FINAL REPORT OF THE WORK DONE

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Title of the Research Project:

"Studies on the larval morphology, cephalic and segmental chaetotaxy, gamma irradiation and chemosterilant-induced histopathological effects on gonads and inherited sterility in fruit piercing moth, *Eudocima materna* (Linnaeus) (Lepidoptera: Noctuidae)"

Introduction:

The fruit piercing moths of Eudocima sp. are a unique noctuid. Adult moths of either sex are well adopted for extraordinary habit of piercing the ripening fruits, causing extensive damage to various fruits. It is a major pest of citrus fruits, particularly oranges (Atwal, 1976). In addition to oranges this pest is recorded to have been attacking mangoes, bananas, grapes and cashew nuts, guavas and custard apples, pomegranates (Yadava, 1969). 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 (Srivastava and Bogawat, 1968). It has created alarming situation in Vidarbha region of Maharashtra due to extensive damage to citrus fruits.

The concept of insect control through autocidal method is credited to E. F. Knipling in (1951), 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 practicability of the autocidal control technique was demonstrated in a series of brilliant experiments with the screw-worm fly (*Callitroga hominivorax*) sterilized by radiation. The species was first eradicated from Florida and the South-eastern United States. (Knipling, 1960).

The sterile-male technique, in which artificially reared male insects would be sterilize by radiation or chemicals and released into infested areas; the direct sterilization technique, in which chemosterilants would be applied like insecticides to sterilize rather than to kill pest insects (Borkovec, 1976). Chemosterilants, i.e., chemical compound that reduce or eliminate the reproductive capacity of the organism to which they are applied. A good number of workers has studied the effect of nonalkylating chemosterilant HEMPA upon the longevity of adults, oviposition and viability of various insect species (Reddy and Sharma, 1987 and Prakash et.al., 1987).

The sterile insect technique is based on a simple but eminently logical assumption that fertile insects confronted with large numbers of predominantly sterile mating partners will not reproduce. The crucial step in the sterile insect technique is the production of large numbers of

sexually sterile males and females insects that would be, from the stand point of the naturally occurring fertile males and females, indistinguishable from the natural insects. Three factors concerning the released insects are important in this regard: changes in normal biological characteristics resulting from mass rearing, physiological and behavioral consequences of the sterilization treatment, and the effects of the release (Knipling, 1968). Chemosterility is of such a technique, which is eco-friendly and effective in controlling pest by depriving them of their ability to reproduce (Mohapatra, 2007). The sterility in insects caused by chemosterilants was the expression of certain morphological, histological and physiological changes which occurred in the gonads (Jalaja and Prabhu, 1976; Taneja et. al., 1979; Mahmood et. al, 1991; Mohapatra, 2007).

The process of the spermatogenesis in some lepidopteran species has been studied by some workers as, *Eurygaster integriceps* (Danilova *et al*., 1984); *Heliothis virescens* (LaChance and Olstad, 1988). However, Toshiaki *et al.* (1988) studied the hormonal regulation of spermatogenesis in the cabbage moth *Mamestra brassicae* and common armyworm, *Leucania separata*, respectively. Loeb *et al*. (1985) have shown the rate of development of spermatogenesis in the larva of the tobacco budworm moth *Heliothis virescens* appears to be modulated by central nervous system i.e. stimulatory factor from the brain and inhibitory factors from the suboesophageal ganglion. Sonoli and Hooli (1992) reported the histological and histochemical studies of the apical cells of *Heliothis armigera.*

Lepidopteran males produced apyrene and eupyrene sperms, both of which were transferred to the females during, mating. The eupyrene sperm was necessary for egg fertilization and thus, for fertility. The apyrene sperm were anucleated and do not fertilize eggs but accompany the eupyrene sperm into the spermatophore and into the spermathecae of the female (LaChance and Olstad, 1988). It is evident from the classical and recent experiments that chemosterilants affect testes and spermatogenesis. Because, the practical application of strong mutagens, as in classical experiments with insects, is out of the question; thus, the possibilities of using chemicals to produce azospermia or inactivation of sperm for male sterilization have been considered lately. The mechanism of these chemicals originate from the papers published so far that spermatogonia and growing spermatocytes undergoing the division are mostly affected (Sengupta, 2013). With the degeneration of spermatogenic cells, the entire testes become smaller (Chandra et. al., 2013).

The use of irradiations in sterility technique has been successfully operated on the insect pests. It is generally accepted that, the fecundity inhibited to great extent by gamma irradiation and neutron flux irradiation (Seth and Sharma, 2001; Ocampo and DeLeon, 2002; Dhouibi and

Abderahmane, 2002). The post irradiation alterations in the male gonads, cytopathological and histopathological effects of radiations in the process of spermatogenesis was reported by various workers (Rimas *et al.,* 2000; Hazaa, 2002). Similarly, histopathological effects of radiation on developing oocytes have been studied (Younes *et al.,* 2009).

Histological examinations of female gonads have revealed degeneration of oocytes and general necrosis following application of these chemosterilants. Mohapatra (2003) 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. Disintegration of chromatin material of the trophocytes, prefollicular tissues and germ cells increased steadily, forming clumps. Mohapatra (2007) has reported the similar effect in *Philosamia ricini* by administering thiotepa.

Classification:

The fruit piercing moth, *Eudocima materna* occupies the following systematic position.

Class - Insecta

Subclass - Pterygota

Division - Endopterygota

Order - Lepidoptera

Suborder - Ditrysia

Superfamily - Noctuidaea

Family - Noctuidae

Genus - ***Eudocima***

Species - ***materna*** **(Linnaeus)**

Field Work: *Eudocima materna* adults were collected from the orange growing areas of Katol and Warud tahasils of Vidarbha region of Maharashtra during the months of August to November,2018 (a season of Ambia bahar). In order to collect the adult males and females of *Eudocima materna*, about 200-300 kms distance was travelled at each field work. Fruit piercing moth, *Eudocima materna* were collected 3 hours after sunset till the midnight with the help of hand nets in the citrus orchards of above said places. Since, these moths are nocturnal in habit and highflyer, it is very difficult to collect them. We could only collect four male and five female moths after successive attempts.

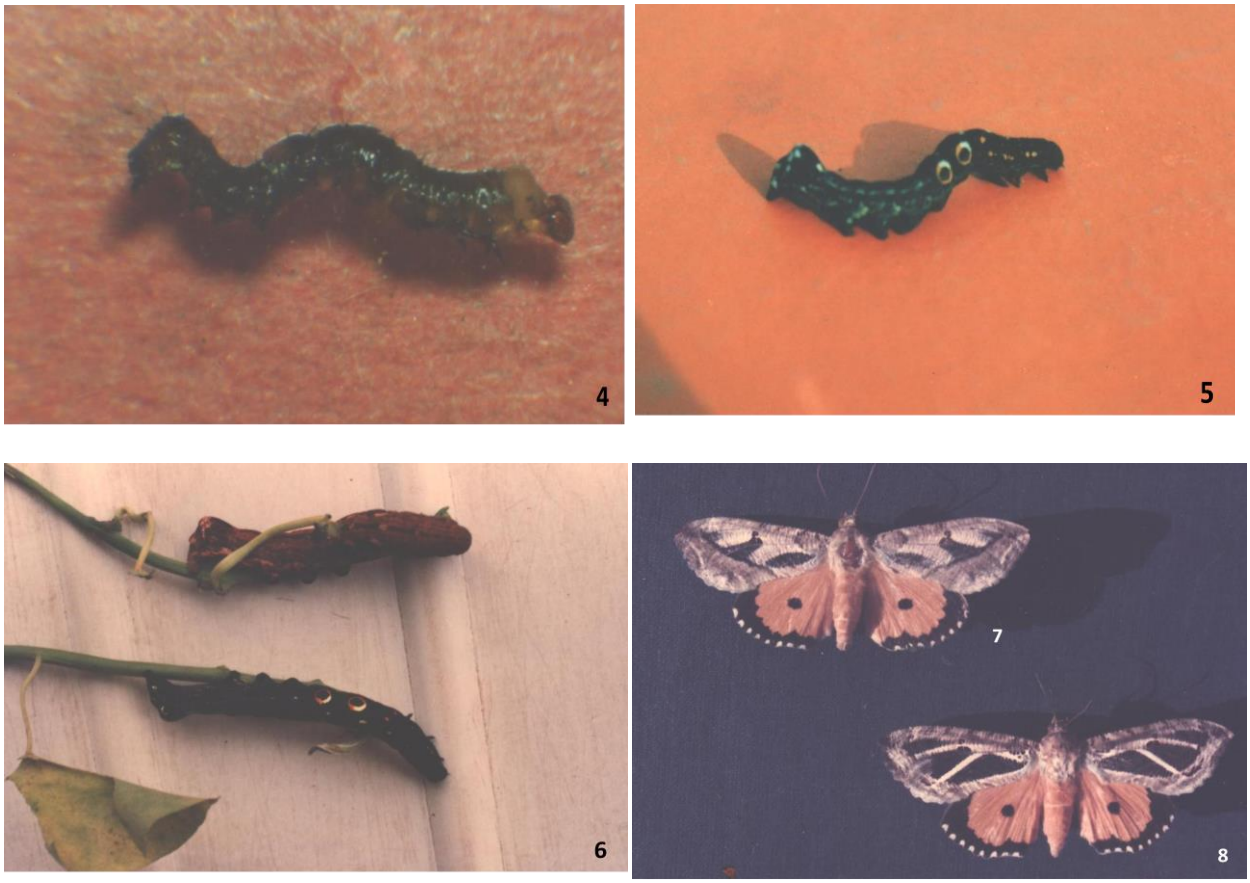
Establishing a Laboratory Culture: After collecting the adult moths from citrus orchards, they were brought to the laboratory and kept in specially prepared cages provided with fine wire grills from all the sides and opening at the bottom for clearing, while other outlet was kept on the side for transfer of moths. The top of the cages was provided with movable glass (Fig.1). As the adults are nocturnal in habit, the cages were closed from all sides by a black cloth. The adult moths were fed with ripen oranges (when available) or 10% sucrose and honey mixture (3:1 proportion). We have successfully reared two generations and life history has also been studied. For rearing of the larvae, plastic trays/tubs were used. Eggs were transferred from black cloth to the trays.

