DJS Vol. 46(1) (2023) –pp.111-126 ISSN: 1012-5965

Delta Journal of Science

Available online at **https://djs.journals.ekb.eg/**

Research Article **ZOOLOGY**

Ultrastructural and Histopathological effect of commonly used pesticide in Egypt on eye, eye stalk and cerebral ganglia of *Theba pisana* land snail (Mollusca, Gastropoda) inhabiting Kafr-El Sheikh, Egypt

Aalaa. I. Atlam[1]*; Fadia N. Heiba[1]; Mohamed M. Mortada[2]; Nglaa S. Geaesa[1] and Sahar I. Abd El-Wahed[2]

[1]Zoology department, Faculty of Science, Tanta University, Egypt
[2]Plant Protection Research Institute, ARC, Dokki, Giza, Egypt
****Corresponding author*:** Aalaa Ibrahim Atlam ***e-mail*:** aalaa.atlam@science.tanta.edu.eg

Received: 23/1/2023 **Accepted**: 25/3/2023

KEY WORDS

Land snails, *Theba pisana*, Biomajic, Agrinate, eye histopathology and cerebral ganglia ultrastructure

ABSTRACT

The land snail *Theba pisana*, is one of the most injurious snails, as it feeds on field crops. The traditional control methods rely on the pesticides. The most commonly used molluscicide in Egypt are Agrinate 24% (Methomyl) and Biomagic (*Metarhizium anisopliae*). The present study aimed to fined and evaluate the effect of pesticide on *T. pisana* land snail in order to predict the hazard effects of these pesticides on the non-target organisms. The exposure of adult *T. pisana* to sub lethal dose of Biomajic (*Metarizhium anisoplae*) and Agrinate caused varying degrees of ulturastrcture and histopathological changes in the examined organs (eyes, tentacles and cerebral ganglia). The exposure to both pesticides for 14 days leads to loss of the architecture of eye and eye stalk in addition, both of them displayed multiple degenerative changes in cereberal gangalia of the snail, but, molluscicide Agrinate 24% methomyl was more effective mollscicide against *T. pisana* than Biomajic.

Introduction

Many land snails (Molluska, Gastropoda) as *Theba pisana, vermiculata, Succinea putris, Helicella vestalis, Cochlicella acuta* and *Deroceras reticulatum* are considered one of the most serious pests attacking fields and agriculture crops around the world **(Heikal, 2015)**, such crops as Egyptian clover, *Trifolium axalendrium*; broad bean, *Vicia faba*; wheat, *Triticum vulgaris*; maize, *Zea maize* and sugar beet, *Beta vulgaris*. Vegetable crops were cabbage, *Brassica oleracea*; lettuce, *Lactuca sativa*; pea, *Pisum sativum*; potatoes, *Solanum tuberosum* and carrot, *Daucus carota*. The ornamental plants and wood trees were nasturtium *Tropaeolum magus*; alocasia, *Alocasia gigantea*; custard, *Monestera acuminate*; casuarinas, *Casuarina equistifolia* and caphor, *Eucalyptus rostrata*. Fruit trees, i.e. grape, *Vitis vinifera*; guava, banana, *Musa* sp.; date palm and citrus. They attack a lot of fields causing great damage to the cultivated plants of economic importance. These mollusks feed on the plants and contaminate them by their bodies, faces or slime resulting in deterioration of the product quality, in addition to the financial loss **(Iglesias *et al.*, 2003)**.

The land snails were mostly controlled chemically by using pesticides **(Geasa *et al.*, 2013)**. On the other hand, the pesticides could cause acute and chronic hazard for each of humans and non-target organisms, environmental pollution and disturb the natural balance **(Zedan *et al.*, 1999)**. Some effective antimicrobial agents were used for controlling pests (land snail, insects, nematods) such as bacteria and fungi, little interest has given to the molluscicidial activity of *Bacillus thuriengensis* against snails or slugs. (**Abd El-Wahed 2014**) Commercial product as Biogard or Dipel 2X and Biomajic were the most common for sale in Egypt the last few years ago for the insect control.

To document the histological alterations caused by the toxic treatment on the cerebral ganglia, eye stalk, and eyes and to demonstrate how those land snails were impacted by various molluscicides, a number of histological, scanning, and ultrastructural experiments were conducted **(Fouad *et al.*, 2004 and Essawy *et al.*, 2009)**. Snails' central nervous systems contain very few, extremely big, and easily recognizable neurons, so they represent a good model for a variety of fundamental neurobiological studies **(Muller *et al.*, 1990)**.

The current study shed light on the most commonly used molluscicide Agrinate 24% (Methomyl) and pesticides;

Biomagic (*Metarhizium anisopliae*) against land snail *Theba pisana* present in Kafr EL-Sheikh Governorate with histopathological and ultra-structural examination. This will be achieved by using Scanning electron microscopic (SEM) investigations to evaluate the effect of pesticides on external feature on eye and eye tentacle of treated snails compared to the control snails *Theba pisana*. Also, the histopathological changes and ultrastructural alterations induced by Agrinate and Biomajic in the cerebral ganglia of treated snails compared to the control snails *Theba pisana* were carried out. So, the snails may be used as bio-indicator of chemical environmental pollution.

MATERIALS AND METHODS

Investigated snails

A ten samples of *T. pisana* were chosen randomly, a terrestrial gastropod, were taken from various crops in the Kafr El-Sheikh Governorate in Egypt (during the spring and autumn seasons from September 2015 to August 2016) .They were kept in open-air cages (40×30×30 cm, with 10 individuals per cage), allowed to feed on fresh leaves of lettuce, and kept to acclimatize under laboratory conditions.

Experimental design

Over 2 weeks prior to the experiment carbamate pesticides, Agrinate (Methomyl 24% SL), (S-methyl-N-[(methyl carbomyl] oxy] thioacetimidate) and fungi biocide Biomajic (*Metarhizium anisopliae* 1.75% WP), the both compounds were provided from Central Laboratory of Pesticides, Agric. Res. Center, Dokki, Giza. A sublethal concentration of 1/8 LC50 for each treatment **(Shahawy, 2018)**, 100 animals were used and kept in 10 plastic boxes (10 animals each), and about 10 g of bait was provided to snails in each box. Another 100 animals in 10 boxes were used as the control group containing wheat bran bait with distilled water only. The poisonous baits were prepared by mixing a known amount of each compound with 5 parts of sugar cane honey, the mixture was incorporated with wheat bran to be finally 100 parts. All boxes were cleaned with water, and the bait was renewed every 3 days till the end of the experiment. For both modes of application, the experiment lasted for 14 days, and a few milliliters of water were added daily into each box to provide suitable humidity for snail activity. The experimental animals were divided into three groups and treated as follows:–

Group 1: Control animals of this group received no treatment (snails received 25 µl of distilled water or fed on bait free of chemicals) and were considered as normal

Group 2: In this group, a single dose of 1/8 LC50 of Biomajic was applied as a poison bait to the animal.

