



Effect of endosulphan on the neurosecretory cells profile of the millipede *Anoplodesmus tanjoricus* (Diplopoda: Myriapodes)

KEYWORDS

Neurosecretory cells, Endosulphan, Millipede, Exposure, Concentration.

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ABSTRACT

LC₅₀ values for the adult millipede *Anoplodesmus tanjoricus* were calculated (0.025 ppm concentration up to 96 hours) after exposure to an organochlorine insecticides endosulphan. The neurosecretory activities of A types of cells. They are differentiation A₁, A₂, A₃, A₄ was observed to be increased at the corresponding concentration for exposure period 24, 48 and 96 hours the cell sizes are increased and 72 hours the cell sizes are decreased. The cytological picture was altered by formation of vacuoles, undulation and distortion of the cell wall in the A cells types. The neurosecretory activity in B cell showed the synthetic activity by 24, 48, 72 and 96 hours, it decreased cell size the cytoarchitecture was disturbed which include clumping of chromatin material, undulation of cell envelop and damage of neurophile. The neurosecretory activity in c cells (C₁ and C₂) showed increased synthetic activity in the 24, 48, and 96 hours and in 72 hours decreased synthetic activity, this may be an initial response in the emergency to pesticidal toxicity.

INTRODUCTION

Endosulphan is a broad spectrum. Non-systemic organochlorine compound of cyclodiene group, extensively used in place of endrin, to control a variety of pests. It is the mixture of two stereoisomers, alpha and beta and later is highly soluble in soil and other media. The chemical nature and solubility of the metabolite, makes endosulphan more potentially toxic to fish and other aquatic organism. The toxicity endosulphan to aquatic invertebrates has been reported. Its effect on oxygen consumption phosphomonoesterases blood glucose and other constituents has been reported. It is known that insecticides cause violent physiological action upon nervous, digestive and reproductive functions of the animals. Uncontrolled release of neurohormones after insecticidal treatment was observed in *Rhodnius prolixus*. Maddrell and Cacida (1971); Maddrell and Reynold (1972) and in *Periplaneta americana*, Garnett and Leeling (1972). Histopathological changes in the neurosecretory cell after insecticidal treatment were reported in *Indonaria caeruleus* Akarte et al. (1982) in *Lamellidens marginalis* and *L.corrianus* Muley (1988) and in *Parreysia corrugates* Thorat (1990). LC₅₀ values for adult prawn, *Macrobrachium kistnesis* were calculated after exposure to an organochlorine insecticide endosulphan. Nagabhushnam; Hanumante, Mirajkar and Kulkarni (1979). The neurosecretory activity of A type of cells was observed to be increased of the corresponding concentration for all exposure period except 3, 6, 12, 24, 48, 72, 96, 7 days and 15 days. The cytological picture was altered by formation of vacuoles, undulation and distortion of cell wall. The neurosecretory activity of B cells showed an initial increase in the synthetic activity by 6 hours, but it increases after acute and chronic treatment up to 96 hours, and 15 days respectively. The cytoarchitecture was disturbed which include clumping of chromatin material.

MATERIALS AND METHODS

Millipede were collected near the Amravati (M.S.) India. The animals were brought to the laboratory and maintained in glass aquaria filled with moist soil containing cowdung and fed with leaf litter carrot, cabbage and cucumber etc. Millipede were acclimatized in the laboratory and maintained at temperature 26°C-28°C. The fully grown millipede used in experimentally work. The animal were divided into two group one kept as control and another for toxicology studies the specimens of millipede segregated into a small glass jar.

Healthy millipede were selected for experiments, millipede were fed on endosulphan treated cucumber (20-25gms) and for each 1.06, 0.42, 0.097, 0.025 ppm the test solution was changed after every 24 hours. The survival percentage for endosulphan was recorded after 24, 48, 72, 96 hours of exposure only actively feeding millipede where choose for experimental and no food was offered 48 hours before the start to experiment to avoid any change in the toxicity to pesticide by excretory product. At the end of 24 and 96 hours exposure the living millipede were removed from test medium along with control and their cerebral, visceral and Suboesophageal ganglia were removed and fixed in aqueous Bouin's fluid for 24 hours. All the ganglia processed as per usual microtechnique method and serial section were cut at 8-10µ thickness and were stained with chrome alum haematoxyline phloxine stain (Gomori 1941). Nuclear as well as cell diameter from control and experimental animals was measured. The average of cell and nuclear diameter of each type of neurosecretory cell was calculated and the ratio of cell diameter to nuclear diameter (C/N) was calculated according to method given by Sarojini and Mirajkar (1982).