- The freshly laid eggs are transparent and creamy white but after 12 hrs they become yellowish and before hatching turn light brown. They are semicircular in shape. The I-instar larva hatches out after a period of 3-4 days.
- The freshly hatched I-instar larva is light brown in colour while it become transparent on the second day. In two days old larva, green coloured alimentary canal can easily be seen from outside (Fig.4).
- The larvae were fed on fresh leaves of gulwel plants, *Tinospora cordifolia* (Figs.2 & 3). In order to check the mortality, I to III- instar larvae were kept in vertical plastic jars closed with black coloured cloth from the top. The IV and V- instar larvae were fed twice a day. As soon as the last instar larvae underwent the pupation (Fig.2) were transferred to the cages for emergence.
- The IV and V- instar larvae are valvety black, the prothorax characteristically bears two spots on anterodorsal orange coloured and other posterodorsal blue. Meso and metathorax possesses 2-3 orange spots laterally.
- abdominal segments are characterized by possessing prominent large eye spots (Figs.5 & 6).
- The forewings of *Eudocima materna* moth exhibit sexual dimorphism. In case of females, large part of each forewing is covered with three brown coloured patches differentiated fully from each other and also distinct from the periphery due to the presence of intermediary white strips. While in the male there are three vertical or oblique brown coloured spots merged into the white area (Figs.7 & 8).

Fig. 1: Photograph showing various types of cages, plastic trays and jars containing developmental stages of fruit piercing moth, *Eudocima materna* stalked on a steel rack in the rearing room.

Fig. 2: Larvae feeding on gulwel (*Tinospora cordifolia*) leaves, kept in plastic trays.

Fig. 3: Pupae of *Eudocima materna*



Figs. 4-8: Developmental stages of *Eudocima materna*.

Fig. 4: I-instar larva.

Fig. 5: IV- instar larva.

Fig. 6: V- instar larva

Fig. 7: Adult male moth

Fig. 8: Adult female moth

Effect of chemosterilat HEMPA: Twenty newly emerged adult males and females were selected for this experiment. 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.

Effect of Gamma-ray Irradiation (Co^{60}): The source (Co^{60}) used for gamma ray irradiation was made available from the Chemistry Department of Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. 50 pupae of approximately 4-5 day old were kept in glass petridish for gamma ray irradiation. A dose of 100 Gy of gamma radiation delivering at the dose rate of 13.5 Gy/min. was applied. Number of emergence (mortality rate) was observed after emergence of adult moths.

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.

Bouin's fluid aqueous - 18-24 hrs.

Picric acid	75 ml
Formaldehyde	20 ml
Glacial acetic acid	5 ml

Fixed tissues were dehydrated in 70%, 90% and absolute alcohol for about 10 min, each. After clearing the material in xylene. (for about 10-15 min.), it was kept in the mixture of xylene and melted paraffin wax (1:1) for 15-20 minutes. There after two changes of about 30 minutes were given to the tissue in paraffin wax at $60^{O}C$ in the oven and later the material was embedded in wax. Sections were cut at 4-6 μ thickness and mounted on the albumenized slides.

The sections were stained with the following histological staining techniques

1. **Haematoxylin – eosin , staning technique stock solutions-**

i. **Delafield haematoxylin solution:-** 4g of haematoxylin was dissolved in 25ml of absolute ethyl alcohol. It was gradually mixed into 400ml of ammonia alum (NH4(SO4)2.H2O) saturated aqueous (Approx. 1 part alum to 11 parts distilled water). It was left exposed to light in a flask with a cotton plug for 3-5 days. Then it was filtered. To the filterate 100ml glycerine and 100ml methyl alcohol was added and allowed to ripen for at least six weeks.

ii. **Alcoholic Eosin**

Eosin	1g
70% alcohol	100 ml
Glacial acetic acid	5 ml

Staining procedure:-

1. The paraffin sections were deparaffinised in xylene and hydrate down to water gradually through various grades of alcohol, absolute alcohol, 90%, 70%, 50%, 30% and distilled water for 4-5 min in each grade.
2. Stained in haematoxylin progressively about 4-5 min.
3. Washed in running water 15-20 min and kept for half an hour.
4. Dehydrated the sections through grades of 30%. 50% alcohols.
5. Counterstained by eosin for 2 min.
6. Dehydrated the slides further in 90%, alcohol and absolute alcohol.
7. Cleared by passing the slides' through the xylene and mounted in DPX.

2. Heidenhain Iron Hematoxylin:

***Solution* A**

Ferric alum 4.0g

Distilled Water 100 ml

Keep in refrigerator to prevent precipitation on sides of bottle.

***Solution* B**

Hematoxylin 10.0g

95% ethyl alcohol 100 ml

Keep it for 4-5 months in dark bottle till deep wine red colour develops. At the time of staining add 4-5 ml of stock solution in 100 ml distilled water. Staining time is 12 hrs. Before staining slides are kept in mordant solution A for 12hrs. Solution A and B are not usually mixed.

Staining procedure:

1. The paraffin sections were deparaffinised in xylene and hydrate down to water gradually through various grades of alcohol, absolute alcohol, 90%, 70%, 50%, 30% and distilled water for 4-5 min in each grade.
2. Transferred the slides to ferric alum (Solution A) for 12 hours.
3. Washed in running water for 2 hours and stained in iron hematoxylin (solution B) for overnight.
4. Washed in running water, differentiated in picric acid solution.
5. Dehydrated the sections through grades of 30%, 50%, 70%, 90% and absolute alcohols.
6. Cleared by passing the slides through the xylene and mounted in DPX

Microphotography: Histological sections were examined under Labomed Research Microscope and microphotography has been done on Labomed Digi-3 compound microscope.

Procedure for Study of Chaetotaxy:

The caterpillars of first, second and sixth instar in laboratory culture were killed in KAAD solution (Peterson, 1962) and stored in 70% alcohol. Heads of freshly killed larvae were made transparent by boiling them in 5% KOH for 2 minutes. The material was washed with 1% glacial acetic acid so as to remove the traces of KOH and then washed in distilled water, dehydrated in ascending grades of ethanol. The larval head then placed in the cavity slide containing glycerine. All observations were done under the stereoscopic binocular microscope. The figures were drawn with the help of camera lucida. Nomenclature proposed by Hinton (1946) has been followed for naming different setae.