Group 3: In this group, a single dose of 1/8 LC50 of Agrinate was topically applied as a poison bait to the tested snails.

Light microscopic investigations

To investigate the histology and histopathology of the cerebral ganglia of *T. pisana* snail exposed to Biomajic and Agrinate, the snails were fixed in 10% formal saline solution. They then passed with conventional ascending steps of dehydration, then cleared, embedded in paraffin wax, sectioned by microtome at 5 µ after that they were stained with Haematoxylin and Eosin Stain (H&E). Finally, the specimens were microscopically examined and photographed by a Zeiss video camera.

Electron microscopic investigations

Scanning electron microscopic (SEM)

After fixation the specimens were washed twice with sodium buffered saline. This was followed by post fixation in 2% osmium tetra oxide. After secondary fixation, specimens should be dehydrated in a series of ascending alcohols (30%, 50%, 70%, 90% (two washes), 100% (3 or 4 washes) each for 15 minutes), in order to eliminate the small amount of water remaining in the tissue. After dehydration the specimens were dried in a Critical point Drying Apparatus, mounted on cupper stubs with carbon conducting paint in the desired orientation. The stubs were placed inside the sputter coater and coated with a thin layer of gold in a Denton Vacuum evaporator (Spi-module). The specimens were viewed in a scanning electron microscope (Jeol JSM 6510L) operated at an acceleration voltage of 20 kv. The results of scanning were preserved as photographs used in this study. The scanning electron microscopic procedure was carried out at the SEM Unit, Faculty of Agriculture, El-Mansoura University, Egypt.

Transmission electron microscopic (TEM)

The head region of *T. pisana* snail, was dissected with sharp scissors, and the cerebral ganglia was obtained using Zeiss binuclear microscope and preserved in formaline- glutraldehyde fixative in phosphate buffer overnight then transferred to 2% osmium tetraoxide (OsO_4) for 2 hours, Samples were washed in the buffer and dehydrated at 4^{o}C through a graded series of ethanol. Specimens were embedded in an Epon-araldite mixture in labeled beam capsules. A LKB ultra microtome was used to cut semithin sections (1Mm thick), which were stained with toluidine blue (60-70 nm thick), and the ultrathin sections were picked upon 200-mesh naked copper grids and were double-stained with uranyl acetate for 1/2 h and lead citrate for 20-30min **(Reynolds, 1963)**. Scoping the grids was achieved by using Jeol 100 CX TEM.

RESULTS AND DISCUSSION

Light microscope investigations

Histopathological changes of cerebral ganglia of *Theba pisana* snails

Snails treated with Biomajic (*Metarhizium anisopliae*) biocide

The central ganglia displayed changes in their cytoarchitecture and secretion activity during the course of treatment, which are visible under a microscope compared with the normal sections (Fig.1). The synthesis of neurosecretory materials increased as a result of exposure to Biomajic 1.75%, as seen in the perikaryon and neurosecretory axons, which are also fully loaded with neurosecretory materials. After 14 days of the experiment, it was noticed that some giant and median-sized neurons had a few vacuoles in different parts of the cytoplasm, a significant accumulation of granules (autophagosomes), eccentric nuclei, and chromatolysis, while some small-sized neurons had obvious damage and cell body dissolution. Cytoplasmic dissolution, certain neurons showing signs of ageing, a frail neuropile with frayed nerve fibers. The cerebral commissure's developing material is getting spongy and showing signs of ageing. The perineurium is separated from the underlying neuronal cells and shown signs of producing vacuoles. The extracellular space has also grown, degenerated into disordered, and lost its neuropile and axons. Degenerative changes, such as the loss of the usual architecture, were detected (Fig. 2).

Snails treated with Agrinate (methomyl 24%) pesticide

After two weeks, exposed snails to Agrinate showed certain histological abnormalities, including disordered and deteriorated nerve fibers in the neuropile as well as varying degrees of degeneration in the perikarya of the nerve cells (neuro-secretory cells). Different neurons' peripheral cytoplasm contains lysed and aggregated autophagosomes. Additionally, some severely afflicted neurons had structural deformities, including abnormal nuclei and lytic chromatin. Some cells with neuronal vacuoles and pyknotic or totally degenerated nuclei displayed cytoplasm dissolution. A frequent histological characteristic is severe extracellular tissue destruction, as shown in Fig. (3 A, B). The nuclear membrane integrity of the large neurons was compromised, giving them a shrunken appearance, and the nuclei lacked the usual rounded or oval morphology. Some neurons displayed full death, and the perineurium around them is noticeably enlarged, detached, and disordered (Fig. 3C)

Due to the fact that Biomajic and Agrinate compounds are found in the environment, consuming them may subject both human and animal bodies to various amounts of exposure **(Niva &Dr Manashi,**

2018) In this context, the objective of the present study was to evaluate the effects of these substances on the nervous system of the land snail *Theba pisana* and to provide information on the potential use of this land snail as a biomarker for environmental contamination **(Gouissi and Adimi, 2019)**. The results of the present study agreed with the observations of size variations noted in the neurons of several pulmonate species, such as the perikaryal region of the cerebral ganglia contains numerous giant, medium, and small neurons (**Kruatrachus *et al*., 1993; Essawy, 2001**). Numerous pulmonates, including *L. natalensis* **Sleem (1993)** demonstrated similar outcomes. The neurosecretory cells, which were larger than other cell types, occupied the majority of the cell space in both species. The light microscope micrographs showed that, Agrinate treatment caused more severe damage to neurons than did Biomajic treatment, as neurons of all sizes had excessive nuclear envelope indentation, eccentric nuclei, and enhanced perikarya shrinkage. **Jones and Cavanagh (1982), Sterman (1982, 1984)** observed pathological changes in cells of all sizes in the ganglia of acryl amide-intoxicated in rats, while the largest cells showed the most prominent changes **Essawy *et al*. (2009)** founded that *Eobania vermiculata* snail treated with methomyl showing completely degenerated large and medium sized neurons, small sized neurons with complete dissolution of cytoplasm and destroyed neuropile. Widened in the intracellular space was observed between the neurons in the cerebral ganglia of treated snails. Reflected a dysfunction of ionic and osmotic balance of these cells **(Cotran *et al*., 1999 and Essawy, 2011).**

