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Neuropathological effect of dimethyl disulfide on neurons of the desert locust *Schistocerca gregaria*

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Abstract

Botanical insecticides have introduced a new concept in insecticide research. In response to insect attacks, some plants can release volatile compounds that alter insect metabolism and nervous system activity. In the present study, changes in the electrical activity of chemoreceptors and alteration of the fine structure of metathoracic ganglia of desert locust were examined after acute exposure to dimethyl disulfide (DMDS), a sulfur compound released from *Allium porrum*. Animals were exposed to 1/4 LC_{50} of DMDS (0.375 µl/L air) and electrophysiological and electron-microscopical studies were carried out. Application of DMDS showed an increase in the activity of deterrent cells present in tarsal chemosensilla of locust. On the other hand, evident degenerative changes in the neurons, neuroglia, neuropile and synaptic vesicles were observed in the metathoracic ganglia of DMDS-treated animals. These findings revealed that pest control using DMDS might be feasible and future work is highly recommended in this respect.

Keywords

Neuropathology, electrophysiology, dimethyl disulfide, chemoreceptors, thoracic ganglia, locust

Introduction

Current public concern about the adverse effects of agricultural chemicals on health and the environment has generated interest in reducing chemical inputs in vegetable growing. Numerous emerging environmental evidence seems to indicate that the use of botanical insecticide represents an exciting alternative way in the biological crop protection (Carlini and Grossi-de-sa, 2002). Some plants can produce, in response to insect attacks, volatile secondary compounds, also known as chemical defensives, which alter insect metabolism and nervous system activity (Rauscher, 1992). Among plants producing the volatile secondary compounds, *Allium* plant species, and particularly the leek *Allium porrum*, can release in the atmosphere, when they are damaged, sulfur volatile compounds such as thiosulfinates that can lead to the formation of disulfides (e.g. dimethyl disulfide (DMDS), tested in this study) (Auger et al., 1989). Available data have shown that sulfur compounds in *Allium* can be classified not only as insecticides, acaricides, nematicides, herbicides, fungicides and bactericides, but also as repellents against arthropods (Auger et al., 2004; Ferry et al., 2009). Because the thiosulfinates are lethal (via a hypothetical neurotoxic activity) for many insect species, they could be used in plant protection and particularly in the seed storage systems as fumigant (Arthur, 1996; Dugravot et al., 2002).

Animals react to chemicals in their environment, primarily through a sensory process called chemoreception. In insects, chemoreception plays a major

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role in a number of behavior, including avoidance (Gaaboub and Tousson, 2005; Newland et al., 2000; Newland and Yates, 2007; Ômura, et al., 2008), detection and selection of food sites (Gaaboub and Hustert, 1998; Tousson and El Atrsh, 2010; White and Chapman, 1990) and selection of egg-laying sites (Tousson, 2004; Tousson and Hustert, 2000, 2009; Newland and Yates, 2007).

Chemoreceptors trap chemical molecules and are transferred to dendrites of chemosensory neurons for recognition, where they specifically depolarize a membrane and elicit a nerve impulse. Accordingly, the present work was designed essentially to evaluate the toxic effects exerted by DMDS on the thoracic ganglia of desert locust *Schistocerca gregaria*

Materials and methods

Experimental animals

Adult female *Schistocerca gregaria* (5–8 days old) were obtained from the Department of Plant Protection, Faculty of Agriculture, Benha University, Benha, Egypt. The animals were fed on lettuce leaves and reared at 25°C and under a 12 h light–12 h dark regime.

The used chemical

DMDS with chemical formula $C_2H_6S_2$ was purchased from Sigma-Aldrich (St Louis, Missouri, USA), and it was of the highest purity grade available (99%).