KAAD (Kerosene-Acetic Acid-Dioxane) solution:

Ethyl alcohol	–	10 ml.
Glacial acetic acid	–	2 ml.
Kerosene	–	1 ml.
Dioxane	–	1 ml

Observations:

Structural Organization of the male reproductive system of *Eudocima materna*.

The internal male reproductive system of *Eudocima materna* consists of a single testis, a pair of seminal vesicle, vasa deferentia, a pair of accessory glands and an ejaculatory duct (Fig.9).

The testis: The testis is a single creamy white and avoid structure, lying in the fifth abdominal segment. It is externally covered with fat bodies, richly supplied with trachea and measures about 2.15 to 2.50 mm in diameter. It is externally covered with a thin peritoneum. The peritoneum is composed of closely fused two membranes enclosing irregularly the masses of nuclei. The internal body of testis is composed of finger shaped follicles which are bounded externally by a thin testicular wall (Fig.10). The follicular wall is composed of an inner layer of epithelial cells having prominent nuclei but ill develop cytoplasm and outer layer of muscle fibers. The apical complex is composed of large spherical cells lodged at the apex just above the spermatogonia (Fig.11). The testicular follicles are completely filled with the cyst containing germ cells in successive stage of development; apical zone of spermatogonia transitional zone of primary and secondary spermatocytes and the posterior zone of spermatids and spermatozoa (Figs.12 & 13). The follicles differ from one another in number of cysts as well as stages of gametes they contain. The posterior region of the follicles is mostly filled with matured spermatozoa. In such follicles the heads of spermatozoa are bound together but the tails remain free forming sperm bundles. Two type of sperms bundles are easily differentiate in thin section, fully developed eupyrene sperm bundles which are characterized by large densely stained nuclei and apyrene bundles which are relatively smaller and faintly stained (Figs.14 & 15).

All follicles independently terminate posteriorly into seminal vesicle. The seminal vesicle opens into the ipsillateral vasa deferentia. Each seminal vesicle is sack like and measure from 2-3.5 mm in length.

Histological Change in the Testes of Chemosterilant HEMPA Treated Adults:

The testis showed many forms of abnormalities as separation of follicular tissue from testicular wall and septa leaving wide space. Disturbed cysts are present in the follicles (Fig.16). They become loosely arrange and large gap are evident on the contrary they are compact in the control testis (Fig.17). Dispersed spermatogenic stages are seen in the follicle. The spermatogonia are without cytoplasm, while compressed spherical nucleic bodies are observed, many vacuolated areas are observed and spermatocytes necrosis is noticed (Fig.18). Some spermatocytes are spherical in shape and some are irregular with condensed nuclei. Different forms of retardation were observed as retardation of sperm maturation, inhibition in growth of spermatids and degenerating spermatozoa (Fig.19).

Histological Changes in the Testes of Gamma Irradiated Adults

Many forms of abnormalities in tesis are seen such as separation of follicular tissue from testicular wall and septa leaving wide space (Fig.20). Disturbed cysts are present in the follicles. They become loosely arranged and large gaps are evident (Fig.21). Spermatogenic stages are disturbed in the follicle. Compressed spherical nucleic bodies are observed in the spermatogonia but of without cytoplasm. Spermatocytes necrosis is evident. Some spermatocytes are spherical in shape and some are irregular with condensed nuclei (Fig.22). Different forms of retardation were observed such as, inhibition in growth of spermatids and retardation of sperm maturation (Figs.23).

Morphology of the Female Reproductive System in Adults:

The female reproductive system of adult moth is composed of the ovaries, the lateral oviducts, the common oviducts, the bursa copulatrix, the spermatheca and the accessory glands (Fig.24). The ovaries are paired and occupy most of the abdominal region lying on side of the alimentary canal. Each ovary consists of four elongated, beaded polytrophic ovarioles. The anterior ends of ovariole of each ovary, the terminal filaments are fused together, forming a small suspensory ligament. Posteriorly the ovaries open into the lateral oviduct. The two lateral oviducts fuse to form a median common oviduct which opens into the vagina. The vagina lies at the ventral side of the abdomen between 7^{th} and 8^{th} segment. A large sacular bursa copulatrix, situated ventrally on the eighth sternum open through the ostium bursae or vulva. The ductus bursae communicate with the posterior part of the common oviduct or vagina by a narrow ductus seminalis. The dorsal single small tubular spermatheca with spermathecal gland also opens into the vagina. A pair of long accessory gland is situated posteriorly. Each accessory gland opens into a common accessory gland reservoir which communicates with the posterior part of the vagina through a short common duct.

The Ovariole:

Each ovariole is divisible into four regions.

a) The terminal filament;
b) The germarium
c) The vitellarium, and
d) The pedicel.

a) The terminal filament:

The terminal filament is the anterior most thread like structure containing stem cells having ovoid nuclei, covered with peritoneal sheath. It is separated from the germarium by a transverse septum.

b) The germarium:

The terminal filament is followed by germarium. It contains cystoblasts and cystocytes. The cystoblasts give rise to cystocytes by undergoing restricted number of mitosis. The posterior region of germarium contains cysts each containing cystocytes which are enveloped by the follicular cells. In each cyst, out of 8 cystocytes, 1 become oocyte and remaining 7 serve as nurse cells during further development in the vitellarium.

c) The vitellarium:

The vitellarium is the largest region of ovariole. It contains large number of follicles arranged in a linear fashion undergoing maturation. Each follicle is composed of 7 nurse cells and an oocyte and is enclosed by a single cell thick follicular epithelium. These follicles become progressively larger towards the end of vitellarium. Each follicle is separated from the preceding and succeeding one by a thin layer of interfollicular tissue. Such histological organization of vitellarium represents the polytrophic type of the ovarioles.

d) The pedicel:

The pedicel is the last part of the ovariole. It is a small tubular structure which connects the vitellarium with the lateral oviduct. The pedicel wall is thrown into numerous folds. It is composed of columnar epithelial cells. The entrance of the last egg into the pedicel is blocked by the epithelial cells, the epithelial plug.

Development of Oocytes and Vitellogenesis:

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 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 (Fig.25).

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.26).

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.27 & 28).

In the maturation stage the nurse cells are completely degenerated. The vitelline membrane and the chorion are fully differentiated (Fig.29 & 30).

Histopathological effects of HEMPA on developing oocytes

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 (Fig.31 & 32). The process of vitellogenesis is highly reduced in the oocyte follicles. The amount of yolk granules in the ooplasm is less (Fig.33). 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 in their cells (Fig.33). 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.34).

Effect of Gamma Irradiations on the Adult Emergence and Fecundity

Whole body irradiation at pupal stage with gamma rays shows effects on the pupal period, fecundity and egg hatchability in *Eudocima materna*. The percentage of adult emergence is decreased in gamma irradiated pupae as compared to emergence in normal pupae. Emergence is only 44% in gamma irradiated pupae for 100 Gy as compared to 82% emergence in normal pupae, pupal period is increased by 2 days when irradiated for the dose of 100 Gy as compared to normal pupal period. Sex ratio is not affected. There is no change in sex ratio, mating behaviour and life span as that of controls (Table 1).

Table 1. Effect of gamma irradiation on pupal period and emergence of *Eudocima materna.*

Dose (Gy)	No. of pupae tested	Pupal period (days)	Emergence (%)	No. of Males (%)	No. of Females (%)
0	50	11	41 (82.00)	23 (56.00)	18 (43.00)
100	50	13	22 (44.00)	6 (54.54)	5 (45.45)

Chaetotaxy of the V-instar larva:

The cranial setae (Figs. 35 & 36): The topographic arrangement of the setae on one lateral half of the head is similar to that of other half. There are 4 setae in frons region, 3 on clypeus region, 7 on epistomal plate of each lateral half of the head. There is no setae on the dorsal side of the mandible. Ventral side of the head shows pair of setae below each mandible and single on stipes.

The thoracic setae: (Fig. 37): The dorsal region of the prothorax possesses about 16 setae, while meso and metathorax possesses 18 setae each. These setae are arranged in two rows. There are no setae on the ventral side of the thorax.

