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Impact of insecticide cypermethrin on the midgut histology of adult flesh fly, *Sarcophaga ruficornis* Fab. (Diptera: Sarcophagidae)

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Abstract
Sarcophaga ruficornis is well-known myiasis causing fly and also act as a vector to transmit disease-causing pathogens. This study evaluates the histological effects of the lethal concentration of the cypermethrin insecticide on the midgut of flesh fly *Sarcophaga ruficornis*. Cypermethrin showed toxic effects at 0.001% after 24 and 48 hours of treatment which causes histological changes. The midgut epithelial cells were disrupted with prominent enlargement in size at 24 hours of treatment, which shows more damage after 48 hours of treatment. More disruption in the basement membrane and outer muscular layer was observed at 48 hours compared to the 24 hours post-treatment. The degenerated peritrophic membrane and cytoplasmic vacuolization also increased with the increase in the exposure time. The obtained results suggest that cypermethrin insecticide can be used to manage the population of this fly.

Keywords: *Sarcophaga ruficornis*, midgut, histopathology, cypermethrin

Introduction
Myiasis is derived from the word 'myia' which means fly in Greek. Hope was the first to introduce the term and described myiasis as a parasitic infestation of humans and vertebrates by larvae of dipterans which for a certain time duration feed and grow on dead or living host tissue, liquid body substances, or food ingested by the host [1, 2]. The disease myiasis is a serious health issue with 'human myiasis' distributed worldwide and in countries where it is not endemic, myiasis is still the fourth most common travel-associated skin disease [3]. Since ancient times the myiasis-causing flies are still considered to be some of the world's most destructive pests, and among them, flesh fly *Sarcophaga ruficornis* is well known for causing myiasis in humans and animals like intestinal myiasis, neonatal myiasis, wound myiasis, cutaneous myiasis, and urinogenital myiasis [4–9]. *Sarcophaga* species are also known to cause anterior poliomyelitis in man, limber neck diseases in fowl and besides this, the flesh fly can also transmit pathogens like helminths eggs, protozoans cysts, and many gastrointestinal disease-causing parasites, by serving as vectors [10–14]. So, to control or manage the population of this pest, insecticide cypermethrin is used to evaluate the toxicity on the midgut of the fly. Cypermethrin belongs to the class II synthetic pyrethroids. It is considered to be one of the most used insecticides globally for vector-borne disease control, domestic and agricultural use. It acts on the central nervous system by the opening of voltage-gated sodium channels which leads to the hyperexcitation of neurons [15–18]. From the various internal systems of flies, the present study focused on the gut which is considered as a chief organ for digestion and absorption and dislodging unused food materials [19]. The digestive system is divided into three main parts namely, the foregut, the midgut and the hindgut, and out of which midgut is taken into the account for this study. Midgut plays an important role in the toxicity of insecticides, as it is known to be the most affected part when comes in contact with other different types of insecticides [20, 21]. So, the main aim of this study is to evaluate the toxic effects on histology of the midgut of flesh fly *S. ruficornis*, as no noticeable work has been done so far on the midgut of *S. ruficornis* treated with cypermethrin despite its abundance and medical and veterinary importance.

Materials and Methods
1. Insects culture
From the campus of Aligarh Muslim University, the adult flesh flies were captured by providing rotten buffalo meat as bait and taken to the insectary of the Department of Zoology for further studies. The colonies of flies were kept in the wooden cages made up of wire mesh and these cages were placed in the incubators maintained at a temperature of 27±2°C and 60±5% relative humidity as per protocol [22].

2. Experimental design
In this experiment, thirty specimens of 7thday adult flies were treated with 0.001% concentrations of cypermethrin insecticide at a time interval of 24 and 48 hours by the method of ingestion. Likewise, a control setup was maintained for comparison. At 24- and 48-hours post-treatment, approximately 10 flies each with

treatment and control were selected for dissection. The midgut from each fly is dissected out in Ringer's solution under a binocular dissecting microscope at 40x magnification and transferred to the cavity block.

3. Histological preparation of midgut for light microscopy

The dissected midgut of treated flies was fixed immediately in Bouin's solution for 18 hours, then washed in tap water and distilled water two times for 15 minutes each and then for 15 minutes each, transferred in an ascending alcohol series for dehydration followed by two times of xylene for 2 minutes. After that incubation was done at 63°C in xylene and paraffin wax in equal ratio for 15 minutes and then in pure paraffin wax for 2 hours. Tissues were then embedded in pure paraffin wax and the sections of 5 µm were cut into a rolling ribbon by microtome. The ribbons were placed on the lubricated glass slide with egg albumin having few drops of glycerine. The slides with sections were warmed lightly so that the creases will straighten and then processed in xylene for 10 minutes each which was followed by descending grades of alcohol and then for 5 minutes each in distilled water. The slides were then stained in Delafield's haematoxylin for 20 seconds and then processed in tap water & distilled water. After dehydration up to 70% alcohol, slides were stained with alcoholic eosin for 20 minutes and then dehydrated in alcohol series up to 100% for 5 min each. The slides were then kept in xylene two times for 10 min each and then finally mounted with DPX. The histological preparation of the midgut of control was also done for comparing the effects. Photography was done using a LEICA compound microscope under proper magnification.

