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ORIGINAL ARTICLE

EFFECTS OF THE PHYTOPESTICIDE NIMBECIDINE ON THE OVARY, BRAIN AND CORPUS ALLATUM OF ADULT FEMALE INSECT *ODONTOPUS VARICORNIS.*

[1*]P. Ramya, [1]G.Sathishkumar, [1]R.Mahesh kumar, [2]M.Loganathan and [1]G.Sridharan

[1*]Department of Zoology, Rajah Serfoji govt.College, Thanjavur -613 005
[2]IICPT: Indian Institute of Crop Processing Technology, Thanjavur- 613 005

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ABSTRACT

The present investigation deals with the histology of ovary, histo morphology of neuroendocrine system and the influence of the phytopesticide Nimbecidine on ovarian tissues and neuroendocrine system, in the twenty day old adult female bug, *Odontopus varicornis*. For understanding the inter – relationships prevailing among the neuroendocrine system and a ovary of the phytopesticide mediated response, studies have been extend to understand the changes in the haemolymph and fatbodies, both in the treated and untreated controls. It has also been observed in *Odontopus varicornis* that a reduction in the number of eggs laid is evident after treatment with the Nimbecidine. Likewise the time of oviposition was also delayed. The hatching of the eggs were also not uniform. The hatched nymphs exhibit perceptible morphological variations.These findings on these aspects of studies of the Phytopesticide was a powerful neurotoxic agent it affects the 'neurosecretory cells in the brain, resulting in the malfunctioning of corpus allatum and the absence of its hormone, inhibits of vitrellogenesis which is observed in the growing oocytes.

Key words: *Odontopus Varicornis*- Test Insect of Red cotton bug, Nimbecidine- phytopesticide, nymphs- unlike a typical larva, overall form already resembles that of the adult.

1.INTRODUCTION

Insects, by virtue of their economic importance have been the object of study for a long time, in particular from the point of view of pest control and management. The common methods employed to combat insect pests include the use of a variety of approaches viz., the application of Phytopesticide, biological control, cultural practices and mechanical devices. The use of Phytopesticides has been the most widely applied single method of pest control.

The casual events leading to reduced fecundity remains unknown and the basic reasons for blocking egg development have not been understood (Lachance and Leverich, 1968). It is doubtful whether the insecticides, or other chemicals applied inhibits egg development strictly by affecting the cells and tissues of the ovaries or whether they act via more indirect path way by interfering with the hormonal regulation of egg development. Pesticides are one of the major xenobiotic substances that have been used in India for a longer period for the management of pest in agriculture fields and control of vectors in public health operations. Most of the insecticides are so hydrophobic that they can be absorbed by soil particles and can migrate to natural water system such as river, lakes and ponds through the run off, causing severe aquatic pollution (Ding, J.Y., and Wu, S.C., 1993).

**Corresponding author: P.Ramya, Department of Zoology, Rajah Serfoji Govt.College, Thanjavur -613 005*

In commonly nimbecidine is used for pest control. It is safe and acceptable to mankind. Nimbecidine contains the azardiechtin properties. Neem products are known to affect particular early larval instars and also young of aphids. The pesticidal activity of neem has a wide spectrum having repellent, phagodeterrent, insect growth regulatory, antiovipositional.

Therefore, it been programmed in the present study to find out the effect of Phytopesticide, Nimbecidine on the ovary corpus allatum and neurosecretory of female insect *Odontopus varicornis.*

2.MATERIALS AND METHODS

Rearing

Adult *Odontopus varicornis*, of both sexes, collected from the field in the agricultural university garden Coimbatore, were used throughout the present investigation. The insects were reared in the laboratory in insect cages measuring 60 x 45 x 45 cm in dimensions and at a temperature of 29° C ±1°C. Equal number of males and females were introduced in the cages and the total number in cages and the total number in each one of them did not exceed twenty. The insect with their natural preferred food, the unripe fruits of cotton. The egg

laid by them was transferred to another cage and thus a continuous culture was maintained. The hatched nymphs were collected, sorted out and reared in separate cages. The adult insect which emerged from the last instar were reared in separate cages and dated. Twenty days old adult insects, from the date of last moult were used for the present investigation.