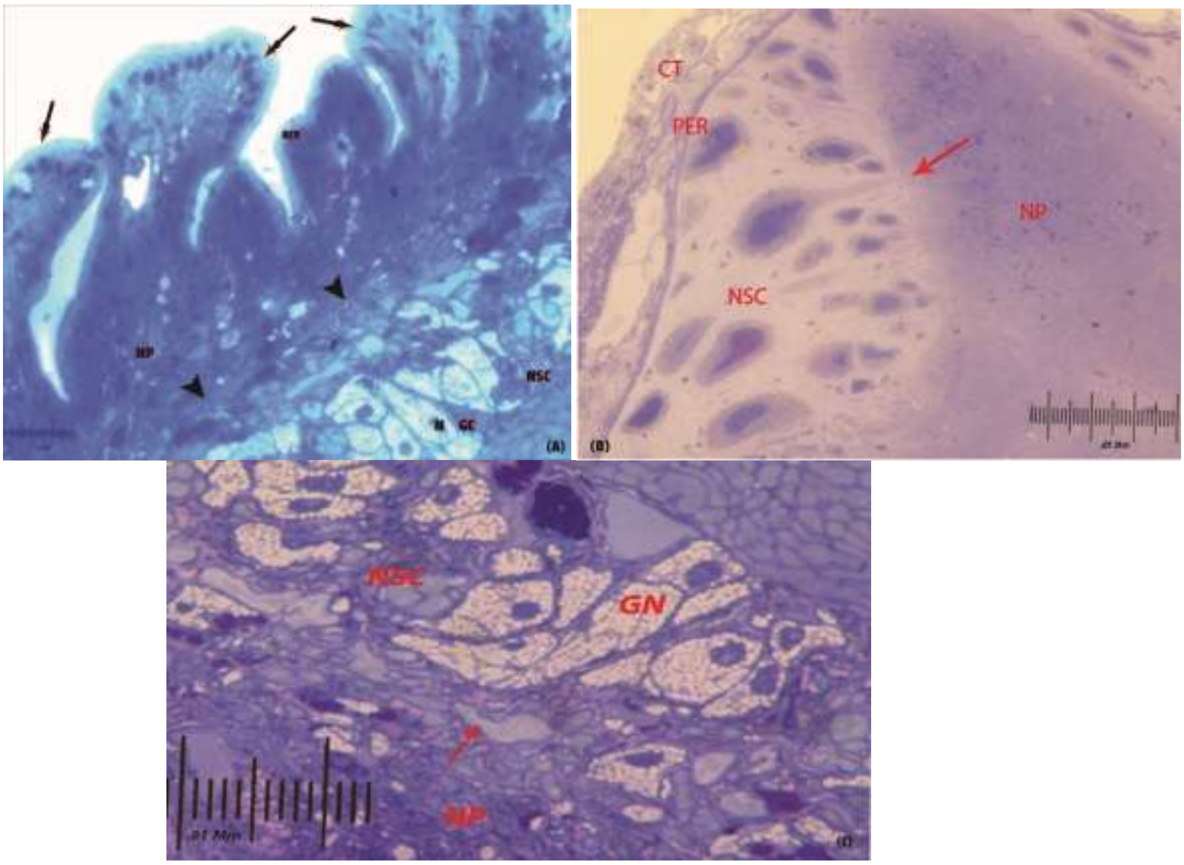

Fig. (1): Light micrograph of histological transverse section of cerebral ganglia of a control snail *T. pisana*. **A-** Showing normal lopes of cerebral ganglia surrounded by a perineurium (PER) with neurosecretory cells (NSC), giant neuron (GN), neuropile (NP), nucleus (N), perineurium (PER) and (arrows) pointed to cortically distributed nerve cell bodies,, and glial cells (arrow head); **B-** Neuropile (NP) , axon (arrow) entering from neurosecretory cells (NSC) to neuropile, connective tissue (CT) composed perineurium (PER); **C-** Showing different neurosecretory cells (NSC), neuropile (NP) and giant neurons (GN).

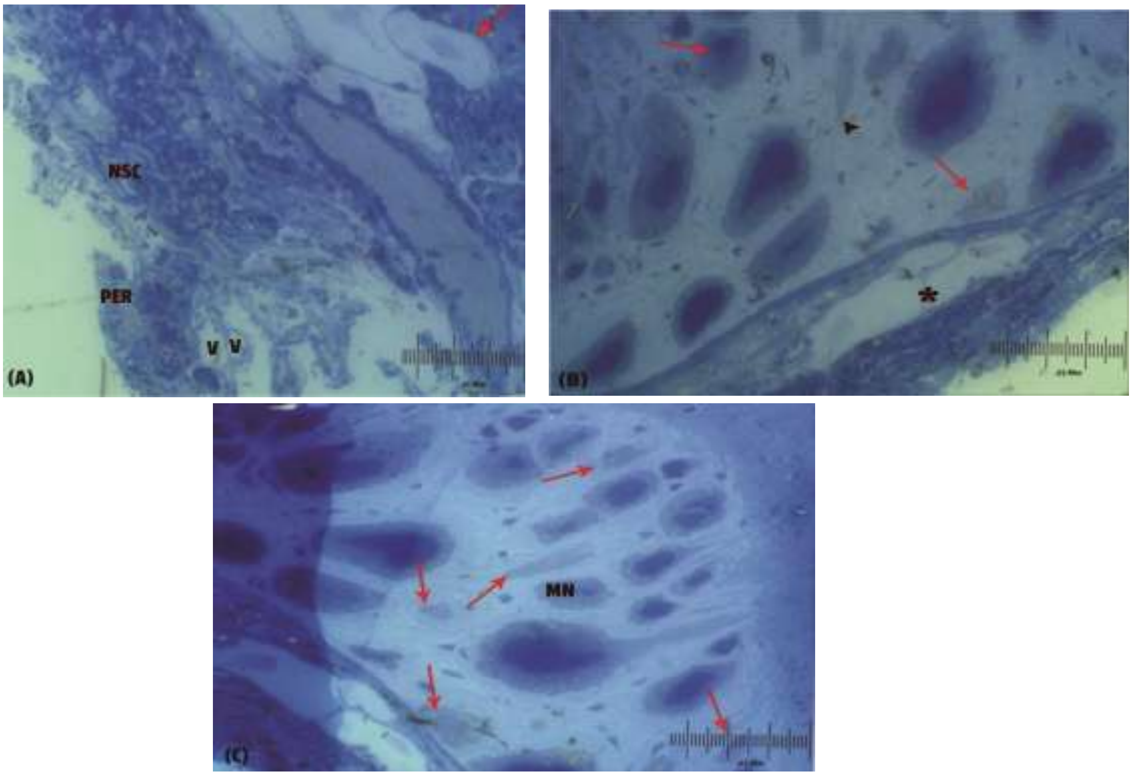

Fig. (2): Light micrograph of histological transverse section through cerebral ganglia of a snail *T. pisana* treated with Biomajic (*Metarhizium anisopliae*) **A-** Showing disorganized perineurium (PER), completely damaged of different neurosecretory cells (NSC), more vacuolation (V) and disturucted cell membrane. **B-** Degeneration of giant neurons with irregular nucleus (arrow), segregation of median neurons with dissolute nuclei (arrow head), disorganized perineurium and damaged extracellular tissue (star). **C-** Showing destroyed median neurons (MN), damaged intracellular tissue and dissolute cytoplasm (arrow).

