INRODUCTION

Pesticides currently in use involve a wide variety of chemicals. Insecticides which can be neurotoxic to humans represent the greatest proportion of pesticides used in developing countries because of their relatively cheaper cost. The major occupational populations at risk are workers in agriculture. Furthermore, pesticide residue, carcinogenicity, and endocrine-disrupting chemicals have recently become major concerns in the world.

The nervous system and endocrine system integrate so as to provide a response to changing environment and biochemical processes. Since nervous system is the sole sensing arm of the integrated reflex, the endocrine organs can be referred as effectors unit of the nervous system. The link between endocrine and nervous system is referred to as central integration and in both vertebrates and invertebrates it consists of the doubly specialized neurosecretory cell (NSC) (Bern, 1963). Fifty percent of the NSCs of the nervous system are composed of non-excitable satellite ganglia cells which are packed between and around neurons. The arrangement of neurons into ganglia allows an increase in number of synaptic contact for many particular cells and thus increases the integrative capacity of a number of neurons.

A systematic scrutiny of publications on neurosecretion has revealed that studies on the neurosecretory system of mollusca were initiated by Scharrer in 1935. Since that time onward number of molluscan species has been reported to exhibit some sign of neurosecretion. There are number of compounds which have potent effect on molluscan neurons. Several neural transmitters have been studied in number of molluscan species
and there is considerable similarity among different species (Zeimal and Vulfins, 1975), Kulkarni (1982) studied in detail the effect of different parameters such as temperature, salinity, neurotransmitter and tranquilizer on the CNS of the slug *Lavicaulis alte*.

Majority of the neurons secrete chemical substances called neurohumours or neurotransmitters which transmit the impulse through synaptic cleft and are then immediately destroyed. Neurons have similar properties throughout the animal kingdom, although their morphology and arrangement may vary. Hagadron (1967) and Highnam and Hill (1978) in their reviews stated that cytological display of neuronal secretory activity is indication of vigorous biosynthetic activity which is said to be associated with the formation of exoplasmic protein. The way in which nervous system operates may differ considerably between animal, depending on the number of animals, depending on the number of nerves involved and the individual shape, size and spatial arrangement of the component. Fifty percent of the NSCs of the nervous system are composed of non-excitable satellite glia cells which are packed between and around neurons. Lubet (1955a) and Umiji (1969) suggested that they transport neurosecretory substances. Leak and Walker (1980) stated that these cells provide physical support for neurons and can modify action of nerve cells as well as act as barrier or reservoir for ions, metabolites and transmitters. The arrangement of neurons into ganglia allows an increase in number of synaptic contact for many particular cells and thus increases the integrative capacity of a number of neurons.

The secretory and transporting nature of chemicals of neurons was discovered by Dahlgren (1914) in the spinal cord of sharks. These observations were later confirmed by Speidel (1919) on Skates. Scharrer (1928) reported the occurrence of secreatory cells like
NSCs in the hypothalamus of vertebrates using staining affinities. Many investigators then began to pay attention to the neurosecretory phenomenon in a wide variety of animals. In invertebrates several investigators reported the occurrence of NSCs; Cheat (1966) in echinoderms, Gabe (1966) in annelids, Highnam and Hill (1978) in insects reported the presence of NSCs. In mollusca, neurosecretory cells were first discovered by Scharrer (1935) in the cerebral and visceral ganglia of *Aplysia limacine* and *Pleurobrancea*. Perusal of literature reveals that the aspect of neurosecretion in bivalve molluscs has been reviewed by Gabe (1965, 1966), Lubet (1966, 1973), Martoja (1972) and Golding (1974). The development of the subject has been hampered by the presence of shell, very small ganglia in small bivalves, diffused distribution of NSCs and by the ignorance of the chemical nature of the neurohormones.

