International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 29-05-2021, Accepted: 13-06-2021, Published: 28-06-2021
Volume 6, Issue 3, 2021, Page No. 192-196

Hexamethylphosphoramide (HEMPA) induced histopathological effects on developing ovarian follicles of the fruit piercing moth, *Eudocima materna* (Linnaeus) (Lepidoptera: Noctuidae)

AS Mohite, AV Dorlikar*, JS Dahegaonkar
PG. Department of Zoology, Sevadal Mahila Mahavidyalaya, Nagpur, Maharashtra, India

Abstract
The effect of chemosterilant Hexamethylphosphoramide (HEMPA) on developing oocyte follicles in the fruit piercing moth *Eudocima materna* were studied. sub-lethal (0.5%) dose of HEMPA was topically applied to the adult female moths to observe the histological changes in the developing oocyte follicles. Effect of chemosterilant resulted in many form of abnormalities as atrophied oocytes and reduced process of vitellogenesis. The amount of yolk granules in the ooplasm is less and numerous vacuoles are seen in the ooplasm. The nurse cells are with disintegrated chromatin material. Disintegration of follicular epithelium and pycnotic nuclei in the follicular epithelial cells have also been evident.

Keywords: *HEMPA, Eudocima, Lepidoptera, fruit piercing moth, vitellogenesis*

Introduction
Among fruit pests, the fruit sucking moths causes extensive damage to various fruits. It is a major pest of citrus fruits, particularly oranges [1, 2]. In addition to oranges this pest is recorded to have been attacking mangoes, bananas, grapes and cashew nuts, guavas and custard apples, pomegranates [3]. Among these, orange is the most preferred host of the fruit sucking moths and consequently, its yield is much retarded. The bionomics of the various fruit sucking moths have been reported by some workers [4]. The concept of insect control through autocidal method is credited to E. F. Knipling [5] who reported the first laboratory study of the method. He has found the most striking application of the concept of decreasing populations of rapidly reproducing organisms by reducing their birth rate rather than by increasing their death rates in the fields. The sterility in insects caused by chemosterilants was the expression of certain morphological, histological and physiological changes which occurred in the gonads [6, 7, 8, 9]. Disintegration of trophocytes, follicular epithelium, break down of the nuclear membrane, clumping of chromatin material of cytoplasm and syncytial appearance of cell wall were reported by treatment with apholate and metepa on the ovarian development of housefly [10]. Inhibition of ovarian growth was noted in *Culex pipiens fatigans* by Grover et. al. [11] with metepa and hempa. Cantwell and Henneberry [12] and Morgan and LaBrecque [13] attributed the decreased ovarian size with tepa, thiotepa and apholate. Studies on the histological changes in the ovaries of *Dysdercus cingulatus* after treatment with metepa revealed that ovariole size as well as oocyte number were induced. Intense disintegration of trophocytes started from the posterior zone of the germarium and progressed forward in two days after injection [6]. This damage was similar to the complete degeneration of egg chamber after treatment with hempa in the housefly [14]. Histological examinations of reproductive tissues have revealed degeneration of oocytes and general necrosis following application of these chemicals. Masner [15] on *Pyrrhocoris apterus*, Sukumar and Naidu [16] on *Dysdercus cingulatus* and Mohapatra [17] on caster silk moth observed severe pathological effects on ovary on the basis of morphological studies after treatment with 6-azauridine, tepa and hempa, respectively. Thus present investigation is aimed to observe the histological changes in the ovaries of adult *Eudocima materna* after treatment with chemosterilant HEMPA.

Materials and Methods
Collection and rearing of *Eudocima materna*:
Adult moths of *Eudocima materna* were collected at night from the citrus orchards of different places of Vidarbha region of Maharashtra with the help of hand nets. The collected insects were brought to the laboratory. The laboratory culture was maintained at natural environmental temperature and relative humidity. Adults were provided with a ripen oranges (when available) or 10% sucrose and honey mixture (3:1 proportion). 0.5% (500mg/100ml acetone) solution of chemosterilant hempa (hexamethylphosphoramide) was topically applied on the lateral (pleurite) sides of the abdomen of newly emerged male adults.

