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

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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 chemosteritants 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).

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Materials and Methods :

The rearing of larvae of castor silk moth was done at 28º C to 30º 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 (30º 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 chemosteritants 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 thiotepa. Different doses of thiotepa 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 thiotepa treated moths. In case of higher dose of thiotepa 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 aphotate induces ultrastructural changes in the presumptive and primary follicles of the adult ovary in *Aedes aegypti*. The present investigation shows that thiotepa 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

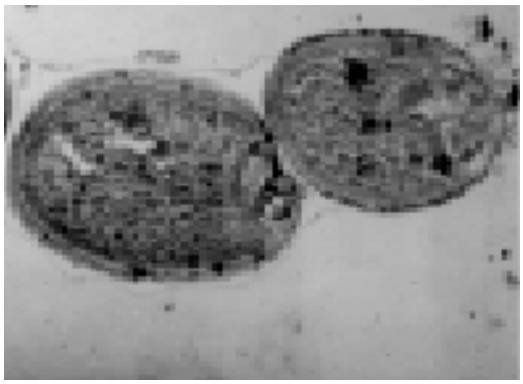
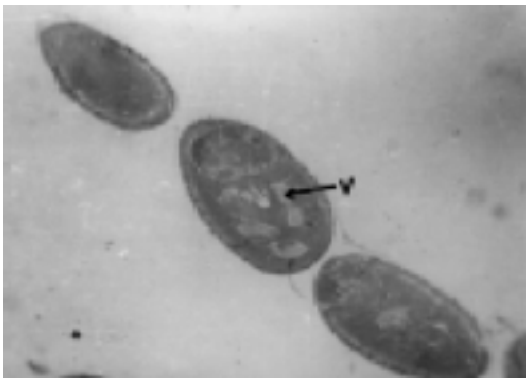


Figure – 1



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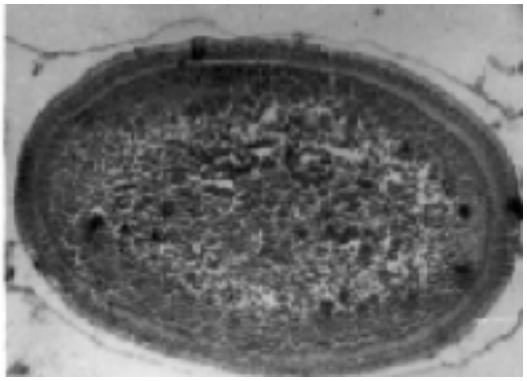

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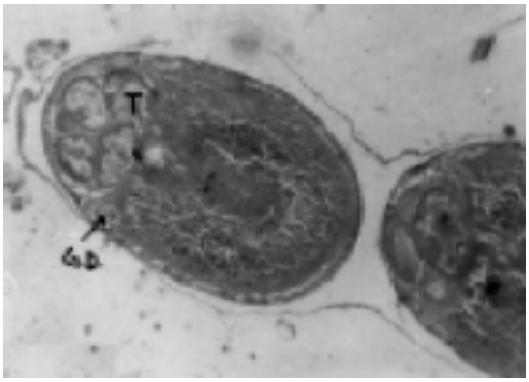

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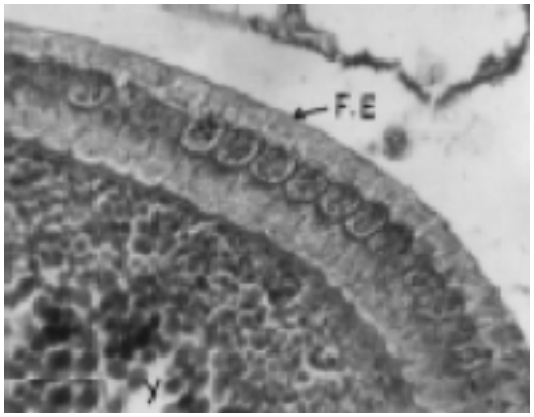

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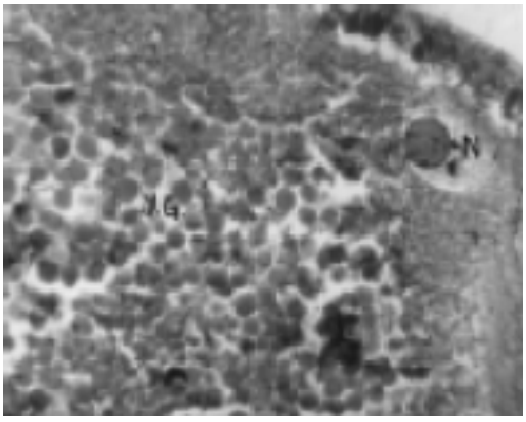
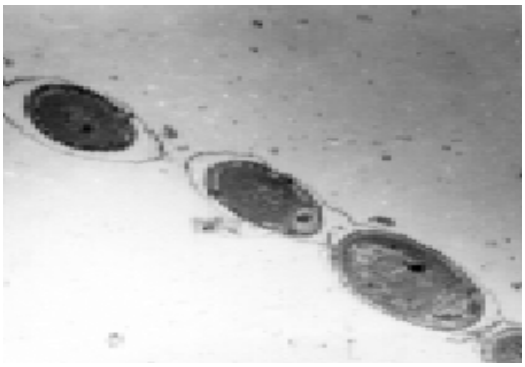


Figure - 6

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.



