Effect of Chemosterilant Hexamethylphosphoramide (HEMPA) on Spermatogenesis in Fruit Piercing Moth *Eudocima materna* (L.) (Lepidoptera:Noctuidae)

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Abstract - The present investigation was carried out to observe the effect of chemosterilant Hexamethylphosphoramide (HEMPA) on spermatogenesis in the fruit piercing moth *Eudocima materna*. Adult *Eudocima materna* were collected from the citrus orchards and reared in laboratory conditions. HEMPA was topically applied to the adult male moths to observe the histological changes in the spermatogenesis. The many abnormalities were observed in spermatogenesis. HEMPA has adversely affected the process of spermatogenesis. Degenerative changes in the testis were, dispersed spermatogenic stages, spermatogonia without cytoplasm, spermatocyte necrosis, condensed nuclei and development of many vacuolated areas. Inhibition of growth and maturation of the sperm was also noted.

KeyWords: chemosterilant, hexamethylphosphoramide, HEMPA, fruit piercing moth, *Eudocima materna*, Lepidoptera.

I. INTRODUCTION

The fruit sucking moth *Eudocima materna* causes extensive damage to various fruits. It is a major pest of citrus fruits, particularly oranges [1, 2]. However it is also a pest of other fruits like mangoes, bananas, grapes and cashew nuts, guavas and custard apples, pomegranates [3] which results in the significant decline in the yield of these fruits. It has created an alarming situations in Vidarbha region of Maharashtra due to extensive damage to citrus fruits [4]. Thus various methods may be followed to control this moth to prevent economic loss by this moth, Artificially reared male insects may be sterilized either by radiation or chemicals and released into infested areas for the effective control of this pest. Reddy and Sharma [5] and Prakash *et al.* [6] has studied the effect of nonalkylating chemosterilant HEMPA upon the longevity of adults, oviposition and viability of various insect species. Cytological aberrant effects of chemosterilants on the gonads of different insects has also been extensively studied [7, 8, 6, 9]. 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 [10] . With the degeneration of spermatogenic cells, the entire testes become smaller [9]. Thus present investigation is aimed to observe the histological changes in the testes of adult *Eudocima materna* after treatment with chemosterilant HEMPA.

II. 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 [11] was used for histological studies. Histological sections were examined and photographed by Labomed Digi-3 compound microscope.

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III. OBSERVATIONS AND RESULTS

Histological structure of testes in control moths:

The testis is a single creamy white and ovoid 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.1). 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.2).

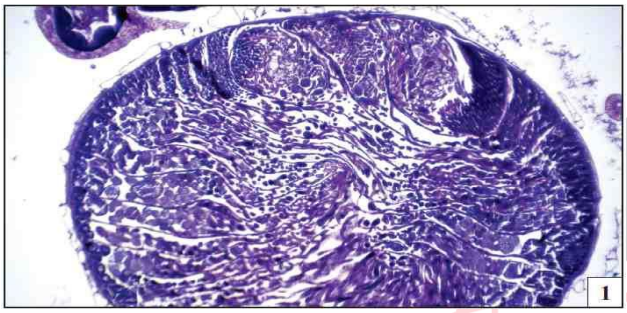

Fig. 1: Sections passing through the testis of control male *Eudocima materna* showing spermatogenic gametes in the follicles (HE X100).

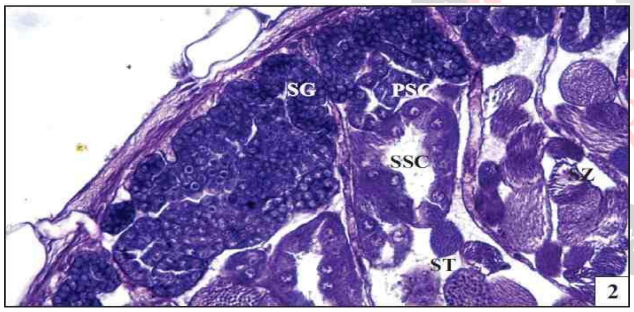

Fig. 2: Sections passing through the testis of control male *Eudocima materna* showing spermatogenic gametes in the follicles (HE X400).

SG- Spermatogonia, PSC- Primary Spermatocytes, SSC- Secondary Spermatocytes, ST- Spermatids, SZ- Spermatozoa

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 (Fig.3 & 4). 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 (Fig.4). 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 changes 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.5). They become loosely arrange and large gap are evident on the contrary they are compact in the control testis (Fig. 6).