Amongst invertebrates, mollusca show great variability in their nervous system, ranging from primitive arrangement in chiton to the complex mass of fused ganglia farming the “brain” of cephalopods. Pharmacological and physiological aspect of mollusca using effector organs has received considerable attention (Huddert, 1955, 1975; Bayne, 1976). Evidence for the occurrence of a wide variety of neurotransmitters in different tissue of bivalve mollusks including the nerve ganglia, has been discussed from the functional point of view by Leak and Walkar (1980). Gabe (1955) reported the presence of neurosecretory cells in 20 marine Lamellibranch mollusks. Lubet (1955) described the relationship between neurosecretion and sexual cycle in *Mytilus* and *Chlamys*. Among fresh water bivalves, Fahrmann (1961) described two types of neurosecretory cells in *Unio tumidus* in its cerebral, visceral and pedal ganglia, while Baranyi and Solanki (1963) observed three types of neurosecretory cell in the three
ganglia of *Anodonta cygnea*. Antheunisse (1963) reported the neurosecretory cell in all three ganglia of *Dreissena polymorpha*. Nagabhushanum (1963) studied the neurosecretory cell in the oyster *Crassostrea virginica* and in the mussel, *Modiolus demissus*. Muley (1988), Thorat (1990) and Waykar (1998) observed two types of cells, cell type I and cell type II in fresh water bivalves *Lamillidens corrianus, Parreysia corrugata* and *Parreysia cylindrica* respectively.

The cell types indentified by several staining techniques appear to have different types of elementary granules. Transport of these granules to neurohaemal areas and release phenomenon (exocytosis) give further indication of their neurosecretory nature. By Alcian blue/Alcian yellow staining technique seven types of neurosecretory cells have been described in *Lymnea stagnalia* (Boir et al., 1977) and three types of NSC’s, in *Helix aspera* (Kaikai and Kerkut 1979). Lever (1957) indentified five cell types in *Ferrissia* species on the basis of size, staining reaction and condition of neurosecretory material in the cerebral ganglia of *Patella vulgate*; two types of NSC’s have been described by Choquet and Lenaire, 1969. Nagabhushanam and Lomte, 1972 identified two types of (A and B) of NSC’s in *Parreysia corrugata*. Recently Motavkin et al., 1988 have classified neurons in ganglia of Japanese Scallop, Mizuhopecten, Yessoensis (Jay) on the basis of morphological, morphometrical and histochemical method into three types large neurons are cholinergic, smaller neurons type I are peptidergic and smaller type II are monoaminergic.

In all major animal groups besides normal neuron, some other nerve cell remarkably of different appearance is called NSC’s. These cells in addition to displaying the cyrtological features common to all neurons generally show prominent indication of
grandular activity, with the light microscope. They are characterized by presence of abundant secretory material in their perikarya. This material is also in the axons which often end blindly adjacent to muscular space rather than innervating their target structure directly. These blindly ending terminals of storage release centre and in the more advanced group such as crustacean, these are termed as neurohaemal organ by Knowles and Carlisle (1956). Well known neurohaemal organ of insects are Carpora cardiaca in crustaceans the sinus glands while in vertebrates the pars nervosa.

The number and location of NSCs vary among different species and the cells are predominantly present in cerebra ganglia in highly evolved bivalves, NSCs are less numerous and more localized. Their location in cerebral ganglia has been reported by Lubet (1955a), Nagabhushanam (1963, 1964), Nagabhushanam and Lomte (1972), Nagabhushanam and Mane (1973) and Nagabhushanam and Kulkarni (1983). A close relationship between neurosecretion and sexual cycle in Dreissena polymorpha (Fahrmann, 1961), Crassostrea virginica (Nagabhushanam, 1963), Katelysia opima (Nagabhushanam and Mane, 1973), Indonaia caeruleus (Pillai, 1984), Lamellidens corrianus (Muley, 1988), Parreysia corrugata (Throat, 1990) and Bhamre (1993) P. favidence and Deshmukh (1995) Parreysia corrugata has been established. A close relationship between secretory activity of NSCs from cerebral ganglia with maturation and release of gamets from gonad has been observed by most of the investigators. However further studies carried out by Herlin-Houtteville and Lubet (1974). Lubet et al., (1976), Whittle et al. (1983), Rao (1988) and Muley (1988) suggested that both cerebral and visceral ganglia in bivalve are responsible for regulation and breakdown of reserve
material and gamete maturation. These authors attributed this to role of neurosecretions from the ganglia.

