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**Mohammad Amir**

Department of Zoology,  
Aligarh Muslim University,  
Aligarh, Uttar Pradesh, India

## Histopathological effect of some insecticides on the Male reproductive system of *Sarcophaga ruficornis* Fabricius (Diptera: Sarcophagidae)

**Mohammad Amir**

### Abstract

Flesh flies belonging to the family Sarcophagidae are of considerable medical and veterinary importance, being responsible for various types of myiasis. During the present study, effect of sub lethal dose of four insecticide Dieldrin (0.0007%), Fenthion (0.01%), Carbaryl (0.01%) and Deltamethrin (0.05%) had been investigated on testis of *Sarcophaga ruficornis*. Spermatogonia and spermatocyte of the testis became pycnotic and gradually degenerated after treatment with sub lethal dose of dieldrin and fenthion but effect was less pronounced after treatment with carbaryl and deltamethrin. The testicular epithelia had gone thinning and folding after treatment with these insecticides. But in case of dieldrin treated testis, the testicular epithelium had undergone detachment from the main testicular tissue, indicating complete lysis of tissue below the epithelium. Sub lethal dose of these insecticides caused degeneration of spermatids and sperm bundle containing spermatozoa of testis. A number of vacuoles or spaces appeared due to lysis of tissue in transformation zone.

**Keywords:** histopathology, testes, *Sarcophaga ruficornis*, insecticides

### Introduction

Flesh flies belonging to the family Sarcophagidae are of considerable medical and veterinary importance, being responsible for various types of myiasis [1-7]. *Sarcophaga* have been associated with anterior poliomyelitis and limberneck diseases of man and fowl respectively [8, 9]. They also serve as vectors of trophozoites or cysts of protozoa, egg of helminths and several intestinal parasites of man and animals [10, 11] and various types of *Escherichia coli* [12, 13]. Effect of different insecticides on testis has been studied by Nath *et al.* [14] in *Locusta migratoria*, Janak [15] in *Poecilcerus pictus*, Fathpour *et al.* [16] in *Blatella germanica*, Ghazawi *et al.* [17] in *Heteracris littoralis*, Shakeet and Bakshi [18, 19] in *Chrotogonus trachypterus*, Reda *et al.* [20] in *Schistocerca gregaria* and Sharaby *et al.* [21] in *H. littoralis*. The above literature shows that very little work has been done on the histopathology of male reproductive system of family Sarcophagidae. Present study investigates the effect of different insecticides on the testis of *Sarcophaga ruficornis* Fabricius to fill lacunae in information on its histopathology.

### Material and Method

The adult flesh flies (*S. ruficornis*) were collected from fields in and around campus of Aligarh Muslim University, Aligarh during September-October, 2103. The flies were kept in cages made of fine wire mesh and ply board measuring 1'x1'x1' in size. The flies were maintained in the laboratory at 27±1 °C temperature and 65-70% relative humidity. They were fed on a mixture of milk, protinex and sugar (2:1:1) soaked in cotton wool. Chopped buffalo meat was provided in petridish as larviposition medium and was replaced daily to maintain hygiene and to avoid contamination. The meat was transferred daily in the glass jar (8''x4'') with extra chopped meat and jar was covered with muslin cloth and tied with rubber band to avoid escape of larvae. A layer of cotton was added in the jar at third instar larvae stage for pupation. The pupae when formed were removed from cotton and kept in meshed cages for adult emergence. During the present study sub lethal dose of four insecticide Dieldrin (0.0007%), Fenthion (0.01%), Carbaryl (0.01%) and Deltamethrin (0.05%) has been investigated on testis of *S. ruficornis*. Effect of insecticides on the histopathology of male reproductive organ (testis) of the adult of *S. ruficornis* was studied by topical application method as per method of Amir [22].

### Correspondence

**Mohammad Amir**

Department of Zoology,  
Aligarh Muslim University,  
Aligarh, Uttar Pradesh, India

Treated flies were kept separately in cages (4''x4''x4'') made of rice paper and cardboard. A few crystals of sugar were added in each cage through a hole which was plugged with moist cotton to provide suitable moisture condition. A control test was also conducted using the acetone only. The experiment was conducted at  $27\pm 1$  °C temperature and 65-70% relative humidity.

