Zagazig University
Faculty of Science
Zoology Department

EFFICIENCY OF CERTAIN CHEMICALS AND BIOCIDES ON CELLS AND TISSUES OF SOME LAND SNAILS INFESTING VEGETABLE CROPS AT SHARKIA GOVERNORATE

A thesis
submitted for the degree of
DOCTOR OF PHILOSOPHY IN SCIENCE

(Zoology)

BY

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Supervised By

Prof. Salem Abd El-Fatah Ahmed
Prof.of Pesticides, Plant Protection, Research Institute, Agricultural Research Center.

Prof. Kamel Zaki Hemmaid
Prof.of Cytology and Histology, Zoology Department, Faculty of Science, Zagazig University.

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Abstract

Controlling the land snails *Eobania vermiculata* and *Monacha cartusiana* by using several chemical compounds and biocides as poisonous baits under laboratory conditions are investigated in the present study. The results revealed that against *Eobania vermiculata* chemical compounds arranged descindingly as follows: Amino, humic acid, cyflu-magic, micronized sulfur, fusillade, k-othrine WG250, potassium thiosulfate, urea and combining Zn comparing with Newmyl, As for biocides they were also arranged descindingly as follows: Vertimec, Protecto, Biover and Bioranza comparing with Newmyl. With *Monacha cartusiana* the chemical compound arranged descindingly as follows: Amino, humic acid, fusillade, cyflu-magic, urea, combining Zn, potassium thiosulfate and k-othrine WG250 comparing with Newmyl. As for biocides toxicity arrangement as follows: Vertimec, Protecto, Bioranza and Biover.

Histological observations of the digestive gland cells in both snails showed the presence of three cell types (a) digestive cells, (b) excretory cells and (c) calcium cells. The intestinal epithelium composed of ciliated and un-ciliated cells, basal cells and mucous cells. Histopathological examination of the digestive gland of both tested land snails, treated with Newmyl (1/2 LC_{50} and LC_{50}) displayed that the digestive cells showed cytoplasmic degenerations. Calcium cells were packed with enlarged calcium spherules and they exhibited pyknotic nuclei. Excretory cells showed increased number of excretory granules. Treatment with Amino (1/2 LC_{50} and LC_{50}) revealed that the excretory cells contained large size of excretory granules. But when 1/2 LC_{50} and LC_{50} of Vertimec were used the digestive gland cells still surrounded by thin membranes.

Moreover, the intestine of snails treated with Newmyl (1/2 LC_{50} and LC_{50}) showed reduction of cilia, fragmentation of muscle tissue and increase in mucus secretion. Amino (1/2 LC_{50} and LC_{50}) caused an increase in the

mucous secretion. The nucleus of the intestinal cells become pyknotic when snails were treated with Vertimec (1/2 LC_{50} and LC_{50}).

Ultrastructural observations of treated digestive gland of both tested land snails displayed that the effective applied chemical compounds (1/2 LC_{50} and LC_{50} of Newmyl, Amino and Vertimec) showed cytological alterations in mitochondria of digestive cells showed a hyperdensity of their matrix and polymorphism, whereas most nuclei showed compaction and margination of the nuclear chromatin with dilation of the nuclear envelope. RER were deformed and exhibited highly electron-dense matrix and disorganized few cristae. The excretory cells showed enlarged nuclei, increase in the size of the excretory vacuoles and increased osmiophilia of their contained excretory granules. Sometimes, the excretory cell vacuoles open together producing a big one occupying nearly the whole cell.

Concerning the effects of the applied compounds on the biochemical parameters of the experimental snails, variable and fluctuated results were recorded. Treatment of *Eobania vermiculata* snails with 1/2 LC_{50} of Newmyl resulted in an increase in the values of glucose, cholesterol, albumin, triglycerides and urea and a decrease in the value of the total proteins. Newmyl LC_{50} caused an increase in all studied biochemical parameters. When the snail was treated with 1/2 LC_{50} of Amino glucose, albumin, triglycerides and cholesterol were increased but urea and total proteins were decreased. Treatment with the LC_{50} of Amino showed an increase in all studied biochemical parameters. Similar findings of decreased biochemical parameters were recorded in case of 1/2 LC_{50} and the LC_{50} of the biocide Vertimec.

Treatment of *Monacha cartusiana* with low concentration of Newmyl (1/2 LC_{50}) showed that the biochemical parameters of glucose and triglycerides, albumin, were decreased. But the total proteins, cholesterol and urea were increased. Treatment of snails with Newmyl LC_{50} showed that

most tested parameters were increased but glucose was decreased. The application of Amino 1/2 LC_{50} led to decrease in all biochemical parameters comparing with control. Urea was the exception as it was increased. Application of Amino LC_{50} caused a decrease in glucose, albumin, triglycerides and cholesterol. But total proteins and urea were increased. When 1/2 LC_{50} of Vertimec was applied to *M. cartusiana* all parameters were decreased. When Vertimec LC_{50} was applied glucose, triglycerides, albumin and total proteins were decreased. But cholesterol and urea were increased.

The field experiment was applied in Egyptian clover field highly infested with *Monacha cartusiana* due to snail wide dispersion in Sharkia Governorate. In this experiment, only the highest concentrations of each compound were applied against *Monacha cartusiana*. The results showed that the micronized sulfur revealed the highest initial effect (the average of reduction percentages after 1and 3 days) while combining Zn showed the lowest initial effect comparing with Newmyl. As for biocides application in the field, Vertimec showed the highest initial effect while Protecto the lowest effective one comparing with Newmyl. The residual effect (the average of reduction percentages after 7, 14 and 21 days) when chemical compound applied urea, the fertilizer was the highest one, while combining Zn still the lowest effective one comparing with Newmyl. As for biocides Biover was the highest one where Vertimec was the lowest.

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Fatma El-Akhrasy

Contents

List of Tabels

List of Figers

Abbreviations

CaC	Calcium cell
CM	Cell membrane
DC	Digestive cell
Dg	Degenration
Diss	Dissosiation
E	Eiosen
E.C.	Emulsifiable concentrate
ExC	Excretory cell
ExV	Excretory vacuole
GABA	Gamma-aminobutyric acid
Go	Golgi apparatus
Hx	Hematoxelin

L	Lumen
La	Lactel
LC_{50}	Lethal concentration causes the death of 50% of test animals
LD_{50}	Lethal dose causes the death of 50% of test animals
LT_{50}	Letahl time causes the death of 50% of test animals
Ly	Lysosomes
M	Mitochondria
Muc	Mucosa
Mv	Microvilli
N	Nucleus
Nu	Nucleolus
Oed	Oedema
RER	Rough endoplasmic reticulum
RH	Relative Humidity
S.L.	Soluble concentrate
T.S.	Water soluble tablets
V	Vills
W.G.	Water dispersible granules
W.P.S.	Water dispersible powder for slurry seed tratment
Zn	Zinc

Chapter I

Introduction

In many parts of the world, the terrestrial molluscs are significantly threat the sustainable agriculture **(Barker, 2002)**. In Egypt, the land snails damage vegetables, field crops, orchard trees and ornamental plants **(El-Wakil *et al*., 2000).** The terrestrial helicid snail *E. vermiculata* was surveyed as agricultural economic pest in Egypt **(El-Okda, 1979; Abo Bakr, 1997; Eshra, 2004 and Abo Bakr, 2011)**.

Land snails are dangerous crop pests and cause effective damage to a wide variety of plants **(El-Okda, 1980)**. Land snails are become one of the serious pests in most of the world countries especially those with moist or rainy climates. They are vegetarians and feed on a wide variety of plant parts both wild and cultivated that including field vegetable and fruit crops as well as ornamental plants **(Godan, 1983)**. **Heiba *et al.* (2002)** reported that *Eobania vermiculata* and *Monacha cantiana* land snails are widely distributed all over the Nile Delta. In Sharkia Governorate the land snail *Monacha cartusiana* reported a relatively high occurrence and population density on economic crops **(Ghamry *et al*., 1993; El-Massry, 1997; Ismail, 1997 and Hegab, 2003)**.

Newmyl was found to have high toxicity to both *Eobania vermiculata* and *Monacha cartusiana* **(Helmy *et al*., 2006)**. The same authors found that Vertimec biocide came secondly to Newmyl in toxicity potential. Moreover, **El-Sayed(2010)** proved that Newmyl in laboratory conditions was highly effective against the above two land snails.

Some natural compounds and biocides were found to have mollucicidal effects on land snails. If compared with pesticides, Vertimec biocide was proved to have effective toxicity **(Abd El-Wahab, 2004 and Helmy *et al*., 2006)**.

Hence, there was a trend to use natural compounds and biocides instead of chemical pesticides **(Ismail and Mohamed, 2009)**.

The digestive gland of molluscs is involved in extracellular digestion of food material, absorption of nutrients, storage of lipids, glycogen and minerals and it plays also a major role in detoxification (**Beeby and Richmond, 1988 and Henry *et al*., 1991)**. It plays the major role of digestion and important in conversion of food resources (**Gerard and Theron, 1996 and Boer and Kits, 2005)**.

The histological and histochemical description of the digestive system of the land snails were investigated by many authors such as **Saad (1988)** and **Saad and Mohamed (1989)** and that investigations help in the reactions of metaldehyde with the digestive tract cells which cause excess mucus production leading to fatal mucus deficiency then death **(Treibskorn, 1989).** Three types of cells can be identified in the digestive gland: digestive cells, excretory cells and calcium cells **(Lopes *et al*., 2001 and Gros *et al*., 2009).**

Treatment of terrestrial snails with chemical compounds pesticides, molluscicides or insecticides in control programs is the main method used for controlling land gastropod pests **(Coupland, 1996; Abdallah *et al*., 1998; El-Shahaat *et al*., 2007and 2009).** The digestive gland of gastropods is one of the most important target organs for toxicity injury **(Abo Bakr, 2011).**

Chapter III: Review of Literature

Chapter III

Review of Literature

1-Snails chemical control:-

El-Sebae *et al.* (1982) tested locally formulated bran baits containing aldicarb, methomyl or dupont–1642 against the land snails *Helicella vestalis, Eobania vermiculata* and *Theba pisana*. Different wheat and rice brans containing 0.5 % aldicarb or methomyl showed high attractant action and toxicity for land snails, represented by their high mortality percentages. Bran baits containing aldicarb mostly gave higher mortalities than methomyl and dupont–1642 after 5 days. The presence of 0.15 % methylene blue in bran baits increased the mortality percentages. The authors suggested that methylene blue increased the toxic potency through its effect as an attractant.

Abd-Allah (1994) evaluated the efficacy of four pesticides (Temik, Furadan, Skipper and Mesurol) against the land snail *Monacha cartusiana* under laboratory conditions. Temik and Furadan potencies significantly exceeded those of Mesurol and Skipper.

Awad (1994) controlled *Monacha cartusiana* and *Succinea putris* by four pesticides under laboratory conditions. The results revealed that the adult *Succinea putris* was more sensitive to pesticide action and the young stages were more susceptible to the pesticidical activities than the adult *Monacha cartusiana*.

Ghamry *et al*. (1994) evaluated fourteen insecticides against *Monacha cartusiana* and *Eobania vermiculata*. Results revealed that methomyl, dithiocarb, carbaryl, chlorpyrifos and dimethoate were more effective in killing snails after 12 days under laboratory conditions. On the other hand, the same trend was observed with these insecticides when

used under field conditions. **Hussien *et al.* (1994)** found that ushanin (oshar plant extract) was 128 times more toxic than methomyl against land snails *Theba pisana*. The LC_{50} was 0.82 µg/ snail for usharin while it was 105µg/ snail in case of methomyl.

Okka *et al.* (1996) tested twenty-two compounds representing different groups: carbamates, organophosphates, pyrethroids, fungicides and fungicide mixtures either as baits or sprays for controlling the land snail *Monacha cantiana* under laboratory conditions. Results revealed that cymbush, dimethoate, Karate, Hostathion, methiocarb and malathion were the most effective applied compounds on *M. cantiana*.

Ismail (1997) tested eight pesticides against *Monacha cartusiana* under laboratory and field conditions. The results revealed that under laboratory conditions oxamayl E.C. was the highest toxic one and fenamiphos was the lowest, while under field conditions the organophosphrus pesticide (fenamiphos) induced the highest effect.

Hanafy *et al.* (1998) tested six methomyl baits against *M. obstructa, E. vermiculata* and *Cochlicella. acuta*. They found that, the first and the second species were highly affected, while *C. acuta* presented the least mortality percentages in this respect.

Shahawy (1998) tested four concentrations of the insecticides fenthion and fenitrothion as well as the acaricides fenpyroximate and bromopropylate as poisonous baits against *Monacha cantiana* under laboratory conditions. Results indicated that bromopropylate was the most toxic compound against the snails followed by fenitrothion and fenthion but fenpyroximate was the least effective compound.

Hussein *et al.*(1999) evaluated the molluscicidal activity of a cardenolide (extract from *Pergularia tomentosa*), methomyl, and methiocarb against the land snail *Monacha obstructa* at 25^{o}C. The LD_{50} value of the plant extract after 24 h of treatment was 60.9 µg / snail,

whereas the LD_{50} values of the two tested carbamate pesticides after 72 hr. of treatment were 11.9 and 27.4 9 μg / snail, respectively.

Lokma and Al-Harby (**1999a)** compared molluscicidal effects of Salut, Ethanox, Sumithion and carbaryl as spray and baits but Troksan used only as bait against *Monacha cartusiana* and *Eobania vermiculata*. Salut was the most toxic compound then Sumithion, Ethanox and carbaryl as spray and the bran baits tested by Salut and Troksan. These tested insecticides were more active when used as baits than spray and *Eobania vermiculata* was less sensitive than *Monacha cartusiana*.

Abd El-Karim (2000) studied the toxic effects of Sumithion as an organophosphrus insecticide and Skipper as a carbamte molluscicide against three land snails, *Eobania vermiculata, Monacha obstructa* and *Theba pisana*. Results revealed that *M. obstructa* was the most sensitive while *E. vermiculata* was the highest tolerant and *T. pisana* exhibited moderate response.

Aioub *et al.*(2000) examined seven pesticides namely, malathion, profenophos, primiphos-methyl, methomyl, oxamyl, trallkoxydim and isoproturoun under laboratory conditions against two land snails, *E. vermiculata* and *M. cartusiana*. Carbamate compounds appeared to be the most highly toxic while organophosphrus and herbicides were the least toxicant. The two pesticides which exhibited higher efficacy (methomyl and oxamyl) were tested under field conditions against the two land snails and methomyl induced a higher effect on the population reduction than oxamyl.

Dimitriadis and Andrews (2000) examined the effects of six nematicides as soil treatment on eggs hatchability of *Eobania vermiculata* and *Monacha cartusiana* and found that carbofuran was the most effective while aldicarb was the least. They also examined 5 pesticides by dipping technique and found that tralkoxydim was the most toxic, while

oxamyl and malathion were the lowest. The same authors also examined 7 pesticides under laboratory conditions and found that carbamates were the most toxic while organophosphrus and herbicides were the least.

Ghamry *et al.*(2000) tested four surfactants namely Solar oil emulsion, nonoditte 20(1:0.5%), nonoditte 40:80 and ethanolamine, against eggs, juveniles and adults of the land snail *Monacha cartusiana* under laboratory conditions. The results showed that Solar oil emulsion gave the highest ovicidal activity followed by ethanolamine, nonoditte 20 and nonoditte 80.

Abd El-Aal (2001) evaluated seven pesticides to reveal their molluscicidal effect as poisonous baits against *M. cartusiana* under field conditions. The molluscicidal efficiency of the tested pesticides were arranged as follows: fenamiphos > sethoxydim > oxamyl > monocrotophos > butachlor > biofly and Seeds guard.

El-Khodary *et al.*(2001) tested the efficacy of four pesticides, Lebaycid, Sumithion (organophosphrus compounds), aurtis (phenoxpyrazole compound), and neuron (benzilate compound) against *Monacha cantiana* under laboratory conditions. Results indicated that the benzilate compound (neuron) was the most potent one against the snail. Organophosphorus and phenoxypyrazole compounds have moderate and lowest potencies against the snail.

