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HISTOLOGICAL EFFECTS OF SELECTED PLANT EXTRACTS ON OVARY DEVELOPMENT OF THE MOSQUITO, *CULEX QUINQUEFASCIATUS SAY* (DIPTERA : CULICIDAE)

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Petroleum ether extract of leaf and seed of *Annona squamosa* (40 ppm) and leaf and seed extract of *Thevetia neriifolia* (200 ppm) treated larvae were reared to adult stage and used in the histological studies as the petroleum ether extracts of these plants were found to be effective compared to chloroform and ethanol extracts. In petroleum ether extract of *A. squamosa and T.neriifolia* leaf treatment, the follicular epithelium showed disruption and pycnosis of the nucleus and yolk granules receded away from the follicular epitheliu[redacted]e vacuolation in the ooplasm and loss of compactness and disintegration of yolk granu[redacted]bserved.

Key words : *Annona squamosa,* Pycnosis, *Ooplasm*, Follicular epithelium.

INTRODUCTION

The wide distribution of *Culex quinquefasciatus* Say in both the northern and southern hemispheres exposes this species to a variety of climatic challenges to its survival. It is able to adjust its seasonal cycle of reproductive activity to environments ranging from temperate continental climates to humid tropics. Examples of the flexibility include reports of peak reproduction in the warmest season in subtropical or temperate continental climates (Brogdone, 1984; Carlson,1982) in the coolest season in deserts (Walters & Smith,1980), in the coolest season in a settling lagoon (O'Meara & Evans, 1982) and in the dry season (De Mellion *et al.*,1967) or the wet season (Barrera *et al.*,1979) in the humid tropics.

In female insects, the reproductive organs are visibly affected either as a direct effect or as an indirect effect of chemosterilants (Labrecque & Smith, 1968).Induced sterility in treated females may be because of the complete cessation of ovarian development resulting in fecundity and non hatchability due to the presence of dominant lethal (Whitten & Foster, 1975; Labrecque & Fye, 1978).

In mosquitoes, alkylating agents caused drastic reduction in the size of the ovary due to the degeneration of follicles (Grover *et al.*,1972; Mathew & Rai, 1975). Sukumar & Naidu (1973) and Jalaja & Prabhu, (1976) observed similar reduction of the ovarian size at the gross level due to resorption of oocytes in *Dysdercus* after apholate treatment. In the developing follicles of *A. aegypti* degenerative changes such as condensation, pycnosis of the nuclei, vacuolization of the cytoplasm and degeneration of follicular epithelium were reported by Rai (1964a & b). Grover *et al.* (1972) showed degeneration of nurse cell and germaria in chemosterilised *Culex pipiens fatigans.*

Ludlum & Seiber (1988) who described the effect of neem products on egg formation in *A. aegypti* and of Hussein (1999) who described the alterations in the ovaries of *C. pipiens fatigans* in treatment with oils of *T. peruvine, D. stramonium* and *Acacia* sp.

Hussein (1999) observed reduced vitellogenesis, reduction in the synthesis of proteins, carbohydrates and lipids, DNA and RNA materials and histological changes in the oocyte of females derived from the treated larvae. In addition, reduced fecundity and fertility were also reported.

MATERIALS AND METHODS

To find out the effect of leaf and seed extracts of *A. squamosa* and *T. neriifolia* on the development of ovarian tissues, larvae of *C. quinquefasciatus* were exposed to different sub lethal concentration of leaf and seed extracts of *A. squamosa* (40 ppm) and *T. neriifolia* (200 ppm). Adults were provided with sucrose solution at the time of emergence and later provided blood meal from 5 to 7 weeks old chicks. Adult females were anaesthetized and fixed in Bouin's fluid. The reproductive tissues were processed for microtome [redacted] following Peterfi's double embedding method. The sections of 6μ thickness were [redacted]ing Weswox rotary microtome and stained with Heidenhein's hematoxylin and eosin. Microscopical examination was carried out to study the histological changes in the ovary. Photographs were taken using a research trinocular microscope (Nikon Lab Phor- 2).

As the petroleum ether extracts of these plants were found to be more effective than other two plant extracts, sub lethal doses of these extracts in which adult emergence could be observed in this study. Larvae reared in water containing 40 ppm of petroleum ether extract of *A. squamosa* leaf and seed and 200 ppm of petroleum ether extract of *T. neriifolia* leaf and seed were used in the histological studies.

RESULTS

Ovary in control

In *C. quinquefasciatus,* the ovaries were of polytrophic type. The ovarioles were bound into a mass constituting the ovary and are covered by a thin membrane (Fig.A). Each ovariole was fusiform structure, consisting of an apical region of undifferentiated cells called germarium and a basal region consisting of a succession of developing follicles, the vitellarium (Fig.B). Each follicle consists of an oocyte or future ovum and three to five nurse cells performing the nutritive function . These were surrounded by a layer of compact follicular epithelium made up of cuboidal epithelial cells with distinct nuclei. The oocyte was almost oval with a conspicuous, compact and centrally placed spherical nucleus and a clear cytoplasm. Yolk granules within the follicle showed compact arrangement .