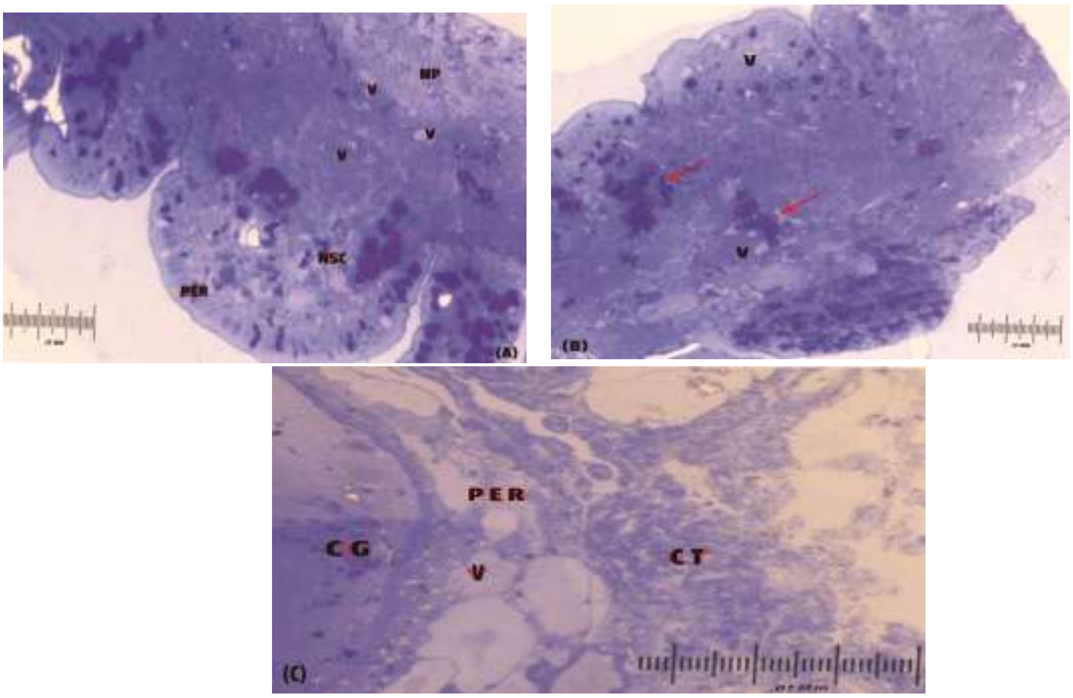

Fig.(3): Light micrograph of histological transverse section through cerebral ganglia of a snail *T. pisana* treated with Agrinate (24 % methomyl) **A-** Showing lyses of neuropile (NP), more degenerated neurosecretory cells (NSC), large number of vacuoles (V), disrupted perineurium (PER). **B-** Showing completely damaged tissue with vacuoles (V) and accumulation of tissue fragments (arrow). **C-** obvious damage of cerebral ganglia (CG), vacuoles (V), destroyed connective tissue (CT); widened and degeneration of the perineurium (PER).

Electron microscopy

SEM investigations:

SEM study on the eye and eye tentacles of *Theba pisana* snail treated with Biomajic *(Metarhizium anisopliae*) biocide

In the snail's normal tentacle was covered by a nearly smooth tegumental surface (Fig.4).While, investigation of the Biomajic-treated *T. pisana* snail showed that there are slight and continued alterations in the eye, which are embedded into the head. In addition the eye tentacles are internally retracted (Fig.5).

SEM study on the eye and eye tentacles of *Theba pisana* snail treated with Agrinate 24%(methomyl) pesticide

The snails exposed to Agrinate exhibit enlarged damage and denaturation in the apical part of the head. Additionally, eyes show signs of erosion and the surface of the optic tentacles on the eyes peels off. (Fig.6).The previous observations showed that the snail's eyes and tentacles were more adversely affected by Agrinate than biocide Biomajic. This finding was agreed with the results of **Eissa *et al.*, (2011)**, who treated ***Schistosoma mansoni, Schistosoma haematobium*** snail hosts, with the medication **Miltefosine**, showed substantial damage to the eyes and tentacles of the head area. The effectiveness of Miltefosine, the first oral medication approved to treat **leishmaniasis**, was confirmed by scanning electron microscopy against various ***Schistosoma*** species life stages and their intermediate snail hosts, *Biomphalaria alexandrina* and *Bulinus truncates*. They founded that there are extensive morphological changes on the soft parts of the snail body especially at its apical part with appearance of sub tegumental tissue in addition to presence of peeling and erosion of its tentacles. **Hemmaid *et al.*, (2017)** studied the cells of digestive gland of land snail *Eobonia vermiculata* treated with biocide and insecticide and they resulted in wide degenerative changes in the cytoplasm of the digestive cells; Mitochondaria were crowded coalesced and strongly osmiphilic heterochromatin clumps in addition to marginated aggregates of heterochromatin. All these results confirm the extent of the harmful effect of pesticides on land snails.

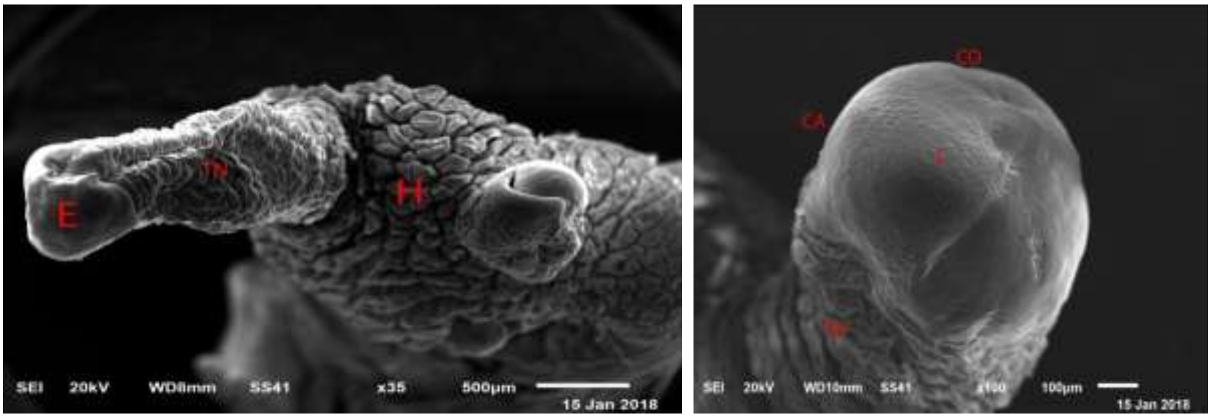

Fig. (4): SEM photos of normal eye and eye tentacles of *T. pisana* land snails. **A-** Snail head (H) showing normal eyes (E) and eye retracted tentacles (RT). **B-** Snail eye (E); lens (L); tentacle (TN); eye capsule (Ca) and cornea (Co).