Youssef (2001) investigated the efficacy of fourteen pesticides against *Monacha cantiana* and *Eobania vermiculata* under laboratory conditions as poisonous baits at three concentrations 2, 4, and 8%. Results revealed that tamaron and Metason (metaldehyde) at concentration of 8% were the most effective pesticides against the two snails, while Tokuthio and diazinon failed to cause any mortality against the two snails.

Heiba *et al.* (2002) tested the efficacy of the insecticide Lannate as a molluscicide for the control of two land snails, *E. vermiculata* and *M. cantiana*. The higher mortality rates were noticed at doses higher than the LD_{50}. However, at all doses, the mortality rates increased by the time and most mortalities occurred over five days post insecticide administration and the maximum mortality rates were observed for *E. vermiculata* and *M. cantiana* after 108 hours.

EL-Deeb *et al.*(2003a) studied the toxicity of two insecticides, methomyl 20% SL. and diazinon 60% EC., used as baits and contact against different ages of *Monacha obstructa*. They found that methomyl was more toxic than diazinon when used as contact for all tested ages (1, 3, 6, 9, 12 and over 24 months) whiles it was less toxic when used as baits.

Later, **EL-Deeb *et al.*(2003b)** revealed that methomyl was more effective than diazinon when used as bait against all ages of *Eobania vermiculata*. Regarded the contact method diazinon was more toxic than methomyl for three months and over 24 months ages.

Hanafy *et al.* (2003) tested two methomyl baits against the land snails *Monacha obstructa*, *Helix vestalis*, *Eobania vermiculata*, *Theba pisana* and *Cochlicella acuta* in some treated orchards of fruit trees. According to the attract ability of each of tested toxic baits the identified species of inspected land snails can be discendingly arranged as follow: *M. obstructa*, *H. vestalis*, *E. vermiculata*, *T. pisana* and *C. acuta*.

Hegab (2003) studied the efficacy of methomyl 90%, 20% dimethoate, Monitor and mixture of Monitor and methomyl against *Monacha cartusiana* as poisonous baits in laboratory conditions. The conc. of 5% from both dimethoate and methomyl caused 100% and 95% mortality, respectively. After 21 days of treatment, Monitor gave 50%, but methomyl was the most effective compound after 3 days of treatment.

Mobarak (2003) mentioned that methomyl was more toxic for *Eobania vermiculata* than *Monacha obstructa* when used as poisonous baits.

Abd El-Wahab (2004) reported that toxicity of Curacron, Dursban, Marshal, methomyl, and Vertimec against *Monacha cartusiana* and *Eobania vermiculata* land snails was studied using bait technique. The results indicated that methomyl exhibited the highest toxic action against *M. cartusiana* land snail followed by Vertimec, Marshal and Dursban while Curacron was the least in activity.

Fouad *et al.*(2004) studied the molluscicidal effects of three pesticides namely: bindiocarb, Sumithion and Machete against the adult stage of three land snails species, *Monacha obstructa, Theba pisana* and *Eobania vermiculata* under laboratory conditions. Results proved that bindiocarb is the most toxic one for the three tested snails, while Machete gave the lowest effect. **Moran *et al.*(2004)** tested the efficacy of Copper hydroxide formulations in controlling land snails on two plots of cut green ornamentals in shade houses. One was a *Ruscus hypoglossum* plot that was infected and damaged by *Monacha syriaca*; the second was an *Aspidistra elatior* plot that was infected and damaged mainly by *Theba pisana* and *M. syriaca*. Two formulations of water dispersible granules were tested. The results revealed that the differences between the efficacies of the two formulations were not statistically significant. Therefore, the 0.1 % concentration of either formulation is sufficient for reducing of land snail populations.

Azzam (2005) evaluated the molluscicidal activity of Gastrotox 5%, Molotov 3%, spintor 24%, Newmyl 20%, Newmyl 90% and Vertimec 1.8% against *Monacha cartusiana* land snails. The results showed that Molotov 3%, Gastrotox 5% and Newmyl 90% exhibited the

highest toxic action while Vertimec, Newmyl 20% and spintor 24% appeared to be the least in activity.

Ebenso *et al.*(2005) investigated the effect of 50, 100, 200, 300, 400 and 500 mg/ml of carbamate molluscicide on the behavioral and macroscopic changes of *Limicolaria aurora* in the laboratory using *Carica papaya* as bait for 120 hr. The data showed that 48 hr after dosing the organism with furadan 60 % mortality was recorded.

Ismail *et al.*(2005) tested the effects of six chemicals namely, methomyl, chlorpyifos, lufenuron, spinosad, copper sulfate and ferrous sulfate against *M. cartusiana* snails under field conditions in two fields cultivated with onion and broad bean. Results revealed that methomyl showed the highest effect while spinosad was the lowest one.

Shahawy (2005) tested twelve pesticides belonging to different chemical groups against *M. cantiana* snails in laboratory conditions and five pesticides as poisonous baits under filed conditions. The results revealed that organophosphrus compounds induced the highest effect followed by trichlorofon then imidacloprid. The molluscicidal action of the carbamates was very greatly reduced under field conditions. Fenobucarb was the least toxic one with a snail reduction average, while methomyl caused higher snail reduction average.

Shetaia (2005) tested some nematicides against the land snail *M. cartusiana* on clover (spraying technique) under field conditions. Nemacur appeared to be more active against *M. cartusiana* snails than Vydate. The same author used methomyl as poisonous baits by three concentrations 0.5, 1, 1.5 % against *Eobania vermiculata* under field conditions. The results revealed that the conc. of 1.5 % was the more effective one.

Salama *et al.*(2005) compared the ability of two carbamate compounds (methomyl and carbofuran), an organophosphorus compound

(chlorpyrifos) and a bipyridylium compound (paraquat) to induce the oxidative stress and affect some biochemical targets in the land snail, *Helix aspersa*. LD_{50} values for these pesticides were determined at 48 hr. following topical application. Some biomarkers of the oxidative stress such as lipid peroxidation (LP), lactate dehydrogenase (LDH) and glutathione (GSH) as well as the inhibitory effects of these compounds against acetylcholinesterase (AChE) were carried out following topical application of 1/4 LD_{50} values. The results showed that carbofuran was the most potent compound in inhibiting AChE in snails, followed by methomyl. It could be concluded that methomyl was the most toxic pesticide followed by carbofuran against the land snail. Chlorpyrifos or paraquat had slightly effects to alter the biomarkers of oxidative stress in the snail.

Shaaban (2005) evaluated four mollucicides against two land snails species, *Monacha cartusiana* and *Succinea putris*. Mollucicides used were methiocarb, aldicarb, carbofuran and Skipper, under laboratory conditions of 18-25°C and 48 to 60 RH %. The obtained results revealed that aldicarb and carbofuran potency significantly exceeded that of methiocarb or Skipper. *S. putris* snail seemed to be more susceptible to the molluscicidal activities than *M. cartusiana*. The response of *M. cartusiana* to aldicarb was quicker than that of *S. putris.*

Abd El-Galeil and Badawy (2006) tested the molluscicidal activities of the essential oils of *Mentha microphlla* and *Lantana camara* against terrestrial snails, *Theba pisana* and *Eobania vermiculata* in comparison with methomyl as a reference. Results indicted that both essential oils were more toxic against *Theba pisana* than *Eobania vermiculata*. The oil of *L. camara* showed moluscicidal activity greater than methomyl, while *M. microphylla* oil showed comparable toxicity with methomyl against the two land snails species.

Abd El-Haleim *et al.* (2006) outlined the molluscicidal activities of indoxacarb, lufenuron and methomyl against glassy clover snails *Monacha cartusiana*. After 3 days of treatment with 0.5 LC_{50} and LC_{50}, aspartate aminotransferases (AST) and alanine aminotransferases (ALT) activities were reduced compared with control animals.

Ismail and Hegab (2006) studied response of juveniles and adults of *E. vermiculata* for abamectin, alphacypermethrin, and methomyl under laboratory and field conditions. Results revealed that juveniles were more sensitive than adults for abamectin and methomyl, while in case of alphacypermethrin the adults were more sensitive than juveniles.

Abd El-Nabey and Shaaban (2006) investigated effects of six pesticides against the two land snails, *Monacha cantiana* and *Eobania vermiculata*, under laboratory and field conditions by using poisonous baits technique. Laboratory results showed that Pyriban exhibited the highest mortality percentage against the two land snail species followed by Ekatin, while Amir and Admiral showed the least mortality percentage. In the same trend, Ekatin, Pyrbian, Polo and Applaud displayed the most mortality percentage (100%) against the same land snails. Admiral showed the lowest mortality percentage. On the other hand, *Eobania vermiculata* was more susceptible than *Monacha cantiana* for the tested insecticides. The field results were in harmony with those obtained from laboratory findings as Pyriban and Ekatin baits were the highest molluscicidal potential against the two land snail species followed by Polo and apploud, while Admiral showed the lowest effect.

Gabr *et al* (2006a) evaluated the molluscicidal effect of spinosad 24 % SC as baits against three land snail species, *Monacha obstructa*, *Eobania vermiculata* and *Theba pisana* under field conditions. The result showed that spinosad was most efficient against *Theba pisana* followed by *Monacha obstructa* and *Eobania vermiculata***.**

Helmy *et al.* (2006) evaluated the activity of certain pesticides against *Monacha cartusiana* and *Eobania vermiculata* land snails under laboratory conditions using poisonous bait technique. Laboratory experiment revealed that Newmyl exhibited the highest toxic action against *M. cartusiana* snails followed by Vertimec, Marshal, Dursban, while Curacron was the least toxic one.

Hussien (2006) evaluated molluscicidal effects of methomyl and two naturally occurring compounds, caffeine and theophylline, in addition to an aqueous tea extract against three land snail species, *Monacha obstructa* , *Theba pisana* and *Eobania vermiculata* under laboratory conditions. Three methods of bioassay were used (contact, leaf-dipping and bait techniques). Results showed that theophylline was the most toxic compound for the three tested snail species followed by caffeine and methomyl while the aqueous tea extract had the lowest effect. The land snail *E. vermiculata* was comparatively less susceptible to the tested compounds than the other two species. Contact technique was the most effective method of application.

El-Masry (2007) tested methomyl, potassium sulfonate solution salt and copper sulfate against the land snail *Monacha cartusiana* which was considered a main pest attacking cucumber crop. Under the laboratory conditions, the hatchability percentages decreased after exposure to tested chemicals methomyl, potassium sulfonate and copper sulfate, comparing to control and the mortality percentages increased. Under field conditions reduction percentages increased as the concentrations increased and reached the highest reduction after 3 weeks of methomyl, potassium sulfonate and copper sulfate treatment .

Hamed *et al.* (2007) evaluated the toxic action of two carbamate molluscicides, methomyl and methiocarb, on the digestive gland of the land snail *E. vermiculata* which is the main site of accumulation and

biotransformation of xenobiotics, using topical application and baiting techniques.

Lokma (2007) evaluated the molluscicidal activity of three pesticides as poisonous baits against *M. cartusiana* under laboratory and field conditions. Results of laboratory experiment indicated that three days after application metaldehyde proved to be the most effective compound followed by fenamiphos, while oxamyl was the least effective one. On the other hand, when metaldehyde was applied at three rates in field experiment, it was found that reduction percentages increased by increasing application rate.

Genena and Mostafa (2008) tested six pesticides namely: bensultap, chlorpyrifos-ethyl, deltamethrin, diazonixy, lambda-cyhalothrin and methomyl laboratory against two land snails, *Monacha cantiana* and *Eobania vermiculata* as poisonous baits at a constant concentration of 2%. Results revealed the toxic effect of all tested pesticide baits with mortality percentage increasing with an increase in the period of exposure. Deltamethrin (pyrethroid) showed high initial toxicity against *M. cantiana* and *E. vermiculata* after three days of exposure. However, methomyl (carbamate) surpassed other pesticides and gave 100 % mortality after seven and 12 days for the two land snails, respectively. On the other hand, chlorpyrifos-ethyl exhibited the lowest mortality percentages against the tested land snails. Obviously, methomyl proved to be the most effective pesticides followed by deltamethrin, bensultap, lambda-cyhalothrin, diazonixy then chlorpyrifos-ethyl against the two land snails. Moreover, *E. vermiculata* showed more sensitivity to the tested pesticides especially methomyl and deltamethrin than the glassy clover snail, *M. cantiana.*

Radwan *et al.* (2008) determined the lethal toxic action of methomyl and methiocarb against the land snail *Eobania vermiculata*

using topical application and baiting techniques. The results showed that methomyl exhibited greater efficacy than methiocarb in both techniques. The authors also recorded higher mortality rates in the topical application technique than in the poisonous baits experiments.

Ismail and Mohamed (2009) evaluated abamectin 1.8% E.C., methomyl 20% S.L. and metaldehyd 5% as poisonous baits against adults of *Monacha cartusiana* under laboratory conditions. The results revealed that metaldehyd was the most effective while abamectin was the lowest one. These compounds could be arranged descendingly according to their efficacy as follow: metaldehyde, methomyl and abamectin.

Ghaly *et al.*(2009) studied the efficacy of methomyl on *Monacha cartusiana* (juveniles and adults) by using dipping and baits techniques under laboratory conditions. The results of dipping technique revealed that Newmyl gave high effect against juveniles and adults but juveniles were more sensitive than adults.

Ismail (2009) found that adding blue color to the poisonous baits increased its attractiveness and Birell syrup increased the efficiency of methomyl when applied as poisonous baits against the two land snails, *Monacha cartusiana* and *Eobania vermiculata* under field conditions.

El-Akhrasy (2010) controlled the land snail *Monacha cartusiana* by the molluscicide metaldehyde in laboratory and field conditions. The reduction percentage increased with increasing molluscicide concentration with the time.

EL-Sayed (2010) investigated the toxic action of three chemical insecticides, Newmyl 90 % W.P.S., solfac 50 % E.C. and Sumithion 50 % E.C. as poisonous baits against two species of the adult land snails *Monacha cartusiana* and *Eobania vermiculata* under laboratory and field conditions. The results revealed that Newmyl was the most effective one;

there was no survivals at highest concentrations after 24-hours post treatment. According to LC_{50}, LC_{90}, toxicity index and relative potency, the descending order of the tested insecticides was Newmyl, solfac 50% E.C and Sumithion. *M. cartusiana* snails were more affected by the tested insecticides than *E. vermiculata* under field conditions.

El-Shafey *et al.*(2010) studied the mortality percentage of the clover snail, *Monacha cartusiana* after certain periods feeding on *Jatropha curcas* hexane and petroleum ether extracts, applied as baits compared with methomyl. It was found that 28 days after treatment methomyl (1%) was the highest toxic compound against *Monacha cartusiana* respected with hexane extract whereas petroleum ether extract was the lowest effective one.

Ismail *et al.*(2010) revealed that metaldehyde recorded highly efficacy than the other tested pesticides (Copper hydroxide, methomyl, and diazinon). However, high mortality percentage (100%) was obtained after 7 days at 2% concentration under laboratory conditions, while under field conditions, methomyl induced a higher effect on the population reduction (73.19%) than metaldehyde. Copper hydroxide was found to be the lowest effective one against *M. cartusiana* snails under both laboratory and field conditions.

Beltagi *et al.*(2011) studied the physiological responses of *E. vermiculata* snails, one of the agricultural pests in Egypt, to sublethal doses (LD_{25} and LD_{50}) of two potent botanical molluscicides (thymol and nicotine) after 1, 7 and 15 days post exposure as a recovery period using the topical application technique. Treated snails showed common signs of toxicity as excessive production of mucus along with specific symptoms as haemolysis in case of snails treated with LD_{50} of thymol or

nicotine in addition to paralysis of the foot in case of nicotine-treated snails.