It is known that insecticides cause violent physiological actions 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 *Periplaneta americana* (Granett and Leeling, 1972). The effects of drugs like acetylcholine and adrenaline on the neurosecretory activity of the oligochaete worm *Perionyx excavates* were studied by Nagabhushanam and Hanumante (1977). Various histopathological changes in the neurosecretory cells after insecticidal treatment were observed in *Odontopus vericornis* by Sabesan and Ramalingam, (1979). Mane *et al.*, (1979) had studied the effect of pesticides and narcotants on bivalve mollusca, *Katelysia opima* and *Donax gcueneatus*. Jadhav (1980) reported the effect of stress factor like temperature, salinity, starvation, desiccation and neurohumours like 5-hydroxy tryptamine, acetylcholine and adrenaline on respiratory activity of the fresh water bivalve, *Lamellidens corrianus*. Akarte *et al.*, (1982) studied the effect of commercial and technical grade malathion on cerebral ganglia of fresh water bivalve, *Indonaia caeruleus*. Nagabhushanam *et al.*, (1982) studied the impact of organophosphates on neurosecretory cells in the cerebral ganglia of fresh water prawn, *Caridina weberi*. Sarojini and Mirajkar (1982) studied the effect of organophosphorus insecticide dimicron on the neuroprofile (brain) of the fresh water prawn, *Macrobranchium kistnensis*. Bodhankar (1984) has studied the effect of pesticides exposure on neurosecretory activity of *Laevicaulis alte* by using pesticides viz. malthion, hygro sevimol, thioden and copper sulphate. Akarte (1985) also has studied the effect of organophosphorus insecticides on bivalve mollusca. Muley (1985) have worked out

Neurohormones from pleuro visceral ganglia have been shown to regulate respiration in gastropod mollusc reported by Hanumante *et al.*, (1980). The role of nerve ganglia in respiration of estuarine clam, *Katelysia opima* was showed by (Mane *et al.*, 1990) and effect of cerebral ganglia removal on the rate of respiration on the fresh water bivalve also reported by Kulkarni (1987) on *Indonaia caeruleus*.

There is no account of the effect of pesticides, indoxacarb and thiamethoxam on neurosecretory cells of the fresh water bivalve, *Parreysia cylindrica*. Hence the present investigation was under taken to study the effect of pesticides like, indoxacarb and thiomethaxin on neurosecretion of the bivalve *Parreysia cylindrica*. 
MATERIALS AND METHODS

Medium sized fresh water bivalve, *Parreysia cylindrica* (50-55mm in shell length) were collected from Jamda dam 30 kilometers away from Chalisgaon, district Jalgaon (M.S., India). Freshly collected bivalves were immediately brought to the laboratory and kept in large glass aquaria. The shells were brushed to remove fouling biomass and mud. They were acclimatized to the laboratory conditions in dechlorinated tap water for four to five days. The air temperature was 31.25°C ± 2.2173°C, water temperature was 27.75°C ± 2.2173°C and its pH was 7.06 ± 0.1699. Since the animals are micro feeders, no special food was supplied during the experiment.

To study the effect of pesticides, indoxacarb and thiamethoxam on the neurosecretory cells of fresh water bivalve, *Parreysia cylindrica*, and the bivalves were exposed to median lethal dose (LC50/2 ppm of 96 hrs) for acute treatment and chronic dose (LC50/10 ppm of 96 hrs) for chronic treatment.