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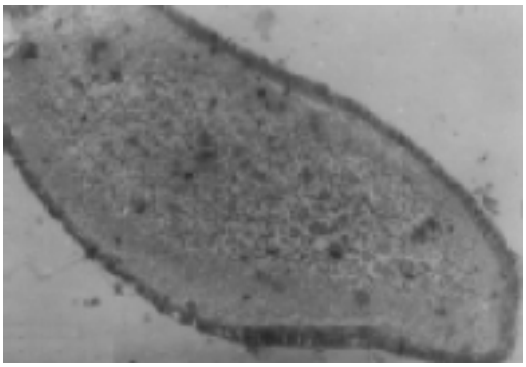

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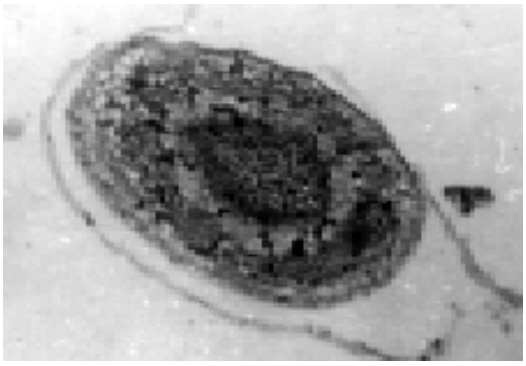

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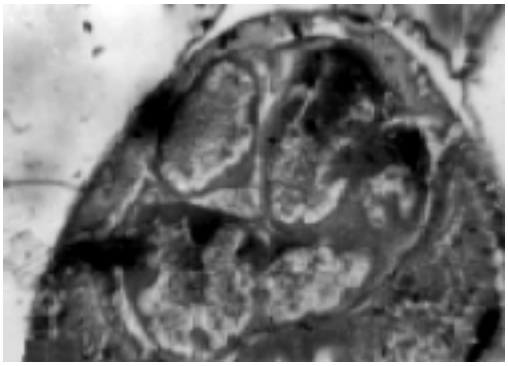

Figure - 10

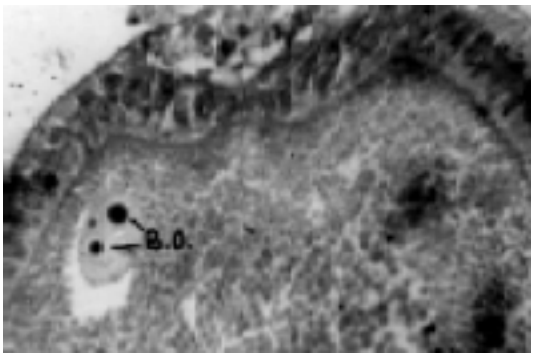

Figure – 11

Abbreviations

F. E. – Follicular epithelium
Y – Yolk granules
V – Vacuole
GD – Germinal disc
T – Trophocyte
N – nucleus
BO – Binucleate oocyte

Plate 2

Photomicrograph of oocytes after treatment with higher dose of thiotepa.

Effect of thiotepa on egg laying and hatching

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

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. Nowdays 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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References :

Anderson L.M. (1971) : Protein synthesis and uptake by isolated cecropia oocytes. *T. Cell. Sci*., **8,** 735-750.

Anderson L.M. and Telfer W.H. (1969) : A follicle cell contribution to the yolk spheres of moth oocytes. *Tissue and cell*, **1,** 633-644.

Beattie G.A.C. (1979) : Oogensis in *Lucilia cuprina* (Wied) (Diptera: Calliphoridae). ii. the effect of Aziridyinyl chemosterlants on oogenesis. *Australia J.Zool*., **27(3),** 349-362.

Bhargava S., Tandon G.N. and Mathur R. (1977): On a possible mode of action of hempa on ovarian tissue of *Periplaneta americana*. *Curr. Sci*., **46(1),** 384-386.

Borkovec A.B. (1962) : Sexual sterilisation of insects by chemicals, *Science*. **137,** 1034.

Campion D.G. (1972) : Insect chemosterilants :a review. *Bull. Ent. Res*., **61,** 577-635.

Cantwell, G.E. and Henneberry T.J. (1963) : The effects of gamma radiation and apholate on the reproductive tissue of *Drosophila melanogaster. J. Insect Physiol*., **5,** 251-264.

Casana – Giner V., Gandia – Balaguer A., Megnod – Puerta C., Primo-Millo J. and Primo-Yufera E. (1999) : Insect growth regulators and chemosterilants for *Ceratitis capitata* (Diptera: Tephritidae). *J. Econ. Ento*., **92(2),** 303-308.

Crystal M.M. and LaChance L.E. (1963) : The modification of reproduction in insects treated with alkylating agents. I. Inhibition of ovarian growth, egg production and hatchability. *Biol. Bull*. **125,** 270-279.