Histological techniques
The male reproductive organs were dissected out from the adult under stereoscopie binocular microscope in Ringer's saline solution. Tissues were fixed in Bouin's fixative for about 18-24 hours for histological studies. Fixed tissues were dehydrated in 70%, 90% and absolute alcohol and cleared in xylene. After clearing, the tissues were embedded in paraffin wax to prepare blocks in 'L'moulds. Sections were cut at 5 µ thickness with the help of rotary microtrome and mounted on the albumenized slides. The Ehrlich's Haematoxylin double staining technique [18] was used for histological studies. Histological sections were examined and photographed by Labomed Digi-3 compound microscope.

Observations and Results

Histological structure of developing oocyte follicles in control moths

During vitellogenesis the developing oocytes show marked changes in their shape, size and cytological organization. For descriptive purpose the development of the oocyte can be divided into five stages – the pre-vitellogenic, the early vitellogenic, the vitellogenic, the late vitellogenic and maturation stage. At the pre-vitellogenic stage the oocytes are not clearly differentiated. Each cyst possesses a group of seven nurse cells and a single oocyte but maximum portion of the follicle is occupied by the nurse cells. The pre-follicular nuclei are present around the cyst.

In the early vitellogenic oocytes, the ooplasmic volume of the follicle is equal or slightly more than the nurse cells. The nurse cells are large with well differentiated ring canals and chromatin material is granular and dispersed. The oocyte nucleus and nucleolus are clearly differentiated. The follicular epithelial cells are double layered and columnar in shape (Figs.1 & 2).

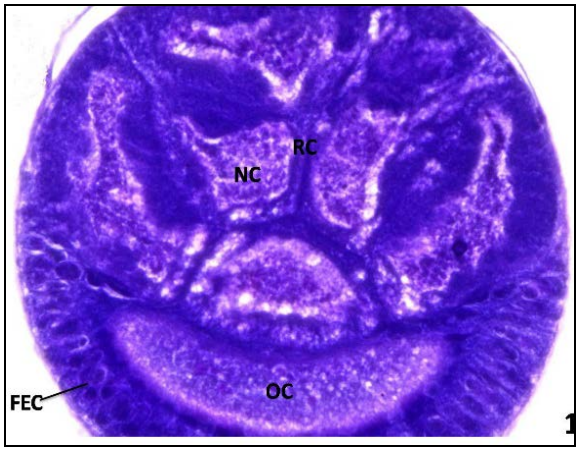

Fig. 1: Section passing through the oocyte follicles of control female *Eudocima materna* of the early vitellogenic stage oocyte follicle (HE X400).

In the vitellogenic stage of oocytes, the ooplasmic volume of the oocytes increases gradually and nurse cells begin to decrease in size. The nurse cells become more active in this stage and the synthesis of nutrient material in the form of yolk spheres takes place. The nutrient material flows into the oocyte through intercellular bridges. Their follicular epithelial cells are single layered and columnar. The interfollicular spaces stain dark with iron haematoxylin (Fig.2 & 3).

At the late vitellogenic stage the vitelline and chorion membrane formation begins. The nurse cells decreases still further and indicate higher concentrated chromatin material. The cytoplasmic transportation from the nurse cells to the oocyte terminates at this stage because of the collapse of the nurse cells. The follicular epithelium is columnar or cuboidal. The yolk material is stored in the form of large yolk granules (Figs. 4 & 5). In the maturation stage the nurse cells are completely degenerated. The vitelline membrane and the chorion are fully differentiated (Fig. 6).

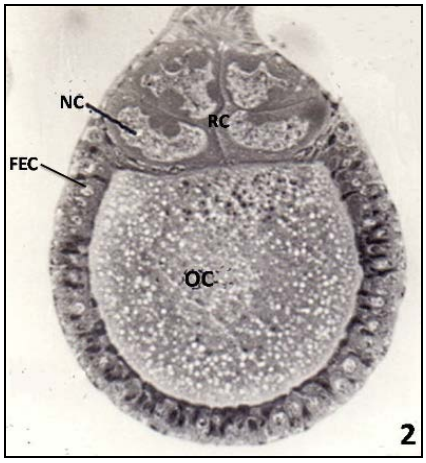

Fig 2: Section passing through the oocyte follicles of control female *Eudocima materna* of the mid vitellogenic stage oocyte follicle (IH X400).