A) **Acute pesticide treatment**:

The acclimatized active bivalves were divided into three groups, one of them was considered as control and the remaining two were considered as experimental. Experimental groups were exposed to median sublethal dose (LC50/2 ppm of 96 hrs) of pesticides i.e. indoxacarb (0.3905ppm), thiamethoxam (14.2114 ppm) respectively upto 96 hours. After 24 and 96 hours treatment, the living bivalves were removed from each test medium along with control and their cerebral, visceral and pedal ganglia were fixed in aqueous Bouin’s fluid for 24 hrs.
B) Chronic pesticide treatment:

The acclimatized active bivalves were divided into three groups, one of them was considered as control and the remaining two were considered as experimental and were exposed to chronic concentrations of pesticides upto 21 days. The concentrations of pesticides (LC$_{50/10}$ ppm of 96 hrs) used, were 0.07811ppm and 2.8422 ppm, for indoxacarb and thiamethoxam respectively. After 7 days and 21 days, the living bivalves were removed from these medium and their cerebral, visceral and pedal ganglia were fixed in aqueous Bouin’s fluid for 24 hrs.

The cerebral, visceral and pedal ganglia of control and experimental (acute and chronic) bivalves were dehydrated in alcohol, cleared in toluene and embedded in paraffin wax (58-60°C). The serial sections were cut at 5µ and stained with Mallory’s triple stain to study the neurosecretory cells in control and experimental animals.
OBSERVATIONS AND RESULTS

Cytological examinations of the sections of cerebral, visceral and pedal ganglia stained with Mallory’s triple stain revealed the presence of group of neurosecretory cells which are cytologically different and larger than ordinary ganglion cells. The chromatin material of control NSCs was intact. They were provided with definite regular envelope and neuropile. Most of the large cells possess large nuclei and abundant cytoplasm, their perikarya and axons are filled with fine granules and showed moderate synthetic activity, which stained conspicuously. Histologically, two types of neurosecretory cells have been distinguished in the ganglia. The size, general shape of the cell body, presence or absence of vacuoles in the cytoplasm and staining properties of the secretory material were used as the basic criteria in distinguishing the type of the NSCs. Figure 1 shows two types of neurosecretory cells in Parreysia cylindrica these are

Cell type I (A cell) or Pyriform cells:

These cells are pyriform in shape ranging from 15 to 18µ in length and 8 to 10 µ in width. The nucleus is round or oval measuring 4 to 8 µ in diameter, it may be either central or eccentric in position. Fine granule of secretory material is present in the cytoplasm. Some I type cells are larger in size in visceral ganglia but smaller in size in pedal ganglia. The nucleus generally contains a large nucleolus but in certain cases and particularly in pedal ganglia 2 or 3 nucleoli appear inside the nucleus. These cells are present in all the ganglia.
Cell type II (B cell) or Oval cells:

These cells are smaller than type I cells and are oval or round in shape, measuring about 9 to 11 µ in diameter. The nucleus is large vesicular and measure about 10 to 13 µ in diameter. Generally nucleus has got one large nucleolus, but 2 to 3 nucleoli are sometimes noticed.

Effect of pesticides on neurosecretory cells in *Parreysia cylindrica*:

Effect of indoxacarb intoxication on cerebral ganglia: (Fig.a and b of plate II):

When compared to control, indoxacarb exposed, neurosecretory cells of cerebral ganglia showed many histological alterations in cell type I and II. After 24 hrs of exposure, the cell and nuclear diameter was increased, also intensity of neurosecretory material was increased. This showed enhancement of synthetic activity. At this stage the rare of transport of secretory material along with axon was very low, compared to rate of synthesis, which causes accumulation of secretory material in axon hillock in pyriform cells.

After 96 hrs of exposure the size of pyriform cell was shrunken and elongated and showed vacuolization and condensation of secretory material. The nuclear diameter was decreased as compared to control. Thus synthetic activity was lowered. Along with these changes, the changes like shrinkage of nuclei, undulation of cell envelope, vacuolization, and change in shape of nucleus becoming oval shaped, and nucleolus became smaller and clumping of chromatin material was observed. After acute exposure, the oval cell diameter and nuclear diameter were increased, the neurosecretory material intensity was
increased thus showing provoked synthetic activity. Neurosecretory material was accumulated and the cells became bulky.

After 7 days of chronic exposure, the cell diameter and nuclear diameter were increased, the intensity of neurosecretory material was increased in pyriform cells indicating enhanced synthetic activity. At this stage the rate of transport must be very less compared to the rate of synthesis, which causes accumulation of secretory material in cells which appear bulky.