Results and Discussion

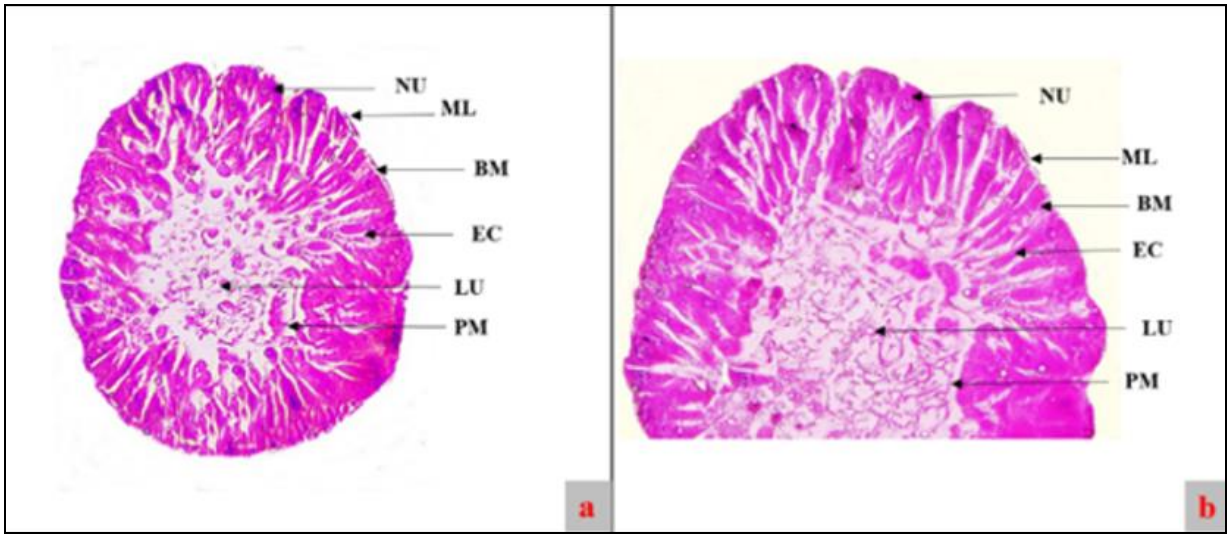
Normal histology of midgut of fly *Sarcophaga ruficornis* (Fig. a, b).