Toxicity study

The toxicity of DMDS was evaluated to determine the median lethal and sublethal concentrations. The tested concentrations were 0.03, 0.06, 0.1, 0.3, 0.6, 0.9, 1, 1.2, 1.6, 1.9, 2.25 and 2.5 µl/L. For each treatment 30 pairs of *S. gregaria* were used and placed in three hermetically sealed glass jars (ten pairs for each) containing the tested concentration of DMDS. After 24 h of exposure, adults still alive were removed and isolated for 48 h in other jars. Control and treated animals were fed on lettuce leaves. The mortality percentages were determined after 48 h and the lethal concentration causing 50% of mortality (LC_{50}) was determined according to Finney (1971).

Electrophysiological study

This study was carried out on five control and DMDS-treated animals. Each tested animal was placed in a Faraday cage and conventional methods were employed to protect the preparation from substrate vibration and to reduce sound reflections from surrounding walls of the Faraday cage.

Responses from individual tarsal chemosensory cells (basiconic sensilla) to chemical stimuli were recorded using the tip-recording technique (Hodgson et al., 1955). A glass microelectrode (tip diameter 40 µm) was filled with saline (0.1 M NaCl) and placed over the tip of the sensillum. DMDS was applied onto the sensillum through an odor delivery system, consisting of three connected flasks (Figure 1). Current of air was allowed to pass through the connected flasks. The first and second flasks contained potassium hydroxide and calcium dichloride for the capture of CO_2 and H_2O, respectively. The third flask contained 0.25 µl/L air DMDS. The current of air was allowed to pass to the third flask to carry the stimulating odor of DMDS and then directed to the tested sensillum in the experimental chamber.

Signals of neural responses to 0.1 M NaCl and 1/4 LC_{50} DMDS were amplified using a high-impedance AC amplifier, filtered, then displayed on an Tektronic 502 oscilloscope and recorded on a magnetic tape. The recorded signals were digitized and stored on a compact disk for further computer analysis. Spike analysis was applied to the discharges after skipping the first 30 ms from the onset of stimulation in order to avoid the baseline shift brought about by the contact artifact and lased for the next first second (Masala et al., 2008).

Ultrastructure study

For electron microscopical study, locusts were assigned into two groups with 10 pairs per group. Animals of first group received no treatment and were considered as controls. In the second group, animals were exposed to 1/4 LC_{50} of DMDS (0.25 µl/L air). Locusts of both groups were placed for 24 h in hermetically sealed glass jars and fed on lettuce. At the end of experimental period, metathoracic ganglia of locusts from treated and control groups were dissected out and immediately immersed in 4F1G mixture for 2 h and then rinsed in 0.1 M phosphate buffer (pH 7.4). Fixed samples were postfixed in 1% OsO_4 for 2 h at 4°C and washed with phosphate buffer for several times for 10 min, dehydrated in graded ethanols, treated with propylene oxide and embedded in Epon. Ultrathin sections were cut on the LKB (Ultramicrotome Nova, LKB, Bromma, Sweden) ultratome with a glass knife, double stained

