Thiotepa Induced Histopathological Changes in the Meroistic Ovary of Castor Silk Moth, Philosamia ricini (L).

Animesh K. Mohapatra
Department of Zoology, Regional Institute of Education (NCERT), Ajmer – 305004; India

Abstract : Sub-lethal doses (20 µg and 40 µg) of thiotepa were administered by injecting in to haemocoel with the help of a micro liter syringe to freshly moulted fifth instar larvae. The effect of thiotepa on ovarian development and ovipositional attributes of Philosamia ricini was studied using standard morphological and histological techniques. Administration of thiotepa induced pathological damage of ovary. These damages comprised reduction in size of ovariole, disintegration of germarium and partial to complete elimination of prefollicular tissues. One of the most striking effects of thiotepa was condensation of chromatin, disruption of nuclear envelope, marked reduction in rate of vitellogenesis as evidenced by extensive vacuolization of oocyte cytoplasm. It also resulted in decreased egg production, reduced egg hatching, abnormal egg size and shape, an increased percentage of unhatched embryonated and sterile eggs.

Key words : Thiotepa, Histopathological Changes, Ovary, Castor Silk Moth, Philosamia ricini.

Introduction :
Use of pesticides is an effective and fastest method of pest control but pesticides, being non-biodegradable, accumulate in the environment, causing lots of pollution and hence disturbing the ecological balance (Kaleka and Parminder, 2003). Chemosterility is of such a technique, which is eco-friendly and effective in controlling pest by depriving them of their ability to reproduce.

A wide variety of chemosterilants have been used to control insect population by affecting fecundity and fertility (LaChance et al., 1968; Campion, 1972; Taneja et al., 1979; Casana – Giner et al., 1999). Most of the studies on this aspect are confined to flies and mosquitoes (Rai, 1964; Landa and Rezabova, 1965; Sukumar and Naidu, 1973; Mathew and Rai, 1975; Mahmood et al., 1991). Beattie (1979) induced inhibition of ovarian development in Lucilia cuprina by two aziridinyl chemosterilants. Casana-Giner et al. (1999) tested ten insect growth regulators (IGRs) as chemosterilizing agent for Ceratitis capitata causing total suppression of egg hatch. There is however dearth of investigation on the effects of these chemicals on female reproductive system of lepidopteran insects. The present investigations have been carried out to study the histopathological effects of the chemosterilant thiotepa on the ovaries of Philosamia ricini (L).

* Corresponding author : Animesh K. Mohapatra, Department of Zoology, Regional Institute of Education (NCERT), Ajmer – 305004; India. E-mail : akmrie@hotmail.com
Materials and Methods:

The rearing of larvae of castor silk moth was done at 28° C to 30° C in the laboratory. Two different doses of thiotepa i.e. 20 µg and 40 µg dissolved in double distilled water were injected into haemocoel by means of a microlitre syringe to two different sets of freshly moulted fifth instar larvae. A control group was also maintained simultaneously.

For histological studies, the ovaries of freshly emerged moths of control and treated groups were dissected out, fixed in Bouin’s fluid, dehydrated and impregnated in wax. The paraffin sections were stained in Harris haematoxylin and eosin.

Observations:

The administration of thiotepa has induced a noticeable change in the size of ovariole, as well as the number of oocytes. There is decrease in length of ovariole in case of lower dose than the control group as less number of oocytes is present in the vitellarium. While in case of higher dose, there is further decrease in the length of ovariole. The germarium is filled with disintegrated tissue in the form of irregular clumps in thiotepa treated groups. Shapes of most of the oocytes have become distorted and they are invariably small in size in case of higher dose. Almost complete elimination of prefolicular tissues is a striking feature of the ovary. As a result, cells of follicular epithelium have disrupted in numerous vacuoles in case of lower dose while in the case of higher dose, follicular epithelium is almost reduced to a thin membrane without any distinct cells.

Some oocytes with apparently two nuclei are also found in some ovariole in case of higher dose. The amount of yolk granules in the ooplasm is less in lower dose treated group as compared to the control group. In case of higher dose, most of the oocytes are almost without any yolk material. Numerous vacuoles are seen in the ooplasm and the oocytes appear empty and stain light colour, which is more prominent in higher dose. In case of lower dose, the trophocytes have become almost functionless with a number of vacuoles in the cytoplasm. The trophocytes of higher dose contain a number of large vacuoles and the chromatin material is disintegrated and is present in the form of small dark granules. In some ovaries of higher dose treated group, the wall of the ovariole have dissolved or broken at various places.

The administration of sublethal doses of thiotepa to silkworm larvae resulted in marked reproductive abnormalities: a decrease in total number of eggs laid; an increase in the number of non-fertilized eggs; the death of embryos in the early developmental stage; the inability of embryos to hatch and the death of newly hatched silk worms. In the control group, hatching started six days after egg laying (30° C) while it was delayed by 36 to 48 hours in thiotepa treated groups.