Annona squamosa leaf and seed extracts

In the leaf extract treatment, the cells of the follicular epithelium showed pycnosis of the nucleus (Fig. C). The cuboidal epithelium became elongated, broken at intervals and the cytoplasm appeared to ooze out and thus showed degenerative changes. The chromatin materials in the nucleus of the nurse cells appeared to be clumped into

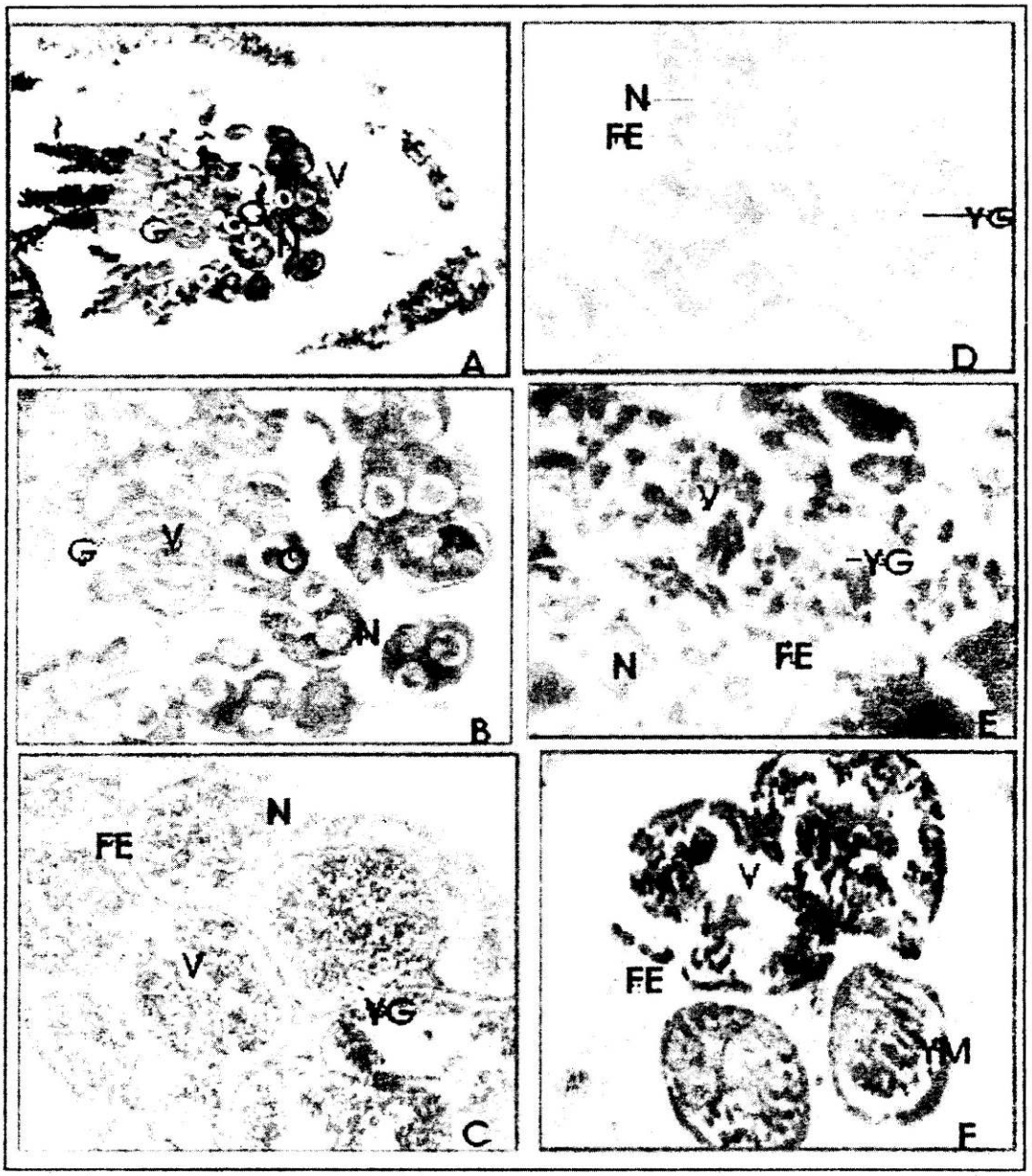

Fig. 1 : Section showing the effect of leaf and seed extract on the ovaries of *C. quinquefasciatus*. ***A.*** L.S of ovary showing ovarioles bound into a mass in control x 25; **B.** Enlarged view of ovarioles in control x 200; **C.** Sectional view of ovarioles in treatment with *A. squamosa* leaf extract x 200; **D.** L.S. of ovarioles in treatment with *A. squamosa* seed extract x 200; **E.** Enlarged view of the follicle showing disruption of the follicular epithelium in treatment with *T. neriifolia* leaf extract x 200; **F.** L.S. of follicules in treatment with *T. neriifolia* seed extract x 200.