Hoque M.R., Bhuiyan N.I. and Borkovec A.B. (1978) : Evaluation of certain chemosterilants for the jute hairy caterpillar, *Diacrisia obliqua* walk. 11. Inducing sterility in the newly emerged moths Bangladesh. *J. Agri. Sci*., **5(2),** 159-171.

Jalaja M. and Prabhu V.K.K. (1976) : Effect of the chemosterilants apholate and metapa on the ovaries of the red cotton bug, *Dysdercus cingulatus Fabr*. *Entomon* **1,** 43-53.

Kaleka APS and Kaur P. (2003) : Chemosterility: A chemico-biological approach for insect control. *Everyman's Science*, **37(4),** 220-223.

Kerns D.R. and Nair K.K. (1972) : Physiological studies on the effects of tepa on *Schistocercea gregaria*. *Ann. Ent.Soc. Amer.*, **65,** 217-221.

Kuribayashi S. (1980) : The ovicidal action of organophosphorousinsecticides administered during the larval stage in silkworm. Proc. 16th Inter. *Cong. Eng., Kyoto*, p. 446.

Kuribayashi S. (1981) : Studies of the effect of pesticides on the reproduction of the silkworm, *Bombyx mori*. L. I.Effects of chemicals administered during the larval stage on egg laying and hatching. *J. Toxic.Sci*. **6,** 169-176.

LaBrecque G.C. (1961) : Studies with three alkylating agents as house fly sterilants. *J. Econ. Entomol.*, **54,** 648-689.

LaChance L.E. and Crystal M.M. (1963) : The modification of reproduction in insects treated with alkylating agents. II. Differential sensitivity of oocyte meiotic stages to the induction of dominant lethals. *Biol. Bull*., **125,** 280-288.

LaChance L. E., North D.T. and Klassen W. (1968) : Cytogenetic and cellular basis of chemically induced sterility in insects, in principles of Insect chemosterilisation (ed. LaBrecque, G.C. and C.N.Smith). *Appleton-Century-Crofts*, 99-157.

Landa V. and Rezabova B. (1965) : The effect of chemosterilants on the development of reproductive organs in insects. *12th Int. Congr. Ent*., 516-517.

Mahmood F., Walters L.L, Guzman H. and Tesh R.B. (1991) : Effect of ivermectin on the

ovarian development of *Aedes aegypti* (Diptera : Culicidae). *J. Med. Entomol.* **28(5),** 701-707.

Masner P. (1971) : The sterilisation effect of 6-azauridine on the ovary and corpus allatum interaction in the bug. *Pyrrhocoris apterus*. *Insect Endocrines*, Suppl. *Actaentomol. bohemoslov*, 25-35, Academic Praha.

Mathew G. and Rai K.S. (1975) : Chemosterilant (apholate) – induced ultrastructural changes during oogenesis in *Aedes aegypti*. *Cytobios.* **12(45),** 45-56. .

Mohapatra A. K. (1993) : Effect of hempa on larval development, fecundity and egg viability in *Philosamia ricini*. Comp. *Physiol. Ecol*, **18(3),** 96-98.

Mohapatra A.K. (2003) : Histopathological changes in the ovary of chemosterilized castor silk moth, *Philosamia ricini*. *Flora and Fauna*, **9(1),** 25-26.

Mohapatra A.K. and Khattar N. (1986) : Effect of thiotepa on larval growth, egg laying and hatchability in *Philosamia ricini* (L). Entomon, **11(1),** 17-19.

Morgan P.B. (1967) : Effects of hempa on the ovarian development of the housefly, *Musca domestica* (Diptera : Muscidae). *Ann. Ent. Soc. Amer*., **60,** 812-818.

Morgan P.B. and LaBrecque G.C. (1962) : The effect of apholate on the ovarian development of house flies. *J. Econ. Ent*., **55,** 626-628.

Morgan P.B. and LaBrecque G.C. (1964) : Effect of tepa and metapa on ovarian development of house flies. *J. Econ. Ent.*, **57,** 896-899.

Ondracek J. and Matolin S. (1971) : Sterilising effects of tepa on the bean beetles *Acanthocelides obtectus* say (coleoptera) *Acta. Entomol. Bohemoslov*., **68,** 209-215.

Rai K.S. (1964) : Cytogenetic effects of chemosterilants in mosquitoes. 2. Mechanism of apholate induced changes in fecundity and fertility of *Aedes aegypti. Biol. Bull*., **127,** 129-131.

Saxena S.C. and Bhatnagar P. (1980) : Histopathological and biochemical changes in panoistic ovary of chemosterilised cockroach, *Periplaneta americana* (L). *Ind. J. Exp. Biol.*, **18,** 35-38.

Sukumar K. and Naidu M.B. (1973) : Inhibition of ovarian growth by tepa in *Dystercus cingulatus. J. Econ. Ent*., **66,** 20-22.

Taneja S.K., Sood V., Taneja S. and Nath A. (1979) : Effect of apholate on neurosecretion of pars intercerebralis, fat bodies and ovarian development of the red cotton bug., *Dysdercus koenigii. Ind. J. Exp. Biol*., **17(2),** 133-138.