Control as well as treated flies was dissected under dissecting microscope in Ringer's solution (0.1g Potassium chloride, 0.0135g Calcium chloride, 0.012g Sodium bicarbonate and 0.75g Sodium chloride in 100ml of distilled water) after 24 hours of treatment. The gonads were excised and fixed immediately in alcoholic Bouin's fixative for 10 to 12 hours. They were then washed several times in 70% alcohol to remove extra fixative, dehydrated in ascending grades of alcohol 70% to 90% for half an hour each and in 100% for an hour, followed by 100% alcohol and xylene solution (1:1) for 15 minutes. Incubation was done at 60 °C in xylene and paraffin wax (1:1) and then in pure paraffin wax for 30 minutes. Testis was then embedded in paraffin wax to make blocks and 5-6µm microtome sections were cut into a rolling ribbon. The ribbon was placed on the glass slide which was lubricated by glycerine and egg albumin solution (1:1). Slides containing section were warmed slightly with a drop of distilled water on stretching board to straighten the creases. Slides were then processed in xylene (2 changes), followed by descending alcohol series of 100% (2 changes), 90% to 30% for 5 minutes each and then in distilled water for 5 minutes. The slides were then stained in Ehrlich's hematoxylin for 2-3 minutes and rinsed in tap water and then counterstained with aqueous eosin for 10 minutes. The slides were then dehydrated in ascending grades of alcohol 30% to 90% for 5 minutes each and then kept in 100% alcohol (2 changes) for 10 minutes, followed by xylene (2 changes) for 10 minutes each. Finally slides were mounted with DPX and cover slip and observed under compound light microscope. Photographs were taken using LEICA-DM compound microscope mounted with LEICA DFC 295 digital camera using appropriate magnification.

## Results and Discussion

### Normal histology of testis (Figs. 1-4)

The male reproductive organs of flesh fly *S. ruficornis* consists of a pair of testes, a pair of vasa deferentia, a pair of accessory glands and the ejaculatory duct with ejaculatory sac which opens to the exterior by aedeagus [23].

The testis in *S. ruficornis* is pyriform in shape and dark brown in colour. The wall of testis consists of two layer viz., a tunica externa and a tunica interna. The tunica externa (Te) is comparatively thin layer and contains a large amount of pigmented granules which impart dark brown colour to the testis. The tunica interna (Ti) is a thick syncytial layer. According to Sukantason *et al.* [24] in almost all diptera, the testes are enveloped by two tissue layers consisting of the outer epithelium (or tunica externa) and the inner epithelium (or tunica interna). The lumen of testis contains the germ cells in various stages of development. According to the stage of development of germ cells, the whole testis is distinguishable into three regions: Zone of spermatogonia (ZSpg), Zone of maturation and reduction (ZMat Red) and Zone of transformation (ZTrans). The apical portion of the testis contains a densely packed mass of primordial germ cells. The Primary germ cells are the spermatogonia (Spg) present in anterior region. The cells produced from each primary cell usually remain attached to one another centrally by

protoplasmic strands and assume a radial position giving a rosette pattern of the spherical mass. The small zone of spermatocyte is followed by a comparatively larger zone of maturation and reduction. Each spermatogonial group of cells undergoes division to form spermatocytes which are enclosed in a cellular envelope called sperm cyst (Cst). The zone of transformation forms the largest portion of the testis. The spermatocytes multiply to form spermatids (Spd) which finally transforms into spermatozoa (Spz) in advancing stage of development.