The abdominal setae (Figs. 38 &39): The setae occur on both the dorsal and ventral surface of the abdomen.

The dorsal abdominal setae (Fig. 38): The dorsal side of the first abdominal segment possesses about 14 setae, which are arranged at randomly. The second and third abdominal segment possesses 12 setae, which are arranged around the eye spot. 14 setae are present on the fourth, fifth and sixth abdominal segments which are arranged in two rows, 6 in first row and 8 in second. Seventh abdominal segment possesses 12 setae, while eighth and nineth segments bear 14 setae each. There are total 6 setae on last abdominal segment. Each proleg is surrounded by 14 setae.

The ventral abdominal setae (Fig. 39): The ventral side of the first abdominal segment possesses 8 setae, which are arranged in two rows. Similarly, 12 setae are present on the ventral region of second and third abdominal segments. There are no abdominal setae on the ventral region of fourth, fifth and sixth abdominal segments. Ventral region of seventh, eighth and ninth abdominal segments possesses 8 setae, 2 in first row and 6 in second row.

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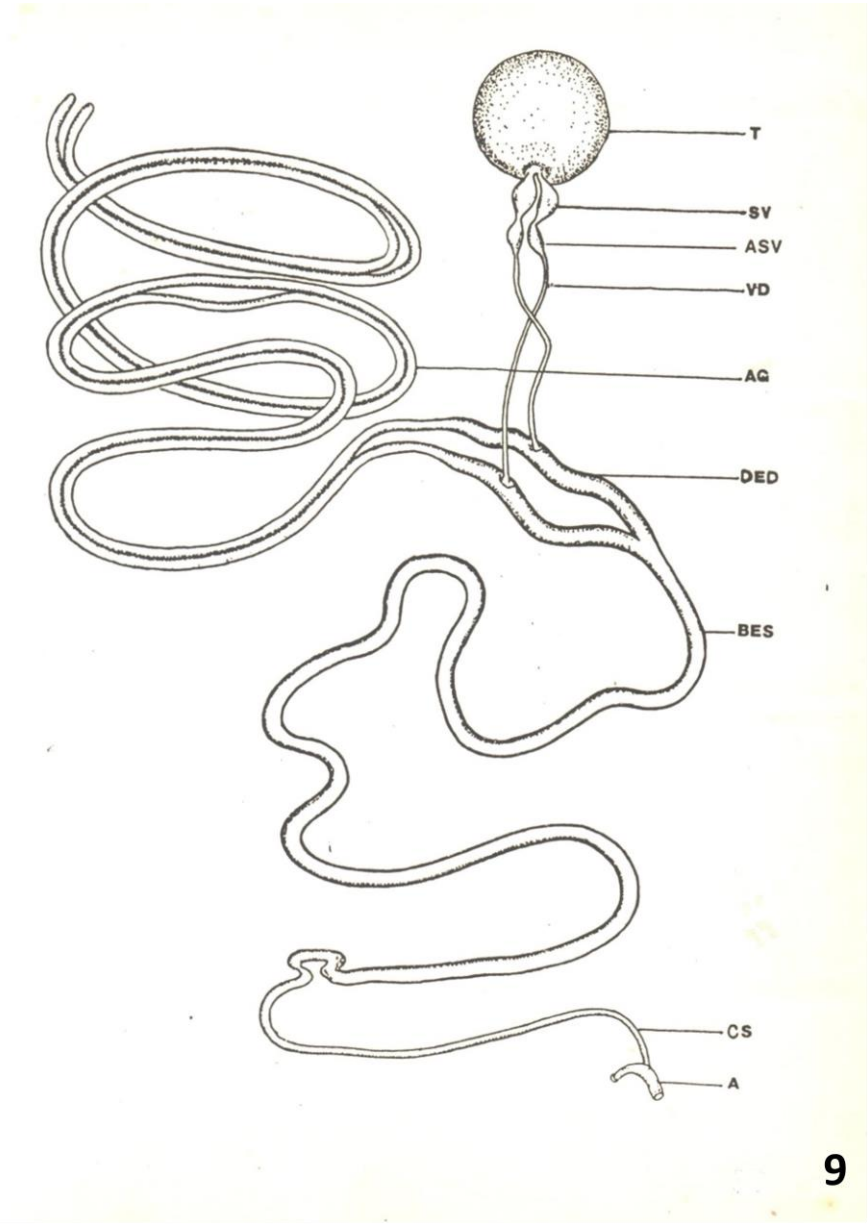
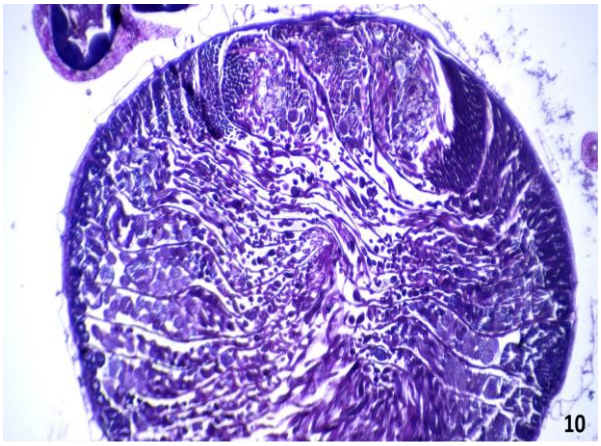


Fig. 9: Anatomical organization of the male reproductive system of *Eudocima materna*.

A	aedeagus
AG	accessory gland
ASV	accessory seminal vesicle
CS	cuticular simplex
DED	ductus ejaculatorious duplex
DES	ductus ejaculatorious simplex
SV	seminal vesicle
T	testis
VD	vasa deferentia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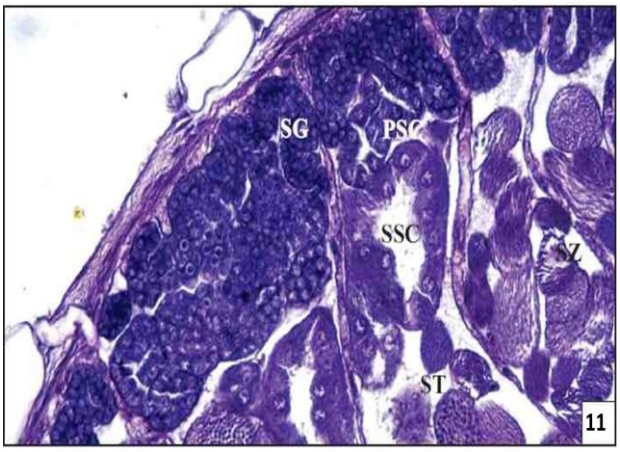

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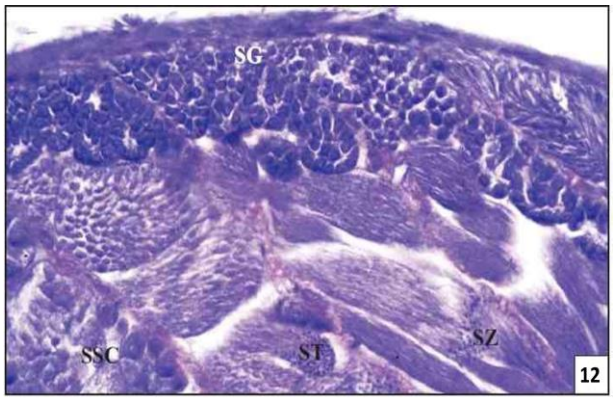

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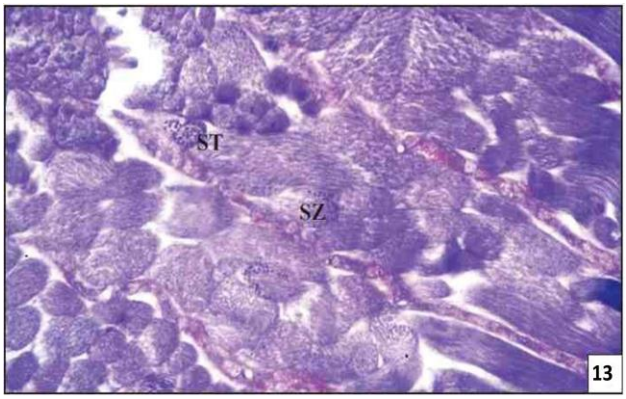