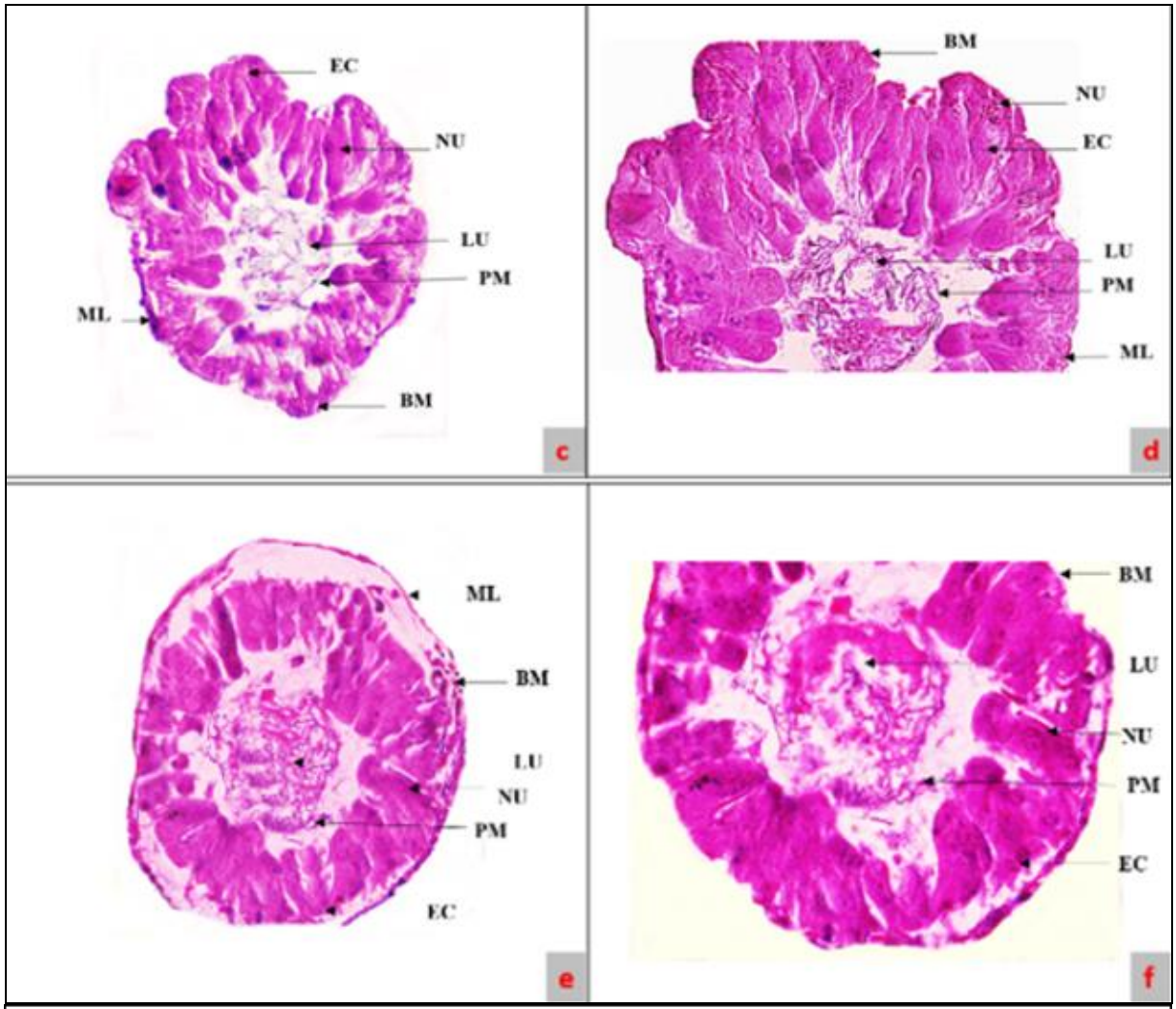
The midgut of fly *Sarcophaga ruficornis* is the longest part out of the other two parts, foregut and hindgut. Histomorphology of the midgut showed well developed outer muscle layer, inner basement membrane, columnar epithelial layer and the thin peritrophic membrane in the lumen of the gut.

The well-developed columnar epithelium showed a nucleus stained with blue dye and have cytoplasm. These epithelial cells rest on the basement membrane, both were seen closely associated with each other, which act as a base for all cells.

Effect of 0.001% concentrations of cypermethrin at 24 and 48 hours of treatment on the midgut of *S. ruficornis* (Fig. c-f).

Results indicate that the cypermethrin affect the morphology of the midgut of myiasis causing fly *S. ruficornis*. The histological changes mediated by the insecticides at 0.001% after 48 hours of treatment are more intense than at 24 hours. These changes include prominent enlargement and distorted shape in columnar epithelial cells at 24 hours of treatment which got severe with more disruption and detaching epithelial cells from basement membrane with increased time of exposure. Similar results were reported in *Spodoptera litura* treated with extract of *Mirabilis jalapa* leaves [23], in *Spodoptera frugiperda* fed with sub-lethal doses of *Azadirachta indica* [24, in the larval of *Chrysomya megacephala* exposed with malathion [25], in *Chironomus calligraphus* larvae fed with cypermethrin [26]. The outer muscular layer and basement membrane at 24 hours of treatment showed slight disruption which was observed prominently with increased exposure time after 48 hours of treatment. The cytoplasmic vacuolization increased with time of treatment and disturbed peritrophic membrane was also observed. Similar findings were reported in *Chrysomya megacephala* and *Dysdercus koenigii* treated with malathion and deltamethrin [22, 25, 27], in *Periplaneta americana* exposed to deltamethrin [28], in *Simulium pertinax* larvae treated with delta-endotoxins of *Bacillus thuringiensis* [29], in *P. americana* treated with N-nitroso-N-methyl urea [30], in *B. germanica* treated with boric acid [31]. Hence, cypermethrin induces deleterious effects on the midgut cells of flesh fly, causing alteration in the morphology of midgut with the time-dependent approach which suggests that cypermethrin can be used to manage the population of *S. ruficornis.*





➢ **Figure showing:-**

T.S. of control midgut of seven days old adults of *S. ruficornis* stained with H&E (a) after 24h (100X), (b) 400X.

T.S. of midgut of seven days old adults of *S. ruficornis* fed with 0.001% concentration of cypermethrin (c) after 24h (100X), (d) 400X; (e) after 48h (100X), (f) 400X. H&E stain.

[ML muscular layer, BM basal membrane, NU nucleus, EC epithelial cells, PM peritrophic membrane, LU lumen]

Fig 1

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