After 21 days of exposure pyriform cells elongated, nucleus became smaller showing lowered synthetic activity. Along with these changes, the changes like, vacuolization, clumping of chromatin material and damage of cell envelope were observed. After chronic exposure, oval cell size was increased indicating increased synthetic activity.

**Effect of thiamethoxam: (Fig. c and d of plate II)**

Neurosecretory cells of cerebral ganglia showed many cytomorphic alterations after thiamethoxam stress as compared to control. Neurosecretory cells of cerebral ganglia were damaged when subjected to acute exposure of thiamethoxam. Both pyriform and oval cells were increased in size. Pesticide stress causes enlargement of cells and undulation of cell envelop. Particularly cell type I (Pyriform cell) showed vacuolization, neurosecretory material was clumped and accumulated in cytoplasm and also near the axon hillock. After chronic exposure, neurosecretory cells were shrunken and became smaller in size. The perikarya showed vacuolization and condensation of secretory material.
Visceral ganglia:

Effect of indoxacarb: (Fig. a and b of plate IV)

The neurosecretory cells of visceral ganglia showed many cytomorphic changes after pesticide stress in pyriform and oval cells. After 24 hrs of exposure, the size of the pyriform cell and nucleus was increased as compared to that of control. The intensity of neurosecretory material was increased, showing increased synthetic activity in pyriform cells along with these changes, neurosecretory cells showed, damaged neuropile and vacuolization. The neurosecretory material was accumulated in axon hillock. After 96 hours exposure pyriform cells elongated, the size of cell and nucleus were decreased, nuclei become shrunken, the synthetic activity was decreased and cell became empty. This shows that synthetic activity was lowered as compared to the rate of transport which causes complete drainage of secretory material. Along with these changes, the pesticide stress caused undulation of cell wall, vacuolization and damage of chromatin material, and neuropile. After 7 days of exposure, the pyriform cell become narrow and elongated, the size of nucleus was increased. The neurosecretory material was condensed in cytoplasm. However after 21 days of exposure nuclear size was decreased, showing decreased synthetic activity in pyriform and oval cells.

Effect of thiamethoxam: (Fig.c and d of plate IV)

Neurosecretory cells from visceral ganglia of thiamethoxam exposed bivalves showed many morphological changes in pyriform and oval cells. When subjected to acute thiamethoxam treatment, neurosecretory cells of visceral ganglia showed enlargement of cell type II and elongation of cell type I, the intensity of neurosecretory material was
increased. This indicates enhanced synthetic activity. At this stage the rate of transport of neurosecretory material must be very low as compared to rate of synthesis, which causes accumulation of secretory material in cells making them bulky and there was vacuolization as well as undulation of cell envelop. After 96 hours of exposure, pyriform cells became elongated showing clumping of chromatin material, accumulation of neurosecretory material and vacuolization.

After chronic treatment, the cell size was increased but the nuclear size was decreased in both cells. Thus synthetic activity was lowered. After 7 days of exposure, pesticide stress caused changes like undulation of cell envelope, damage of neuropile, clumping of chromatin material and vacuolization in pyriform cells. After 21 days of exposure, pyriform cells became elongated and neurosecretory material was accumulated around the nucleus. The pyriform cells showed the vacuolated texture. The oval cells showed changes like, vacuolization, damage of chromatin material and nucleolus became smaller.

**Pedal ganglia:**

Effect of indoxacarb: (Fig a and b of plate VI)

In the acute exposure to indoxacarb, NSCs of pedal ganglia showed some morphological changes in the pyriform and oval cells. When exposed to indoxacarb neurosecretory cells showed marked reduction in their size. The pyriform cells became narrow and taller, oval cells became shrunken. The neurosecretory material was accumulated in cytoplasm. The perikarya became highly vacuolated; cell envelope and neuropile were damaged. The nucleus became oval in shape, nucleolus became smaller, and the chromatin material was condensed. After chronic exposure along with changes in
the acute exposure, the changes like vacuolization in cytoplasm, smaller nucleolus, less chromatin material, with vacuoles appearing in the nucleus were observed.