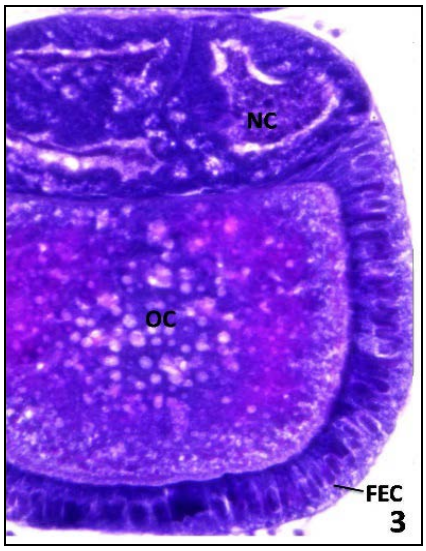

Fig 3: Section passing through the oocyte follicles of control female *Eudocima materna* of the vitellogenic stage oocyte follicle (HE X400).

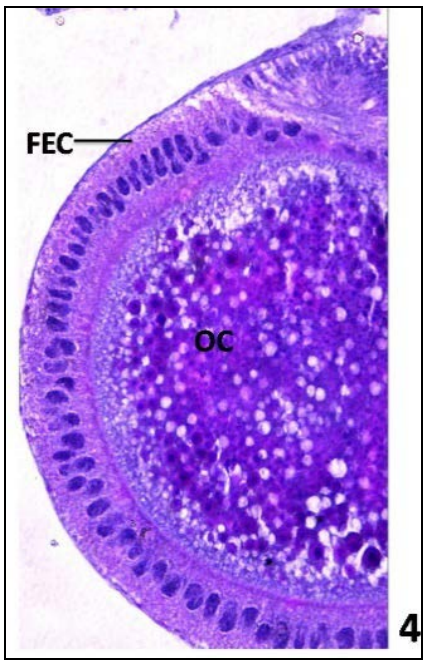

Fig 4: Section passing through the oocyte follicles of control female *Eudocima materna* of the late vitellogenic stage oocyte follicle (IH X400).

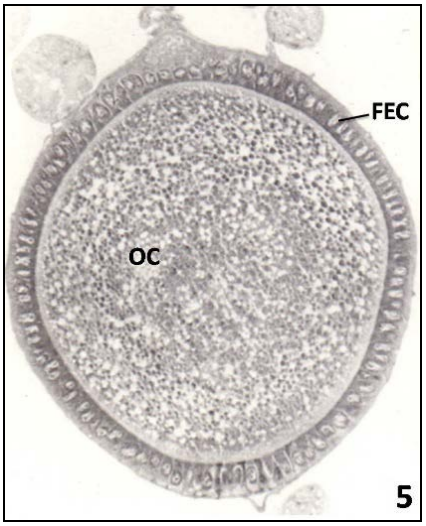

Fig. 5: Section passing through the oocyte follicles of control female *Eudocima materna* of the late vitellogenic stage oocyte follicle (IH X400).

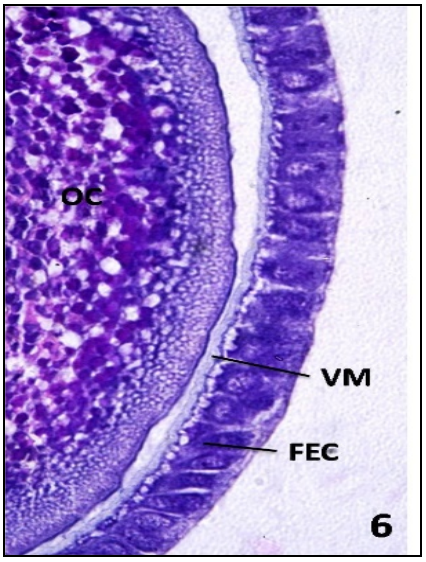

Fig 6: Section passing through the oocyte follicles of control female *Eudocima materna* of the late vitellogenic stage oocyte follicle showing columnar follicular epithelial cells and formation of vitelline membrane (HE X400).