OBSERVATION AND RESULTS

Millipede *Anoplodesmus tanjoricus* effect of endosulphan showed greater amount of discharge of neurosecretory material in comparison with control. Nuclei of neurosecretory cells of treated millipede displayed enlargement over those of control and scanty neurosecretory material was spotted in their perikarya and axon. The (C/N) ratio showed pronounced alteration, staining property and neurosecretory activity were also affected. Pesticidal stress, severally affected the pyriform cells as compare to oval cells in brain, when compared with of control. Changes in histomorphology of subtypes of A and C cells are found to be more or less similar. The cell size and nuclear size of all A and C cells was found to be increased up to 48 hours and decreased in 72 hours and again increased 96 hours of exposure. Reverse changes were noticed in histology of B cell of the brain. There size was found to be decreased up to 96 hours of exposure. Alteration in A₁, A₂, A₃ and A₄ neurosecretory cells of millipede *Anoplodesmus tanjoricus* exposed to 0.025 ppm of endosulphan for 24, 48, 72, and 96 hours. The neurosecretory cells of cerebral ganglia were disturbed to great extent. They show large number of vacuoles in the cell perikarya along with loss in compactness, acute cellular degeneration, clumping of chromatin

material, undulation of cell envelope and loss in damage of neuropile .The C/N ratio decreased and it is inversely proportional to rate of synthesis.

In the B cell the chromatin material was affected and was not intact but pesticide caused clumping of chromatin material in B cell. During long term exposure, formation of vacuole as well as undulation and distortion of cell wall was observed. The C/N ratio increased and it is inversely proportional to rate of synthesis.

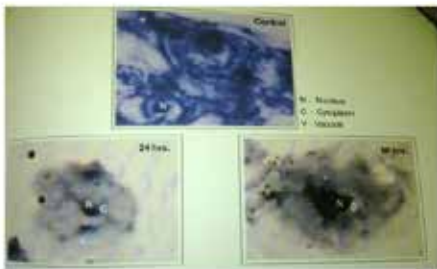
In C₁ and C₂ cells, there was a significant increase in cell as well nuclear diameter. The C/N ratio decreased and it is inversely proportional to rate of synthesis. The neurosecretory activity which includes synthesis as well as transport of neurosecretory material was thus accelerated under the influence of endosulphan. The chromatin material inside the nucleus was intact but the cell wall and neuropile region was slightly distorted and vacuoles were visible.

DISCUSSION

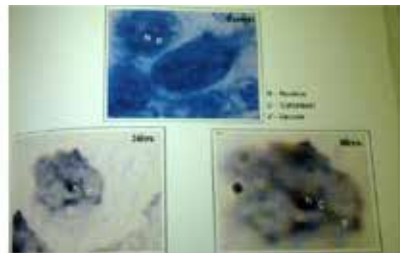
In the present investigation the effect the endosulphan on the oval, pyriform, spherical cells from cerebral , visceral, suboesophageal, ventral ganglion were studied in relation to pesticide . Number s if worker (Nanda.1974; Nagabhushan et.al.,1892) have observed various histopathological changes like vacuolization in the perikarya, undulation of the cell boundries, clumping of the chromatin material, altered compactness of neurosecretory cells, alteration of nuclear and cytoplasmic area. staining properties and neurosecretory activity of NSCs.

The identification of the histological section of the NSCs found in the brain Visceral, suboesophageal and ventral ganglion reveals some interesting features. In arthropods it is a well established fact that neurosecretory products function as neurohormones which synchronize various physiological activities. This is further strengthened by the facts that one or more types NSCS may be responsible for the increase in the size of the reproductive and associated structures.