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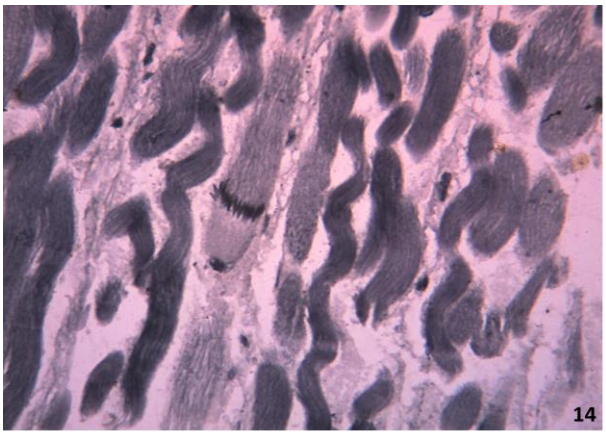
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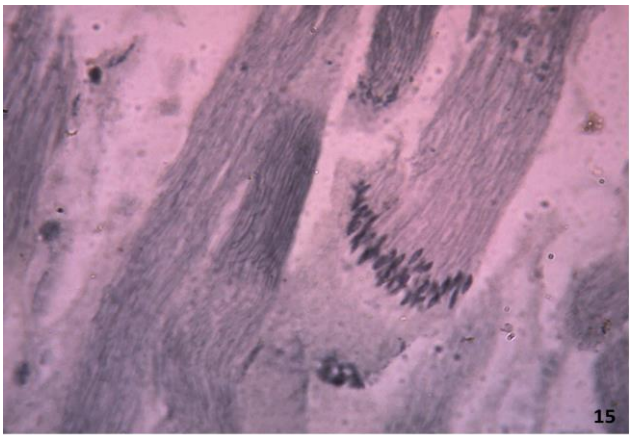
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Figs. 25 – 30: Sections passing through the oocyte follicles of control female *Eudocima materna*.

Fig. 25: Photomicrograph of the early vitellogenic stage oocyte follicle (HE X400).

Fig. 26: Photomicrograph of the mid vitellogenic stage oocyte follicle (IH X400).

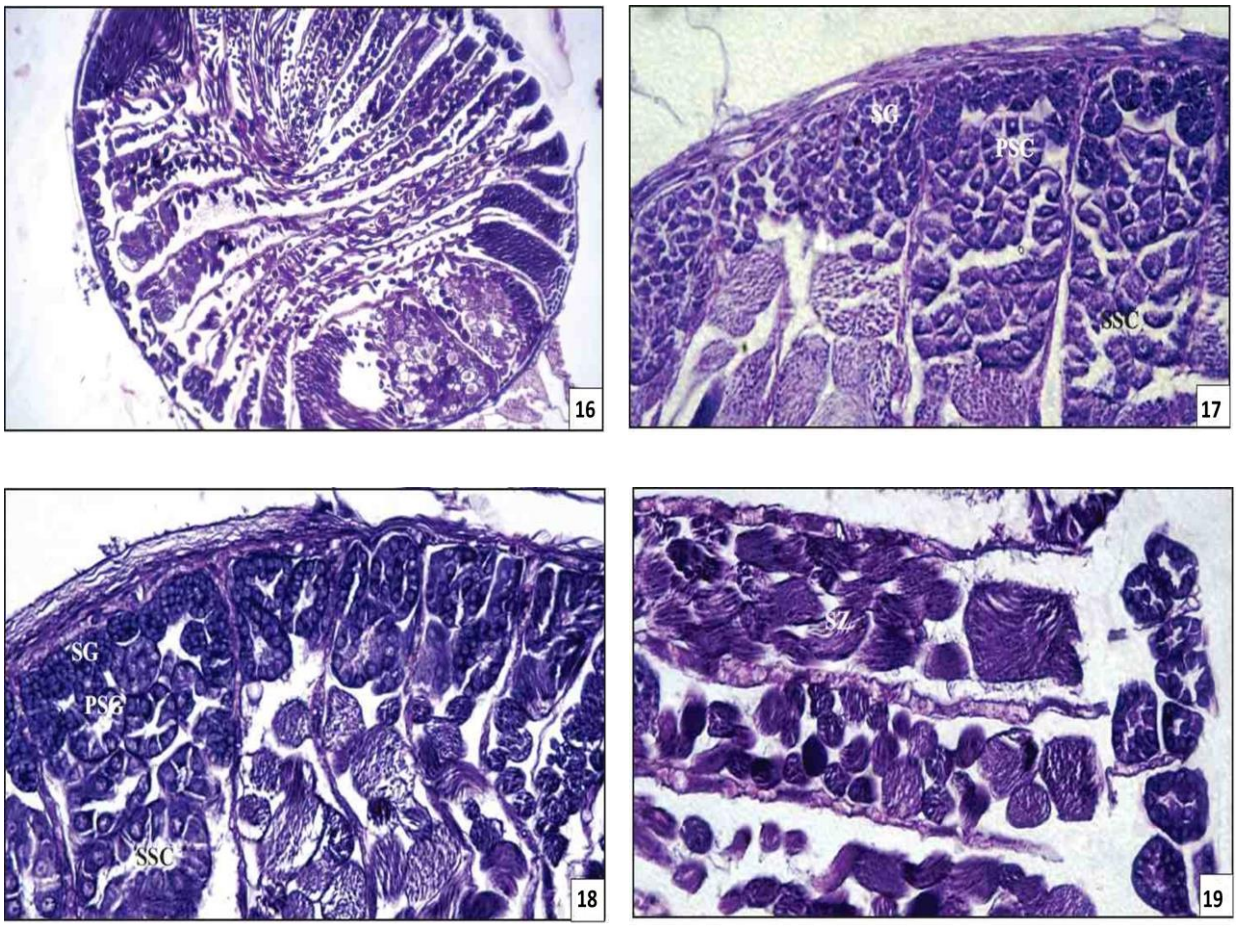
Fig. 27: Photomicrograph of the vitellogenic stage oocyte follicle (HE X400).

Fig. 28: Photomicrograph of the late vitellogenic stage oocyte follicle (IH X400).

Fig. 29: Photomicrograph of the late vitellogenic stage oocyte follicle (HE X400).

Fig. 30: Photomicrograph 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



Figs.16-19: Sections passing through the testis of Chemosterilant Hexamethylphosphoramide (HEMPA) treated male *Eudocima materna*.