Ismail and Abdel Kader (2011) evaluated the potential of flower-bud powder and commercially available eugenol of *Syzygium aromaticum* against juveniles and adults of *Monacha cartusiana* using baiting technique. The results revealed that the molluscicidal concentrations of both poisoning baits proved to be effective in killing both juveniles and adults *M. cartusiana* snail according to the period of exposure. Consequently, clove bud powder and essential oil (eugenol) of *S. aromaticum* appears to have a potential importance for land snails control in Egypt.

Awad (2013) conducted studies on *M. cantiana* and *Succinea putris* for measuring the influence of copper sulphate, super phosphate 15% and low moisture of substrate comprised by Lannate 90 as recommended insecticides under laboratory conditions. The obtained data revealed that Lannate 90 was more effective on *S. putris* than in case of *M. Cantiana*, then copper sulphate followed by super phosphate. The lowest number of dead individuals was recorded in dry substrate or low moisture for *S. putris* and *M. cantiana*. So, using safe materials such as copper sulphate and super phosphate 15% were more effective and they also act as fertilizers, improving crop fields and quality without environmental pollution.

Eshra (2014) investigated the molluscicidal activity of methomyl, urea and copper hydroxide against the brown garden snail, *Eobania vermiculata* and the small white garden snail, *Theba pisana*. The obtained results indicated that copper hydroxide was the most toxic compound against *E. vermiculata* followed by methomyl and urea. Methomyl was also the most toxic compound when it was tested against *T. pisana* followed by copper hydroxide followed by urea. Brown garden snail was

more susceptible to copper hydroxide and methomyl than *T. pisana*. There was no difference in the susceptibility of the two tested snails to urea. The toxicity of the compounds was enhanced by increasing the exposure time. This study showed that the land snails may be controlled using urea fertilizer.

Ismail *et al.*(2014) studied the effect of attractive intervals distances and some control application methods on the efficacy of methomyl against *Monacha cartusiana* snails under field conditions. Result revealed that poisonous baits of methomyl containing wheat bran and sugar cane syrup can attractive *M. cartusiana* snails far from 100 cm. These results included that bait stations of poisonous bran baits for control land snails could be applied on two meters intervals between one station and the other. Regarding the evaluation of two application (methods spray and poisonous baits) results indicated that poisonous baits were more effective than spray technique.

Kandil *et al.*(2014) determined the toxicity effects of acetylsalicylic acid, methomyl, abamectin bioinsecticide and the joint action of methomyl or abamectin with acetylsalicylic acid against two land snail species, *E. vermiculata* and *M. obstructa* under field conditions, the high efficiency concentrations of tested compounds were tested as a spray. Results revealed that the combination of methomyl and acetylsalicylic acid enhanced the molluscicidal activity against both land snail species.

El-Sayed *et al*. (2015) performed chemical control in a field infested with three land snails species, *Theba pisana*, *Helicella vestalis* and *Monacha obstructa* on pear trees. The obtained results exhibited the comparative higher and faster molluscicidal activity of methomyl and boric acid and mixture against *T. pisana* than boric acid alone.

Samy *et al*.(2015) used leaf dipping technique in lettuce and cabbage infested by the land snail *Monacha* Spp. The result showed that Newmyl

exhibited the highest toxic effect in lettuce and cabbage followed by dipel2x, agree, Protecto and voliam flexi.

2-Snail biocide control:-

Lokma (1998) estimated the efficacy of Protecto biocide together with 5 molluscidides against *Monacha cartusiana* and *Rumina decollate* as poisonous baits. Protecto was the least effective compound and the land snail *Monacha cartusiana* was more sensitive to all tested compounds than *Rumina decollate*. All compounds gave high effect when used as poisonous baits more than when used on filter papers and the mortality increased with increasing the concentration and exposure period. **Lokma and Al-Harby** (**1999b)** estimated the efficacy of Protecto (9.4%) containing *Bacillus thuninglensis* to reduce the chemical pollution of edible crops in laboratory against *Monacha cartusiana* and *Rumina decollate*. The authors found that spraying was more effective than bran baits, and *Monacha cartusiana* was more sensitive than *Rumina decollate*. The mortality in *Monacha cartusiana* was higher than *Rumina decollate* with spraying but the bran baits and the field experimental mortality were the same for both.

Zedan *et al.*(1999) tested the bactericidal activity of *Bacillus thuningensis*t Israelensis serotype H-14 primary powder and Lannate LC_{50} in laboratory against *Monacha obstructa* using leaf dipping technique. The results revealed that the bacterial formulation was the most effective against these land snails compared with Lannate LC_{50}.

Youssef (2001) evaluated the effect of Vertimec against the two land snail species *Monacha cantiana* and *Eobania vermiculata* as poisonous baits at three concentrations 2, 4 and 8% under laboratory conditions. Results showed that percent of mortalities were (90, 100), (100, 100) and

(l00, l00) for the three tested concentrations, respectively, against the two tested land snail species after 9 days exposure periods.

In a study carried out by **Abd El-Wahab (2004)** Vertimec biocide came secondly to methomyl insecticide in toxicity to *Monacha cartusiana* and *Eobania vermiculata*. Other used insecticides (Curacron, dursban and Marshal) came after Vertimec in efficiency**.**

Shetaia (2005) compared in a field experiment the initial effect, residual effects and general reduction of dry leaf powders of enab-eldeeb, datura and damsisa as poisonous baits against *Eobania vermiculata*. The result revealed that damsisa was the most toxic powder and enab-eldeeb was the lowest.

Arafa (2006) controlled *M. cartusiana* by certain plant seed powders (cauliflower and bitter apple) under laboratory conditions and results revealed that after 24 days materials cauliflower powder gave the highest mortality and bitter apple was the lowest. The author also used the plant seeds as poisonous baits in an Egyptian clover field and tested other four biocides. The results revealed that the mortality percentage of the rest biocides increased by increasing concentrations and exposure period.

Abd El-Galeil and Badawy (2006) evaluated acaricidal and molluscicidal activities of essential oil isolated from *Eucalyptus camaldulensis* and the essential oils of *Mentha microphylla* and *Lantana camara* L. against terrestrial snails, *Theba pisana* and *Eobania vermiculata*. The results showed that the essential oils of *L. camara* and *M. microphylla* exhibited pronounced molluscicidal activity against *T. pisana* snails. The oil of *L. camara* showed molluscicidal activity greater than methomyl as a reference at all of the tested concentrations.

Gabr *et al.*(2006b) evaluated the effects of two natural compounds, Vertimec 1.8 % biocompound (abamectin) and neemix 4.5 % (plant

extract) in addition to the molluscicide compound Cekumeta 5.0 % (metaldehyde) as a bait or contact (thin film) against two land snail species, *Monacha obstructa* and *Eobania vermiculata*. Results showed that when the three tested compounds used as bait, Cekumeta was the most toxic one against the two snail species followed by Vertimec, while neemix was the lowest effective one. On the other hand, *Monacha obstructa* was more susceptible for neemix and Vertimec than *Eobania vermiculata*. In case of contact method, neemix compound was more toxic to *Eobania vermiculata* than *Monacha obstructa*. In contrast, *Monacha obstructa* was more sensitive to Vertimec than *Eobania vermiculata.*

Also**, Helmy *et al.*(2006)** used Vertimec biocide and Newmyl to control *Monacha cartusiana* and *Eobania vermiculata* under laboratory using poisonous bait technique. Laboratory experiment revealed that Newmyl exhibited the highest toxic action against *M. cartusiana* snails followed by Vertimec, whereas against *E. vermiculata* Vertimec exhibited the highest toxic action then Newmyl.

In an experiment carried out by **Abd El-Aal (2007)** to compare Protecto biocide efficacy with other molluscidies against *E. vermiculata* and *M. cartusiana*. The author found that Protecto was the least effective compound and the molluscicidal efficiency could be arranged as follows: methomyl > glyphosate > fenamiphos > sethoxydim > malathion > Protecto.

Salem *et al*. (2007) evaluated certain pesticides including the biopesticide Protecto as poisonous baits during the activity period of land snails *E. vermiculata* and *M. cartusiana*. Results revealed that the highest values of percent reduction were detected with methomyl and glyphosate in *E. vermiculata* and *M. cartusiana*, while fenamiphos, sethoxydim and malathion gave moderate effects. However, Protecto was the least effective one in this respect.

El-Said (2009) evaluated the efficacy of Protecto and Biovar (biocide) compared with Newmyl on juveniles and adults of *Monacha cartusiana* snails under laboratory conditions. The results showed that mortality percentages for the highest concentrations after 21 days of treatment Newmyl was the effective insecticide followed by Biovar then Protecto for juveniles and adults, respectively.

Ismail *et al.* (2010) evaluated the effect of neem extract (neemazal T.S.) on the eggs hatchability and adult snails of two land snail *M. cartusiana* and *E. vermiculata* under laboratory conditions. Results revealed that the hatchability of the two tested snail eggs decreased to reach 50, 42.5 % on the highest concentration (400 ppm.). Regarding the effect of neem extract (neemazal T.S.) against the adult snail *Monacha cartusiana* mortality percentage reached 20, 40 and 55 % in the end of experiment for the three tested concentrations 0.625, 1.250 and 2.5 %.

Al-Sarar *et al.* (2012) evaluated the molluscicidal activity of two cardenolide extracts from *Adenium arabicum*. The benzene (B) and methanol (M) extracts, one cardenolide extract from *Calotropis procera* Aiton (extract C), and methomyl against the harmful land snail *Monacha cantiana* (Montagu). The results revealed that contact LD_{50} value for methomyl was the most toxic molluscicide than the above mentioned plant extracts.

Dawidar *et al.* (2012) studied the molluscicidal activity of seed oil and seed glycosides of *Balanites aegyptiaca* against *Monacha cartusiana*. Results revealed that the bioassay of *B. aegyptiaca* against the land snail *M. cartusiana* indicated the activity of the seed oil and the high activity of the seed glycosides.

Farag (2012) studied the molluscicidal effect of some natural components: fresh and expired oils (soybean oil, sunflower oil, sesame oil, castor oil, maize oil and linseed oil), natural fatty acids(citric acid,

oleic acid and linoleic acid) used as dipping or baits techniques and methomyl and metaldehyde pesticides against *Monacha cartusiana* and *Eobania vermiculata* under laboratory conditions. Experiments results showed that the highest mortality percentages were methomyl followed by castor oil (fresh and expire) for the two tested land snails.

Abo Elnaser (2013) studied the control of *M. obstructa* and *E. vermiculata* under laboratory conditions by Kuik, Proclam, pestaban, radical, radiant and *Bacillus thuringiensis* (Bt). The results revealed that Kuik was the toxic pesticide against the two land snail species followed by Proclam, pestaban, Bt. but the lowest one was radiant. Furthermore, *E. vermiculata* was more sensitive than *M. obstruct*a to the tested pesticides except in case of radiant. Under field conditions the Kuik 90 % EC (3000 ppm.) showed variable toxicity to *M. obstructa* followed by Prolclam 5 % EC and Bt. 9.4 %.

Sabha *et al.* (2013) controlled *Monacha cartusiana* by *Bacillus thuringiensis* (Bt). The results revealed that (Bt) caused 100% mortality to *M. cartusiana* in laboratory treatment. Field application of *B. thuringiensis* as toxic spray on citrus trees infected with *M. cartusiana* snails by using 2 x 103 c.f.u/ml showed that mortality reached 89 % of snails within 21 days.

Shetaia *et al.* (2013) studied the effects of certain insecticides (agrinate, Vertimec and actara) and biocides (zantara, Biover and Bioranza) on *M. cartusiana*. Results revealed that agrinate recorded the highest mortality percentages against *M. cartusiana*, while actara was the lowest one under laboratory conditions. Under field conditions, it was noticed that agrinate was more effective than any insecticide or biocide where reduction percentage reached 77.17 %, while Biover gave 15.98 %. Generally, agrinate was highly efficient against *M. cartusiana* (Müller) snails under laboratory and field conditions.

Lokma (2013) evaluated molluscidal activity of four pesticides (methomyl, predalil, bentazon and Biovar) under laboratory and field conditions. Results showed that methomyl was the most effective compound followed by predalil, bentazon, while Biover was the least effective one. The investigator also showed that solar oil emulsion had weak effectiveness on land snail *M. cartusiana* under field conditions when used in irrigation water, while it gave 100% mortality for eggs.

Mourad (2014) evaluated the molluscicidal effects of five ethanolic crude extracts of cumin (*Cuminum cyminum*), golden shower (*Cassia fistula*), umbrella tree (*Melia azedarach*), olive (*Olea europaea*) and pomegranate (*Punica granatum*) against the two land snail species, *Monacha obstructa* and *Eobania vermiculata,* under laboratory conditions. Three methods of bioassay including contact, leaf-dipping and bait techniques were used. The results indicated that the ethanol crude extract of cumin was the most toxic extract for the two tested land snail species followed by golden shower, umbrella tree and pomegranate extracts while olive extract had the lowest effect. The land snail *E. vermiculata* was comparatively less susceptible to the tested plant extracts than the land snail *M. obstructa.* Results showed that contact technique of the tested plant extracts was the most effective method of application.

El Sayed (2014) investigated *in vivo* effects of abamectin, emamectin benzoate and methomyl on gamma-Aminobutyric acid (GABA) and glutamate decaboxylase (GAD) of *Eobania vermiculata* and *Theba pisana.* The results revealed that methomyl clearly inhibited GAD activity, abamectin and emamectin benzoate stimulated markedly the GAD activity in both types of the used land snails. The inhibitory effect of methomyl was dose dependent where the activity of GAD enzyme increased by decreasing the dose treatments in

both types of snails. However, the inhibition of GAD activity was more pronounced with *E. vermiculata* than *T. pisana*. Abamectin and emamectin benzoate induced a significant GAD stimulatory effect for both type of snails.

3-Snail histology and ultrastructure

The fine structure of the calcium cells in the hepatopancreas of *Helix pomatia* was studied by **Abolins (1965)**. The nucleus of the calcium cells was large and lobulated. Large masses of supranuclear chromatin were present. The mitochondria appeared as elongated dense bodies with barely recognizable cristae. A rough-surfaced endoplasmic reticulum was only sparsely present. The Golgi complex was voluminous, especially the Golgi membranes. The calcium spherites occupied the region between the nucleus and the Golgi complex within the cell. The organic matrix of the spherites consisted mainly of fibrillar protein. In a few cases, the periodic band structure of the fibers was recognized in EDTA-decalcified sections. In undecalcified spherites the grains of calcium salts were deposited along the fine fibrils. The role of the Golgi complex in the secretion of the organic matrix of the spherites was discussed.

Abolins (1970) demonstrated that the hepatopancreas of the snail *Helix pomatia* produces proteinaceous particles (termed *b*-granules), which are involved in the process of calcification. In calcium cells of this organ, calcium spherites arise from these granules. The study retraces the intracellular development of *b*-granules from Golgi vesicles and from Golgi saccules encircling the cytoplasmic areas up to the formation of calcium spherites. It was suggested that the mature *b*-granules are well-defined cytoplasmic units with calcifying capacity. The fine structure of *b*-granules and calcium spherites and the possible function of various

cell organelles in the formation of the *b*-granules and spherites were discussed.

Trevor (1976) investigated the structure and function of the digestive gland of the gastropod mollusc, *Bithynia tentaculata*, using ultrastructural, histochemical and cytochemical techniques. The digestive gland was shown to be composed of two main cell types, the digestive cells and secretory cells. The digestive cells appeared to be concerned with the absorption and digestion of nutrients, while secretory cells produced digestive enzymes and calcareous concretions. Undifferentiated cells were scattered between these two main cell types.

Rajalakshmi *et al.* (1982) described the histology and histochemistry of the albumen and capsular glands of *Thias bufo*. Histochemical tests revealed the high proteinaceous nature of these glands. The secretion of the albumen gland is rich in carbohydrates beside protein whereas that of the capsular gland is a mucoprotein.