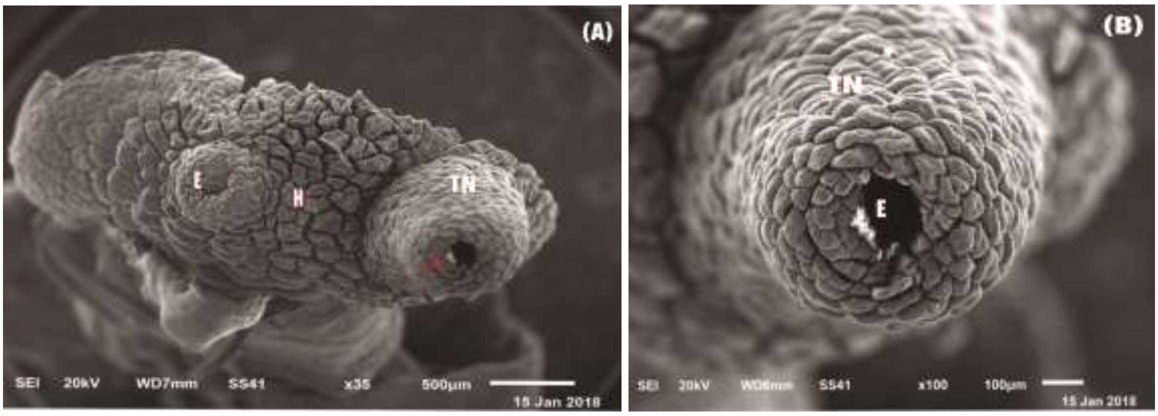

Fig. (5): SEM images the eye and eye tentacles of *T. pisana* land snails treated with Biomajic, **A-** snail head (H) showing fine affected eyes (E) and eye retracted tentacles (arrow). **B-** Snail eye (E) and compressed tentacle (TN).

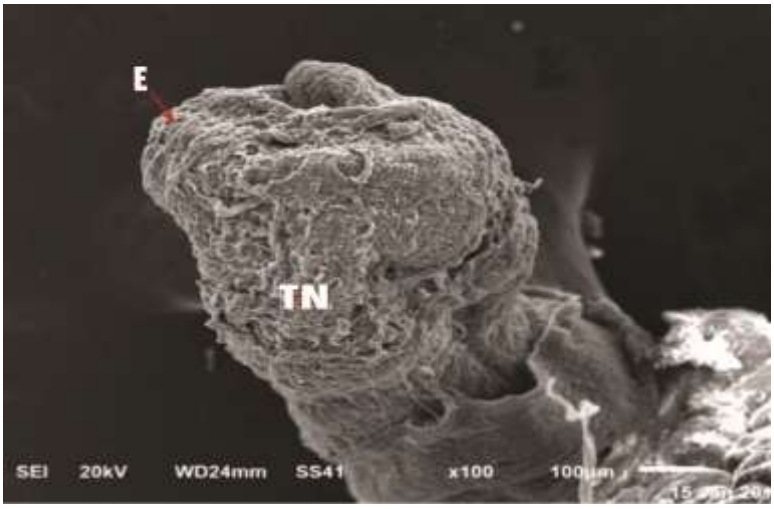

Fig. (6): SEM photo of the eye and eye tentacles of *T. pisana* land snails affected by Agrinate, snail head (H) showing deep affected enclosed eyes (E) and destructed tentacles (TN).

TEM investigation

TEM of cerebral ganglia of *Theba pisana* snails treated with Biomajic (1.75% Metarhizium anisopliae)

The nuclei in the snail cerebral ganglia that had received Biomajic treatment are greatly shrunk, with an eccentric location and an uneven nuclear envelope, this was the most obvious ultrastructural observation compared with control snail (Fig.7). Rough endoplasmic reticulum, tiny electron-dense granules dispersed throughout the nerve cell, and swollen mitochondria with partial dilatation and loss of their cristae were also noted. The profile of the nerve fibers also appeared larger (Fig. 8).

TEM of cerebral ganglia of *Theba pisana* snails treated with Agrinate (24% methomyl)

The neurosecretory cells nuclei are highly shrunken, appeared eccentric, breakdown of chromatin from the nuclear membrane as well as loss of the nuclear membrane neurons noticed with irregular out line, degeneration and alternation of cytoplasm fragmentation of rough endoplasmic reticulum, large numbers of vacuoles found in the cytoplasm. Agrinate treated cerebral ganglia indicated a marked damage of nerve fibers and degeneration of the dense and clear electron synaptic vesicles (Fig.9).

The TEM micrographs of the cerebral ganglia of *Theba pisana* treated by Biomajic and Agrinate showed that the nuclei of neurons appeared highly shrunken, karyolitic eccentric chromatic, with peripherally located nucleoli and irregular contour nuclear envelope. Similar findings were estimated in the cerebral ganglia neurosecretory cells of *Biomphalaria glabrata* treated with herbicide atrazine **(Eissa and Omran, 2007)** and in the neuron of buccal ganglia treated by methiocarb and methomyl **Essawy *et al*., (2009)** and in cerebral ganglia of *Eobania vermiculata* treated with TBT **Essawy *et al*., (2011).** Breakdown of the nucleus may be a sign of the cell responses to molluscicides toxication this is in agreement with the results of **Bayne *et al*., (1985)** who described karyolysis as a late reaction to toxication in the vertebrates and invertebrate.

The changes primarily were with increase the number of free ribosomes. Similar alternations were described in the buccal ganglia of *Eobania vermiculata* treated with carbamate molluscicides **Essawy *et al*., (2009)** and in the cerebral ganglia **Essawy *et al*., (2011).** While the dilatation and degranulation of the rough endoplasmic reticulum are discussed as general changes of the cell response to toxicants **Hamed *et al*., (2007).** All these effect led to osmotic effect and finally cell death.

