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Research Article

HISTOPATHOLOGICAL EFFECT OF SOME TOXICANTS ON THE FEMALE REPRODUCTIVE SYSTEM OF *SARCOPHAGA RUFICORNIS* FABRICIUS (DIPTERA: SARCOPHAGIDAE)

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ABSTRACT

The ovariole of *S. ruficornis* consists of polytrophic ovarioles and the vitellarium contains an average of two egg chambers in which both the developing oocyte and trohpocytes or nurse cells are enclosed by cuboidal epithelium of follicle cells. The trophocytes are generally triangular shaped cells containing spherical nucleus. A number of nucleolus is seen within each trophocyte nucleus. Sub lethal dose of dieldrin caused considerable damage to the nucleus of the trophocytes leading to the pycnosis or clumping of the nucleus, but no such change has been seen in the nucleus of trophocytes treated with sub lethal dose of cypermethrin and malathion. Sub lethal dose of dieldrin, cypermethrin, and malathion affect the normal vitellogenesis resulting in reduced deposition of yolk in ooplasm which show vacuolization at number of places. Follicular epithelial cells treated with sub lethal dose of dieldrin, cypermethrin and malathion show necrosis and thinning at number of places. The nuclei of follicular epithelial cells show pycnosis due to the effect of insecticides. The thinning of follicular epithelial cells cause detachment from the oocyte in case of ovarioles treated with cypermethrin.

Keywords: *Histopathology, Ovariole, Sarcophaga Ruficornis, Dieldrin, Cypermethrin, Malathion*

INTRODUCTION

The family Sarcophagidae is of considerable medical and veterinary importance, being responsible for various types of myiasis (Dutto and Bertero, 2010; Gupta *et al.*, 2010; Sukontason *et al.*, 2010; Ahmad *et al.*, 2011; Ricket *et al.*, 2011 and Zagool *et al.*, 2013). *Sarcophaga* have been associated with anterior poliomyelitis and limberneck diseases of man and fowl respectively (Bishopp, 1923; Melnick, 1949). They also serve as vectors of trophozoites or cysts of protozoa, egg of helminthes and several intestinal parasites of man and animals (Chang, 1943; Pipkin, 1949) and various types of *Escherichia coli* (Shura-Bura, 1952; Graczyk *et al.*, 2005). Exposure to sub lethal doses of insecticides on ovariole of the insect has been studied by Saxena and Aditya (1974), Chaudhry and Tripathi (1976), Saxena and Bhatnagar (1980), Bhide (1986), Jain and Bhide (1990), Mahmood *et al.*, (1991), Janak (1992), Fathpour *et al.*, (2007), Ghazawi *et al.*, (2007), Senthil *et al.*, (2008), Shakeet and Bakshi (2009 a & b), El-Bokl *et al.*, (2010), Rai *et al.*, (2011), Sharaby *et al.*, (2011) and Habes *et al.*, (2013). From the literature it is evident that very scanty work has been done on the histopathology of toxicants on the reproductive system of dipteran insects. The present study deals with the histopathological effect of some toxicants on the ovariole of *S. ruficornis.*

MATERIALS AND METHODS

The adult flesh flies (*S. ruficornis*) were collected from fields in and around campus of Aligarh Muslim University. 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±1ºC temperature and 65-70% relative humidity. They were fed on a mixture of milk, protienex 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

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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. Effect of insecticides on the histopathology of female reproductive organ (ovary) of the adult of *S. ruficornis* was studied by topical application method. Flies used for the experiment were taken out using an aspirator from the cage. The flies were anaesthetized by carbon dioxide for proper handling during testing period. Sub lethal dose of insecticides almost equivalent to LC_{50} (Amir, unpublished data) were applied on the dorsum of the 5 day old female flies. Measured drop (1µl) of insecticide solution in acetone was applied on the dorsum of each fly by means of micropipette. 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±1ºC 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%, 80%, 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 60ºC in xylene and paraffin wax (1:1) and then in paraffin wax only for 30 minutes. Ovariole and 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%, 70%, 50% and 30% for 5 minutes each and then in distilled water for 5 minutes. The slides were then stained in Ehrlich's haemotoxylin 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%, 50%, 70%, 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

The female reproductive system of *S. ruficornis* consists of a pair of ovaries, a pair of lateral oviduct, a median oviduct, three spermathecae, a pair of accessory gland and a uterus communicating with exterior through gonopore (Amir, 2013). Each ovariole is elongated in shape with broad base and tapering apical region. Diptera have typical meriostic polytrophic ovarioles (Telfer, 1975; Kokwaro, 1983; King and Buning, 1985, 1994). The ovariole of *S. ruficornis* consists of polytrophic ovarioles and the vitellarium contains an average of two egg chambers in which both the developing oocyte and trohpocytes or nurse cells are enclosed by cuboidal epithelium of follicle cells (Figures 1 & 2). The trophocytes are generally triangular shaped cells containing spherical nucleus. A number of nucleolus is seen within each trophocyte nucleus. According to Kokwaro (1983) the oocyte occupies almost half of the follicle space on the 5th day of follicle development with considerable amount of yolk in *Sarcophaga*. Each ovariole of *S. ruficornis* is of polytrophic type in which the trophocyte or nurse cells are found closely associated with the oocyte of each follicle. Similar observations were recorded by Miller (1950) in *Drosophila melanogaster*, Chaudhry and Tripathi (1976) in *S. ruficornis*, Spradbery and Sands (1976) in *Chrysomya bezziana*, Ansari and Murad (1981) in *Hippobosca maculata*, Kokwaro (1983) in *S. tibialis*, Bansal and Murad (1987) in *Chrysomya megacephala*, Pellegrini *et al.*, (2011) in *Glossina morsitans morsitans* and Amir (2013) in *S. ruficornis*.