**Effect of thiamethoxam : (Fig c and d of plate VI)**

Thiamethoxam exposed neurosecretory cells of pedal ganglia showed some morphological changes in pyriform and oval cells. When subjected at 24 hours the cell and nuclear size was increased. This indicates increase in synthetic activity of the pyriform and oval cells. After 96 hours of exposure cells and nuclear size was decreased, thus synthetic activity was lowered. Along with these changes, the changes like vacuolization, clumping of chromatin material, and shrunken nuclei were also observed.

After chronic exposure, along with changes of acute exposure the changes like vacuolization, damage of chromatin material, undulation of cell envelope were observed.

Thus during the present study it has been observed that in the initial stage of toxication (at 24 hours of exposure) there was increase in synthesis of secretary material and its accumulation in cells, but as the exposure period was prolonged, a gradual release of secretary material was observed which become vigorous at further prolongation of exposure leading to complete drainage of secretary material from neurosecretory cells.
PLATE –I

Microphotographs of cerebral ganglia of control bivalve *Parreysia cylindrica*
showing neurosecretory cells X 1000

**Abbreviations:**

AX - Axon

N - Nucleus

NU - Nucleolus

NM - Neurosecretory material
PLATE –II

Pesticide induced changes in neurosecretory cells of cerebral ganglia in *Parreysia cylindrica* X1000

a) Microphotographs of cerebral ganglia from bivalve *Parreysia cylindrica* exposed to indoxacarb for 96 hours

b) Microphotographs of cerebral ganglia from bivalve *Parreysia cylindrica* exposed to thiamethoxam for 96 hours

c) Microphotographs of cerebral ganglia from bivalve *Parreysia cylindrica* exposed to indoxacarb for 21 days

d) Microphotographs of cerebral ganglia from bivalve *Parreysia cylindrica* exposed to thiamethoxam for 21 days

Abbreviations:

A  - A cell
B  - B cell
AX - Axon
N  - Nucleus
NU  - Nucleolus
NM - Neurosecretory material
V  - Vacuole
PLATE –III

Microphotographs of visceral ganglia from control bivalve Parreysia cylindrica showing neurosecretory cells X 1000

Abbreviations:

A - A cell
AX - Axon
N - Nucleus
NU - Nucleolus
NM - Neurosecretory material
PLATE –IV

Pesticide induced changes in neurosecretory cells of visceral ganglia in Parreysia cylindrica X1000

a) Microphotographs of visceral ganglia from bivalve Parreysia cylindrica exposed to indoxacarb for 96 hours

b) Microphotographs of visceral ganglia from bivalve Parreysia cylindrica exposed to thiamethoxam for 96 hours

c) Microphotographs of visceral ganglia from bivalve Parreysia cylindrica exposed to indoxacarb for 21 days

d) Microphotographs of visceral ganglia from bivalve Parreysia cylindrica exposed to thiamethoxam for 21 days

Abbreviations

B - B cell
N - Nucleus
NU - Nucleolus
NM - Neurosecretory material
Microphotographs of pedal ganglia from control bivalve, *Parreysia cylindrica* showing neurosecretory cells X 1000.

**Abbreviations:**

A - A cell  
N - Nucleus  
NU - Nucleolus
PLATE –VI

Pesticide induced changes in neurosecretory cells of pedal ganglia in *Parreysia cylindrica* X1000.

a) Microphotographs of pedal ganglia from bivalve *Parreysia cylindrica* exposed to indoxacarb for 96 hours

b) Microphotographs of pedal ganglia from bivalve *Parreysia cylindrica* exposed to thiamethoxam for 96 hours

c) Microphotographs of pedal ganglia from bivalve *Parreysia cylindrica* exposed to indoxacarb for 21 days

d) Microphotographs of pedal ganglia from bivalve *Parreysia cylindrica* exposed to thiamethoxam for 21 days

**Abbreviations:**

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<thead>
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<td>A</td>
<td>-A cell</td>
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<td>NU</td>
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DISCUSSION

It is well known that neurosecretory system controls the physiological processes and the pesticide stress interferes with the normal functional processes and ultimately an imbalance in the hormonal system is created (Nagabhushanam et. al., 1982).