Fig.1 ***Odontopus Varicornis Odontopus Varicornis*** **Test Insect of Red cotton bug**

Administration of Nimbecidine

By using the higher concentration of the pesticide absolute mortality at 24 hours was established. By using a low concentration of the pesticide the mortality at 72 hours was also established. The median lethal dosage was derived using the nomograms(Lichfield and Wilcoxan, 1949) and lethal

dosage was established per weight of the insect for a particular time interval (Nayer *et al*., 1996).

Seventeen day old adult insects, after their last moult, were sprayed with a 4 percent of Nimbecidine using a sprayer bottle. The amount of phytopesticide Nimbecidine which is an Azardiraetin compound, was administrated to the insects. Each insects received 4ml of the Nimbecidine in one litre of distilled water to dissolve the desired amount of the phytopessticide Nimbecidine, preliminary studies have shown that this has produced any drastic effect on the insect. At the end of 72 hours of treatment the insects were dissected in insect ringer solution. The tissues such as ovary, corpus allatum and brain were fixed in Bovin's fluid and used for histological studies.

Following the standard procedures mentioned earlier for processing, embedding sectioning and staining, the stained sections were observed for the histological changes due to Nimbecidine treatment. Determination of nuclear and cytoplasmic volumes were also done to compare them with the normal untreated controls.

Histology

Insect collected from the cages were sacrified by ether anesthetization for the normal histological studies of the ovary, corpus allatum and neurosecretory cells.

Determination of the nuclear volume of the Corpus allatum

Measurement of the diameter of the corpus allatum were made with a caliberated eye – piece micro – meter.

Secretory activity of the Median Neuro Secretory Cells (MNSc)

The secretory activity of the MNS cells were determined from the volume of their nuclei and cells and their nucleoplasmic index values (NP) were calculated following the method adopted by Tan 1976.

The NP index value was calculated by using the formula = Vn

Vc - Vn

Where 'Vn' is the volum of the nucleus and 'Vc' is the volume of the cell.

3.RESULTS AND DISCUSSION

Effect of phytopesticide Nimbecidine on ovary

Due to treatment with the phytopesticide Nimbecidine, the colour of the ovary changes from brown to pale black. The treated ovarioles are shorter in length than normal and shows shrinkage. The effect of various chemosterilants on the oogenesis of insects has been investigated by a large number of workers. In has been recorded theat ovarian development is generally inhibited due to the direct action of chemosterilants (Campion, 19730), and by pesticides (Coehoun, 1959; moriarity, 1969; misra, 1981; Rizada *et al*., *1982 and* Hughes and Penton, 1983). The histopahtological changes due to the treatment of Nimbecidine are described below.

Histology of the ovary -
External sheaths

The entire ovariole is covered by an outer epithelial sheath and an inner layer of cells. The epithelial sheath was a syncytium. The anterior part of the sheath was thicker having two or three rows of nuclei while the posterior part was thinner having only one row of nuclei. The nuclei are elliptical having coarse chromatin granules mitotic activity is rare.

The lamellae of the inner envelop were displaced with the result, these cells eventually come to lie in groups in the inner envelope were so closely adpressed to the follicles that the cells enclosed between them were displaced with the result, these cells eventully come to lie in groups in the constriction between the follicles.

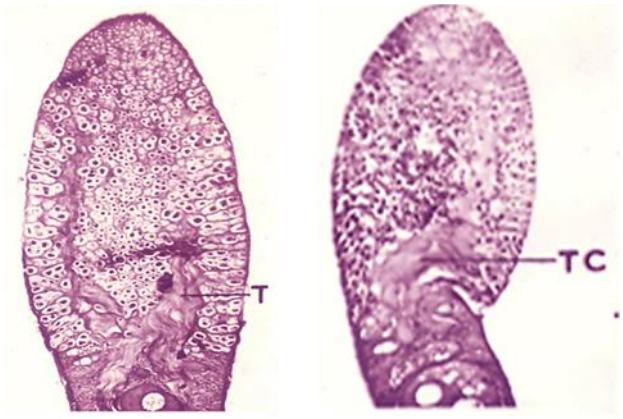
Terminal filament

The terminal filament was an elongated, slender flask shaped structure, having a broad base and a tapering end. The terminal filament was separated from the germarium by a constricted transverse septum. It has oval shaped nuclei distributed in a haphazard fashion. The cytoplasm of the bulbous base shows the presence of a number of lacunae. There is no cell boundary between the cells and cell divisions are not encountered. The entire terminal filament has covered by epithelial sheaths and the inner envelope forms collar around the base of the filament. Since the type of ovary was telotrophic, the major portion of the germarium was occupied by the trophic tissue. It has a central cytoplasm which is surrounded by densely packed cells. (Fig.2A & 2B).