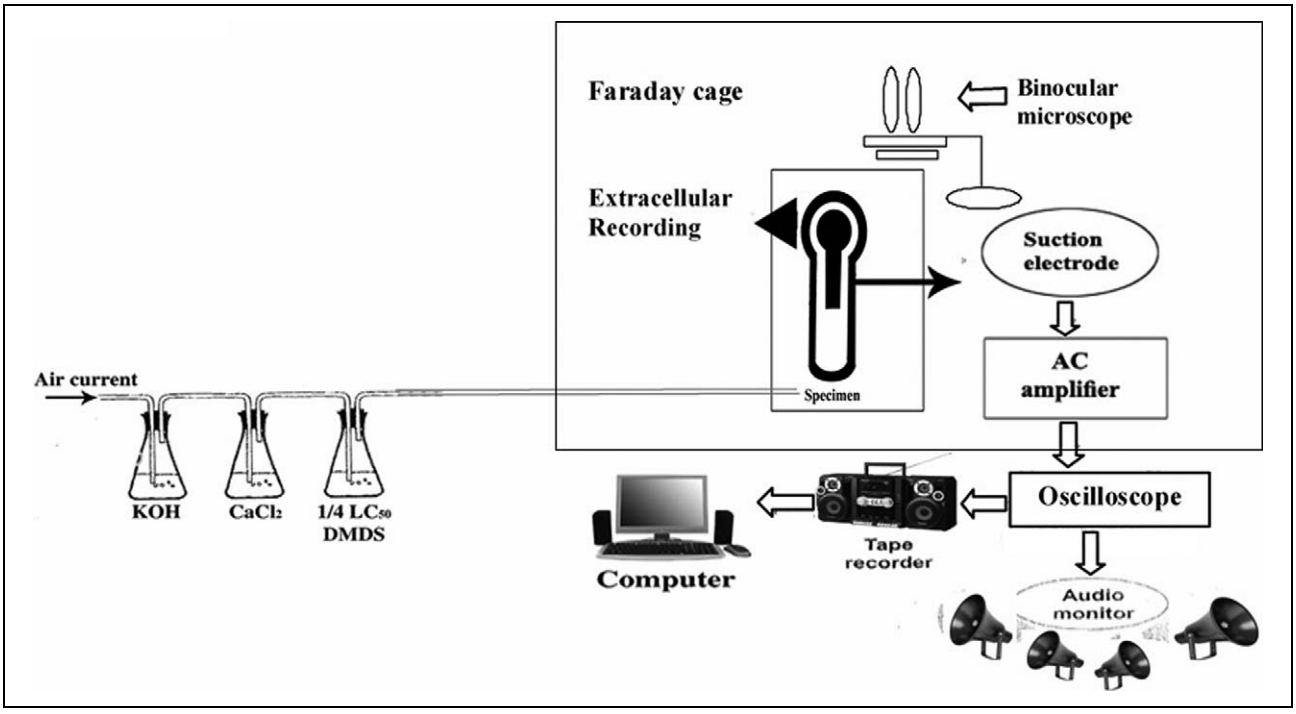

Figure 1. Electrophysiological unit.

with uranyl acetate and lead citrate and examined by a Jeol 100 CX electron microscope.

Results

Effect of DMDS on the mortality of S. gregaria

Observation of the mortality rate over a period of 24 h in *S. gregaria* treated with various concentrations of DMDS showed that the percentage of mortality in the locusts depended on the concentration of DMDS. The probit analysis of the results showed that the LC_{50} of DMDS for *S. gregaria* was 1.00 µl/L air.

Effect of application of 1/4 LC_{50} DMDS on the activity of antennal and tarsal chemosensilla in locust

In control animals, stimulation of tarsal basiconic sensilla by 0.1 M NaCl evoked discharges of three different sensory neurons (water, salt and fifth (deterrent) cells) as identified by Liscia et al. (2004). In animals exposed to DMDS, an increase in the activity of deterrent cells was observed compared to control. However, a decrease in the firing activity of both water and salt cells was recorded (Figure 2).

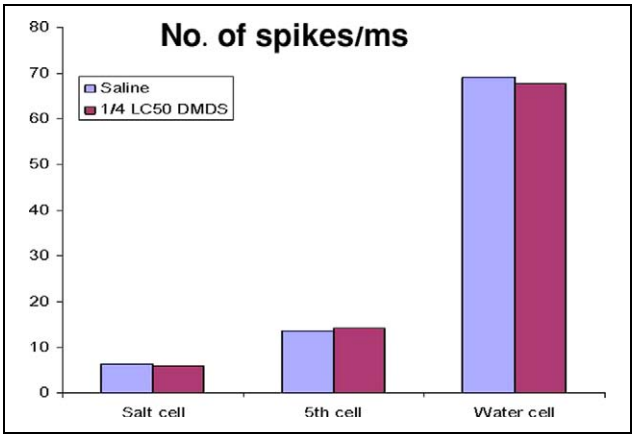

Figure 2. Effect of DMDS on the spike firing frequencies from salt, water and deterrent cells of tarsal chemosensilla. DMDS: dimethyl disulfide.