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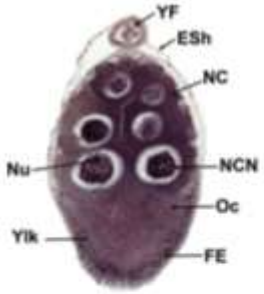

Fig. 1

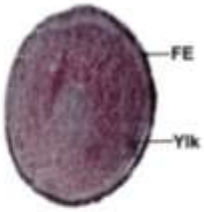

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Figure 1: Sagittal section of normal/ untreated ovariole of *S. ruficornis* (X400)
Figure 2: Transverse section (T.S.) of normal/ untreated ovariole of *S. ruficornis* (X400)
Figure 3: Sagittal section of ovariole of *S. ruficornis* treated topically with sub lethal dose of 0.0007% Dieldrin (X400)
Figure 4: Sagittal section of ovariole of *S. ruficornis* treated topically with sub lethal dose of 0.02% Cypermethrin (X400)
Figure 5: Sagittal section of ovariole of *S. ruficornis* treated topically with sub lethal dose of 0.06% Malathion (X400)
Abbreviations of figures 1-5: CN- clump nucleus, ESh- epithelial sheath, FE- follicular epithelium, NC- nurse or trophocyte cell, NCN- nurse cell nucleus, Nu- nucleolus, Oc- oocyte, S- space or vacuole, YF-young follicle, Ylk- yolk

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Sub lethal dose of dieldrin caused considerable damage to the nucleus of the trophocytes (Figure 3) leading to the pycnosis or clumping of the nucleus in *S. ruficornis*. No such change has been seen in the nucleus of trophocytes treated with sub lethal dose of cypermethrin and malathion indicating that these insecticides do not affect the nucleus of the trophocytes (Figures 4 & 5). Similar effect of thiotepa in the trophocytes of *Philosamia ricini* has been reported by Mohapatra (2007). Follicular epithelial cells of ovariole of *S. ruficornis* treated with sub lethal dose of dieldrin, cypermethrin and malathion show necrosis and thinning at number of places. The nuclei of follicular epithelial cells show pycnosis due to the effect of insecticides. The thinning of follicular epithelial cells cause detachment from the oocyte in case of ovarioles treated with cypermethrin. Similar observations in the follicular epithelial cells of the ovarioles have been reported in *Poecilocerus pictus* treated with chemosterilants (apholate and tepa) by Saxena and Aditya (1974), in *S. ruficornis* treated with thiourea by Chaudhry and Tripathi (1976), in *Periplaneta americana* treated with chemosterilants by Saxena and Bhatnagar (1980), in *P. americana* treated Y-BHC by Bhide (1986), in *P. americana* treated with sub lethal dose of BHC and DDT by Jain and Bhide (1990), in *P. pictus* treated with ivermentin by Mahmood *et al.,* (1991), in *P. pictus* treated with endosulfan by Janak (1992), in *Blattela germanica* treated with pyriproxyfen by Fathpour (2007), in *P. ricini* treated with thiotepa by Mohapatra (2007), in *Heteracris littoralis* treated with azadirachtin by Ghazawi *et al.,* (2007), in *Chrotogonus trachypterus* treated with cypermethrin and monocrotophos by Shakeet and Bakshi (2009 a & b), in *Rhynchophorus ferrugineus* treated with neem plant extract by El-Bokl *et al.,* (2010), in *P. pictus* treated with fenoxycarb by Rai *et al.,* (2011), in *H. littoralis* treated with alcoholic extracts of plants by Sharaby *et al.,* (2011) and in *B. germanica* treated with boric acid by Habes *et al.,* (2013).

Present study reveals that sub lethal dose of dieldrin, cypermethrin, and malathion affect the normal vitellogenesis resulting in reduced deposition of yolk in ooplasm of *S. ruficornis* which show vacuolization at number of places. Such observations were also made in *P. pictus* treated with chemosterilants (apholate and tepa) (Saxena and Aditya, 1974), in *P. americana* treated with chemosterilants (Saxena and Bhatnagar, 1980), in *P. americana* treated with Y-BHC (Bhide, 1986) and in *P. americana* treated with sub lethal dose of BHC and DDT (Jain and Bhide, 1990). Degeneration of follicle and reduction in vitellogenesis were also reported in ivermectin treated mosquito *A. aegypti* (Mahmood *et al.*, 1991). Vacuolization of ooplasm in *P. pictus* and arrested vitellogenesis has been reported when gonads were treated with endosulfan (Janak, 1992). Ovaries revealed morphological alteration in both vitellogenic and previtellogenic stage at follicular and germinal level in *D. limbata* when treated with botanical insecticide neem Azal (Habluetzel *et al.*, 2007). Damage to the oocyte and reduced vitellogenesis has also been reported in *H. littoralis* treated with azadirachtin by Ghazawi *et al.,* (2007), *C. trachypterus* treated with cypermethrin and monocrotophos by Shakeet and Bakshi (2009 a & b), in *P. pictus* treated with fenoxycarb by Rai *et al.,* (2011), in *H. littoralis* treated with alcoholic extracts of plants by Sharaby *et al.,* (2011) and in *B. germanica* treated with boric acid by Habes *et al.,* (2013).

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