The histological features of the terminal filament of the ovary of *Odantopus varicornis* are, in general, similar to those

described for *Oncopeltus fasciatus* (Bonhay and Wick, 1953); *Odantopus varicornis* (Kamalakannan, 1977). Marked histological changes are not evident during the reproductive maturation of the ovary. These observations, therefore, indicate that the terminal filament does not serve any function other than a mechanical one, i.e., to hold the ovarioles in position.

Fig. 2.Effect of phytopesticide Nimbecidine on germarium



2A 2B

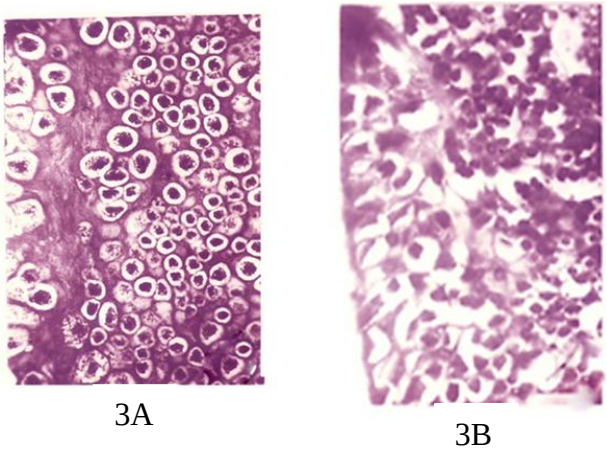
Pedicel

The pedical was tubular in shape. It has single row of columnar epithelial cells each with its distinct cell boundary. The anterior cells of the pedicels form the transverse septum between the follicular tissue and the pedical lumen. The nucleus was found at the base of the cells. The free end of the pedical facing the lumen was irregular in outline.

Effect of phytopesticide Nimbecidine on germarium,

Studies on the histology of the ovary of *Odontopus varicornis* have revealed that the trophic tissue occupies the major portion of the germarium. The cell cytoplasm exhibits vacuoles. Cytological abnormalities noticed in zone I due to Phytopesticide Nimbicidine treatment are also evident in zone II. The arrangement of cells is improper. The size and shape of the cells are reduced. (Fig. 3B) (2A & 3A Untreated) (2B & 3B Treated). A similar fusion of the trophic nuclei has been reported for *Oncopeltus fasciatus* (Bonhag, 1955) and in *Odontopus varicornis*b (Kamalakannan, 1977).