In the present investigation, the effects of two pesticides, indoxacarb and thiamethoxam on the neurosecretory system of the medium sized bivalve, Parreysia cylindrica were studied. Cell type I and cell type II NSCs from cerebral, visceral and pedal ganglia were studied in relation to pesticide stress photomicrographs revealed that there was, elongation of pyriform cells enlargement of oval cells, vacuolization, acute cellular degeneration, clumping of chromatin material, undulation of cell envelope, damage of neuropile, and pronounced changes in the nucleo-cytoplasmic ratio. The staining properties as well as neurosecretory activity of neurosecretory cells were also drastically altered.

Gundevia and Ramamurthis (1972) have first of all observed various histopathological changes like vacuolization in the perikarya, undulation of the cell boundaries, and clumping of the chromatin material in the neurosecretory cells of Hydrophilus olivaceous when exposed to different pesticides like dimecron, diazinon and dieldrin. Nanda (1974) studied the effect of endrin and sumthion on the brain neurosecretory cell of Periplaneta americana and reported the impairment in both inter and intracellular structure of neurosecretory cells and also noted the various grades of disturbances in the compactness of neurosecretory elements and undulation in the periphery of cell boundaries along with the appearance of small to large number of vacuoles inside the perikarya.
Similar changes were also recorded by Hanumante et al. (1979) in snail *Indopanorbis exustus* under pesticides stress. Nagabushanam *et al.*, (1982) studied the impact of organophosphates on neurosecretory cells in the cerebral ganglia of fresh water prawn, *Caridina weberi* and reported different cytomorphic changes like vacuolization, degenerative changes in the neurosecretory cells and the neuropilar tissue, such as undulation of the cell boundaries, loss of compactness and necrosis. Sarojini and Mirajkar (1982) studied the effect of organophosphorus insecticide dimicron on the neuroprofile (brain) of fresh water prawn, *Macrobranchium kistnensis* and reported different histopathological changes like vacuolization in cytoplasm, undulation of cell wall and clumping of chromatin material. Thorat (1990) studied the impact of pesticides and heavy metals on the bivalve, *Parreysia corrugate* and reported different histopathological changes such as vacuolization, cellular degeneration, and alterations in cytoplasmic and nuclear areas and altered staining properties and neurosecretory activities of NSCs. These alterations are in agreement with the present study.

In the present study, short treatment enhanced synthetic activity with accumulation of neurosecretory material and after prolonged treatment inhibition of synthetic activity and the depletion of neurosecretory material in the cells were observed. These results are in agreement of with the previous findings (Nanda, 1974; Nagabhushanam *et al.*, 1982; Mirajkar and Sarojini, 1985; Patil, 1986: Muley, 1988, Thorat, 1990 and Deshmukh, 1995).

In the present study, it was observed that in the initial stage of poisoning there was a gradual synthesis of secretory material but as the incubation period was prolonged a gradual release of secretory material started. This discharge became more vigorous and
finally the entire secretory material got drained off. Sabesan and Ramalingam (1969) reported the accelerated synthesis after short duration and release of secretory material after long duration of the endosulfan intoxication in the median neurosecretory cells of *Odontopus varicornis*. Various histopathological changes were observed for the first time in the neurosecretory cells of *Hydrophilus olivaceous* after treating them with dimecron, diazion and dieldrin (Gundevia and Ramamurthi, 1972). In this case it was shown that, short exposure periods with these insecticides trigger the synthetic activity of the neurosecretory cell of brain. Sarojini and Mirajkar, (1982) reported that after acute exposure neurosecretory activity was increased while decreased after chronic exposure. The initiation of synthesis, its gradual acceleration and ultimately accumulation of secretory material by pesticides is indicative of the fact that the accelerated pace of synthesis may be an initial response to the emergency caused by the pesticide action.