Bolognani *et al.* (1987) studied the metabolic pathways of glucose by histochemical reactions in some species of gastropods living in different habitats. The glycolytic pathway was histochemically indicated by positive results for glucose-6-phosphate isomerase, fructose-1,6-biphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, and D-lactate dehydrogenase. The enzymes of the Krebs cycle gave different responses: isocitrate dehydrogenase and L-malate dehydrogenase were positive, whilst succinate dehydrogenase was constantly negative. Malate synthetase activity, phosphoglucomutase and glucose-6-phosphate phosphatase were also positive.

Treibskorn *et al.* (1989) followed the passage of a C^{14}-labeled carbamate through the alimentary system of *Deroceres reticulatum*. Firstly, the molluscicide penetrated the cells of the esophagus and the

crop transported by the hemolymph to the periphery of the body and re-entered the cells of the digestive tract and the mid-gut gland. In the second step, the connective tissue cells were the major storage sites of the labeled material exceration while feces and secretion in mucus were thought to be the routes of C^{14} elimination.

Treibskorn and Kunast (1990) fed *Derocores reticulatum* on lethal or sublethal conc. of the carbamate to investigate the influence of the chemical on the ultrastrcture of the cells of the digestive tract as oesophagus crop, stomach, intestine and hepatopancreas. They concluded that the higher conc. of the pesticide not necessarily produce stronger effects.

El-Mahrouki (1991) revealed the histological structure of the digestive gland in *E. vermiculata* by the presence of two cell types digestive and excretory cells. The digestive cells were less abundant in the dark banded morphs than in the unbanded ones in comparison with the excretory cells. The follow up of the histochemical patterns of distribution and mode of occurrence of certain essential chemical components in the digestive gland of two phenotypes discerned certain points of similarities and discrepancies between the two types.These components included the general carbohydrates, total proteins and nucleic acids. These similarities and discrepancies were interpreted in the light of how histochemical differences could play a role in the maintenance of polymorphism in this snail.

Bourne *et al*. (1991) used horseradish peroxidase (HRP) and lanthanum nitrate markers to demonstrate the uptake of endogenous material in the crop of the gastropod *Deroceras reticulatum*. Pinocytosis of HRP was found to occur within 5 min of feeding in slugs that had previously starved for 48 hr. similar rates of uptake recorded for

lanthanum, but this marker confined to a paracellular route. The effects of Metaldehyde and methiocarb following ingestion were studied using electron microscopy. At sub-lethal doses morphological damage to the surface epithelium of the crop was attributable to both molluscicides. In addition, intracellular vacuoles appeared within crop cells from 24 h after treatment with methiocarb.

El-Banhawy *et al.* (1991) investigated histologically the digestive gland of *Eobania vermiculata* and concluded that it comprised of two lobes each formed of a complicated system of branching tubules harboring two main cell types, digestive and excretory. **Henry *et al.* (1991)** stated that the digestive gland of *Pecten muximus* consists, as in other lamellibranchs, of numerous blind ending tubules which communicate with the stomach by partially ciliated main ducts and non ciliated secondary ducts. The non-ciliated cells of the main ducts arc characterized by a well developed brush border constituted by high and dense microvilli and a strong pinocytotic activity. Ciliated and nonciliatcd cells have a very similar fine structure. The digestive tubules have a large lumen and contain digestive cells at different stages of absorption, digestion and excretion, one part of the tubules being functional while the other is disintegrated. The dark crypts contain the flagellated secretory cells, characterized by a well developed granular endoplasmic reticulum, and the young immature cells which may replace both the secretory and digestive cells. The numerous lipid droplets occurring in the digestive duct cells and in the digestive cells reveal the lipid storage function of the digestive gland. Some intracellular peptidases and glycosidases have been localized in the cells of the digestive gland, especially in the brush-border cells of the ducts and in the functional part of the tubules. These results showed that the main role of the digestive gland is absorption and intracellular digestion.

Treibskorn (1991a) described cellular reactions in two epithelial cell types as muscle and nerve tissue of the digestive system of both *Derocerase reticulatum* and *Arion lusitanicus* treaded by carbamates. The results included destructive effect on nerve and muscle in all kinds of cells when metaldehyde attacked the mucous cells including an increase in the production and extrusion of mucous leading to the destruction of mucous cell ultrastructure. **Treibskorn (1991b)** investigated the influence of three types of molluscicide pellets, cloethocarb, methiocarb and metaldehyde, on the activity of six enzymes in the hepatopancreas of *Deroceras reticulatum* by light and electron microscope, histochemistry as well as by photometric studies. In the digestive cells, enzymes catalysing energy-producing digestive processes were induced, while in the crypt cells enzymes related to energy-consuming metabolic pathways often involved in detoxification were activated.

Ulrich (1991) mentioned that digestive gland consisted of numerous tubules, connected to the stomach by primary and secondary ducts. The epithelium of the tubules is made of digestive cells and basophilic cells. The author reported that the height of the predominant digestive cells number and form of their vacuoles changed according the food type.

Almendros and Porcel (1992) investigated phosphatase acid (PhA) activity in the digestive gland (hepatopancreas) of the common garden snail *Helix aspersa* using cytochemical methods. The results showed that all the cells composing this gland show PhA activity, the distribution pattern differed according to the cell type. The digestive cells showed the most widely distributed reaction product (brush border, phagolysosomes, multivesicular bodies and autophagic vacuoles). In the excretory cells, this activity appeared in large sacs, while in the calcium cells the reaction

product was abundant in the calcium granules. The cellular digestion processes performed by each of these cell types was discussed.

Flari and Charrier (1992) found that the carbohydrates activity in the digestive tract of *Helix lucorum* and the main digestion of carbohydrate takes place in esophagus, crop, stomach, intestine and digestive gland. The enzymatic activities varied according to the physiological state of the animal examined and it was found that starch, xylan, lichenine and saccharose were the least hydrolyzed substrates.

Dimitriadis *et al.* (1992) found that seven days hibernation resulted in significant changes in the morphology of the digestive gland epithelium of *Helix lucorum* compared to control animals. The number of the digestive cells significantly decreased, but the number of the other cell types of digestive gland, calcium and excretory cells, significantly increased. The apical granules and the cisternae with dense cores of the digestive cells significantly increased in number and size, while the calcium granules of the calcium cells increased in number and showed more intense concentric rings compared to those of the control animals.

Brooks and White (1995) exposed the terrestrial snail *Helix aspersa* to food containing elevated levels of aluminium for up to 33 days and the digestive gland examined by light and electron microscopy as well as X-ray microanalysis. Four types of cells were found in the digestive gland (digestive, excretory, calcium and thin cells).The aluminum was localized in the 'yellow' or excretory granules that are a characteristic feature of the excretory cells. Sulphur, phosphorus and calcium were detected in granules from all snails. The presence of sulphur may indicate protein residues. The amount of aluminum and phosphorus in the granules increased over the experimental period but the number of granules did not change. Levels of aluminum in the granules decreased when the snails

were given control food. The role of the excretory granules in the localization, detoxification and excretion of aluminum was discussed.

Dimitriads and Domoucht (1995) indicated that the mucous cells of the intestinal epithelium of *Helix lucorum* produce granules give positive reaction for periodate reactive carbohydrate (PAS). Glycogen particles were present in close relation to lipid inclusions in ciliated and unciliated cells of the intestinal epithelium, whereas hibernation and starvation didn't alter the chemistry of carbohydrates secreted by the intestinal cells. The amount of glycogen particles and lipid inclusions decreased in columnar cells in hibernated and starved snails compared to controls.

Gerard and Theron (1996) reported that the role of molluscan digestive gland was important for digestion processes and conversion of food resource. Therefore, a study was performed to determine the influence of the nutrition and assimilation on the digestive gland volume of *Biomphalaria glabrata*. Results showed that the nutrition and assimilation kinetics of juveniles and adult snails were linked to the variation of the digestive gland volume.

Treibskorn *et al.* (1998) investigated the light and electron microscopy in combination with histochemical and immunocytochemical techniques, the impact of orally and dermally applied metaldehyde on mucous cells in the digestive tract, the skin, and the salivary gland of the slug *Deroceras reticulatum*. The study showed that metaldehyde induced severe alterations and damage in mucocytes even under low temperature and humid conditions when sufficiently high doses were applied. After metaldehyde application, not only the quantity of mucus produced by slugs but also its quality was modified. Structural, enzyme-histochemical and immunocytochemical investigations revealed that metaldehyde

induced effects in mucocytes would be related to influences of the molluscicide on serotonin and on energy metabolism.

Boer and Kits (1999) studied the alimentary tract of the freshwater snail *Lymnaea stagnalis*, with histochemical and ultrastructural methods. The main focus was on the epithelia. Results indicated that the digestive gland, where intracellular digestion occurs, plays the major role in the digestion process. The main cell types of the tract are ciliated cells and cells with microvilli. The presence of ciliated cells reflects transport of food particles and faces. Both cell types showed signs of endocytosis, lysosomal breakdown of substances, and storage of reserve materials (glycogen, lipid, protein). These features indicated that absorption of energy-rich substances can occur in the whole tract.

Brackenbury (1999) applied a lethal concentration of a crude aqueous extract of *Agave attenuata* as a contact poison to *Bulinus africanus*, the intermediate host of *Schistosoma haematobium*, for 24 hrs period to investigate the gross histopathological effects of the extract on the epithelium of the digestive tract. A graded series of cellular injuries to the epithelial layer was observed along the length of the tract. These included the loss of cilia and brush border, disruption of the epithelial layer, cellular vacuolation, swelling and rupture, and the discharge of secretory products from mucous gland cells. The results showed that epithelial tissue is probably a primary target of the molluscicide. The cytological injuries induced by extracts of *A. attenuata* indicated that the molluscicide acts by disrupting the osmoregulatory mechanisms of the epithelial cells.

Taïeb and Vicentea (1999) found that the crypt cells lining the *Aplysia punctata* digestive tubules are compound of three types of cells: calcium, excretory and thin cells. The calcium cells play a role in

osmoregulation, mineral storage, exocrine secretion, iron detoxification and excretion processes. They possess well- developed microvilli and a basal labyrinth, suggesting a role in absorption. The Golgi apparatus is involved in the production of two main components of calcium spherules: fibrillar material and mineralized granules. Golgi complex, rough endoplasmic reticulum (RER), ribosome and mitochondria are involved in the formation of calcium spherules. Secretory activity is indicated by the formation of dense granules containing iron and calcium salts. Lipofuscin pigments have been found in large concretions which may arise from cytoplasmic areas surrounded by endoplasmic reticulum (RER) and Golgi tubules. There are three stages of excretory cells, called early, mature, and post-excretory cells.

Zedan *et al.* (1999) tested the bactericidal activity of *Bacillus thuningensis*t Israelensis Serotype H-14 primary powder and Lannate toxicity against *Monacha obstracta* land snails using leaf dipping technique. Histological examination of stomach fundus of treated animals revealed different mild pathological changes which represented by production of acute erosion in the mucosa of the fundic region of the stomach at concentration of LC_{50} and degenerative changes in the inner part of the mucosa at 1/2 LC_{50} conc.

Aioub *et al.* (2000) studied the effect of oral administration of three doses (0.5, 1, and 1.5%) of oxamyl carbamate insecticide, on the digestive glands of *Eobania vermiculata* snails. Treatment with 1.5% dose of oxamyl led to more drastic alterations in the digestive gland. The distinction between tubules of the gland became hard to demonstrate. The excretory substance has been greatly lost and many of these cells appeared empty of any excretory material. Numerous lymphocytes were seen in the intertubular connective tissue. The coat cells appeared

suffering from hypertrophy while their nuclei were less enlarged than the previous case. Derangement of muscle fibers shown histologically could be considered responsible for readily detachment of soft parts. This may partially explain the observed low activity of treated snails. Such alteration in digestive gland coat may lead to disfunction of the epithelial cells coat.

Lobo (2000) cited that the digestive cells are the most abundant cell type in the digestive diverticula of *Aplysia depilans*. These are columnar cells, covered with microvilli. A large number of endocytic vesicles containing electron-dense substances, heterolysosomes of large diameter, glycogen particles and some lipid droplets were also observed. Peroxisomes with a circular or oval profile were common. These organelles were strongly stained after cytochemical detection of catalase activity. The Golgi stacks are formed by 4 or 5 cisternae, with dilated zones containing electron dense material.

Keiichiro *et al.* (2000) studied the dorsal and ventral epithelia of the terrestrial slug, *Incilaria fruhstorferi*, which is simple and consists of five cell types: microvillous, ciliated, round mucous, tubular mucous and channel cells. Microvillous cells were similar to human intestinal epithelial cells morphologically and functionally. At the base of microvilli, pinocytic vesicles which ultimately fused to form larger vacuoles or multivesicular bodies were present. Mucous secretory cells were either tubular or round and their granules were membrane-bound and secreted by exocytosis. Granules of round mucous cells were proteinaceous but those of tubular cells were acidic mucopolysaccharides. Channel cells were elongate U-shaped and the central lumen was filled with a large amount of fluid (hemolymph). The function of channel cells is thought to remove hemolymph accumulated during hyperhydration.

Lopes *et al.* (2001) studied a detailed characterization of the different types of cells comprising epithelium lining the digestive gland of *Orychilus atlarzricus* by light and scanning electron microscope (SEM). Three types of differentiated cells can be identified in the digestive gland: digestive cells, excretory cells and calcium cells. Digestive cells were the most numerous, and were present in two forms one believed to be responsible for absorbing food material and the other for secreting material. Excretory cells were distinguished by having a large central vacuole, containing excretory granules. Calcium cells contained spherules of calcium salts.

Taïeb (2001) studied the distribution of digestive tubules of *Aplysia punctata* in animals under experimental feeding conditions. Histological analysis of the digestive gland revealed the presence of two types of tubules, called tubules A and B. Tubules of type A were composed of basiphilic cells (calcium, excretory and thin cells) and tubules of type B were lined by large digestive cells and basophilic cells. The latter occur in small groups, usually in the corners of the tubules. Type A tubules are involved in ion metabolism and show a diphasic cycle (absorptive and reconstitutive) according to the height and the stage of calcium cells. Type B tubules are involved in digestive processes and display a tetraphasic cycle (holding, absorption, fragmentative and reconstitutive) depending upon the height and the stage of the digestive cells. The tetraphasic cycle was compared with the four categories of tubules in bivalves. It has been proposed that digestive processes may be continuous in digestive cells of *A. punctata*.

Dimitriadis and Konstantinidon (2002) examined the digestive gland cells of *Helix lucorum* with light and electron microscopes after starvation and periods of feeding. The digestive cells contained

heterolysosomes and residual bodies while the excretory cells contained excretory vacuoles and the decrease in number and size of heterolysosomes was accompanied with an increase in number and size of excretory vacuoles.

Heiba *et al.* (2002) treated two species of land snails, *E. vermiculata* and *M. cantiana,* with Lannate and recorded pathological alterations in the digestive gland. The vacuolated and swollen digestive cells and presence of numerous yellowish brown granules (residual bodies) in the cells were the most pathological changes observed. These findings indicated that Lannate have toxic effects in cellular damage of the digestive glands of the snails which could be correlated with the disturbed enzyme activities.

Hemmaid and Mohammadein (2003) studied the effects of feeding the land snail, *Eobania vermiculata* with 1.5 parts of oxamyl (24%/l Vydate) for one week ultrastructurally in cells of the digestive gland. Tubules if the digestive glands of treated snails showed hypertrophied cells on the expense of the intertubular connective tissue. All cellular types of the glands were enlarged and most nuclei were hypertrophied. The digestive cells (the most abundant) display degenerative enclosing areas. Some of the vacuoles were enlarged enclosing strongly osmiophilic aggregations acquiring a bunch-like appearance. Nuclei of the digestive cells were hypertrophied and occupied with numerous clumps of heterochromatain and some of the nuclei were indented. The apical pole of digestive cells revealed the presence of a huge number of mitochondria, whereas the excretory cells of treated snails exhibited a clear increase in the size of their characteristic excretory vacuole. Their cytoplasm was just a rim and the nucleus was elongated and was pressed to one side of the cell. Three

morphological types of the excretory granules were described. Calcium cells of the treated snails contained scattered vacuoles of variable size. Spherical granules with flocculent osmiophilic boundaries and central osmiophilic cores were seen. Hypertrophied nuclei of some cells revealed a bizarre shape with long protrusions and condensations of strongly osmiophilic euchromatin.