Effect of DMDS on ultrastructural pattern of the metathoracic ganglion locust

The structure of the metathoracic ganglion was examined at ultrastructural level. Normal neurons, neuroglia and neuropile ultrastructure was observed in the control animals (Figure 3(a) to (c)). In locust treated with 1/4 LC_{50} DMDS, the nuclei of the neurons appeared highly shrunken with irregular contour and with signs of chromatolysis (Figure 4(a)). An

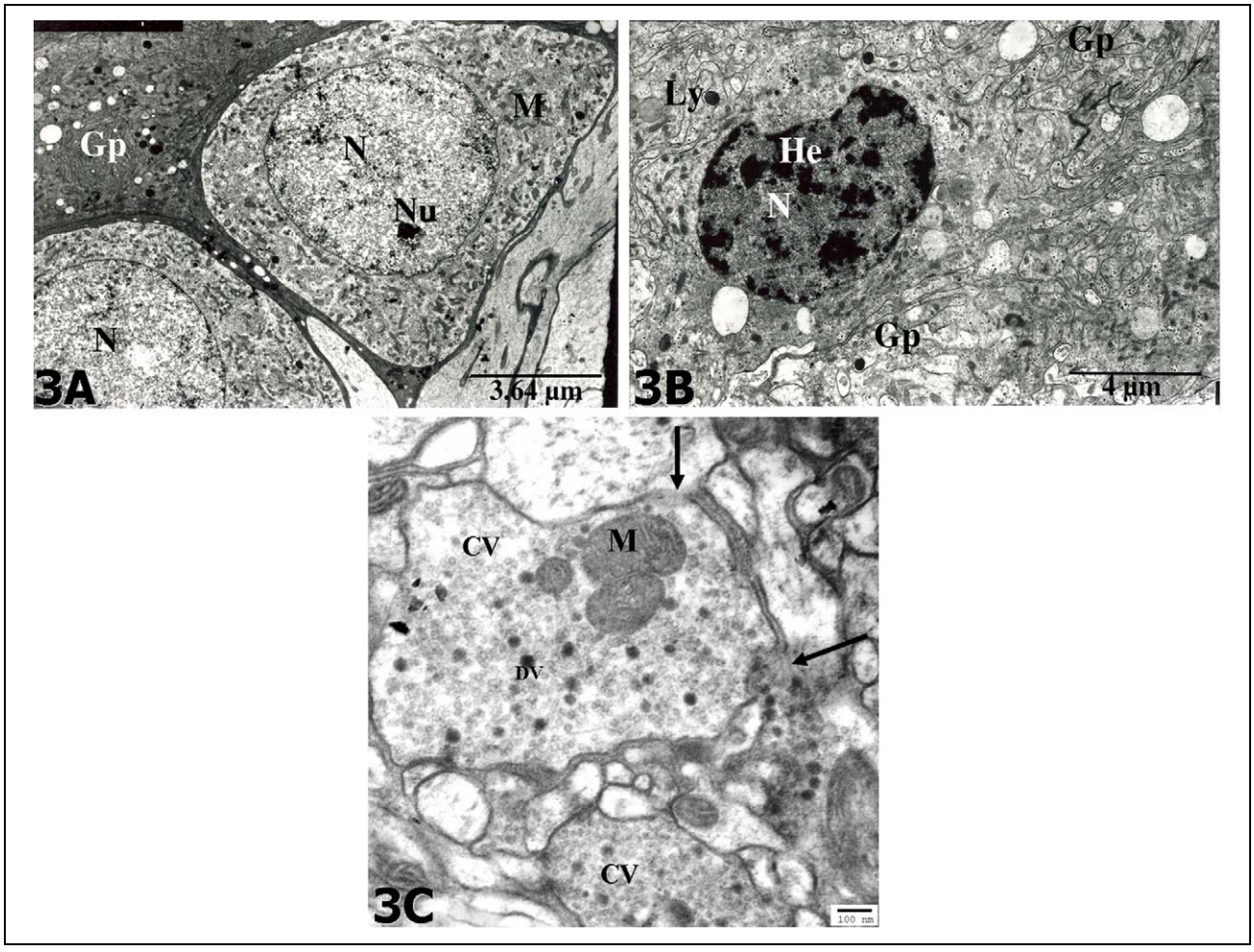

Figure 3. Electron micrographs of cross section of ganglia of control locust. (a) A cortical neuron with nucleus (N), distinct nucleolus (Nu) and large number of mitochondria (M). The perikaryon is surrounded by glial processes (Gp). (b) A glial cell with nucleus (N), lysosomes (Ly), mitochondria (M) and numerous glial processes (Gp). He: heterochromatin. (c) Neuropile with nerve fibers containing mitochondria (M) and filled with dark (DV) and clear (CV) synaptic vesicles. Arrows point at synaptic zones.

increasing in the electron opacity of some neurons was also noticed (Figure 4(b)). Moreover, severe cytoplasmic alterations were recorded including loss of cytoplasmic texture, hypertrophy of Golgi body, polymorphic degenerated mitochondria and presence of cytoplasmic vacuoles, autophagosomes and large dense lysosomes (Figure 4(c)). Neuroglia cells (Figure 4(d) to (e)) appeared with divided nuclei or with nuclei showing severe indentations. Vacuolated cytoplasm containing large number of dense lysosomes and degenerated mitochondria and glial processes were also recorded in these cells.

In addition to these findings, compared to the control group, neuropile in DMDS-treated animals revealed a marked degeneration of nerve fibers, loss of cytoplasmic texture of some axons, damage of mitochondria and degeneration of synaptic vesicles and synaptic membranes (Figure 4(f)).

Discussion

The results of the current study demonstrated that DMDS is a neurotoxic plant insecticide that affects the electrical activity and fine structure of the neurons in the nervous system of the desert locust *S. gregaria*. Exposure of locust to DMDS increased the activity of deterrent cells of tarsal basiconic sensilla which means that DMDS is repellent to insects. Using electrophysiological techniques, it was found that toosen, a tetranortriterpenoid isolated from the bark of *Melia toosendan*, exerts its feeding repellent activity by stimulating a deterrent receptor cell located in the