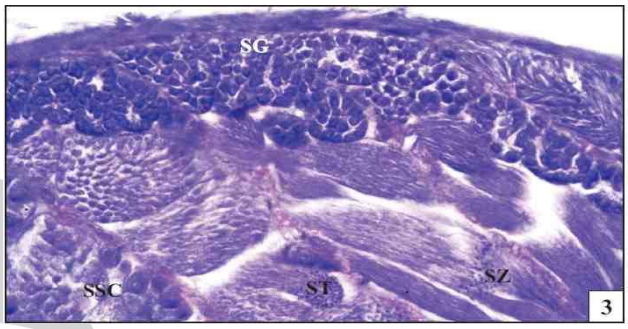

Fig. 3: Sections passing through the testis of control male *Eudocima materna* showing spermatogenic gametes in the follicles and formation of spermatozoa from spermatids (HE X400).

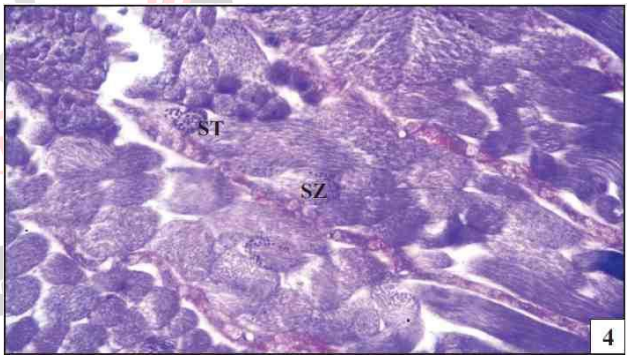

Fig. 4: Eupyrene and apyrene sperm bundles (HE X400).

SG- Spermatogonia, PSC- Primary Spermatocytes, SSC- Secondary Spermatocytes, ST- Spermatids, SZ- Spermatozoa

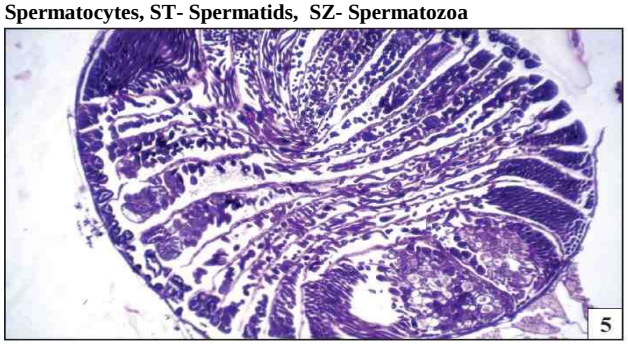

Fig.5: Sections passing through the testis of Chemosterilant Hexamethylphosphoramide (HEMPA) treated male *Eudocima materna* showing separation of follicular tissue from testicular wall and disturbed cysts (HE X 400).

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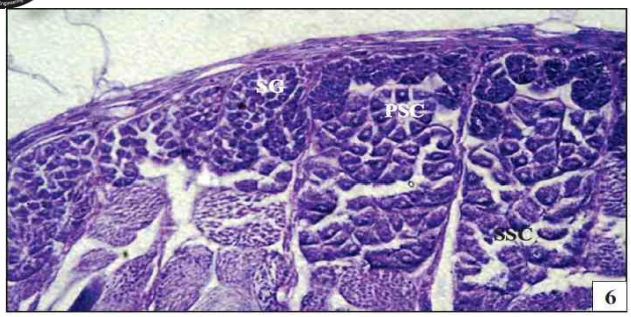

Fig. 6: Sections passing through the testis of Chemosterilant Hexamethylphosphoramide (HEMPA) treated male *Eudocima materna* showing loosely arranged cysts and dispersed spermatogonia (HE X400).

SG- Spermatogonia, PSC- Primary Spermatocytes, SSC- Secondary Spermatocytes, ST- Spermatids, SZ- Spermatozoa

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.7). 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.8).

Fig. 7: Sections passing through the testis of Chemosterilant Hexamethylphosphoramide (HEMPA) treated male *Eudocima materna* showing sperm bundles with vacuoles and condensed nuclei (HE X400).