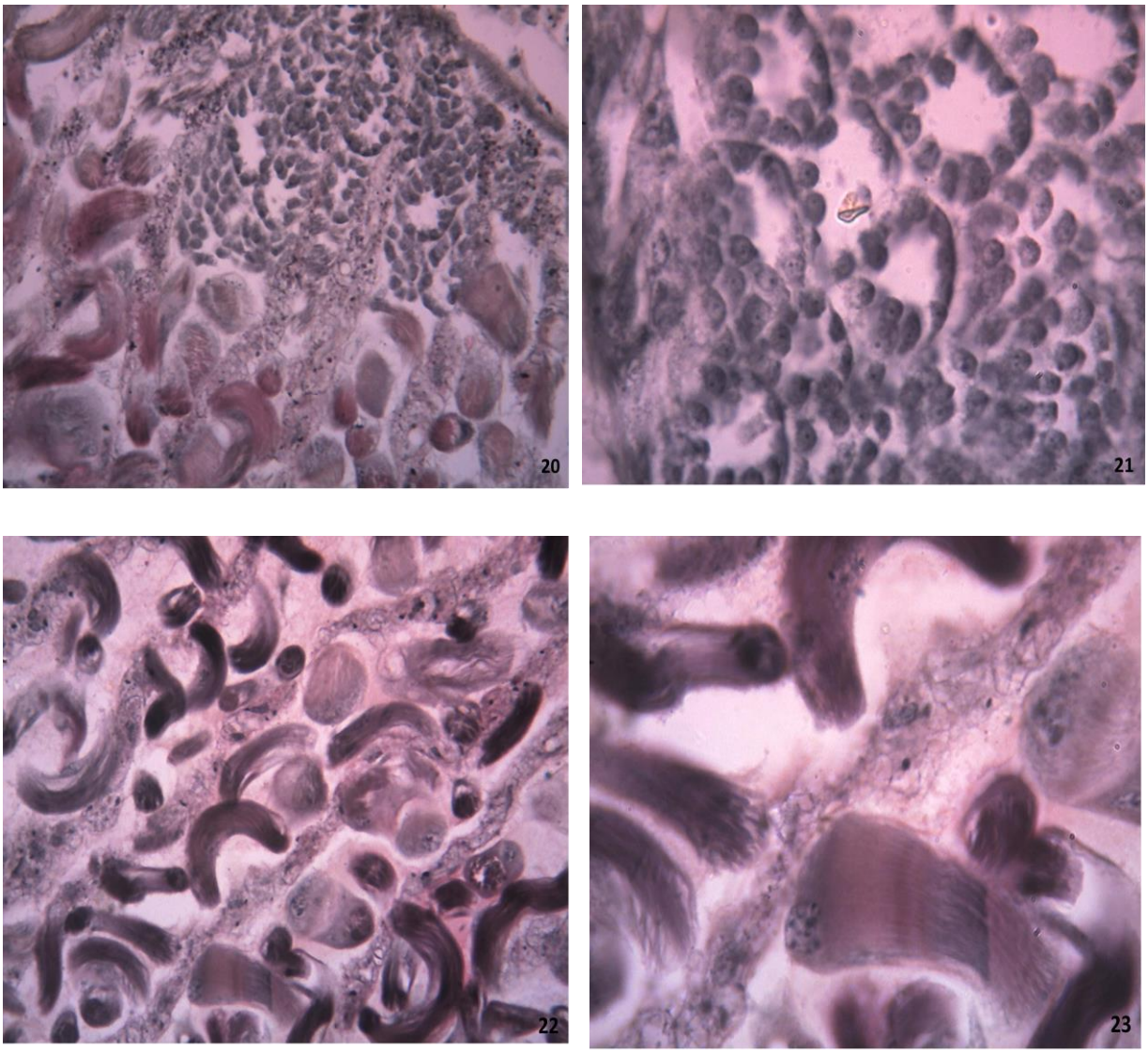
Fig. 16:Section showing separation of follicular tissue from testicular wall and disturbed cysts (HE X 400).

Fig. 17: Section showing loosely arranged cysts and dispersed spermatogonia (HE X400).

Fig. 18: Section showing sperm bundles with vacuoles and condensed nuclei (HE X400).

Fig. 19: Magnified view showing retardation of sperm maturation and condensed nuclei (HE X400).

SG- Spermatogonia
PSC- Primary Spermatocytes
SSC- Secondary Spermatocytes
SZ- Spermatozoa



Figs. 20-23: Sections passing through the testis of 5 K-rad gamma irradiated male *Eudocima materna*.

Fig. 20: Section showing separation of follicular tissue from testicular wall and disturbed cyst (HE X 160).

Fig. 21: Section showing loosely arranged cysts and dispersed spermatogonia (HE X 400).

Fig. 22: Section showing sperm bundles with vacuoles and condensed nuclei (HE X 400).

Fig. 23: Magnified view showing retardation of sperm maturation and condensed nuclei (HE X 1000).

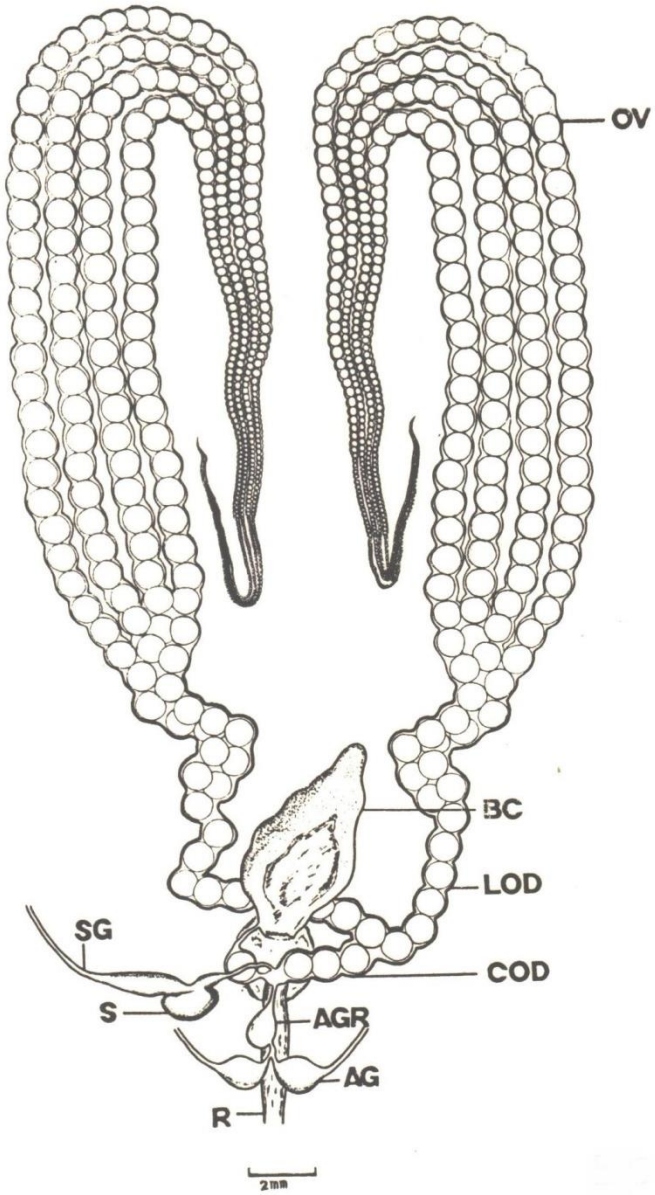
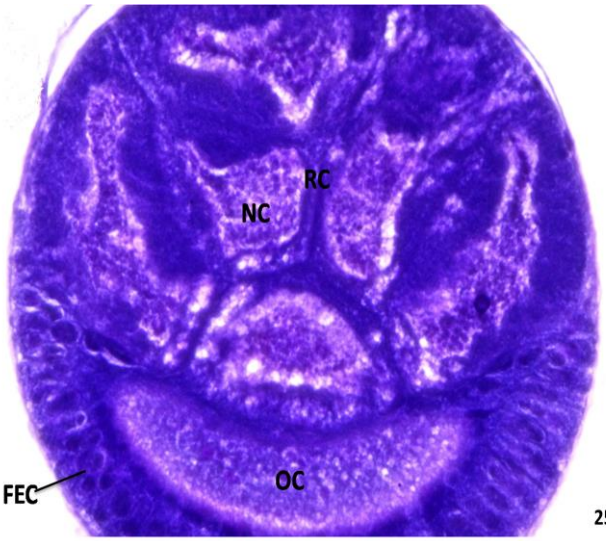


Fig. 24: Diagramatic representation of the female reoroductive system in *Eudocima materna.*

AG accessory gland
AGR accessory gland reservoir
BC bursa copulatrix
COD common oviduct
OV ovariole
R rectum
S spermatheca
SG spermathecal gland



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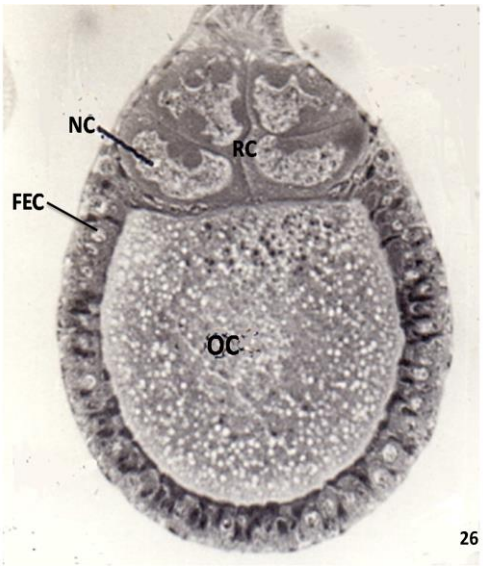