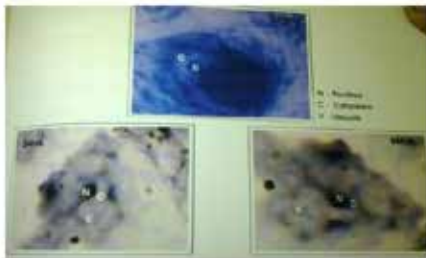
In the present investigation it was observed that pesticidal stress altered C/N ratio drastically. Study of C/N ratio NSCs is the best parameter to know the responses of the cells to the pesticidal stress. It was also observed that in the initial stage of poisoning the C/N ratio was decreased, indicating increases in synthetic activity. An enhanced synthetic activity may be correlated to extent of pollution stress and to maintenance of homeostasis in the internal environment. However , as exposure period was increased , the C/N ratio was increased over the control NSCs. This indicates that long duration of pesticidal stress hampared the synthetic activity of neurosecretory cells. The functional status of neurosecretory element is linked with changes in the size of the nucleus and nucleolus and may be considered as the index of cell activity (Ortman, 1960 and Ghosh et al., 1968). In the present investigation it was noted that the areas of nucleus and nucleolus is altered. The chromatin material in the nuclei of the neurosecretory cells treated with pesticides, become so immobilized after clumping that it was unable to act with other cellular constituents.



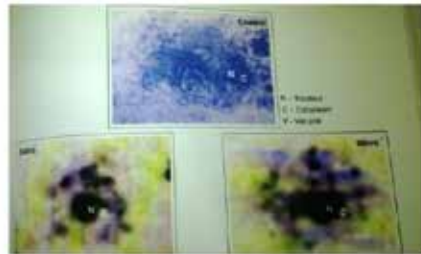
Photographs of A₁ neurosecretory cell of cerebral ganglion of control and endosulphan treated (0.025ppm) mullipede for 24 and 96 hrs. 1000 x



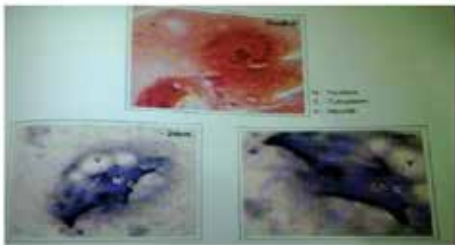
Photographs of A₂ neurosecretory cell of cerebral ganglion of control and endosulphan treated (0.025ppm) mullipede for 24 and 96 hrs. 1000 x



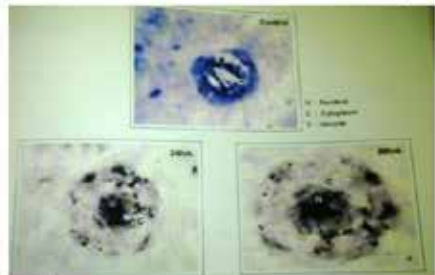
Photographs of A₃ neurosecretory cell of cerebral ganglion of control and endosulphan treated (0.025ppm) mullipede for 24 and 96 hrs. 1000 x



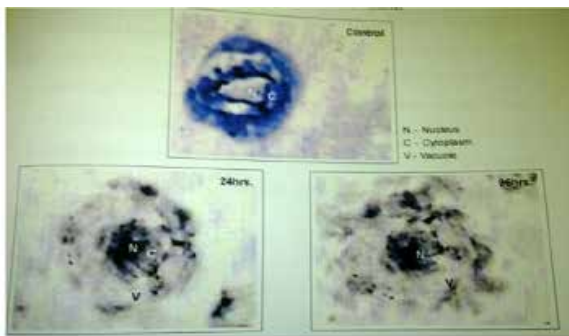
Photographs of A₄ neurosecretory cell of cerebral ganglion of control and endosulphan treated (0.025ppm) mullipede for 24 and 96 hrs. 1000 x



Photographs of B neurosecretory cell of cerebral ganglion of control and endosulphan treated (0.025ppm) mullipede for 24 and 96 hrs. 1000 x



Photographs of C₁ neurosecretory cell of cerebral ganglion of control and endosulphan treated (0.025ppm) mullipede for 24 and 96 hrs. 1000 x



Photographs of C₁ neurosecretory cell of cerebral ganglion of control and endosulphan treated (0.025ppm) millipede for 24 and 96 hrs. 1000 x

After endosulphan exposure in *Anoplodesmus tanjoricus* the cell and nuclear size in A and C cells was increased at initial stage followed by decrease in middle stage and again increase at the final stage. The chromatin material of these cells was intact and almost unaffected it was interesting to note that reverse changes were noticed in *Anoplodesmus tanjoricus* in B cell, after endosulphan exposure cell and nuclear size was decreased. The chromatin material of B cell was significant as there was formation of big vacuoles as compared to A and C cells. The alteration noticed in the neurosecretory cells of *Anoplodesmus tanjoricus* during present study are almost similar to that reported in Prawn, *Macrobrachium kistnesis*, Mirajkar and Sarojini (1985).

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