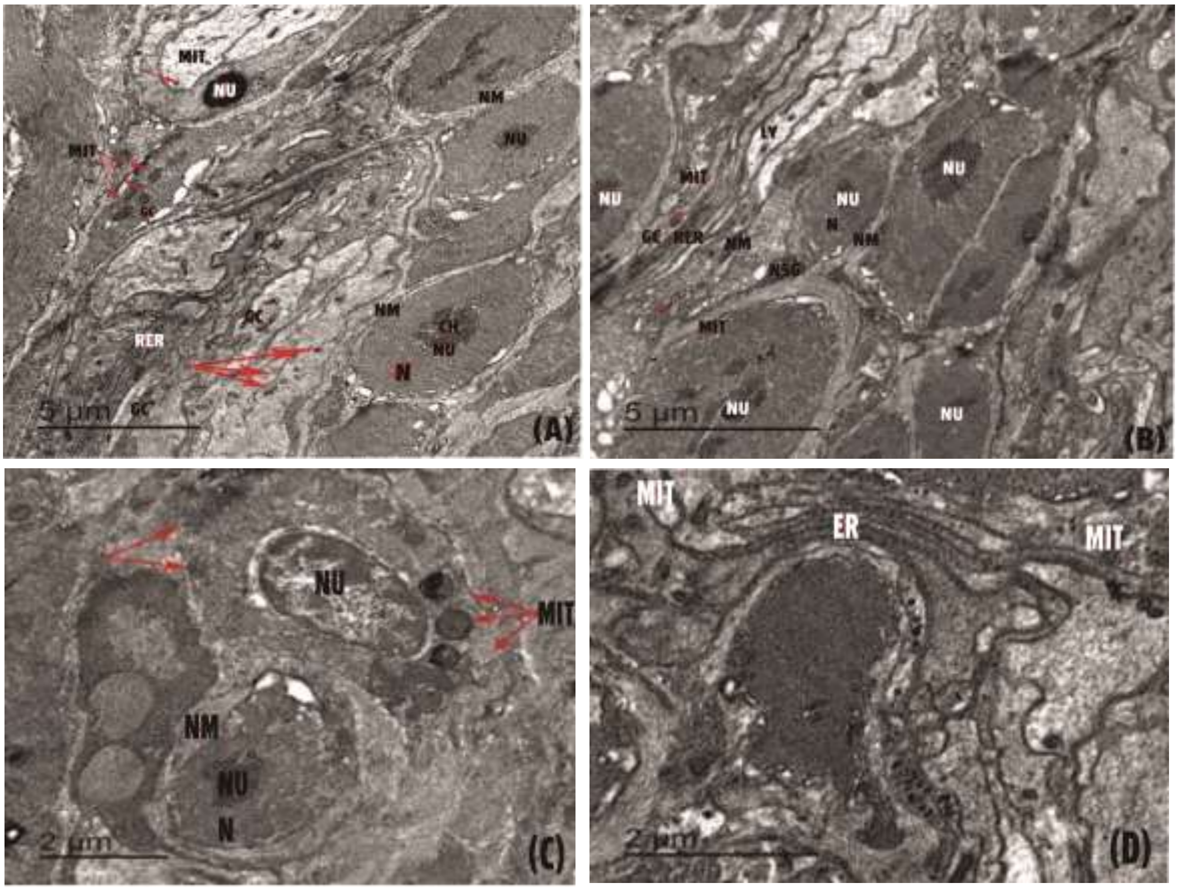

Fig. (7): TEM photomicrographs showing transverse sections of control cerebral ganglia of *T. pisana* snail. **A-** Showing normal neurosecretory cells with its nucleus (N), nucleolus (NU) with chromatin (CH) enveloped by nucleus membrane (NM), Golgi complex (GC), rough endoplasmic reticulum (RER), mitochondria (Mit) and free lysosomes (arrows). **B-** Showing median and small neurosecretory cells with Golgi apparatus (GC), rough endoplasmic reticulum (RER), mitochondria (Mit}, lysosomes (LY), extracellular space (*), nucleus (N), nucleus membrane (NM), nucleolus (NU), free ribosome (arrow) neurosecretory granules (NSg). **C-** Numerous mitochondria (Mit), nucleolus (NU), nucleus (N), nucleus membrane (NM). **D-** Well developed endoplasmic reticulum (ER), and mitochondria (Mit).

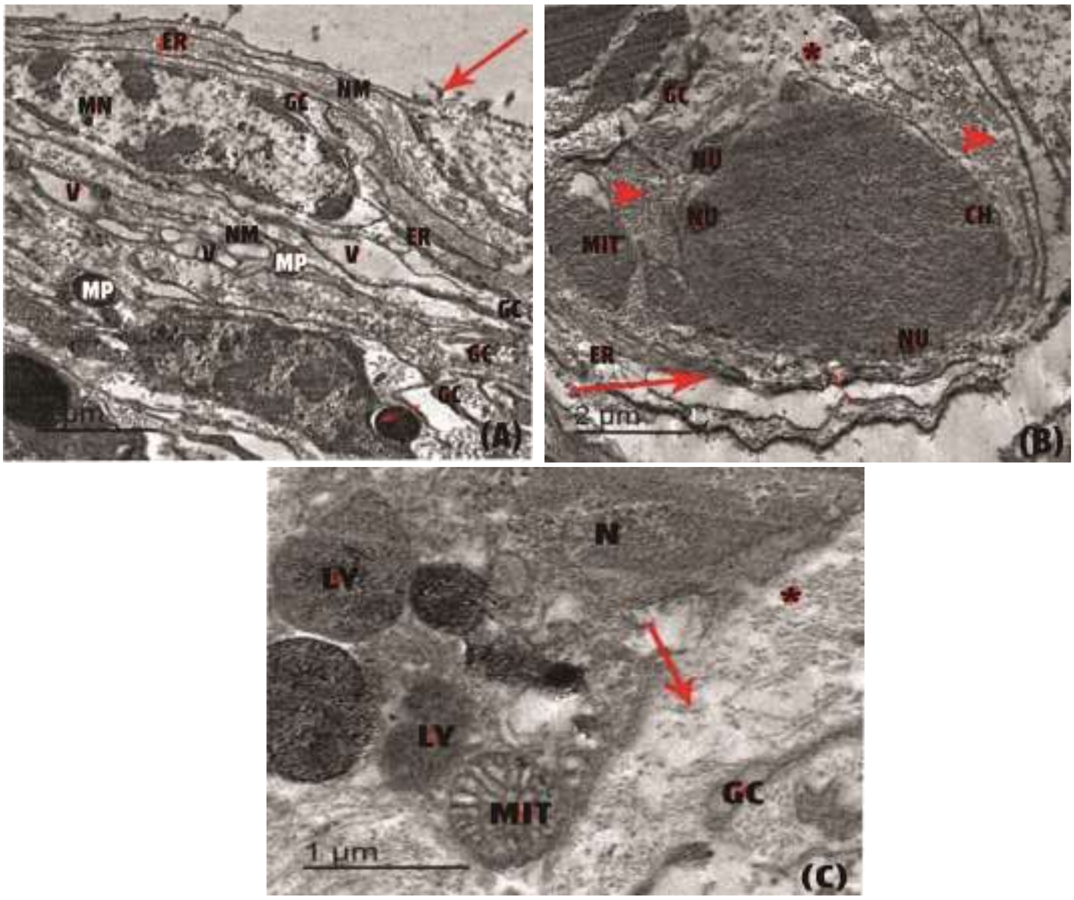

Fig. (8): Transmission electron micrographs showing transverse sections of a cerebral ganglia of *T. pisana* snail treated by Biomajic, **A-** Break down of all organelle cells such as mitochondria (Mit), endoplasmic reticulum (ER), Golgi complex (GC), large number of vacuoles (V), aggregation of some desterioed cell organelle in the form of myelin bodies (Mp), diffused and ruptured perineurium (large arrow). Shrinkage of chromatin of the nucleolus (small arrow). **B-** Neuron showing nucleus with three peripherally located nucleoli (NU), rupture in nuclear envelope (*), autophagosomes (small arrow), peripheral accumulation of Nissl substance (head arrow), displaying loss of endoplasmic reticulum (ER), widened extracellular space (large arrow), lyses of mitochondria (Mit) and Golgi complex (GC). **C-** Large number of axon containing desterioed synaptic vesicles (large arrow), damaged mitochondria (Mit), segregated nucleus (N), aggregation of dense lysosomes bodies (LY), numerous clear vesicles (*), distinct effect in the Golgi complex (GC).