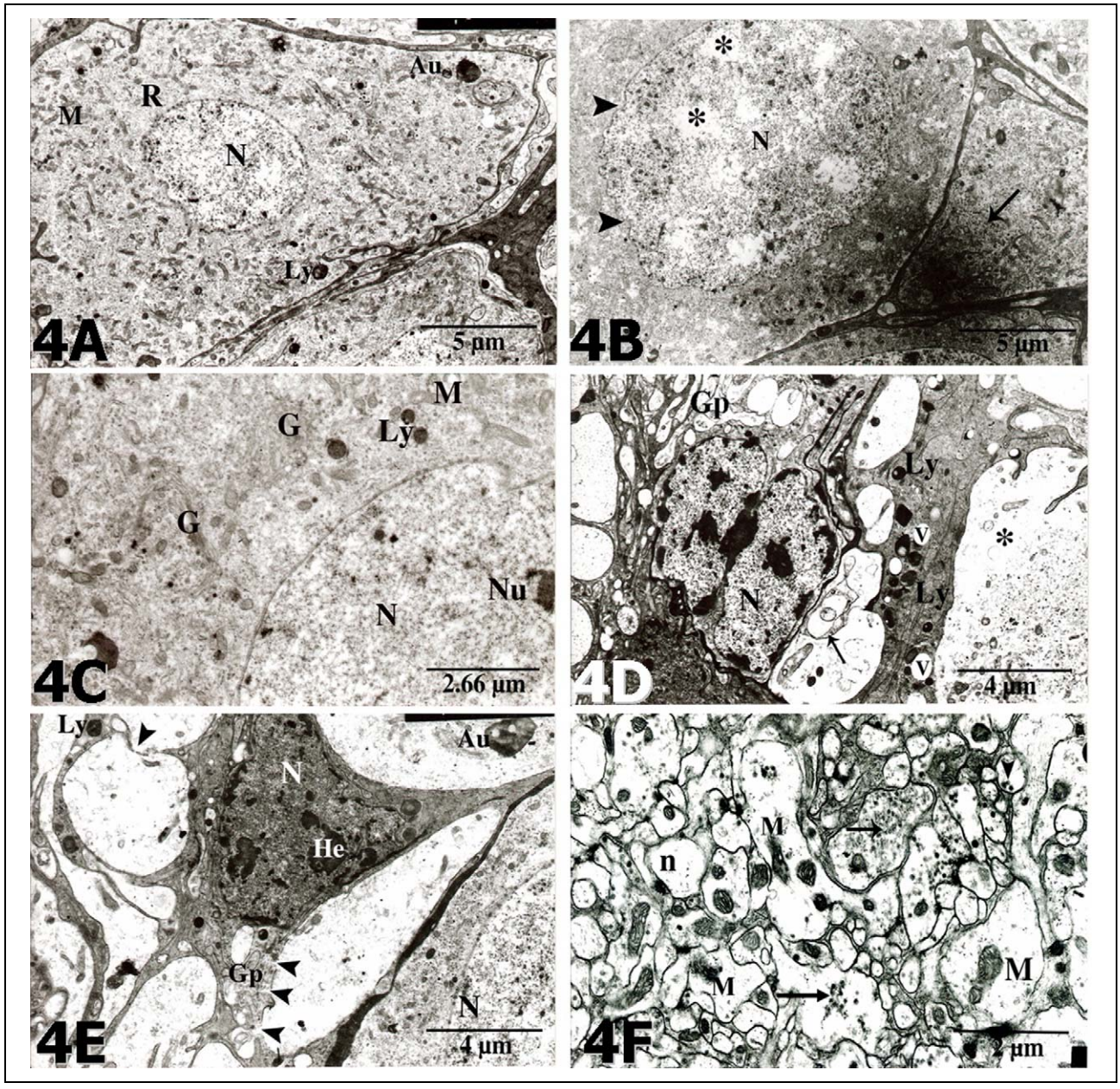

Figure 4. Electron micrographs of cross section of metathoracic ganglion of locust treated with DMDS. (a) A neuron with irregular-shaped nucleus (N) and signs of chromatolysis. The cytoplasm is destructed with large number of free ribosomes (R), lysosomes (Ly) and small damaged mitochondria (M). V: vacuoles; Au: autophagosome. (b) A neuron with irregular-shaped nucleus (N) and corrugated nuclear membrane (arrowhead). Note: abnormal distribution of chromatin material with areas of chromatolysis (asterisks), and destructed cytoplasm with increasing electron opaque (arrow). (c) A neuron with part of nucleus (N) and nucleolus (Nu). Note: cytoplasm with lysosomes (Ly), damaged mitochondria (M) and hypertrophied Golgi complex (G). (d) A glial cell with divided nucleus (N) and degenerated glial processes (GP). Degenerated areas in the nerve fibers with degenerated organelles indicated by arrow. V: vacuoles; Ly: lysosomes. (e) glial cell with irregular shaped nucleus (N) having few patches of heterochromatin (He) and cytoplasm with lysosomes and degenerated organelles. Arrowheads point at degenerated areas in nerve fibers containing degenerated organelles. Gp: degenerated glial processes; Ly: lysosomes; Au: autophagosome. (f) Neuropile region showing large number of axons with depleted vesicles and damage mitochondria (M) with indistinct cristae and ruptured mitochondrial membrane. Synaptic vesicles under degeneration are indicated by arrows. n: neurotubules. DMDS: dimethyl disulfide.

medial maxillary sensillum styloconicum (Schoonhoven and Luo Lin-er, 1994). In their study on the insecticidal effect of DMDS, Dugravot et al. (2003) reported that DMDS exerts insecticidal neurotoxicity through mitochondrial dysfunction and activation of insect K_{ATP}.