Discussion:

Progress of research on the concept of insect control through the use of chemicals to induce sterilisation has raised many questions concerning the cytological effects of chemosterilants (Campion, 1972; Mohapatra 2003). LaChance and Crystal (1963), Morgan
(1967), Morgan and Labrecque (1962, 1964), Rai (1964), Landa and Rezabova (1965), Bhargava et al. 1977, Taneja et al. (1979) and Mahmood et al. (1991) have studied the effects of certain chemosterilants on reproductive organs of houseflies, mosquitoes and other insects showing that ovarian development is inhibited. Histological examinations of reproductive tissues have revealed degeneration of oocytes and general necrosis following application of these chemicals. Masner (1971) on Pyrrhocoris apterus, Sukumar and Naidu (1973) on Dysdercus cingulatus and Mohapatra (2003) on castor silk moth observed severe pathological effects on ovary on the basis of morphological studies after treatment with 6 azauridine, tepa and hempa, respectively. Jalaja and Prabhu (1976) observed that there is disintegration of germarium and follicular epithelium as well as reduction in size of oocytes and their resorption in Dysdercus cingulatus after treatment with metapa and apholate. The present investigation on Philosamia ricini (L) shows similar results after treatment with thiopeta. Different doses of thiopeta produced changes, which range from partial to complete necrosis of the ovary resulting in the production of more abnormal eggs. There was a gradual reduction in the size of the ovariole from control to lower and higher doses of thiopeta treated moths. In case of higher dose of thiopeta treated moths, the percentage of emergence is low with a remarkable decrease in size of ovariole, fewer oocyte and heavily disintegrated germarium. The most striking feature is the complete elimination of prefollicular tissue, as a result of which the follicular epithelium is almost reduced to a thin membrane without any distinct cells. The amount of yolk granules in the ooplasm is also less. The trophocytes have become functionless as indicated by disintegrated chromatin and large vacuoles in the cytoplasm. Production of oocytes is inhibited by the sterilant. This is due to the disintegration of posterior zone of the germarium where oocytes are differentiated as also shown by Cantwell and Henneberry (1963) in Drosophila and Jalaja and Prabhu (1976) in Dysdercus cingulatus. Morgan and LaBrecque (1962, 1964), Landa and Rezabova (1965), LaChance et al (1968), Ondracek and Matolin (1971) and Saxena and Bhatnagar (1980) observed that the effective doses of chemosterilants cause severe pathological damages to ovary such as degeneration and dissolution of ovariole cytoplasm, pycnosis and fragmentation of chromatin material in oogonia and follicular cells, which resulted in complete degeneration of cellular material. Mathew and Rai (1975) have shown that chemosterilant apholate induces ultrastructural changes in the presumptive and primary follicles of the adult ovary in Aedes aegypti. The present investigation shows that thiopeta caused condensation of chromatin, disruption of nuclear envelope and extensive degeneration as evidenced by numerous myelin figures and residual bodies in the oocytes. Mohapatra (2003) has shown similar effects in castor silk moth by administering hempa.

Kerns and Nair (1972) and Sukumar and Naidu (1973) observed that the lower
Plate 1

Photomicrographs of the oocytes.
Fig. 1, 2 and 3: oocytes of control group
Fig. 4, 5 and 6: oocytes after treatment with lower dose of thiotepa.
Plate 2

Photomicrograph of oocytes after treatment with higher dose of thiotepa.
Effect of thiotepa on egg laying and hatching

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average length of ovariole (cm)</th>
<th>Eggs laid</th>
<th>Normal eggs</th>
<th>Non-fertilized eggs</th>
<th>Death at an early embryonic stage</th>
<th>Death just before hatching</th>
<th>Death just after hatching</th>
<th>Percentage of normal eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.4</td>
<td>458</td>
<td>431</td>
<td>17</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>94.1</td>
</tr>
<tr>
<td>Thiotepa (lower dose)</td>
<td>7.4</td>
<td>207</td>
<td>67</td>
<td>64</td>
<td>47</td>
<td>17</td>
<td>12</td>
<td>32.3</td>
</tr>
<tr>
<td>Thiotepa (higher dose)</td>
<td>4.3</td>
<td>91</td>
<td>0</td>
<td>45</td>
<td>38</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The concentration of haemolymph proteins in the sterilant treated insects is one of the factors that contribute to the resorption of oocytes. Anderson (1971) observed that poor deposition of yolk in the oocytes of treated insects is due to failure of follicular cells to differentiate properly. The sensitivity of follicular epithelium to the sterilant 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 (1969). The administration of thiotepa in *Philosamia ricini* (L) resulted in marked reproductive abnormalities, significant reduction in egg production, reduced egg hatching; abnormal egg size and shape, increased percentages of unhatched embryonated and sterile eggs, and death of newly hatched silk worms. Similar results have also been reported in other insects (LaBrecque, 1961; Borkovec, 1962; Crystal and LaChance, 1963; Hoque et al., 1978; Kuribayashi, 1980, 1981; Mohapatra and Khattar, 1986; Mahmood et al., 1991 and Mohapatra, 1993).

**Conclusion:**

Although chemosterility technique has been one of the potent ways for controlling insects, but till now it has not emerged as a method of extensive usage. It is probably because of high cost and laborious work. Other important factor responsible is the possible hazard of mutagenic, carcinogenic, teratogenic types with these chemicals to higher animals including man. Nowadays there are more than a thousand of chemosterilants but a very limited of these are on the verge of practical application. Invention of less hazardous chemosterilants offers a great possibility in future.

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