Fig.3 Effect of phytopesticide Nimbecidine on Oocytes

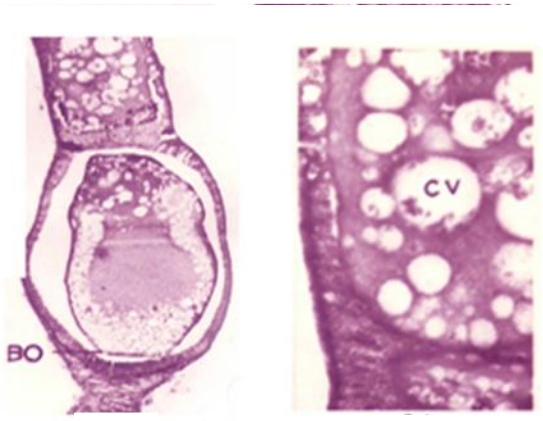


3A 3B

Effect of phytopesticide Nimbecidine on Oocytes

Nimbecidine treatment has altered the histological organization of oocytes in the vitellarium. To begin with, a reduction in the number, shape and size of the primary oocyte is evident. The succeeding oocytes also show several changes. Cytoplasmic vacuolization, occurrence of empty spaces in the pheriperal region were some of the characteristic features noticed in the oocytes (Fig. 4B) The germinal vesicles loose their spherical shape and appear elliptical. The amount yolk material in the ooplasm was also reduced. The condensation of the oocypte material leaves a space between the follicular epithelium and the ooplasm. (Fig. 4A) Mature oocytes appear degenerated and most of them have atrophied. The primary oocyte measures about 0.16mm in length and 0.05mm in width and the fully mature alpha oocyte measures about 0.199mm in length and 0.58mm in width. Studies on the histology of the ovary of *Odontopus varicornis* have revealed that thetrophocte of zone I and zone II of the germarium have distinct cell boundaries. Similar observation has been in *Musca domestica* (Thakur and mann, 1982)

Fig.4 Effect of phytopesticide Nimbecidine on germarium



4A 4A

It has been observed in the twenty day old normal adult *Odontopus varicornis* the oocyte is fully laden with yolk. During this stage the nucleus in the alpha and beta oocytes are large and these have migrated from the centre to the periphery of the ooplasm. A similar observation in the progressive increase in oocyte nuclear size has been reported for insects such as culex fatigans (Tenebrio molitor schottman and Bonhag, 1956).

It has also been observed in *Odontopus varicornis* that oviposition time is delayed due to the treatment with nimbecidine. Similar observation has been done in micro bracon habster (Grosch, 1959) When fed with antimitotic substance. The longevity also reduced in *Odontopus varicornis* due to the nimbecidine treatment. Such an observation has been reported in Housefly (Afifi and Kndfss, 1956); pink boll worm (Adkisson and wellso, 1962); Cole onigla maculate (Atallah and Newson, 1966).

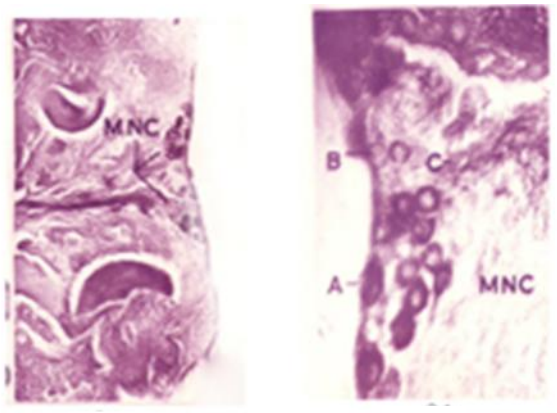
It has also been observed in *Odontopus varicornis* that a reduction in the number of eggs laid is evident after treatment with the Nimbecidine. Likewise the time of oviposition is also delayed. The hatching of the eggs were also not

uniform. The hatched nymphs exhibit perceptible morphological variations.

Neurosecretory cells

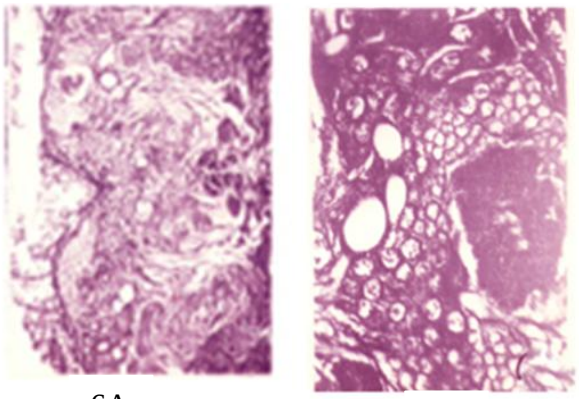
The median group of 45 to 48 cells in the pars intercerebralis region was characterized by the occurrence of three distinct cell types of cells viz., A, B and C. All these three type of cells occur scattered in the middle region (Fig. 5A & 5B). A similar occurrence in the number of 'A' cells have been reported for *Aulacophora foveicoollis, Belostoma indica* (Dogra,1969), Pyrilla perpussilla (Krishnandam and Ramamurthy, 1971).

