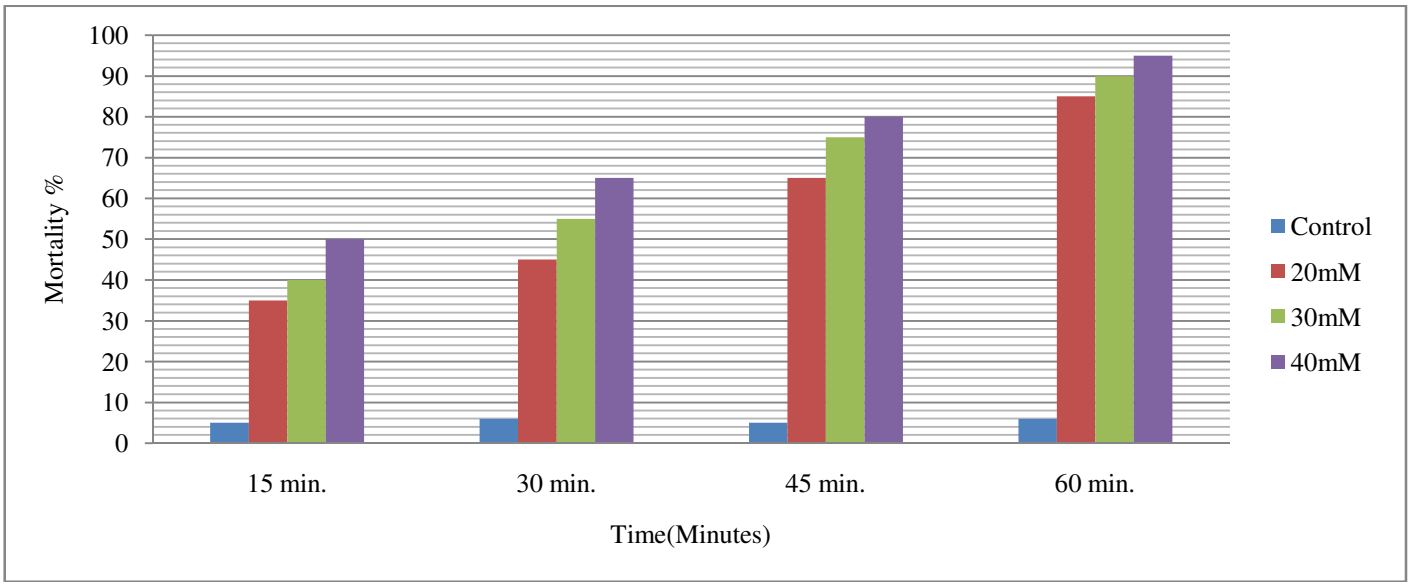

Figure-1: The mortality rate of *S. ruficornis* larvae after Thiourea treatment.

Discussion: Present work shows that Thiourea is toxic chemical which causes mortality in *Sarcophaga ruficornis*. It also causes stress effect on larval tissues. In *S. ruficornis* larvae the gut tissues viz. gastric caeca, midgut, hindgut and malpighian tubules are more susceptible regions not only to one chemical but to any type of stress as compared to non gut tissues i.e. brain ganglia and salivary gland[11,13].

In *Drosophila* also the gut tissues appear to be the most sensitive to heat shock and the slowest to produce heat shock protein (HSP)[12,14]. Krebs and Feder[12] predict that increasing heat shock protein level experimentally ought to increase the tolerance of these tissues more than others. The physiological response to any type of stress correlates with expression of a set of heat shock gene whose proteinaceous product i.e. Heat Shock Protein (Hsp) presumably contributes to protection from injury. Heat shock protein induction is often accompanied by tolerance to these stresses[15-20]. The author came to the opinion that in *S. ruficornis* there is tissue specific expression of heat shock proteins. There is a close link between stress protein expression and tissue damage as revealed by trypan blue staining[12].

Conclusion

In *S. ruficornis* trypan blue staining in non gut tissues was moderate or spotty, suggesting that tissue damage occurred in small proportion of cells. The gut tissues, however, incorporated darkest staining suggesting extensive necrosis. It can be concluded that in *S. ruficornis* the heat shock proteins are expressed early in non-gut tissue than the gut tissues.

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