FEC- Follicular Epithelial cells, NC- Nurse cells, OC- Oocyte, RC- Ring canal, VM- Vitelline membrane

Histopathological effects of HEMPA on developing oocytes of chemosterilant HEMPA treated adults

The topical application of HEMPA has induced a noticeable changes in the size of ovarioles and developing oocytes. The germarium is filled with disintegrated tissue in the form of irregular clumps. Histopathological effects on HEMPA indicated atrophied oocytes. Disintegration of follicular epithelium of the early and mid vitellogenic oocytes and they are with condensed nuclei (Figs.7 & 8). The process of vitellogenesis is highly reduced in the oocyte follicles. The amount of yolk granules in the ooplasm is less (Fig. 9). Numerous vacuoles are seen in the ooplasm. The nurse cells have become almost functionless with the number of vacuoles in the cytoplasm. Disintegrated chromatin material have been evident in the nurse cells of HEMPA treated oocytes. Vitellogenic stage oocyte follicle showed malformation and epithelial cells around the oocytes, pycnotic nuclei.

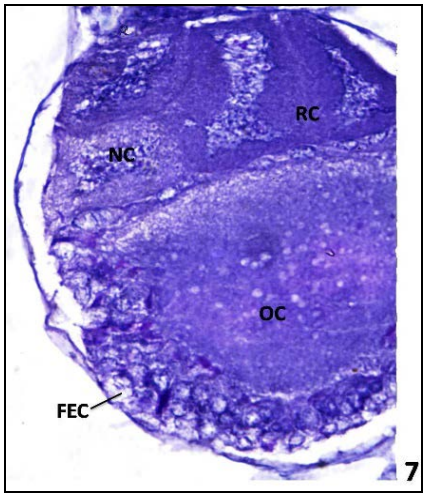

Fig 7: Section passing through the oocytes of chemosterilant HEMPA treated female *Eudocima materna* showing the disintegration of follicular epithelial cells and disturbed nurse cells early-vitellogenic stage oocytes follicle (HE X400).

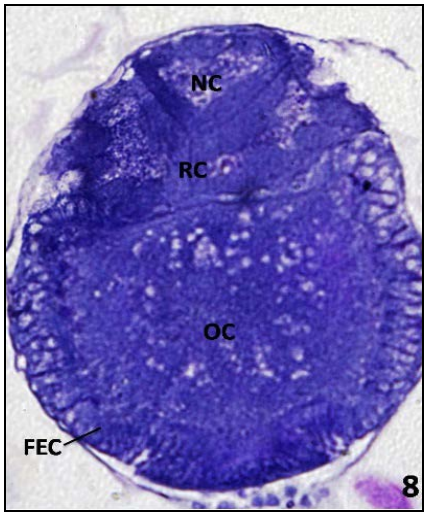

Fig 8: Section passing through the oocytes of chemosterilant HEMPA treated female *Eudocima materna* showing the malformation and vacuoles m the follicular epithelial cells of mid-vitellogenic stage oocyte follicle (HE X400).

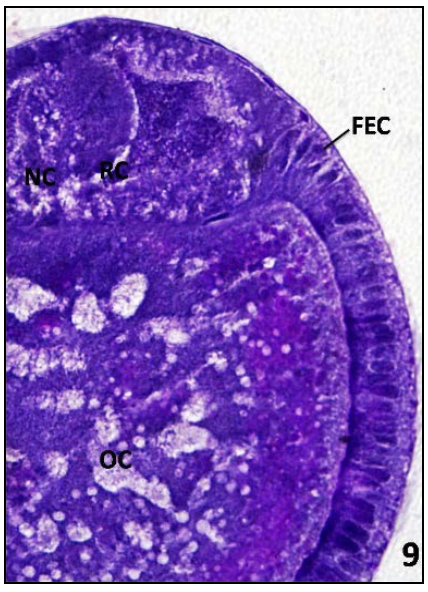

Fig 9: Section passing through the oocytes of chemosterilant HEMPA treated female *Eudocima materna* showing atrophied oocytes and disintegrated chromatin material of nurse cells of vitellogenic stage oocyte follicle (HE X400).