Fig.5 Effect of phytopesticide Nimbecidine on neurosecretary cells; Changes in the Secretory content



5A 5B

Fig.6 Effect of phytopesticide Nimbecidine on neurosecretary cells; Cytomorphological changes



6A 6B

It was reported in this study that three types of neurosecretory cells namely A, B and C cells are recognized in *Odontopus varicornis* on the basis of their staining properties. The presence of four types of neurosecretory cells have been recognized in the brain of *Schistocerca orecaria* (Highnam, 1961); *Odontopus varicornis* (Kamalakannan, 1977); two types of neurosecretory cells have been recvognizzed in the brain of *Iphita limbata* (Nayar, 1955); *Pyticus marginalis* (Abraham, 1966); and only one type in *Belostoma indica* (Dogra, 1969); *Melanoplus sanguinipus* (Dogra and Ewen, 1970) and *Pyrilla perpusilla* (Krishnanandam and Ramamurthy, 1971).

Effects of Nimbecidine on the median neuro secretory cells

Changes in the Secretory content

All types of median neuro secretory cells, after 72 hours of treatment with the Nimbecidine, exhibit a feeble reaction with chrom alum Haematoxylin phloxin indicating the occurrence of a low concentration of the secretory substances (Fig. 6A).

The median neuro secretory cells of control insects, on the other hand contain moderate concentration of the secretory substance in their cytoplasm.

Cytomorphological changes

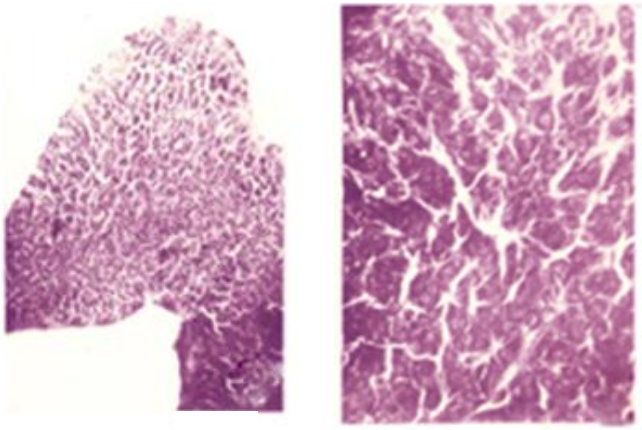
The median neuro secretory cells, treated with the insecticide for 72 hours, exhibit considerable shrinkage in their size. Vacuolization in the cytoplasm has also been noticed. The packed cells, after treatment, become loosely arranged and in certain regions 'A' cells show disintegration. Clumping of chromating material and also the disintegration of nucleolus has been noticed in some cells (Fig. 6B).The median neuro secretory cells of control insects, on the other hand, does not show any histological changes.

Corpus allatum

Effects of Nimbicidine on corpus allatum

Due to the Nimbecidine treatment the colour of the corpus allatum appears some what darker compared to the normal insect. It is reported in glanted in general, the smaller the gland, with closely packed nuclei, reflects its state of inactiveness. Similar observations have been reported by various authors(Nayar, 1956; Engelmann.)

Fig.7 Effects of Nimbicidine on corpus allatum



7A 7B

Histological changes

The cells of the corpus allatum, after 72 hours of treatment, shows signs of vacuolization in their cytoplasm some of the secretory cells have become degenerated. The degenerated cells have pycnotic nuclei and chromat in clumping. In a few cells the nuclei have disintegrated (Fig. 7A).

Affinity to CAHP or AF was less in treated insects compared to untreated controls. The quantity of secretory material localized in the cells of the corpus allatum of treated insects were sparse, even the secretory product exhibit feeble reaction with chrom alum heamatoxylin phloxin and aldehyde fucshin (Fig. 7B).

The cells of the corpus allatum of control insects, on the other hand, exhibit an increase in the concentration of the chromalum haematoxylin phloxin and aldehyde fucshin positive substances in their cytoplasm during the period of 72 hours of treatment.

4.CONCLUSION

On the basis of these findings it is concluded that the application of Phytopesticide Nimbecidine exhibits characteristics histopathological changes in ovarian follicle cells and in neuroendocrine complex. As the Phytopesticide is a powerful neurotoxic agent it affects the 'A' types of median neurosecretory cells in the brain, resulting in the malfunctioning of corpus allatum and the absence of its hormone, inhibits of vitrellogenesis which is observed in the growing oocytes.

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