The functional status of the neurosecretory elements 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). Thus the chromatin material in the nuclei of the neurosecretory cells, treated with pesticides, became so immobilized after clumping, that it was unable to act with other cellular constituents and it was possible that in this state the DNA content of such nuclei became quite diminished leading to a loss in production of an optimum amount of RNA. In this way the inhibition synthetic activity after prolonged incubation period may be assumed as a failure of the RNA synthetic machinery which inhibits the further synthesis of secretory material (Ghosh *et. al.*, 1968).

Highnam and Hill (1969) observed that the activity of neurosecretory cells is affected by three factors.
1) Rate of synthesis of secretory material.

2) Rate of transport of secretory material along with axons and,

3) The rate of release of transported material into the circulatory system.

The comparative study of alterations in the cytoarchitecture of NSCs due to different types of pesticide stress revealed that all the pesticides did not show the same effect because no pesticides is specific in its action and they vary greatly in their toxicity and persistence (Moore, 1969).

Accumulation of secretory in the NSCs of *Samiacynthia ricini* and *Poekilocerus pictus* due to application of pesticides like BHC and endrin was noted by Srivastava (1981). Similar result due to different pesticide stresses were also observed by Nagabushanam *et al.*, (1982); Sarojini and Mirajkar (1982); Mirajkar and Sarojini (1985) and Nagabushanam and Reddy (1985).

Cooke (1977) suggested a working hypothesis. The pesticides like, lindane acts on neurosecretory cells and other neurons by rendering the plasma membrane very permeable to Ca++. The role of Ca++ ions is linked with the stimulus and it was studied in detail by Fingerman *et al* (1977).

Methomyl and methiocarb are carbamate pesticides were known to act as nerve poisons by the inhibition of cholinesterase (Matsumura 1985; Eldefrawi and Eldefrawi 1990). They were also shown to alter the activity of other nonspecific serine-containing enzymes or nonenzymatic biochemical constituents of land snails (Radwan and Salama 1999; Salama *et al.* 2005; Radwan *et al.* 2008). Carbamates such as methiocarb were found to cause a loss of muscle tonus in terrestrial gastropods (Godan 1983) and exhibit neurotoxic effects on the nerves controlling the locomotion and feeding behavior (Wright
and Williams 1980; Bailey 1989; Bailey et al. 1989). According to McIlwain and Hoke (2005), shrinkage of cell bodies, eccentric nuclei, terminalized nucleoli, and crenations of the nuclear envelope could be attributed to the effect of the two tested carbamate molluscicides on the cytoskeleton of affected neurons. Penjun et.al (2010) investigated the histological features of neuronal ganglia and the localization of serotonin in the unionid mussel *Hyriopsis (Hyriopsis) bialata* in Thailand. Singh and Singh (1984) reported that insecticide treatment induced enhanced transmitter release and mitochondrial damage resulting in the accumulation of damaged membranes in the neuropile and nerve cell bodies in the metathoracic ganglion of *Periplaneta americana*.

In the present study the pesticides toxicity can be explained by the hypothesis of Cooke (1977). Pesticides might be causing hormonal release due to excessive entry of Ca\(^{++}\) inside the NSCs. Thus it can be concluded that pesticides might be exerting their effects on the neurotransmitters which in turn give messages to neurosecretory cells for the release of neurohormones.
SUMMARY

1. The fresh water bivalve, *Parreysia cylindrica* were collected from Jamda dam, near Chalisgaon, to study the impact of pesticides on neurosecretory cells (NSCs) in cerebral, visceral and pedal ganglia.

2. Two types of cells have been observed in cerebral, visceral and pedal ganglia. They have been designated as cell type I and cell type II. The cells differ in shape, size, cytometric parameters and staining properties.

3. The bivalves were exposed to acute and chronic treatments of pesticides, indoxacarb and thiamethoxam in order to assess the pesticide impact on the neurosecretory cells of cerebral, visceral and pedal ganglia.

4. Pesticide stress caused, vacuolization, acute cellular degeneration, clumping of chromatin material, undulation of cell envelope, damage of neuropile, staining properties and neurosecretory activity among the neurosecretory cells.

5. Pesticide stress, severely affected the pyriform cells more than oval cells.
REFERENCES


*Original not referred.*