Amaral *et al.* (2004) chemically analyzed the digestive glands of adult land snails, *Helix aspersa*, sampled from four different sites in São Miguel Island (Azores). Autometallography and haematoxlyin/eosin staining were applied in order to quantify the relative abundance of heavy metals, calcium cells and connective tissue cells. Metals were visualized, through light microscopy, as black silver deposits mostly in the connective tissue cells. Metal levels, essentially of Cu and Fe, were related to the relative volumetric density of connective tissue cells but not to the relative volumetric density of calcium cells from the digestive gland epithelium. Thus, the connective tissue index presented had been suggested as a biomarker of Cu exposure in terrestrial mollusks.

Birgül *et al.* (2004) determined the histopathological effects of endosulfan on the digestive gland, foot and mantle of the great ramshorn snail (*Planorbarius corneus*), under laboratory conditions. Endosulfan caused significant histopathological alterations in the digestive gland, foot and mantle tissues of the snail, irrespective of concentration of the pesticide and its exposure periods.

Christiane and Plawen (2005) investigated the digestive tract of 2 species of the genus *Helicoradomenia* with special focus on the ultrastructure and histochemistry of epithelia and glandular organs. The preoral cavity and foregut epithelia are composed of microvillous main cells, secretory cells producing protein-rich substances and sensory cells

with specialized cilia. The foregut bears a pair of glands with 3 types of extremely long-necked glandular cells surrounded by musculature. The midgut has a narrow, mid-dorsal tract of ciliary cells, but most of the epithelium is composed of digestive cells with a highly developed lysosomal system. The hindgut is lined by ciliated cells and free of glands. The digestive tract is not adapted to microvory and there is no indication of a symbiosis with chemoautotrophic bacteria.

Fouad (2005) demonstrated histopathological effects of malathion and methomyl compounds on the mucous gland and the body wall of the land snail *E. vermiculata* at 0.5% level after 24 hours post-treatment.

Lobo and Batista (2005) described that the oesophagus and crop epithelium of *Aplysia depilans* consist of a single layer of columnar cells with apical microvilli, and some of them also possess cilia. Cell membrane invaginations, small vesicles, multivesicular bodies and many dense lysosomes were observed. Histochemicaly methods showed that the secretion stored in these cells contains acidic polysaccharides. Secretory vesicles with thin electron-dense filaments scattered in an electron-lucent background fill most of these cells, and the basal nucleus is surrounded by dilated rough endoplasmic reticulum cisternae containing small tubular structures.

Histopathological and ultrastructural alterations in the digestive gland of *E. vermiculata* were more obvious after topical application of methiocarb and methomyl than after baiting technique **Hamed *et al.* (2007)**. These alterations included hemocyte infiltration, bizarre nuclei that ranged in their degenerative changes from karyolysis to severe karyorrhexis and complete pyknosis, after methomyl treatment. Extensive destruction and disorganization of the intertubular connective tissue were demonstrated after methiocarb treatment. In addition, severe cytoplasmic

vacuolization, disruption and reduction of microvilli and formation of surface blabs, increased number of calcium spherules in calcium cells and an aberrant increase in the number of excretory cells containing large number of excretory granules or residual bodies were observed after treatment with both molluscicides.

Radwan *et al.* (2008) investigated effects of sublethal dose of methomyl and methiocarb on the digestive gland of *E. vermiculata* under laboratory conditions using topical application and baiting techniques. The results showed that methomyl exhibited greater efficacy than did methiocarb against the snails in both techniques. Biochemical and histochemical examinations revealed that treatment of the snails with methomyl and methiocarb caused significant decrease in carbohydrate, lipid and protein contents. This decrease was also more obvious after topical application than after baiting technique, and methomyl found to be more toxic than methiocarb.

El-Said (2009) indicated that following oral administration of three doses of Protecto, Biover pesticides and *Aspergillus flavus* against *Monacha cartusiana* the general architecture of the digestive gland lost its normal appearance. The damaged acini in the treated snails included narrowing in acinar lumen, atrophied acini which became adherent to each other with disturbed architecture, disappearance of glandular and calcium cells. Snails stopped feeding and lost the appetite with very sluggish movement.

Gros *et al.* (2009) described the structure of the digestive gland of *Strombus gigas* in individuals from Guadeloupe and discussed the function of its cell types. Three cellular types were found in the epithelium of the blind-ending tubules of the digestive gland according to

histological and transmission electron microscopy (TEM) observations; these were: digestive cells, pyramidal crypt cells and vacuolated cells.

Hesham (2009) revealed a pronounced decline of carbohydrates in the digestive gland cells of *Monacha cartusiana* snails after starvation (15 & 30 days). Severe decline in carbohydrate content was observed especially after 30 days of starvation. Moreover, protein inclusions have exhibited week stainability in the digestive gland cells of these snails as a consequence of starvation.

El-Akhrasy (2010) performed a control study on the land snail *Monacha cartusiana* by the molluscicide metaldehyde in laboratory and field conditions. The LC_{50} was calculated after 48 hrs with value of 4.421 % and LC_{99} was 277.347 %. The reduction percentage increased with increasing molluscicide concentration with the time. After treatment with 0.1 % of the metaldehyde LC_{50}, the reactions of metaldehyde with the digestive tract cells caused excess mucus production leading to fatal mucus deficiency then death. Moreover, protein inclusions have exhibited weak satinability in the digestive gland cells of these snails.

Lobo *et al.* (2011) studied the stomach and intestine of *Bulla striata* with light and electron microscopy. A 3D-model of the stomach and its connections with the posterior oesophagus, digestive gland ducts and intestine was created from a series of histological sections. Mucus-secreting cells were abundant in the intestine and all of them stained with alcian blue. However, most mucus-secreting cells of the intestine were not significantly stained by PAS reaction, but contain more proteins than the mucus-secreting cells of the stomach. The granular cells with a large number of small electron-dense secretory vesicles containing proteins and neutral polysaccharides were found only in the intestine.

Hamlet *et al.* (2012) estimated the effect of aneonicotinoid insecticide (thiametoxam) and investigated the histopathological perturbations in the hepatopancreas in adult snails, *Helix aspersa*. The treated snails showed alterations as a response to all the treatments, and revealed the degeneration of the digestive tubules and the breakdown of the basement membrane in a dose-dependent manner, leading to a severe deterioration of the tissues at a concentration of 200 mg/L thiametoxam.

Aukkanimart *et al.* (2013) studied the histopathological changes in tissues of *Bithynia siamensis* (Gastropoda, Bithyniidae) incubated in crude extract solutions of camellia (*Camellia oleifera*) seed and mango-steen (*Garcinia mangostana*) pericarp, and also estimated the molluscicidal effects of the 2 plant substances. Substantial numbers of *B. siamensis* snails were incubated in various concentrations of tested 2 plant solutions for 24 hrs. The positive control snails were incubated in various concentrations of Niclosamide, a chemical molluscicide as a reference for comparison. The histopathological findings showed that both camellia and mangosteen extracts had molluscicidal effects at 24 hr with 50% lethal concentration (LC_{50}) at concentrations of 0.003 and 0.002 g/ml, respectively, Whereas niclosamide had LC_{50} at concentration of 0.599 ppm. After treatment of *B. siamensis* snails, the tissues including the digestive system showed irregular apical and calciferous cells, dilatation of the digestive gland tubule, and presence of large hemolymphatic spaces.

Sharaf *et al.* (2013) investigated the impact of two pesticides namely: Methiocarb and Chlorpyrifos against histological aspects of the helicid land snail, *Helicella vestalis*. Many histological changes were observed in the digestive gland of *H. vestalis* after exposure to sublethal concentrations of both pesticides. These alterations included severe

tubular disruption, vaculation, nuclear pyknosis and necrosis of tubules. Moreover, this study revealed that Chlorpyrifos was much more toxic to the tested snail than Methiocarb.

Kandil *et al.* (2014) studied the histopathological effect of methomyl, abamectin and their mixtures with acetyl salicylic acid as well as acetyl salicylic acid a lone on *E. vermiculata* and *M. obstructa*. The effect of LC_{50} of acetylsalicylic acid on the tissues of mucus gland was studied in both snail species. There was partial as well as complete disappearance, necrosis and atrophy of mucus glandular tissue of *E. vermiculata*. It also caused focal necrosis especially underneath necroses destructed covering epithelium in association with degeneration in case of *M. obstructa*. Under field conditions, the high efficiency concentrations of methomyl, acetyl salicylic acid and abamectin bioinsectidide were tested as a spray. Results revealed that the combination of methomyl and acetylsalicylic acid enhanced the molluscicidal activity against both land snail species.

Histological changes which observed in digestive gland of snail *Helix aspersa* following treatment by industrial metal dust were atrophy of the connective tissue, membrane destruction, cell necrosis and appearance of inflammatory infiltrates **(Mounir *et al.*, 2015).**

Besnaci *et al.* (2016) estimated the effect of Iron oxide nanoparticles onadult snails, *Helix aspersa* on histological changes in the hepatopancreas after treatment for six weeks. Snails wereexposed by ingestion and contact to wheat flour, which contai ns NPs powder. The doses of ferric oxide nanoparticles were 0, 1, 2 and 3 g/kg of wheat flour. The histological examination of the hepatopancreas of snails showed alterations as a response to all treatments.These included narrowing of thetubular lumen, degeneration of some digestive cells, tubule degeneration and necrosis of the intertubular connective

tissue, which occurred from the second dose. The appearance of some inflammatory infiltrates, leading to sever tissue damage was recorded at the third dose (3 g/kg) of iron oxide nanoparticles.

4-Snail biochemical parameters

Mohamed *et al*. (1981) examined the effect of prolonged exposure (l, 3, 5, 10, 17, 24 and 30 days) of *Biomphalania Alexandrina* to low concentration (1/10 LC_{50}) of nidosamide, Frescon and copper sulphate on the total proteins and lipids. Results indicated that Frescon and copper sulphate led significant reduction in total proteins and lipids content from the first and third day of exposure. Nidosamide treatment showed decrease in protein content after 17 and 24 days and in lipids after 24 days.

EL-Emam and Ebeid (1989) tested the effects of natural molluscicide (Plant extract) of *Calendula micrantha* and synthetic molluscicide (Mokotox) on total protein in the heamolymph of *Biomphalaria alexandrina* snail. Both mollusciciades had similar effect as they produced an increase in the total protein level.

El-Wakil and Radwan (1991) studied the effects of methomyl, thiodicarb and metaldehyde at 0.2 w /w in bran baits after 1 -10 days on AST and ALT enzymes in *Eobania vermiculata*. Methomyl and thiodicarb led to significant increases in the two enzymes activity, while metaldehyde showed no significant effect on AST level but it caused a significant increase in the ALT activity.

Radwan *et al.* (1992) found that methomyl and oxamyl insecticides led to significant increase of AST and ALT activity while thiofanox reduced the activity of the two enzymes in tissues of the land snail Theba pisana.

Chaudhari and Kulkarni (1994) estimated alterations in the lipid metabolism of the land snail, *Zootecus insularis* after 1, 7 and 14 days of

sublethal dose of monocrotophos intoxication. The total lipid content was found to be decreased at all exposure periods. The cholesterol content increased initially, but afterwards the level declined. The phospholipids level after initial increase declined at the end of the 14^{th} day. The free fatty acids were found to be initially decreased followed by an increase.

Abd Allah *et al*. (1998) studied the activities of some enzymes of *Eobania vermiculata* and *Theba pisana* after *in vivo* effects of aldicarb and metaldehyde. The activities of acetylcholinesterase (AChE), lactate dehydrogenase (LDH), glucose 6-phosphate dehydrogenase (G6PDH), glutamate oxaloacetate transaminase (GPT) and protein metabolism revealed that the fast toxic effect was that of aldicarb compared with metaldehyde.

Chaudhari *et al*. (1999) tested the toxic effect of Roger (dimelhoate) to fresh water snail *Thiara lineata* and its impact on biochemical composition. Snails were exposed to the LC_{50} for 29, 48, 72 and 96 hours. Biochemical constituents (total protein, total lipid and cholesterol) were estimated following the different exposure periods and were found to change substantially.

El-Deeb *et al.* (1999) tested the mollusicidal activity of khella fruits ethanotic extract, *Santonica* hexanic extract, Lebaycide and Osbac pesticides under laboratory conditions against the land snail *Monacha cantiana*. The *in vivo* effects of these compounds on five snail enzyme systems (peroxidase, catalase transaminases, GPT, GOT and acetylcolinesterase) were assayed after 1, 3, and 7 days of exposure periods to 1/4 LC_{50} of the tested compounds. Osbac pesticide was the most effective one against snails followed by Lebaycide pesticide, khella ethanoilc extract while *Santonica* hexane extract was the least effective one.

Kumari *et al*. (1999) studied the lipid metabolism profiles in tissues of the fresh water snail, *Pila globosa*, following exposure to sublethal (48 ppm. for 48 h) and lethal (13.18 ppm. for 48 h) concentrations of phenthoate. Total lipids, phospholipids, and cholesterol were increased, whereas neutral lipids, glycerol and free fatty acids were decreased in the exposed tissues. The activity of phospholipids decreased in all the major tissues during phenthoate exposure.

Salama *et al.* (2005) compared the ability of two carbamate compounds (methomyl and carbofuran), an organophosphorus compound (chlorpyrifos) and a bipyridylium compound (paraquat) to induce the oxidative stress and affect some biochemical targets in the land snail, *Helix aspersa*. Some biomarkers of the oxidative stress such as lipid peroxidation (LP), lactate dehydrogenase (LDH) and glutathione (GSH) as well as the inhibitory effects of these compounds against acetylcholinesterase (AChE) were evaluated following topical application of 1/4 LD_{50} values for these pesticides. The results showed that carbofuran was the most potent compound in inhibiting AChE in snails, followed by methomyl, where the enzyme activities dropped to 9.86 and 28.82% of the control activity, respectively. It has been concluded that their mode of action could be due to the induction of oxidative stress in addition to their anticholinesterase potencies. chlorpyrifos or paraquat had slightly effects to alter the biomarkers of oxidative stress in the snail.

Hussein and Gabr (2006) studied the biochemical effects of the two natural products neemix 4.5 % (plant extract) and Vertimec 1.8% (biocide abamectin) on the snail *Eobania vermiculata*. Snails were treated with sub-lethal concentration (1/4 LC_{50}) of each tested compound using contact (thin film) technique. Some enzymes activities: liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total

protein and total lipid were determined. Results showed that the two tested compounds reduced the activity of AST gradually with the progression of periods after treatment, while the contrary occurred with ALT as both compounds enhanced its activity than control. Regarding total lipids, both compounds had the same effects as they raised total lipid percentage while their effect differed on total proteins especially in the third and fourth days of treatment, whereas neemix 4.5 % induced a marked reduction in the total protein percentage comparatively with increase in case of Vertimec.

Gabr *et al.* (2007) studied the biochemical effect of sublethal concatenation (1/4 LC_{50}) of carbofuran on land snail *Monacha obstructa*. Results indicated that total protein level increased after 24 hrs then reduced gradually after 48 and 72 hrs post treatment comparatively with control. The level of the total protein was fluctuated as it decreased after 42 hrs but it raised post 48 hrs then reduced after 72 hrs.

Yasser *et al.* (2007) tested methomyl and a cardiac glycoside extract isolated from *Calotropis procera*, against *Eobania vermiculata* by the contact method. The LD_{50} values of tested materials were 153.51 and 13.87 µg/ gm of body weight, respectively, which means that the extract is 11 folds more toxic to this snail than methomyl. The spectrophotometric analysis of the cardenolide extract content proved that it is equivalent to 95 % ouabain, which is a good indication on the purity of isolated group. At sublethal doses of tested extract, changes of alanine transaminase (ALT) level were higher than those of aspartate transaminase (AST). The results of this work proved that *C. procera* is an important source for new and strong molluscicidal compounds that could be exploited against *E. vermiculata* and other species of harmful land snails.