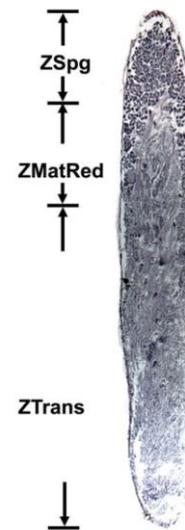


Fig 1: Sagittal section of normal/untreated testis of *S. ruficornis* (X100)

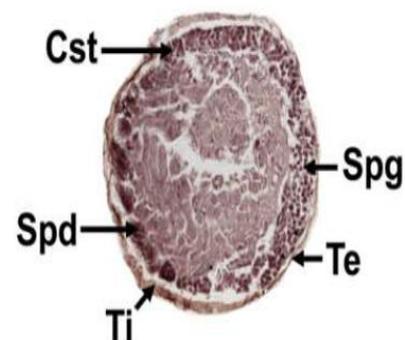


Fig 2: Transverse section of normal/untreated testis of *S. ruficornis* (X100)

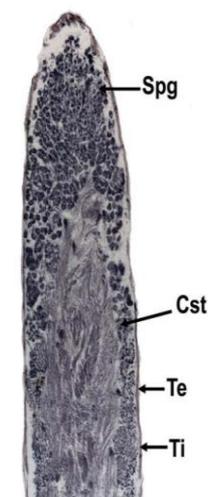


Fig 3: Sagittal section of normal/untreated testis (anterior region) of *S. ruficornis* (X400)



**Fig 4:** Sagittal section of normal/untreated testis (posterior region) of *S. ruficornis* (X400)

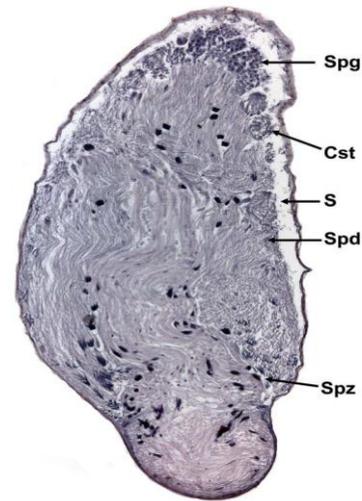
#### Histopathology of testis (Figs. 5-8)

Present study reveals that the spermatogonia and spermatocyte of the testis of *S. ruficornis* become pycnotic and gradually degenerated after treatment with sub lethal dose of dieldrin and fenthion. The effect of sub lethal dose of carbaryl and deltamethrin on spermatogonia and spermatocyte of the testis was less pronounced. The apical region of testis shows vacuolization and spacing due to degeneration of spermatogonia and spermatocytes. Similar observations were made in testis of *P. pictus* after treatment with chemosterilants (apholate and tepa) by Saxena and Aditya [25, 26] and in testis of *L. migratoria* after treatment with tepa and apholate by Nath *et al.* [14, 27]. Pycnosis occurred in the spermatogonia and spermatocyte of *P. pictus* when gonads were treated with endosulfan [15]. The azadirachtin treated apical region of testis of *H. littoralis* showed swelling of the dividing cells and cells in the central part were smaller in size [17]. Similar findings have been reported by Shakeet and Bakshi [18, 19] in the testis of *C. trachypterus* treated with cypermethrin and monocrotophos respectively. The number of spermatogonia and spermatocytes were severely reduced in the case of testis of *R. ferrugineus* treated with the neem extract and flufenoxuron [28]. Sharaby *et al.* [21] reported similar findings in the testis of *H. littoralis* treated with alcoholic extracts of five medicinal plants where spermatocytes showed abnormal shape and disruption and disintegration of cytoplasm resulting into formation of vacuoles.

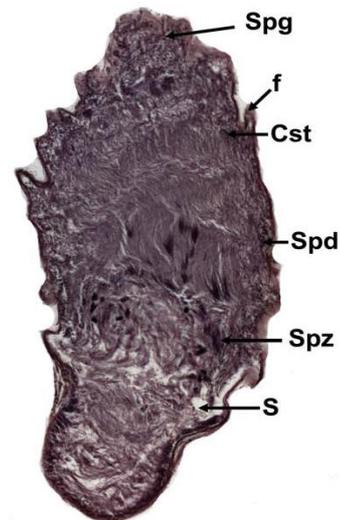
Experimental study reveals that the testicular epithelia of *S. ruficornis* has gone thinning and folding after treatment with sub lethal dose of dieldrin, fenthion, carbaryl and deltamethrin. In case of dieldrin treated testis, the testicular epithelium has undergone detachment from the main testicular tissue, indicating complete lysis of tissue below the epithelium. Similar observations have been reported by Nath *et al.* [14] in testis of *L. migratoria* treated with apholate, by Shakeet and Bakshi [18, 19] in *C. trachypterus* treated with cypermethrin and monocrotophos and by Ghazawi *et al.* [17] in *H. littoralis* testis treated with azadirachtin.