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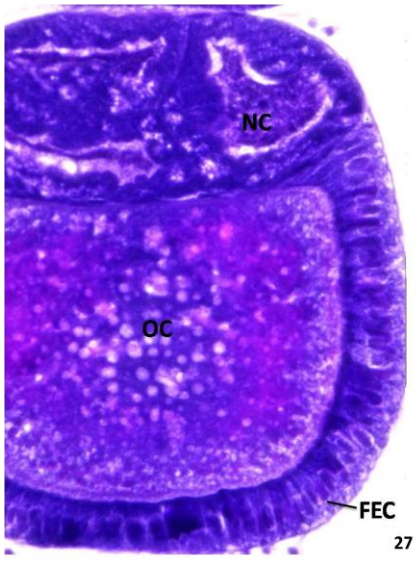

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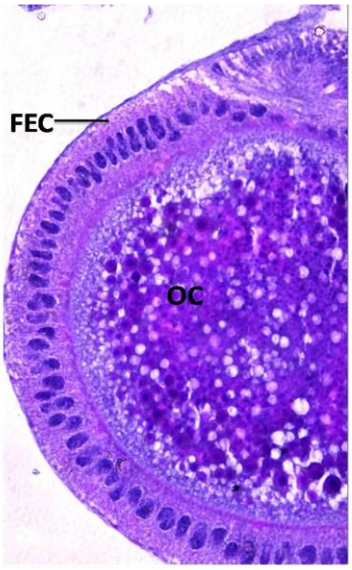

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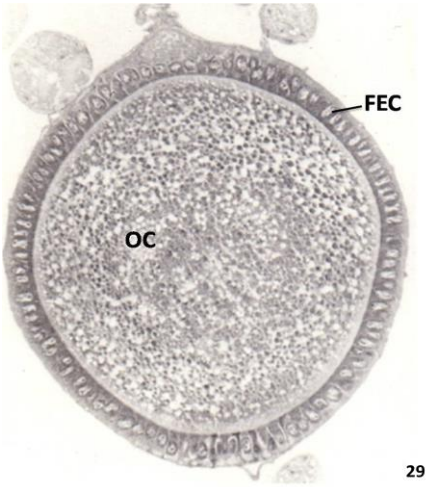

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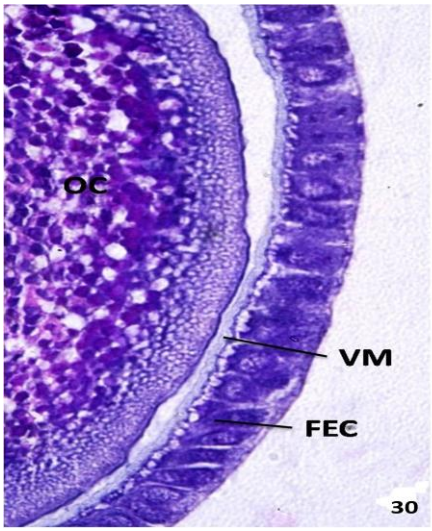

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Figs. 10-15: Sections passing through the testis of control male *Eudocima materna*.

Fig. 10: Transverse section passing through the testis showing spermatogenic gametes in the follicles (HE X100).

Fig. 11: Magnified view showing spermatogenic gametes in the follicles (HE X400).

Fig. 12: Magnified view showing spermatogenic gametes in the follicles and formation of spermatozoa from spermatids (HE X400).

Fig. 13: Spermatids and sperm bundles (IH X400).

Fig. 14: Epyrene and apyrene sperm bundles (IH X400).

Fig. 15: Magnified view showing Epyrene and apyrene spermatozoa (IH X1000).