El-Gendy *et al.* (2009) examined the *in vivo* evaluation of oxidative stress biomarkers in the digestive gland of *Theba pisana* exposed to sublethal doses (40 % and 80 % of LD_{50} after 48 h) of Copper -based pesticides: copper oxychloride, copper hydroxide and copper sulphate. Lipid peroxidation (LPO) was also evaluated as marker of cell damage. The results indicated that copper sulphate was the most potent compound against this snail followed by copper hydroxide and copper oxychloride. copper -based compounds resulted in a significant increase in the level of LPO and the activation power of these compounds was in the following order: copper sulphate > copper hydroxide > copper oxychloride.

Kandil *et al.* (2009) studied the biochemical impacts of methomyl (20 %), abamectin (1.8 %) and their mixtures with acetylsalicylic acid against two land snails *Eobania vermiculata* and *Monacha obstructa*. Snails were tested with LC_{50} of each tested compound and their mixture using baiting technique. Some biochemical parameters related to mucus excretion and important for synthesis of shell were determined to emphasize the role of acetylsalicylic acid. These parameters included activity of alkaline and acid phosphatase, total protein and total lipid and cholesterol contents. Results showed that the acetylsalicylic acid alone exhibited clear influence on all tested biochemical parameters while its effect was more pronounced when mixed with tested compounds especially with methomyl. In addition, it caused complete inhibition of enzyme activities post three days of treatment in tested land snail species, *Eobania vermiculata* and *Monacha obstructa*.

Beltagi *et al.* (2011) studied the physiological responses of *E. vermiculata* snails to sublethal doses (LD_{25} and LD_{50}) of two potent botanical molluscicides (thymol and nicotine) after 1, 7 and 15 days. A general significant increase in levels of some biochemical parameters

(total proteins, total lipids, total cholesterol and glucose levels) of the haemolymph were detected in most cases. Both examined materials caused a marked enhancement in the activity of acid phosphatase (ACP), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) enzymes in the haemolymph; while the activity of alkaline phosphatase (ALP) exhibited a significant suppression. In addition, the examined biochemical parameters (total soluble proteins, total lipids and glycogen content) in the digestive gland exhibited marked reduction in response to both examined materials at the three post exposure periods.

El-Gohary and Genena (2011) investigated the biochemical effect of three molluscicide namely: Gastrox, Molotov and Mesurol in the tissues of the two land snails, *Monacha cantiana* and *Eobania vermiculata*. Total proteins and total lipids were laboratory tested. The results revealed that the levels of total proteins and total lipids were increased after treatment with all tested molluscicides when applied against *E. vermiculata* and decreased when applied against *M. cantiana* compared with control.

Hasheesh *et al.* (2011) studied the effects of sublethal concentrations of methanol extract of *Sesbania sesban* plant on *Bulinus truncatus* snails on certain physiological parameters of treated snails. The glucose level in haemolymph of exposed snails was elevated while the glycogen, protein content and the activities of hexokinase, pyruvate kinase and lactate dehydrogenase showed a decrease in soft tissues when compared with the control group. It was concluded that the application of sublethal concentration of methanol extracts of *Sesbania sesban* may be helpful in snail control as it interferes with the snails' biology and physiology.

Hamlet *et al.* (2012) used adult snails, *Helix aspersa* to estimate the effect of aneonicotinoid insecticide (thiametoxam) on biochemical parameters in the hepatopancreas of this gastropod after treatment for six weeks. During this period, snails were exposed by ingestion and contact to fresh lettuce leaves which were soaked with an insecticide solution. The thiametoxam test solutions were 0, 25, 50, 100 and 200 mg/L. The results of the biochemical dosages (total carbohydrates, total proteins and total lipids) showed significant decreases at two concentrations (100 and 200 mg/L) of thiametoxam.

Industrial metal dust causes a significant increase in proteins with a significant reduction in the carbohydrates and lipids on digestive gland of the land snail *Helix aspersa* **(Nedjoud *et al*., 2012).**

Kandil *et al.* (2014) determined the effect of Acetylsalicylic acid, methomyl, abamectin and the joint action of methomyl or abamectin with acetylsalicylic acid on some biochemical parameters (alkaline, acid phosphatase, total protein, lipid and cholesterol) against two land snail species, *Eobania vermiculata* and *Monacha obstructa* under laboratory conditions. Snails were tested with LC_{50} of each tested compound and their mixture using contact technique. Results showed that the combination of acetylsalicylic acid and methomyl exhibited the highest effect on all biochemical parameters. The LC_{50} of acetylsalicylic acid caused desiccation and adhesive for snail body of both species.

Kemajl *et al.* (2015) evaluated the effect of industrial pollution from Trepça smelter in Mitrovica in some biochemical parameters of snail hemolymph. About 30 individuals of natural populations of the garden snail, *Helix pomatia* taken in two regions with different levels of pollution. The first group (test) is taken near the Trepça smelter in Mitrovica, while the control group from the vicinity of Peja (a locality

unaffected by the industrial pollution). An approximately equal amount of hemolymph was extracted from each individual to estimate the follwing: glucose (Glc), total protein (TP), albumin (Al), triglycerides (Tgl) and total lipids (TL). Results indicated that there was a significant decrease ($P<0.001$) in the concentration of glucose, total proteins, albumins, total lipids and triglycerides in the hemolymph of the snails taken near the locality Trepça smelter compared to the control group taken from the vicinity of Peja.

Industrial metal dust treatment caused a significant increase in the levels of total protein, carbohydrate and lipids in the digestive gland of the snail *Helix aspersa*, along with less effect on the carbohydrates in kidney when compared with their controls **(Mounir *et al.*, 2015).**

Besnaci *et al* (2016) estimated the effect of iron oxide nanoparticles on adult snails, *Helix aspersa* on biochemical parameters ofthe hepatopancre as aftera treatment forsix weeks.The results of the biochemical assays (tot al carbohydrates, total proteins and total lipids) showed significant increa ses of total carbohydrates and total proteins at three doses (1, 2 and 3 g/kg) of Fe2O3 and significant decreases of total lipids at two doses (2 and 3 g/kg) of Fe2O3 nanoparticles.

Chapter IV

Materials and Methods

I. Toxicity test

1. The Tested land snails:-

1. 1. *Eobania vermiculata*

- Description:

The color of the shell is very variable, whitish to greenish yellow, often with colour bands or spots. Lower side is frequently with two brown bands and whitish between lowest band and umbilicus. The shell has 4-4.5 whorls and the last whorl is descending abruptly below periphery. The apertural margin is white, reflected in adult shells. The width of the shell is 22–32 mm. The height of the shell is 14–24 mm. **(Godan 1983) Fig. (1)**

-Classification:

Phylum :Mollusca
Class :Gastropoda
Subclass : Prosobranchia
Order : Stylommatophora
Superfamily : Helicoidea
Family :Helicidae
Genus :*Eobania*
Species :*Eobania vermiculata*

1.2. *Monacha cartusiana*

- Description:

The shell diameter of adult *Monacha cartusiana* is 10-12.5 mm. height of shell 6.5-7.3 mm. height. Slightly translucent thin-walled, milky- or creamy white, with faint transverse 5.5-6.5 convex whorls

rising in a flattened cone. Sometimes a paler band is found at the periphery, aperture elliptical rounded-crescentic and the peristome (shell mouth edge) is sharp. Scarcely expanded, with a strong internal rib; young animals are slightly hairy umbilicus minute, partly obscured by reflected columellar lip **(Godan 1983). Fig. (2).**

- Classification:

Phylum : Mollusca
Class : Gastropoda
Subclass : Prosobranchia
Order : Stylommatophora
Superfamil : Helicoidea
Family : Helicidae
Subfamily : Monacheae
Genus : *Monacha*
Species : *Monacha cartusiana*

1.3. Rearing of Land Snails:

Culture of the tested land snails were collected from two districts in Sharkia Governorate *Monacha cartusiana* was collected from untreated clover fields at Malames village, Menia El-kamh district, while *Eobania vermiculata* was collected from untreated orchards in Anshas district. Chosen snails of nearly equal shell diameter were directly transferred to the laboratory. The healthy individuals of both tested snails were kept for acclimatization in two glass cages (50 x30 x 30 cm) containing moist clay soil to a depth of about 10 cm and were provided daily with fresh cabbage leaves for two weeks before any tests (**El-Okda 1984**). The cages were kept clean by removing left-over food when the new food was introduced and the soil was adjusted at 75 ± 5 % relative humidity.

Fig. (1): photograph of the land snail *Eobania vermiculata* shell

Fig. (2): Photograph of the land snail *Monacha cartusiana* shell

Before starting the experiment, the snails were starved for 48h. Concentrations of chemical compounds and biocides were prepared as poisonous baits. A summation of 258 round plastic boxes [13 cm in diameter] were prepared. The following table indicates the number of boxes used for each snail.

Table (1) The number of boxes used for each snail

Chemical compounds or biocides	No. of concentrations	No. of replicates	Summation
10 chemical compound	3	3	10x3x3=90
4 biocides	3	3	4x3x3=36

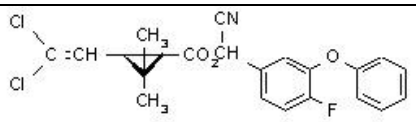
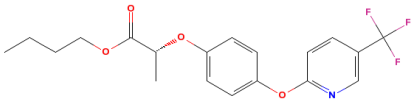
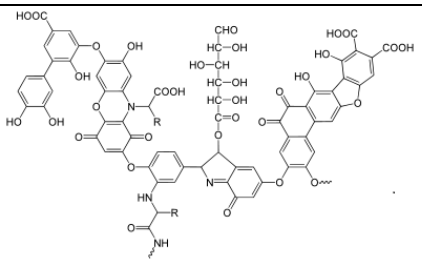
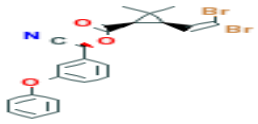
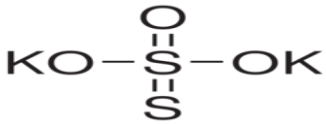
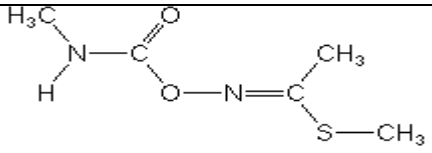
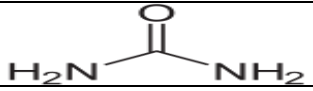
Additional 6 boxes were prepared for control (3 replicates for each snail). These boxes were left without chemical compounds or biocides.

Each one of the plastic boxes contained 1/4 kg of clay soil to form a layer of 10 cm depth. The soil moisture contents were adjusted at 75 ± 5% of water field capacity.

2-The pesticides used

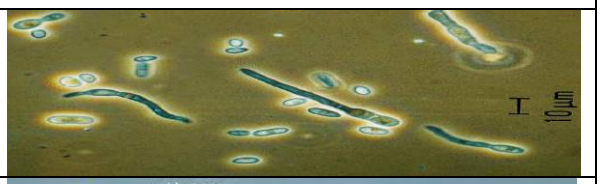
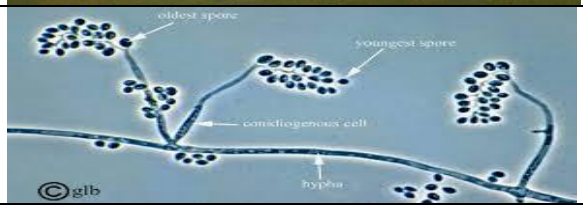
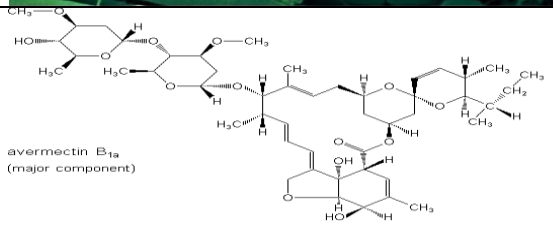
2.1. The chemical compounds

Table (2) The chemical compound, Chemical class, Chemical name, Formula and Structure

The compound		Chemical class	Chemical name	Formula	Structure
1	Amino (Biotech)	Fertilizer	Combining(Fe, Zn, Mn, Mg)		
2	Cyflu-magic 5%Ec (Magico)	Insecticide (pyrethroid)	Beta-cyfluthrin	$C_{22}H_{18}Cl_2FNO_3$	
3	Fusilade (PPRI)*	Herbicide	Fluazifop-p-butyl	$C_{19}H_{20}F_3NO_4$	
4	Humic acid (Biotech)	Fertilizer	85 % Humic acid	----	
5	K-othrine WG250 (Bayer)	Pyrethroid Insecticide	Deltamethrin	$C_{22}H_{19}Br_2NO_3$	
6	Potassium thiosulfate (Biotech)	Fertilizer	Potassium thiosulfate	$K_2S_2O_3$	
7	Micronized sulfur (Agrochem)	Fungicide	Sulfur		
8	Newmyl (KZ)	Insecticides carbamate	Methomyl	$C_5H_{10}N_2O_2S$	
9	Urea (PPRI) *	Fertilizer		$(NH_2)_2CO$	
10	Combining Zn (Biotech)	Fertilizer	Zn	Zn-EDTA	

2.2. The biocides used

Table (3) The biocide, Chemical class, Chemical name, Formula and Structure

The compound		*Chemical Class*	*Chemical name*	*Formula*	*Structure or image*
1	Bioranza (PPRI)*	Insecticide	*Metarthizium anisopliae*		
2	Biover (PPRI)*	Insecticide	*Beauvaria bassiana*		
3	Protecto (PPRI)*	Insecticide	*Bacillus thuigenisis*		
4	Vertimec (PPRI)*	Acaricide, insecticide	Abamectin	$C_{48}H_{72}O_{14}$	

* PPRI=Plant Protection Research Institute

2.3. Preparations of the poisonous baits

Table (4) The chemical compounds and the biocides used state and concentrations

Compounds		State	Concentrations
A	**The chemical compounds**		
1	Amino	Liquid	1,2 and 4 %
2	Cyflu-magic 5%Ec	Liquid	0.5, 1 and 2 %
3	Fusilade	Liquid	2 , 4 and 8 %
4	Hummic acid	Liquid	1,2 and 4 %
5	K-othrine WG250	Powder	1, 2 and 4 %
6	Potassium thiosulfate	Powder	1.25, 2.5 and 5 %
7	Micronized sulfur	Powder	0.5 , 1 and 2 %
8	Newmyl	Liquid	1,2 and 4 %
9	Urea	Pellets	0.6, 1.2 and 2.4 %
10	Combining Zn	Powder	0.1 , 0.2 and 0.4 %
B	**The biocides**		
1	Bioranza	Powder	1,2 and 4 %
2	Biover	Powder	1,2 and 4 %
3	Protecto	Powder	1.5,3 and 6 %
4	Vertimec	Liquid	1,2 and 4 %

The poisonous baits were prepared by mixing appropriate amount of each pesticide with five parts of black sugarcane syrup molasses then the mixture was incorporated with wheat bran to be finally 100 parts. The bait was moistened with appropriate amount of water to form a crumbly mash mixture. Ten snails were exposed to 5 grams bait on a plastic sheet in the surface of the soil in each box. Control treatment bait was prepared using wheat bran mixed with molasses without any pesticide. Boxes were covered with holes muslin secured with rubber bands for ventilation and prevention of snails from escaping. The mortality percentages were calculated after 1, 3, 7, 14, 21 and 28 days post treatment and obtained data were subjected to statistical analysis and L.S.D. calculation using **COSTAT program (1986)** Snails considered dead if they did not

response to prodding with stainless steel needle **(WHO, 1965).** Dead snails were recorded and removed at each test.