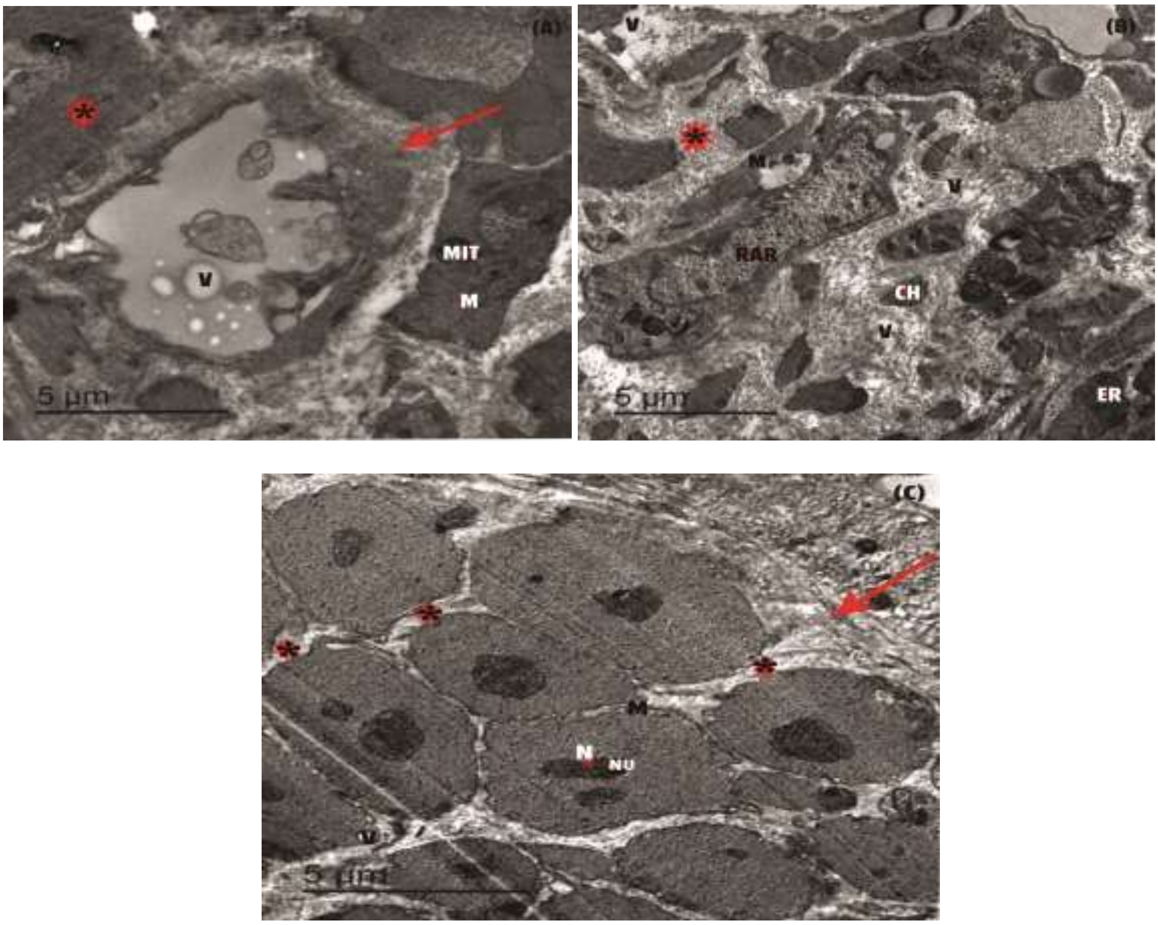

Fig. (9): Transmission electron micrographs showing transverse sections of a cerebral ganglia of *T. pisana* snail treated by Agrinate. **A-** Disturbance of all cell organellae including cytoplasm (*), mitochondria (Mit), endoplasmic reticulum (ER), and lysosomes with appearance of large number of vacuoles (V), **B-** Deprise fragment of Golgi element (double arrow), partly disappearance of endoplasmic reticulum (RER), swollen mitochondria (Mit), segregation of nucleus component (large arrow) with degenerated nucleolus (NU) with completely disappearance of chromatin (CH) and vacuoles were founded (V). **C-** Disorganized extracellular space (*), fragile glial processes (arrow), some nucleus appear without nucleoli (N), fragmentation of rough endoplasmic reticulum (ER), ruptured nucleus membrane (M).

The present results demonstrated that, treatment with Biomajic and Agrinate produced damage and loss of the cristae of mitochondria in the perikarya of the neurons. In treated cerebral ganglia, ultrastructural analysis of the neurosecretory cells revealed the disappearance of neurosecretory granules and the formation of large vacuoles in the cytoplasm, which led to the destruction of the cytoplasmic organelles. Vacuolation of the neurosecretory cells was also observed in *B. glabrata* treated with atrazine by **Eissa and Omran (2007).**

In conclusion, the white garden snail may be used as a bioindicator to detect chemical pollution of terrestrial ecosystem, by chemical compounds. In addition these results show that Agrinate was more affected than Biomajic and both of them are neurotoxic compounds.

References:

Abd El- Wahed, M.I.S. (2014). Biological and pathological studied on some land snail species and their control at Kafr El-Sheikh Governorate. M. Sc. Thesis, Fac. Sci. Tanta Univ., 149pp**.**

Bayne, B. L. O.; Clarke, K. R.; Dixon, D. R.; A. Ivanovici; Livingstone D.R., and Lowe, D. M. (1985). The effects of stress and pollution on marine animals. New York: Praeger p. 384.

Cotran, R.; Kumar, V. and Collins, T. (1999). Robbins Pathologic basis of disease (6th ed.). Philadelphia, PA:WB. Saunders Company.

Eissa, M.M.; EL-Bardicy, S. and Tadros, M. (2011). Bio activity of miltefosine against aquatic stages of *Schistosoma mansoni*, *Schistosoma haematobium* and their snail hosts, supported by scanning electron microscopy. Licensee *Bio. Med. Central, Ltd*., 11(5): 4-73.

Eissa, S.H. and N.E. Omran (2007). Effect of the herbicide atrazine on the cerebral ganglia neurosecretory cells of *Biomphalaria glabrata. J. Egypt. Ger. Soc. Zool*., 35: 78-92.