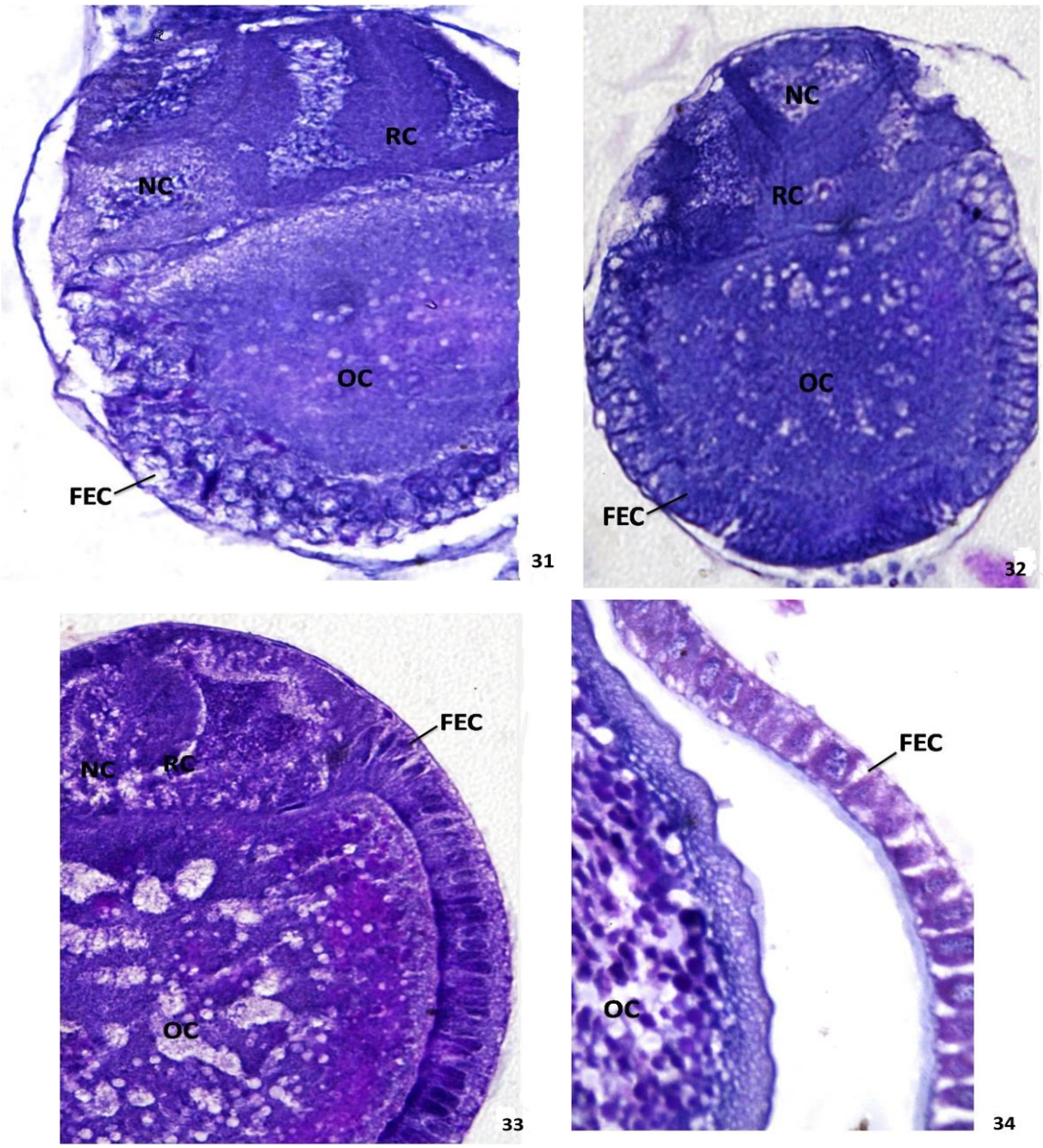
SG- Spermatogonia

PSC- Primary Spermatocytes

SSC- Secondary Spermatocytes

ST- Spermatids

SZ- Spermatozoa



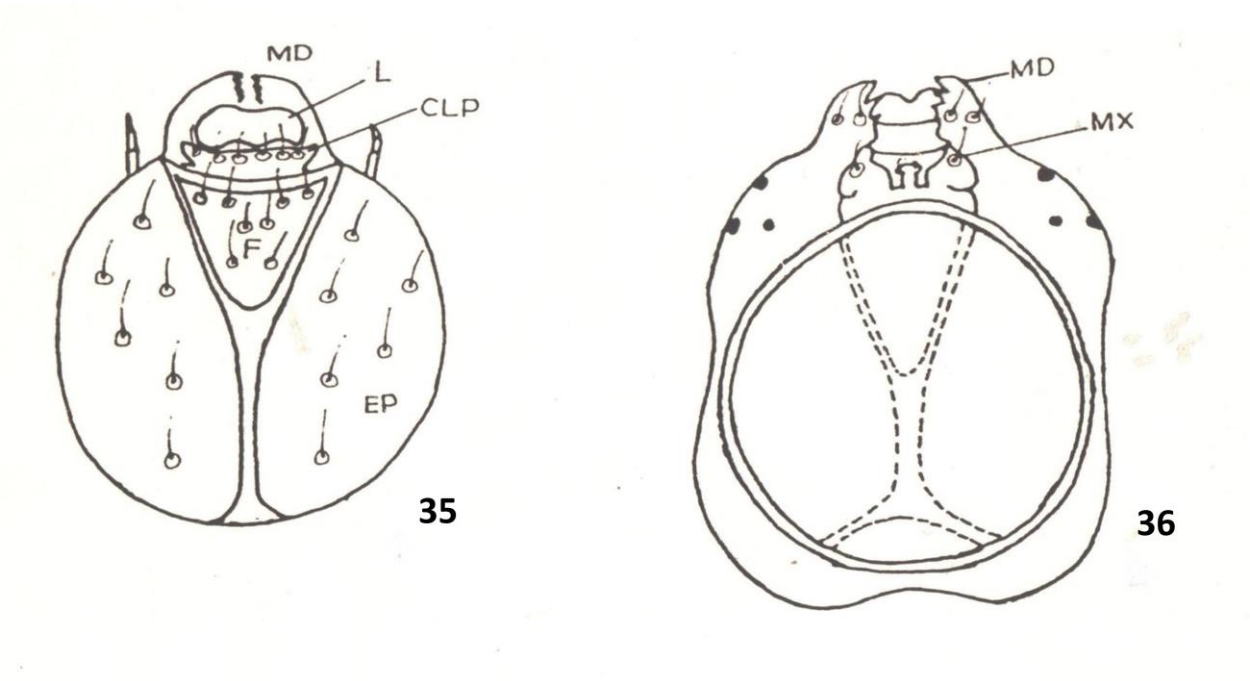
Figs. 31-34: Sections passing through the oocytes of chemosterilant HEMPA treated female *Eudocima materna*.

Fig. 31: Section showing the disintegration of follicular epithelial cells and disturbed nurse cells early-vitellogenic stage oocytes follicle (HE X400).

Fig. 32: section showing the malformation and vacuoles in the follicular epithelial cells of mid-vitellogenic stage oocyte follicle (HE X400).

Fig. 33: section showing atrophied oocytes and disintegrated chromatin material of nurse cells of vitellogenic stage oocyte follicle (HE X400).

Fig. 34: section showing atrophied oocytes, detachment and disintegration of follicular epithelium of late-vitellogenic oocyte follicle (HE X400).



Figs. 35-36: Chaetotaxy of V-instar larva of *Eudocima materna.*
Fig. 25: Dorsal view of head
Fig. 26: Ventral view of head
CLP- clypeus, EP- epicranial plate, F- frons, L- labrum, MD- mandible, MX- maxilla

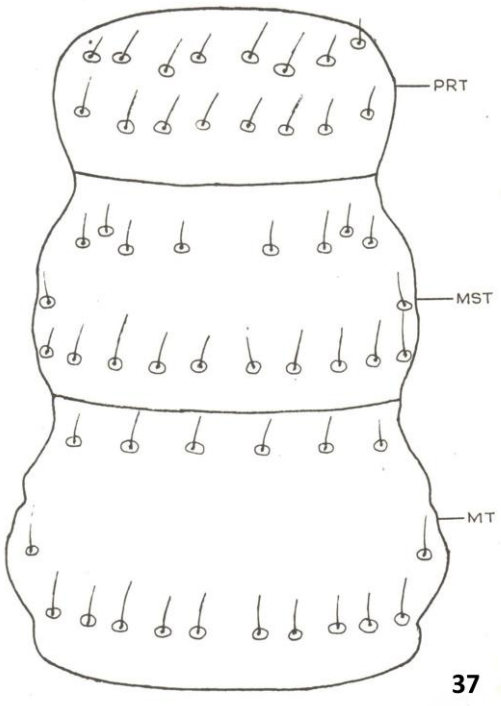
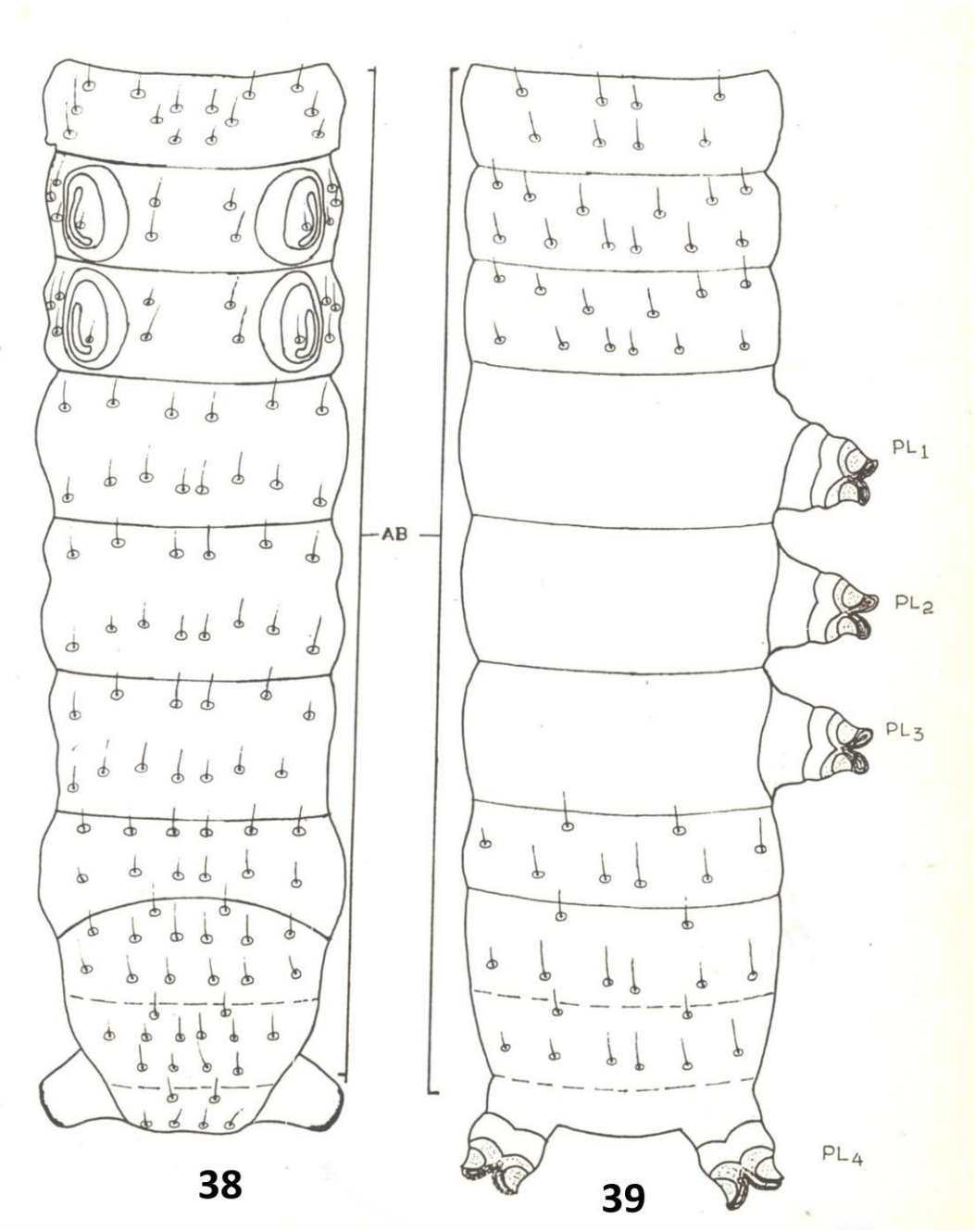


Fig. 37: Chaetotaxy of dorsal side of thorax of V-instar larva of *Eudocima materna.*
PRT- prothorax, MST- mesothorax, MT- metathorax



Figs. 38-39: Chaetotaxy of abdomen of V-instar larva of *Eudocima materna*.
Fig. 38: Dorsal view of abdomen
Fig. 39: Ventral view of abdomen
AB- abdomen, PL1-4- prolegs

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