Abbreviations : G : Germarium; O : Oocyte; YM : Yolk mass; YG : Yolk granules; N : Nurse cell; FE : Follicular epithelium; V : Vitellarium.

irregular mass. The yolk granules receded away from the follicular epithelium. Another noteworthy feature was the extensive vacuolation in the ooplasm, loss of compactness of yolk granules and the disintegration of yolk granules. In *A. squamosa* seed extract treatment, the germarium appeared to be poorly differentiated. (Fig. D). The follicular epithelium became thin and nuclei were not very distinct. The chromatin materials of the nurse cells showed condensation and heterochromatization. The nurse cells lost their compactness and were found to recede away from the follicular epithelium.

***Thevetia neriifolia* leaf and seed extracts**

In *T. neriifolia* leaf extract treatment the cells of the follicular epithelium were pycnotic, the nurse cells showed extensive vacuolizaation (Fig. E). and yolk granules showed degeneration. In seed treatment, the follicular epithelial layer showed disruption or breakage and the cells showed distinct vacuolization throughout the layer (Fig. F).The nurse cells became clumped and showed heavy heterochromatization, yolk granules showed extensive degeneration and disintegration and spread into a thin layer of yolk mass.

DISCUSSION

Changes affecting the reproductive system have direct implications on the reproductive potential. Pest control strategy explores the possibility of managing the pest either through toxic effects leading to mortality or through suppression of the reproductive potential. In the present study, treatment of larvae with leaf and seed extracts of *A. squamosa* and *T. neriifolia* brought forth considerable changes in the ovary of adult mosquito, such as breakage of follicular epithelial layer, pycnosis of the nucleus of the epithelial cells, vacuolization in the follicle and nurse cells, condensation of chromatin material in the nurse cells and loss of compactness of yolk granules.

According to Bell and Bohm (1975), oosorption is a means by which insects, when conditions are unfavourable for oviposition resorb their developing eggs and recirculate the nutrients. Thus oosorption is a specific type of reproductive strategy in insects by which the oocyte degenerate instead of being laid as eggs in response to behavioral, ecological and physiological factors. Histological changes observed in the ovary in the present study may be attributed to the process of oosorption of ovarian tissues in response to the physiological stress induced by the leaf and seed extracts. Shyamala *et al.* (2003) observed a decline in follicular length in adult mosquitoes emerging out from larvae that were treated with neem oil and neem seed kernel aqueous extract from first instar onwards. In the present observation also the ovaries appeared to be shrunken in treatment with leaf and seed extracts.

The effect of alkylating agents causing degenerative changes in ovarian follicles in mosquitoes has been reported by several workers (Grover *et al.*, 1972; Mathew and Rai, 1975). Rai (1964 a,b) described the degenerative changes such as condensation, pycnosis of the nuclei, vacuolization of the cytoplasm and degeneration of follicular epithelium in the developing follicles of *A. aegypti* in treatment with alkylating agents. Grover *et al.* (1972) reported reduction in the gross size of the ovary and cytogenetic damage in the nurse cells and germaria after treatment with alkylating agents in *C. p. fatigans.* Apholate treatment studies of Mathew & Rai, (1975) in *A. aegypti* showed suppression and inhibition of follicular differentiation and striking changes in the ultra structure of ovarian

tissue such as condensation of chromatin in the nuclei, nucleolar fragmentation and abnormal appearance of nuclear envelope accompanied by complete degeneration of organelles. In the present investigation, similar histological changes have been observed in treatment with *A. squamosa* and *T. neriifolia* leaf and seed extracts.

Degeneration of follicles in the *A. aegypti* and *A. gambiae* in treatment with Thiotepa had been reported by Betram (1963). Murray & Bickley (1964) observed severe abnormalities in the follicles of *C. p. quinquefasciatus* in apholate treatment. Kawatra (1981) analysed the histopathological effects of tepa on oocyte development of *C. p. fatigans* and found various degrees of degeneration of the follicles such as vacuolation of the cytoplasm of follicular epithelium, deformed nuclei and clumping of chromatin in the nuclei of nurse cells. In the present investigation, although plant extracts have been used several similar histological changes such as breakdown of follicular epithelium, loss of compactness in the yolk, disintegration of yolk granules and degeneration of nurse cells had been observed in treatment with leaf and seed extracts. In the present study, the direct effect of leaf and seed extracts could be evaluated on the basis of larval and pupal mortality and percentage of adult emergence. The delayed insecticidal effect which operates indirectly by inhibiting reproduction could be assessed by observing the histological changes in the ovary of the newly emerged adult mosquitoes which may inhibit the production of viable eggs.

Conclusion : From the study, it is concluded that, the leaf and seed extracts of *A. squamosa and T.neriifolia* exhibited strong toxic effects on larvae of *C. quinquefasciatus*.Hence, the plant could be suggested as one of the agents in the integrated management of mosquito population.

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