Essawy, A. (2001). Mapping of buccal neurons innervating the feeding apparatus of *Eobania vermiculata* (Gastropada, Pulmonata). *J. Biol. Sci*., 1: 645–50.

Essawy, A. E; EL Sherief, S. S.; Sadek, I. A. and Soffar, A. A. (2011). Neuropathological effect of tributyltin on the cerebral Ganglia of the land snail, *Eobania vermiculata. Inter. J. Zool. Res*., 7(3): 252-262.

Essawy, A.E; N.E. Abdel Megeed; M.A. Radwan; S.S. Hamed and A.E. Hegazy (2009). Neuropathological effect of carbamate molluscicides on the land snail, *Eobania vermiculata*. *Cell–Biol. and Toxic.* 25(3): 275-290.

Fouad, M. M.; K. F. K.; Ibrahim, I.K. and Soliman A.M.A., (2004). Laboratory studies on the molluscicidial effect of sumithion, bendiocarb and machete pesticides against three land snail species. *J. Agric. Sci*., 29 (1): 451- 455.

Geasa, N. S.; Heiba, F.N.; M.M. Mortada and Mosbah, I.S. (2013). Molluscicidial activity of certain Biological insecticides against land snails *Monacha cartusiana* and *Succinea oblonga* in laboratory and field conditions. *Egy. J. Zool*., 60: 179 – 188.

Gouissi F. and Adimi A. (2019). Bioavailability of Agricultural Pesticides on the Land Snails in the Commune of Kérou in the North of Benine. *Open J. oil Sci*., 9 (7):103-112.

Hamed, S.S.; Abdel-meguied, N.E.; Essawy A.E.; Radwan, M.A. and Hegazy, A.E. (2007). Histological and Ultrastructural changes induced by two carbamate molluscicides on the digestive gland of *Eobania vermiculata*. *J. Biol. Sci*., 7(6): 1017-1037.

Heikal, M.H. (2015). Biological aspects and population dynamics of three terrestrial snails infesting fruit trees in Egypt. *Int. J. Adv. Res. Boil. Sci*., 2(1):169 – 180.

Hemmaid, K. Z.; Ahmed, S. A. and El-akhrsay, F.I. (2017). Ultrastructural alternations in cells of the digestive gland of *Eobania vermiculata* (Muller) treated with three chemical compounds. *Middle East J. of Appl. Sci.,* 7(3):595-612.

Iglesias, J.; Castillego J. and Castro R. (2003). The effects of repeated application of molluscicides metaldehyde and the biocontrol nematode *Phasmarhabditis hermaphrodita* on Mollusca, earth worms, nematodes, acarids and collembolans two study in north-west spain. *Pest. mang. sci*., 59(12):17-21.

Jones, H.B. and Cavanagh, J.B. (1984). The evolution of intracellular responses to acrylamide in rat spinal ganglion neurons. *Neuropath. Appl. Neuro. Boil*., 10:101–21.

Kruatrachue, M.; Upatham, E.S.; Seehabutr, V.; Chavade, j.; Sretarugsa, P. and Sobhon, P. (1993). General anatomy and histology of the nervous system in Achatina fulica. *J. Sci. Soc. Thail*., 19:1–24.

Muller, L.J.; Moorer-van Delft, C.M.; Zijl, R. and Roubos, E.W. (1990). Use of snail neurons in developing quantitative Ultrastructural parameters for neurotoxic side effects of Vince antitumor agents. *Cancer Res*., 50: 1924-1928.

Niva Dutta and Dr Manashi Bortamuly (2018). Pesticides and its effect on Health and Environment. *IJRAR*.,4(5):57-59.

Shahawy, W.A. (2018). Biochemical Effects of Mullicicides against the Land Snails, *Helicella vestalis* and *Theba pisana* Using Sublethal Doses., *J. Plant Prot. and Path*., Mansoura Univ., 9(4): 261-264.

Sleem, H.S. (1993). Studies on the reproductive biology and some factors affecting reproduction of certain freshwater snails. Ph.D. thesis, Faculty of Science, Ain Shams Univ. Cairo. Egypt, 145 pp.

Sterman, A.B. (1982). Acrylamide induces early morphologic reorganzation of the neuronal cell body. *Neurology*, 32:1023–6.

Zedan, H.A.; Saleh, A.A. and Abd El-Aal, S.M. (1999). Bactericidal activity of *Bacillus thuriengensis* against snails toxicological and histological studies the 2 Int. Conf. of Pest Control, Mansoura, Egypt, 489-497.

التركيب الدقيق والتأثير المرضي على الانسجة لمبيدات الآفات شائعة الاستخدام فى مصر على العين ، وساق العين والعقد المخية للقوقع الأرضى ثيبة بيسانا (موللاسكا، جاستروبودا) من كفر الشيخ ، مصر

آلاء إبراهيم عتلم[١]، فادية ناجى هيبة[١]، محمد محمد مرتضى[٢]، نجلاء شوقى جعيصة[١]، سحر إبراهيم عبد الواحد[٢]
[١]قسم علم الحيوان ، كلية العلوم ، جامعة طنطا
[٢]قسم بحوث الحيوانات الضارة ، معهد بحوث وقاية النباتات، مركز البحوث الزراعية، كفر الشيخ

يعتبر الحلزون البري ثيبا بيسانا من أكثر الحلزونات انتشارا وضررًا ، حيث انه يتغذى على المحاصيل الحقلية، وتعتمد طرق المكافحة التقليدية على المبيدات، ومن أكثر مبيدات الرخويات شيوعًا في مصر هي أجرينيت ٢٤% (ميثومايل) و بايوماجيك (ميتاريزيام انيسوبليا) .ولقد هدفت الدراسة الحالية إلى دراسة وتقييم تأثير المبيدات المستخدمة على الحلزون الأرضي ثيبا بيسانا من أجل التنبؤ بالآثار الخطرة لهذه المبيدات على الكائنات غير المستهدفة. وقد خلصت الدراسة الحالية الى أن تعرض القواقع البالغة محل الراسة ثيبا بيسانا لجرعة شبه مميتة لكلا من المبيدات (اجرينيت وبايوماجيك) أدى بدرجات متفاوتة إلى تشوهات بالغة وتغيرات نسيجية مرضية في الأعضاء التي تم فحصها وهى (العيون ، لوامس الاعين والعقد المخية). وتسبب التعرض لمدة ١٤ يومًا لكلا من المبيدات الحشرية المستخدمة إلى فقدان بنية العين وساق العين بالإضافة إلى ذلك فقد أظهر كلاهما تغيرات تنكسية متعددة في العقد المخية للحلزون ، وقد اثبتت الدراسة أن مبيد الرخويات أجرينيت ٢٤% كان أكثر فعالية ضد الحلزون البرى ثيبا بيسانا من البايوملجيك.