In the current work, electron microscopical study revealed the impact of DMDS on the ultrastructure of neurons and glial cells in the metathoracic ganglion of *S. gregaria*. The nuclei of neurons appeared highly shrunken with irregular contour and an increase in the electron opacity of nucleoplasm and cytoplasm were noted. According to Mcllwain and Hoke (2005), changes in the size and position of the nucleus could be attributed to the effect of the neurotoxin on the cytoskeleton and increased electron opacity of nucleoplasm and cytoplasm of affected neurons could be due to an enhanced affinity of these cells to osmium tetroxide used as the electron stain. Obsorne (1979) mentioned that, an increase in electron opacity is usually associated with events underlying necrosis, which may result in the death of affected cells.

The present results demonstrated large dense lysosomes and autophagosomes in the cytoplasm and axons of DMDS-treated neurons. Autophagy is a cellular degradation pathway particularly important during development stages and under certain environmental stress conditions (Klionsky and Emr, 2000). This phenomenon was observed in *Hoplias malabaricus* exposed to inorganic lead and may be related to an autophagic degeneration of the cells as reported by Clarke (1990). The multivesicular bodies and/or lysosomes may perform a scavenging function thereby cleaning the products of DMDS-induced destruction in the neuropile. Our results suggest, therefore, that neuronal lesions involving degeneration of cytoplasmic organelles occur in the neuropile of DMDS-treated ganglia; the cellular organelles undergo destruction and digestion and undigested materials accumulate as multivesicular bodies. The formation of these structures is therefore an indication of insecticide-induced stress in treated nerve cells. Nevertheless the increased number of these bodies accompanied by depletion of synaptic vesicles and mitochondrial and glial cell damage is certainly a sign of DMDS-induced neuronal fatigue consequently leading to the failure of nervous transmission in treated ganglia (Singh and Singh, 1984).

In DMDS-treated ganglia, the cytoplasm and nucleoplasm of glial cells became extremely damaged. Chemical insecticides like dichlorodiphenyltrichloroethane was also found to impair glial cells in the thoracic ganglia of the house fly (Lane, 1973). Singh and Singh (1984) reported that damaging glial cells could disturb the neuronal function leading to the failure of nervous transmission with lethal consequences

Moreover, the present results clearly indicated that treatment with DMDS induced damage and loss of the cristae of mitochondria in the perikarya of the neurons as well as in the axons of the neuropile. Some previous studies have also indicated that insecticide treatment might damage mitochondria in the nervous tissue. These alterations resemble those described in insects, snails and vertebrates as cellular stress symptoms after intoxication (Triebskorn, 1991; Wang et al., 1991). At cholinergic presynaptic terminals, mitochondria are the main source of acetyl-CoA which is utilized for the synthesis of acetylcholine. Thus, DMDS, by damaging mitochondria at presynaptic nerve terminals, may block processes involved in the synthesis and transport of the transmitter. This, when combined with enhanced transmitter release, will eventually lead to the failure of synaptic transmission (Singh and Singh, 1984). In addition to mitochondrial damage, a significant reduction in synaptic vesicle population was also recorded in the neuropile of treated ganglia. In agreement with our results, Singh and Singh (1984) reported that dieldrin caused a notable depletion of synaptic vesicles from presynaptic terminals, suggesting enhanced transmitter release. In conclusion, our results demonstrated that DMDS has an insecticidal neurotoxicity, and pest control using DMDS is feasible.

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