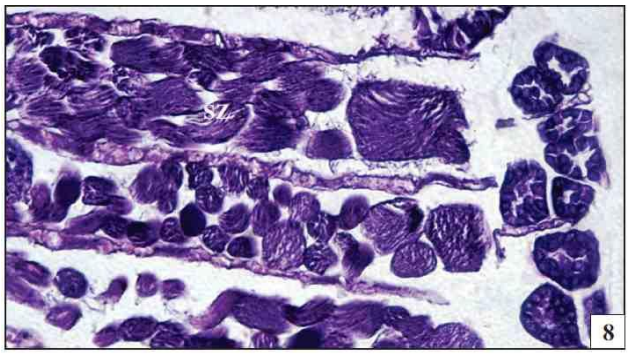

Fig. 8: Sections passing through the testis of Chemosterilant Hexamethylphosphoramide (HEMPA) treated male *Eudocima materna* showing retardation of sperm maturation and condensed nuclei (HE X400).

SG- Spermatogonia, PSC- Primary Spermatocytes, SSC- Secondary Spermatocytes, ST- Spermatids, SZ- Spermatozoa

IV. DISCUSSION

The germ cells of all the stages of spermatogenesis develop successive zones. Such a type of gametic maturation and their sequential arrangement is a peculiar feature in most of Lipidoptera [12]. In *Eudocima materna* also the follicles shows the development of gametes in sequential progressive stages of development.

The process of the spermatogenesis in some lepidopteran species has been studied by some workers as, *Eurygaster integriceps* [13] ; *Heliothis virescens* [14]. However, Toshiaki *et al.* [15, 16] studied the hormonal regulation of spermatogenesis in the cabbage moth *Mamestra brassicae* and common armyworm, *Leucania separata*, respectively. Loeb *et al*. [17] 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. The role of the testis sheath in the spermatogenesis in lepidopteran larva was studied by Giebultowicz *et al.* [18]. Sonoli and Hooli [19] reported the histological and histochemical studies of the apical cells of *Heliothis armigera.*

The chemosterilant hempa is found to affected longivity, oviposition and viability of eggs in different insect species [5,6]. Khan and Khan [20] and Tan and Mordue [21] observed weak sterilizing activity with hempa than other chemosterilants tested on *Dacus cucurbitae* and *Ephestia kuhniella,* respectively. But on the contrary, Hafez *et al.* [22] reported hempa to be superior than other chemosterilant in sterilizing both sexes of *Musca sorbens*. Cytological aberrant effects of chemosterilants such as general atrophy condensation and pycnosis of nuclei, vacuolation of cytoplasm, suppression of gonial cell division entire testes become smaller [9]. In classical experiment, Hamilton and Sutter [32] reported the effect of apholate on accessory gland in *Diabrotica undecimpunctata* Howardii barbar (cucumber beetle). The accessory glands degenerate (as they are androgen dependent) and the transfer of sperm during copulation are blocked [32]. Earlier experiments on the inductions of dominant lethal mutations also reported that the effects of chemosterilants on spermatogenesis are often followed by lowered are reported on the testes of some insects by a number of workers [14, 23, 24, 25, 26, 27]. The most common effect of chemosterilants on the male insects is found to be aspermia and the lack of spermatozoa mobility beside the induction of dominant lethal mutation in the sperms [28]. LaChance and Leopold [8] and Landa and Metwally [25] have found that sterility in the male *Musca domestica* and *Trogoderma granarium*, respectively due to induction of lethal mutation in the spermatozoa. In *Eudocima materna*, the effects of chemosterilant-hempa showed atrophy, vacuolation, and suppression of gonial cells division and

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thus confirm the observations of other workers. In 1955, Knipling [29] proposed eradication of screw worm, *Cochliomyia hominivorax*, by suppressing their reproduction through release of a large number of sterile males into the natural environment to compete with wild males for mating. Eradication of the fruit piercing moth, *Eudocima materna* can also be achieved through the release of sterile males in the environment since a single male moth can inseminate numerous females. This sterility can be introduce either by application of irradiation at pupal stage, or by application of chemosterilant hempa at adult stage just after the emergence. The potentialities of chemosterilants for control of insect pests have been elaborated widely [29, 30. 31].

It is evident from the classical and recent experiments that chemosterilants affect testes and spermatogenesis. The practical application of strong mutagens, as in classical experiments with insects, is out of the question; thus, the possibilities of using chemosterilants to produce azospermia or inactivation of sperm for male sterilization have been considered lately. The spermatogonia and growing spermatocytes undergoing the division are mostly affected [10]. With the degeneration of spermatogenic cells, the vitality of sperm, which may lead to its immotility or even death [33, 34].

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