Fig. (3**)**: Photograph of round plastic boxes covered with holes muslin

II-Histology

2.1. Separation of the snail alimentary system:

The shell of snails was carefully broken and the soft tissues dissected out. Digestive gland and the intestine of snails were placed in a Petri dish containing isotonic buffer. The digestive gland and the intestine were separated and fixed in 10 % formalin **(Bezerra *et al*. 1999).**

2.2. The histological stains:

For histological studies, the organs were dehydrated through ascending series of ethanol alcohol, cleared in xylene for 2 minutes, then immersed in three successive solutions: The first consists of a mixture of xylene and wax in ratio 1: 1, the second and the third ones were pure wax each for 1/2 hour in an oven at 56^{o} C. Embedding in paraffin and blocking was carried out under vacuum. Serial transverse sections of 5/ 6 µm were mounted on clean glass slides without using any adhesive material. Ehleish's haematoxylin and eosin method **(Drury and Wallington, 1980)** was employed for general histological studies of the digestive gland and intestine.

III- Electron Microscopy

3.1. The electron microscope chemicals

1- Formalin-glutaraldehyde fixative (4F:1G)

2- Phosphate buffer solution (pH 7.2)

- Solution (A)

Disodium hydrogen phosphate (NaHPO.2Ho)	35.61 gm
Dist. water to	1000 ml

- Solution (B)

Sodium Dihydrogen phosphate (Na H_2O_4.2H_2OA)	31.21gm
Dist. water to	1000 ml

- Solution (C)

Solution (A)	40.5 ml
Solution (B)	9.4 ml
Dist. water to	100 ml

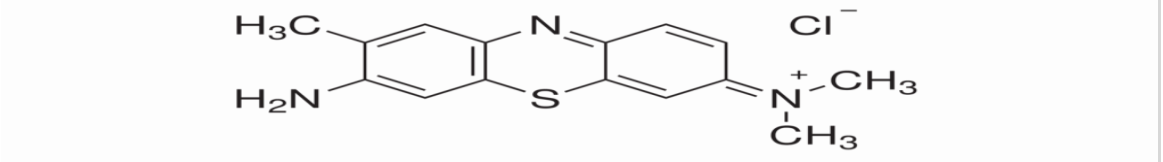
3- 2% Osmium Tetroxide solution (OsO_4)

Osmium Tetroxide	1.0 gm
Dist. water	50 ml

4- Epon-araldite mixture

Embed-812	25ml
Araldite 502	15ml
DDSA	55ml
DMP-30	1.5 – 1.9ml

5- Toluidine blue stain ($C_{15}H_{16}ClN_3S$)



6- Uranyl acetate stain

($C_4H_6O_6U \cdot 2H_2O$)

7- Reynold's Lead Citrate Solution:

Lead nitrate	1.33 g
Sodium citrate, dihydrate	1.76 g
1N NaOH	5 ml
Dist. water	30 ml

3.2. The electron microscope procedure:

For electron microscop, small pieces of fresh specimens of digestive gland were removed from the two tested land snails before treatment and after 3 days of treatment. The tissues were fixed by immersing them immediately in formalin-glutaraldehyde fixative (4F:1G) then in phosphate buffer solution (pH 7.2) at 4°C for 3 h. Specimens were then postfixed in 2 % OsO_4 in the same buffer at 4°C for 2 h. Samples were washed in the buffer and dehydrated at 4°C through a graded series of ethanol. Specimens were embedded in Epon-araldite mixture in labeled beam capsules. Semi thin sections (1μm thick) were stained with toluidine blue. Ultrathin sections (60-70 nm thick) were picked upon 200 mesh naked copper grids and double stained with uranyl acetate for ½ h and lead citrate for 20-30 min **(Reynolds, 1963).** Specimens were examined under Jeol 100 CX TEM at the department of Histology, Faculty of medicine, Zagzig University.

IV-Biochemical parameters

4.1. Hemolymph sampling

The snail's shell was first cleaned with a paper towel and the hemolymph was collected with a Pasteur pipette inserted through a tiny hole made in the shell above the pericardial region **(Bezerra *et al*., 1999).**

4.2. The evaluation of biochemical parameters:-

4.2.1. Glucose

Reagents :- (Vitro Co.)

R 1 (buffer)	Glucose standard	100 mmol/L
R 2 (enzymes)	Phosphate buffer, pH 7.0	100 mmol/L
	Phenol	11 mmol/L
	Glucose oxidase	15 KU/L
	Peroxidase	2 KU/L
	4-Aminoantipyrine	0.4 mmol/L

Preparation:-

Working reagent (WR): dissolve the contents of one vial R 2 Enzymes in one bottle of R 1 Buffer. Cap and mix gently to dissolve contents **(Keilin and Hartree, 1948)**

Procedure:-

1. Assay conditions:

Wavelength: . . . . . 505 nm (490-550)

Cuvette: . . . . . 1 cm light path

Temperature. . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 10 min at 37ºC or 15-20 min at room temperature (15-25ºC).
5. Read the absorbance (A) of the samples and standard, against the blank. The color is stable for at least 30 minutes.

Calculations:-

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 100\ (\text{Standard conc.}) = \text{mg/dL glucose in the sample}$$

Conversion factor: mg/dL x 0.0555= mmol/L.

4.2.2. Total proteins

Reagents:- (Diamond Co.)

R Biuret	Sodium potassium tartrate	15 mmol/L
	Sodium iodide	100 mmol/L
	Potassium iodide	5 mmol/L
	Copper (II) sulphate	5 mmol/L

Preparation:-

The reagents are ready to use **(Doumas *et al*., 1981)**

Procedure:-

1. Assay conditions:

   Wavelength: . . . . . 540 (530-550) nm

   Cuvette: . . . . . 1 cm. light path

   Temperature . . . . . .37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard (µL)	--	25	--
Sample (µL)	--	--	25

4. Mix and incubate 5 min at 37ºC or 10 min at room temperature.
5. Read the absorbance (A) of the samples and standard, against the blank. The color is stable for at least 30 minutes.

Calculations:-

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 7 \text{ (Standard conc.)} = \text{g/dL of total protein in the sample}$$

4.2.3. Albumin

Reagents: - (Diamond Co.)

R	Bromcresol green pH 4.2	50 mmol/L

Preparation:-

Reagent and calibrator are ready to use **(Reinhold, 1953)**

Procedure:-

1. Assay conditions:

   Wavelength: . . . . 630 nm (600-650)

   Cuvette: . . . . . 1 cm light path

   Temperature 15-25ºC

2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard (µL)	--	5	--
Sample (µL)	--	--	5

4. Mix and incubate for 10 min at room temperature (15-25ºC).
5. Read the absorbance (A) of the samples and standard, against the blank. The color is stable 1 hour at room temperature.

Calculations:-

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 5\ (\text{Standard conc.}) = \text{g/dL albumin in the sample}$$

Conversion factor: g/dL x 144.9 = µmol/L

4.2.4. Urea

Reagents: - (Diamond Co.)

R 1 Buffer	TRIS pH 7.8 -Ketoglutarate Urease	80 mmol/L 6 mmol/L 75000 U/L
R 2 Enzymes	GLDH NADH	60000 U/L 0.32 mmol/L

Procedure

1. Assay conditions: **(Fearon, 1939)**

Wavelength: . . . . 340 nm

Cuvette: . . . . .1 cm light path

Temperature . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard(Note 2-3) (L)	--	10	--
Sample (L)	--	--	10

4. Mix and read the absorbance after 30 s (A1) and 90 s (A2).

5. Calculate: A= A1 – A2 .

Calculations :-

$$\frac{(\Delta A)\text{Sample}}{(\Delta A)\text{Calibrator}} \times 50\ (\text{Calibrator conc}) = \text{mg/dL urea in the sample}$$

Conversion factor**:** mg/dL x 0.1665 = mmol/L.

4.2.5. Triglycerides

Reagents:- (Spinreact Co.)

R	GOOD pH 6.3 p-Chlorophenol Lipoprotein lipase (LPL) Glycerol kinase (GK) Glycerol-3-oxidasa (GPO) Peroxidase (POD) 4 – Aminophenazone (4-AP) ATP	50 mmol/L 2 mmol/L 150000 U/L 500 U/L 3500 U/L 440 U/L 0.1 mmol/L 0.1 mmol/L

Preparation:-

Reagent and calibrator provided are ready to use **(Kaplan, 1984)**

Procedure :-

1. Assay conditions:

Wavelength: . . . . . 505 nm (490-550)

Cuvette: . . . . . 1 cm light path

Temperature . . . . . 37ºC / 15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard (L)	--	10	--

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.

5. Read the absorbance (A) of the samples and calibrator, against the blank. The color is stable for at least 30 minutes.

Calculations:-

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times \text{Standard conc.} = \text{mg/dL triglycerides in the sample}$$

Conversion factor**:** mg/dL x 0.0113= mmol/L.

4.2.6. Cholesterol

Reagents:- (Spinreact)

R	PIPES pH 6.9	90 mmol/L
	Phenol	26 mmol/L
	Cholesterol esterase (CHE)	1000 U/L
	Cholesterol oxidase (CHOD)	300 U/L
	Peroxidase (POD)	650 U/L
	4 – Aminophenazone (4-AP)	0.4 mmol/L

Preparation:-

All the reagents are ready to use **(Burtis, 1999).**

Procedure:-

1. Assay conditions:

   Wavelength: . . . . 505 nm (500-550)

   Cuvette: . . . . 1 cm light path

   Temperature . . . . .37ºC /15-25ºC

2. Adjust the instrument to zero with distilled water.

3. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard[(Note 1-2)] (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 min at 37ºC or 10 min at 15-25ºC.

5. Read the absorbance (A) of the samples and calibrator, against the blank. The color is stable for at least 60 minutes.

Calculations:-

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 200\ (\text{Standard conc.}) = \text{mg/dL cholesterol in the sample}$$

Conversion factor**:** mg/dL x 0.0258= mmol/L.

V- Field experiment

The field experiment was carried out on *Monacha cartusiana*. The molluscicidal activity of the tested pesticides were tested in Egyptian clover field highly infested with land snail *Monacha cartusiana* at Malames village, Menia El-kamh district, Sharkia governorate. Areas divided into plots each of about 25 m^2, from the whole area of the field, which was controlled. The field was irrigated one day before treatment. The tested pesticides poisonous baits were prepared as mentioned before with the highest concentration of each pesticide as well as control baits without pesticides. Baits were offered on plastic sheet each one contained 100 gm. The snail number in each concentration was counted before the experiment begins (t_1) and after 1, 3, 7, 14 and 21 days of application. Reduction percentages were calculated according to the formula of **Henderson and Tilton (1955)** as follows:

$$\% \text{ Reduction} = \left[1 - \frac{t_2 \times r_1}{t_1 \times r_2} \right] \times 100$$

Where;

r_1 = number of alive snails before treatment in untreated plots.

r_2 = number of alive snails after treatment in untreated plots.

t_1 = number of alive snails before treatment in treated plots.

t_2 = number of alive snails after treatment in treated plots.

Data were statistically analyzed using F test and L.S.D. values were calculated at 0.001 % according to **COSTAT (1986).**

Chapter V

Results

I-Toxicity test

1.1. Toxicity of chemical compounds baits against *Eobania vermiculata*:-

The results in table (5) show the toxicity of chemical compounds used baits on *Eobania vermiculata* under laboratory conditions. Newmyl was the highest toxic compound which gave 100 % mortality after 14 days of treatment with all concentrations used (1, 2 and 4 %). Also, Amino produced complete mortality (100 %) after two weeks of all used concentrations (1, 2 and 4 %).

Humic acid with concentrations 1, 2 and 4% gave 100 % mortality after 21 days, but cyflu-magic reached 100 % mortality after using the highest concentration (2 %) after 2 weeks but the other concentrations used (1 and 1/2 %) gave 100 % mortality after 3 weeks.

Fusillade gave the high peak of mortality (100 %) with the highest concentration (6 %) after 2 weeks and the other two concentrations (2, 4 %) gave 100 % mortality after 3 weeks. Micronized sulfur displays the highest mortality percent (100 %) after 4 weeks when concentration of 1/2 % was used, where the other concentrations (1 and 2 %) reached 100 % mortality after 3 weeks of treatment.

K-othrine did not reach 100 % mortality but gave 33.3 and 40 % mortalities after 28 days of using 2 and 4 %, respectively. The lowest concentration of K-othrine did not give any mortality. Potassium thiosulfate gave no mortality (0 %) when concentrations of 1.25 and 2.5 % were used but concentration of 5 % gave 13.3 % mortality after 28 days of treatment.

Both combining Zn and urea gave the same result where no mortality (0 %) was recorded to the end of experiment when the two lowest concentrations were used (0.1 and 0.2 %) and 0.6 and 1.2 %, respectively. Treatment with the highest concentrations of the two chemical compounds (0.4 %) and (2.4 %) produced mortality reached 6.7 % after 28 days in case of combining Zn but the same mortality percentage (6.7 %) was recorded after 2 weeks in case of urea.

Table (5) Toxicity of chemical compound as baits against *Eobania vermiculata* under laboratory conditions

Chemical compound	Conc.	Mortality percentage after indicated days					
		1	3	7	14	21	28
Control	0	0	0	0	0	0	0
Newmyl	1	10	30	80	100	-	-
	2	10	40	85	100	-	-
	4	20	60	90	100	-	-
Amino	1	0	10	70	100	-	-
	2	0	10	80	100	-	-
	4	0	30	90	100	-	-
Humic acid	1	0	10	60	90	100	-
	2	10	15	70	90	100	-
	4	0	20	80	95	100	-
Cyflu-magic	1/2	0	0	10	40	100	-
	1	0	10	20	70	100	-
	2	0	20	66.6	100	-	-
Fusillade	2	0	0	20	60	100	-
	4	0	10	30	80	100	-
	6	0	10	40	100	-	-
Micronized sulfur	1/2	0	0	20	70	90	100
	1	0	0	40	80	100	-
	2	0	0	55	90	100	-
K-othrine wg250	1	0	0	0	0	0	0
	2	0	0	0	0	6.7	33.3
	4	0	6.7	6.7	13.3	20	40
Potassium thiosulfate	1.25	0	0	0	0	0	0
	2.5	0	0	0	0	0	0
	5	0	0	6.6	6.6	6.6	13.3
Combining zn	0.1	0	0	0	0	0	0
	0.2	0	0	0	0	0	0
	0.4	0	0	0	0	0	6.7
Urea	0.6	0	0	0	0	0	0
	1.2	0	0	0	0	0	0
	2.4	0	0	0	6.7	6.7	6.7

1.2. Toxicity of biocides baits against *Eobania vermiculata*:-

As shown in table (6) when *E. vermiculata* treated with the applied biocides no mortality occurred in the first day of treatment except with 6 % of Protecto and 4 % of Vertimec where mortality reached 10 %. Vertimec produced 100 % mortality when concentration of 4 % was used for 7 days while snail treatment with concentrations 1 and 2 % produced 100 % mortality after 2 weeks. Bioranza was the less effective biocide where at 1 % concentration mortality reached 10 % after 2 weeks and the highest concentration (4 %) produced 10 % mortality after 7 days and 70 % mortality were attended after 28 days.

Table (6) Toxicity of biocides as baits against *E. vermiculata* under laboratory conditions

Biocides	Conc.	Mortality percentage after indicated days					
		1	3	7	14	21	28
Control	0	0	0	0	0	0	0
Newmyl	1	10	30	80	100	-	-
	2	10	40	85	100	-	-
	4	20	60	90	100	-	-
Vertimec	1	0	10	60	100	-	-
	2	0	10	80	100	-	-
	4	10	20	100	-	-	-
Protecto	1.5	0	0	0	70	100	-
	3	0	0	20	80	100	-
	6	10	30	40	100	-	-
Bioranza	1	0	0	0	10	30	50
	2	0	0	0	20	30	60
	4	0	0	10	30	40	70
Biover	1	0	10	10	40	40	70
	2	0	10	20	60	60	80
	4	0	0	30	40	70	80

Protecto concentration of 6 % ended the experiment (100 %) mortality after 2 weeks and concentrations of 1.5 and 3 % the 100 % mortality was attained after 3 weeks.