In their cells (Fig.9). Detachment of epithelial cells from the ooplasm and poor yolk material in the oocyte follicles. Late vitellogenic oocytes follicles are changed into the resorptive bodies (Fig.10).

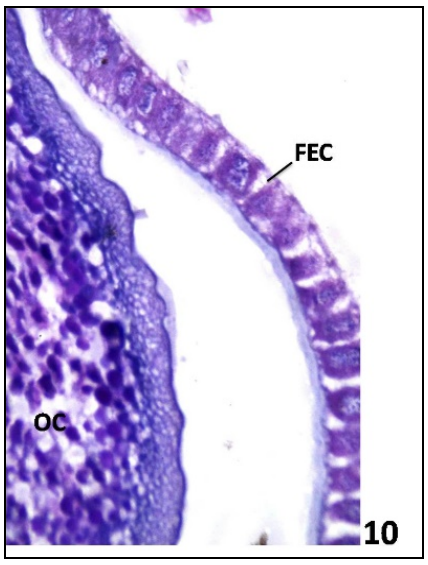

Fig. 10: Section passing through the oocytes of chemosterilant HEMPA treated female *Eudocima materna* showing atrophied oocytes, detachment and disintegration of follicular epithelium of late-vitellogenic oocyte follicle (HE X400).

Discussion:

The process of oogenesis is often evident in the terminal oocyte of the ovariole of the insect ovary. During vitellogenesis the developing oocytes show marked changes in their shape, size, and cytological organization. Vitellogenesis takes place through five consecutive vitellogenic stages; pre-vitellogenic, early vitellogenic, late vitellogenic and maturation. The terminal oocytes follicle of noctuid moth consists of a group of seven nurse cells dorsally and an oocytes ventrally [19].

During early vitelloigenic stage the follicular epithelium of *Eudocima materna* showed mitotic activity, but in later stages this was not evident, the follicular epithelial cells are columnar up to first five days. In sixth day the old adult terminal oocytes showed columnar epithelial cells. Similar observations were also described in *D. melanogaster* [20], *Musca domestica* [21], *L. exiqua* [22]. The growth of oocytes is due to transfer of the cytoplasmic contents from the nurse cells via ring canals to the oocytes. Thus, the nurse cells strongle support oocytes development by contributing cytoplasmic [20, 23, 24, 25].

Histopathological examination of developing oocytes have revealed degeneration of oocytes and general necrosis following application of chemosterilant [7, 8, 17]. The present investigation on *Eudocima materna* shows similar results after treatment with HEMPA. The amount of yolk granules in the ooplasm is less. The nurse cells have become functionless as indicated by disintegrated chromatin and large vacuoles in the cytoplasm pycnosis and fragmentation of the chromatin of follicular epithelial cells, as also reported by Morgan and LaBrecque [13] and Nath *et al*. [26]. Disintegration of nurse cells consequently failed to synthesize the yolk material resulting in the formation of ill-developed eggs, similar observation has reported by Jalaja and Prabhu [6] in *Dysdercus cingulates*. Morgan and LaBrecque [10] Saxena and Bhatnagar [27] and Mohapatra [9] observed that the effective doses of chemosterilants causes severe pathological abnormalities. Landa and Matolin [28] observed that poor deposition of yolk in the oocytes of chemosterilant treated insects is due to failure of follicular epithelial cells to differentiate properly. The sensitivity of follicular epithelial cells to chemosterilant HEMPA observed during the present study is specifically significant in the light of the findings that follicle cells play an important role in incorporating yolk into the oocytes as observed by Anderson and Telfer [29].

Acknoledgements

Authors are thankful to the University Grants Commission for financial support under Minor Research Project (No. F. 47-954/14/ General/48/WRO/XII plan).

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