Present investigation points that sub lethal dose of dieldrin, fenthion, carbaryl and deltamethrin causes degeneration of spermatids and sperm bundle containing spermatozoa in *S. ruficornis*. A number of vacuoles or spaces appeared due to lysis of tissue in transformation zone. Similar findings were reported by Saxena and Aditya [25, 26] in testis of *P. pictus* treated with apholate and tepa and by Nath *et al.* [27] in the testis of *L. migratoria* treated with tepa and Nath *et al.* [14] in

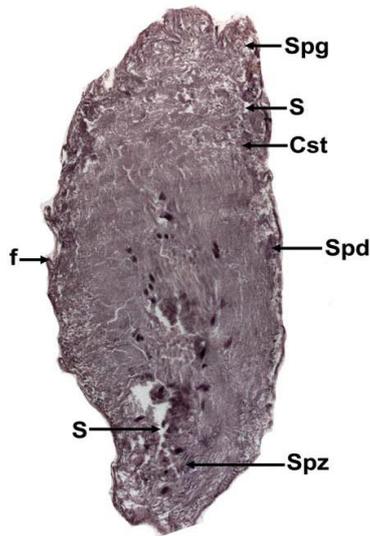
*L. migratoria* treated with apholate. Similarly, Fathpour *et al.* [16] found that pyriproxifen disrupted spermatogenesis leading to the incomplete development of sperm in the testis of *B. germanica* and Shakeet and Bakshi [18, 19] found hypertrophied spermatozoa called elongated cells in the testis of *C. trachypterus* treated with cypermethrin and monocrotophos. Reda *et al.* [20] found degeneration and necrosis in spermatogenic stage and inhibition in the formation of sperm bundles of the *S. gregaria* treated with Consult and Lufox (IGR). Similar findings were reported by Sharaby *et al.* [21] in testis of *H. littoralis* treated with five medicinal plant extracts where transformation zone shows hyperplasia and clumping of abnormal spermatids and spermatozoa. It may be concluded that dieldrin and fenthion gives more pronounced effect as compared to carbaryl and deltamethrin on testis of *S. ruficornis*.



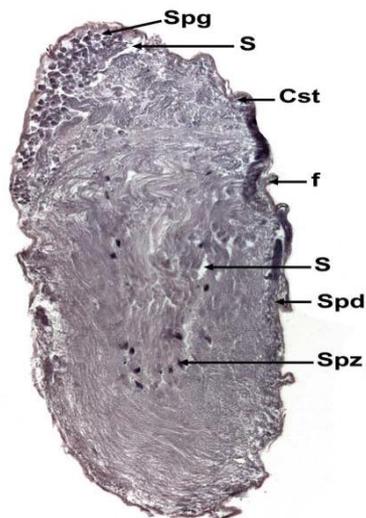
**Fig 5:** Sagittal section of testis of *S. ruficornis* treated with sub lethal dose of 0.0007% Dieldrin (X400)



**Fig 6:** Sagittal section of testis of *S. ruficornis* treated with sub lethal dose of 0.01% Fenthion (X400)



**Fig 7:** Sagittal section of testis of *S. ruficornis* treated with sub lethal dose of 0.01% Carbaryl (X400)



**Fig 8:** Sagittal section of testis of *S. ruficornis* treated with sub lethal dose of 0.05% Deltamethrin (X400)

**Abbreviations:** Cst, Spermatocyte; f, fold; S, space; Spd, Spermatids; Spg, Spermatogonia; Spz, Spermatozooids; Te, Tunica externa; Ti, Tunica interna; ZSpg, Zone of spermatogonia; ZMatRed, Zone of maturation and reduction; ZTrans, Zone of transformation

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