Biover gave 80 % mortality with the two highest concentrations (2 and 4%) after 28 days of treatment but this percent was 70 % when concentration 1 % was used in the same period (28 days).

1. 3.Toxicity of chemical compounds against *M. cartusiana*:-

The toxicity of the chemical compounds used as baits against *M. cartusiana* under laboratory conditions is illustrated in table (7) it could be shown that after one day of treatment with Newmyl (the reference mollusicicide) there was high toxicity. The mortalities started to occur after one day of treatment by the three applied concentrations (1, 2 and 4 %) and the first two concentrations caused 100 % mortality after only one week. The lower concentrations produced 100 % mortality after 2 weeks. In case of Amino which was the highest toxic compound comparing with Newmyl, 100 % mortality was recorded after 2 weeks of treatment when 1 and 2 concentrations were used. The same compound when employed at a concentration of 4 % showed 100 % mortality after only one week of treatment. At all concentrations expertised of Amino, mortality of snails started after 3 days of treatment. Results showed that Humic acid came secondly in toxicity after Amino, where mortality begun after 3 days and reached 100 % mortality after 3 weeks in all the applied concentrations (1, 2 and 4 %).

All concentrations of cyflu-magic (1/2, 1 and 2) produced 10 % mortality after 3 days and the one hundred percent of mortality occurred after 3 weeks (21 days). Combining–Zn mortality started after 3 days of treatment with all concentrations (0.1, 0.2 and 0.3) and reached a high

peak after 28 days but no one hundred percents were attained as the 3 concentrations recorded 63.3, 77.7 and 86.7 %, respectively.

Fusillade at concentrations 2, 4 and 6 % gave 100 % mortality after 28 days of treatment in case of the first two concentrations (2 and 4 %). The highest concentration (6 %) produced 100 % mortality after 3 weeks. K-othrine gave fluctuated result where concentration of 2 % induced snail mortality after one day whereas the highest concentration (4 %) did not. The latter concentration produced mortality after 3 and 4 weeks. the lowest concentration (1 %) started to cause mortality after one week.

Micronized sulfur showed that the concentrations of 1/2 and 1 % produced 100 % mortality after 3 weeks. The highest concentration (2 %) produced only 80 % mortality after 7 days and no other mortalities were recorded after that time.

The lowest potassium thiosulfate concentration (0.125 %) caused no mortality after 1, 3 or 7 days of treatment. After 2 weeks of administration mortality of snails started and reached to 53.3 % after 28 days. The other two concentrations (2.5 and 5 %) did not produce 100 % mortality till the maximum experimentation period (28 days). Urea gave results similar to those in case of potassium thiosulfate but the lowest concentration (0.6 %) caused mortality earlier (after 3 days).

Table (7) Toxicity of chemical compounds as baits against *M. cartusiana* under laboratory conditions

Chemical compound	Conc.	Mortality percentage after indicated days					
		1	3	7	14	21	28
Control	0	0	0	0	0	0	0
Newmyl	1	5	35	85	100	-	-
	2	5	45	90	100	-	-
	4	10	60	100	-	-	-
Amino	1	0	25	75	100	-	-
	2	0	45	90	100	-	-
	4	0	50	100	-	-	-
Humic acid	1	0	20	60	90	100	-
	2	0	30	65	95	100	-
	4	0	35	75	95	100	
Cyflu-magic	1/2	0	10	55	80	100	-
	1	0	10	75	90	100	-
	2	0	10	80	95	100	-
Fusillade	2	0	5	35	90	95	100
	4	0	10	60	65	90	100
	6	0	10	60	70	100	-
Micronized sulfur	1/2	0	5	30	75	100	-
	1	0	10	40	85	100	-
	2	0	20	80	90	100	-
K-othrine wg250	1	0	0	6.7	6.7	46.7	60
	2	0	0	10	16.7	53.3	70
	4	3.3	10	15	20	62.3	80
Potassium thiosulfate	1.25	0	0	0	7.3	30.3	53.3
	2.5	0	3.3	4.4	8	43.3	63.3
	5	0	3.3	6.6	13.3	54.4	84.4
Combining Zn	0.1	0	3.7	6.7	10	33.3	63.3
	0.2	0	3.3	6.7	13.3	66.7	77.7
	0.3	0	6.7	10	16.7	46.7	86.7
Urea	0.6	0	3.3	3.3	10	50.7	60.3
	1.2	0	3.3	3.3	6.7	53.3	76.6
	2.4	6.7	6.7	13.3	16.7	56	80

1.4. Toxicity of biocides baits against *M. cartusiana*:

Table (8) showed toxicity of the applied biocides as baits against *M. cartusiana* under laboratory conditions. After one day of treatment, no mortality was recorded with any of the applied biocides except in case of the highest concentration of Vertimec (4 %). In this case, 40 % mortality was recorded. Also, 100 % mortality was attained with all concentrations (1, 2 and 4 %) of Vertimec after only one week.

Mortality results proved that Protecto came secondly beyond Vertimec in toxicity. One hundred mortality percent was reached with the highest concentration (6 %) after two weeks, with the intermediate concentration (3 %) after 3 weeks, while the lowest concentration of Protecto (1.5 %) did not produce 100 % mortality even after 28 days of treatment.

Table (8) Toxicity of Biocides as baits against *M. cartusiana* under laboratory conditions

Biocide	Conc.	Mortality percentage after indicated days					
		1	3	7	14	21	28
Control	0	0	0	0	0	0	0
Newmyl	1	5	35	85	100	-	-
	2	5	45	90	100	-	-
	4	10	60	100	-	-	-
Vertimec	1	0	35	100	-	-	-
	2	0	50	100	-	-	-
	4	40	65	100	-	-	-
Protecto	1.5	0	10	45	70	90	95
	3	0	15	50	85	100	-
	6	0	15	80	100	-	-
Bioranza	1	0	5	45	70	95	100
	2	0	5	40	65	95	100
	4	0	15	40	80	100	-
biover	1	0	20	45	90	100	-
	2	0	15	50	95	100	-
	4	0	15	75	95	100	-

Biover caused 100 % mortality after 21 days with all expertised concentrations (1, 2 and 4 %). Likewise, the highest concentration of bioranza (4 %) produced 100 % mortality after 21 days. The other two concentrations of Bioranza (1 and 2%) caused (100 %) mortality after 28 days of treatment.

1.5. Determination of LC_{50} values after 72 hr. of treatment

1.5.1. *Eobania vermiculata*

1.5.1.1. LC_{50} values of chemical compounds

The data in table (9) show that amino gave the lowest LC_{50} value (11.19 %) with lower confidence limit of 6.140 % and the upper limit recorded 72.683 %.

Humic acid showed LC_{50} value of 56.507 % with the lower confidence limit was 10.636 and the upper was 100000002.004e+12. The reference insecticide Newmyl revealed LC_{50} value of 2.733 % with a lower confidence limit of 2.111% and the upper limit reached 4.133%.

Table (9) The LC_{50} values after 72hr. of treatment the land snail *E. vermiculata* with the chemical compounds

Chemical compound	LC_{50}	Confidence Limits 95 %	
		Lower	Upper
Newmyl	2.733	2.111	4.133
Amino	11.190	6.140	72.683
Fusillade	18.057	-	-
Cyflu-magic	28.042	-	-
Humic acid	56.507	10.636	100000002.004E+12
K-othrine	-	-	-
Micronized sulfur	-	-	-
Combining zn	-	-	-
Potassium thiosulfate	-	-	-
Urea	-	-	-

Micronized sulfur k-othrine, potassium thiosulfate, urea and combining Zn showed no 72 hrs LC_{50} values.

1.5.1.2. LC_{50} values of biocides

Table (10) indicateds the biocides LC_{50} values, where Vertimec had the lowest LC_{50} value (2.75 %) with lower confidence limit of 2.320 % and upper confidence limit of 3.442 %.

Table (10) The LC_{50} values after 72hr. of treatment the land snail *Eobania vermiculata* with the biocides

Biocide	LC_{50}	Confidence Limits 95 %	
		Lower	Upper
Vertimec	2.750	2.320	3.442
Protecto	9.994	-	-
Biover	22.736	-	-
Bioranza	-	-	-

The highest LC_{50} was that of Biover (22.736 %) with no confidence limits. While Bioranza gave no LC_{50} values. Protecto LC_{50} value came secondly to Biover (9.994 %) but also with no confidence limits.

1.5.2. *Monacha cartusiana*

1.5.2.1. LC_{50} values of chemical compounds

The data in Table (11) show the LC_{50} values after 72hr of treatment the *Monacha cartusiana* land snails with chemical compounds.

Table (11) The LC_{50} values after 72hr. of treatment *Monacha cartusiana* with the chemical compounds

The pesticide	LC_{50}	Confidence Limits95 %	
		Lower	Upper
Newmyl	2.412	1.748	3.896
Amino	3.469	2.512	7.383
Micronized sulfur	8.515	3.889	177.528
Humic acid	12.039	5.051	67003.500
Potassium thiosulfate	130.748	-	-
Fusillade	234.102	-	-
Urea	829.488	-	-
Combining Zn	-	-	-
K-othrine	-	-	-
Cyflu-magic	-	-	-

Amino has the lowest LC_{50} value (3.469 %) with lower confidence limit of 2.512 % and upper limit of 7.383%. Urea gave the highest LC_{50} value 829.488 % with no confidence limits comparing with Newmyl LC_{50} value (2.412 %) with lower confidence limit 1.748 % and upper limit of 3.896%. Combining Zn, cyflu-magic and finally k-othrine gave no LC_{50} values.

1.5.2.2. LC_{50} values of biocides

Data in table (12) show that Vertimec had the lowest LC_{50} value (2 %) with lower confidence limit of 1.492 and upper limit of 2.681. Biover gave the highest LC_{50} value (1406.82 %) with no confidence limits.

Table (12) The LC_{50} values after 72hr. of treatment *Monacha cartusiana* with biocide

The biocide	LC_{50}	Confidence Limits 95 %	
		Lower	Upper
Vertimec	2.00	1.492	2.681
Bioranza	38.775	10.785	2105222.500
Protecto	84.755	15.955	100000002.004 E+12
Biover	1406.820	-	-

Bioranza had the second order of LC_{50} value (38.775) while its lower confidence limit was 10.785 and the upper confidence limit was 2105222.500. Protecto came secondly to Bioranza with LC_{50} value of (84.755 %) and lower confidence limit of 15.955 % and upper confidence limit of 100000002,004 E+12.

II-Histological and histopathological observations

2.1. The digestive gland

2.1.1. Digestive gland of control *Eobania vermiculata*

The digestive gland is a bilobed tubulo-acinar gland located in the dorsal portion of the animal in bath sides of the stomach. It is surrounded by a thin membrane composed of a single layer of short columnar cells resting on basal membrane underlied with circular muscle fibers (Fig 4). The digestive gland tissue consists mainly of spherical digestive tubules separated by loose connective tissue containing hemolymphatic vessels and hemocytes. Each tubule is provided externally with a circular muscle layer. Different cell types were observed in the epithelium lining the lumen of the digestive gland tubules. These cells are: digestive cells, calcium cells and excretory cells.

(a) Digestive cells:

They constitute the major cellular population of the digestive tubule. These cells are columnar with flattened or slightly rounded apical surfaces bearing well-developed brush border. The nuclei of them are often basally-located and are rounded or oval in outline with condensed chromatin and mostly have a single nucleolus (Fig. 4).

(b) Calcium cells:

They are fewer than the digestive cells and occur either singly or in pairs in the corners of the tubules. They have pyramidal shape with narrow distal end and a marked broad base. These cells have Calcium spherules which are round and also contain rounded lightly refractive bodies. Calcium cells possess apical secretary granules and large rounded nuclei. Nuclei of these cells are rich in heterochromatin and possess conspicuous nucleolus. Nuclei are usually located near the center or in the basal half of the cell (Fig. 4).

(c) Excretory cells:

These cells have globular shape. They are characterized by the presence of a single large vacuole filling nearly the whole volume of the cell. The excretory products are accumulated in the vacuole often in the form of a large brown body. The apical end of the cell possesses a well developed brush border. The nucleus is small and usually pressed flat against the cell base (Fig. 4).

2.1.2. Digestive gland of treated *Eobania vermiculata*:-

After the toxicity test, the concentrations applied in this part involved half of LC_{50} and LC_{50} of the highly toxic compounds. These included Newmyl as a reference pesticide and Vertimec as a biocide and Amino as a fertilizer. In the following table (13) chemical compounds and their half LC_{50}^{s} and the LC_{50}^{s} used with *E. vermiculata* are shown.

Table (13) The chemical compounds 1/2 LC_{50} and LC_{50} used with *E. vermiculata*

The compound	*Eobania vermiculata*	
	1/2 LC_{50}	LC_{50}
Newmyl	1.493	2.986
Amino	2.1035	4.207
Vertimec	1.375	2.750

a-Newmyl changes

Effects of 1/2 LC_{50} of Newmyl :

The digestive gland tissue exhibited marked histopathological alterations in response to treatment with 1/2 LC_{50} of Newmyl. Many tubules suffered from severe atrophy which was mingled in some places with some degenerations (Fig. 5). As for calcium cells they showed some vacuolation and their nuclei were mostly pyknotic. In the excretory cells conspicuous excretory vesicles increased in number and larger excretory granules were detected (Fig. 5).

Effects of LC_{50} of Newmyl:

Exposure of snails to LC_{50} of Newmyl exhibited hemocytic infiltration which was frequently observed and the basement membrane of tubules appeared irregular and relatively thickened. The digestive cells showed accumulation of large number of granules and undergone extensive breakdown into membrane-bound vesicles. Calcium cells were packed with enlarged calcium spherules and they exhibited pyknotic nuclei. The cytoplasm of most calcium cells was replaced by large vacuoles containing granules. Excretory cells showed increased number of excretory granules. They were loaded with huge number of such granules and the degeneration was increased (Fig. 6)

b-Amino changes

Effects of 1/2 LC_{50} of Amino:

Cytoplasmic degenerations were observed in digestive cells of such treated snails. Treatment with 1/2 LC_{50} of Amino led to an increase in the excretory cells number which contained excretory granules of large size and the cell nuclei were suffered from pyknosis. The accumulated excretory granules exhibited increased size and number. There was a conspicuous degeneration and dissociation at some places of the luminal surface of cells (Fig. 7).

Effects of LC_{50} of Amino:

The cytoplasm of digestive cells is filled with cell organelles where the excretory cells increased in number and the vacuoles showed large size. Wide degenerative regions were demonstrated in digestive cells. There was a decrease in the spherules of calcium cells (Fig. 8).

c-Vertimec changes

Effects of 1/2 LC_{50} of Vertimec:

After treatment with Vertimec 1/2 $LC_{50,}$ the cell membranes were hardly detected. Digestive cells revealed that the cellular vacuoles and

degenerative cytoplasm increased in size and number. The residual bodies in the excretory cells appeared larger and darker. The number of excretory vacuoles increased. Calcium cells displayed the presence of spherules but vacuoles were also detected (Fig. 9).

Effects of LC_{50} of Vertimec:

Vacuolar degeneration in tissue of the digestive gland was obvious. The cytoplasm of digestive cells was highly degenerated. The whole tissue was perforated due to such degenerations. The excretory granules were seen larger in size and darkly stained (Fig.10).

2.1.3. Digestive gland of control *Monacha cartusiana*

Nearly, the histological investigation revealed that the histology architecture as well as the cellular composition of *M. cartusiana* similar to those described in *E. vermiculata* (Fig. 11).

2.1.4 Digestive gland of treated *Monacha cartusiana*

Below is table (14) summarizing the1/2 LC_{50} and LC_{50} concentrations of the insecticide, biocide and fertilizer applied in the treatment experiment used with *M. cartusiana*.

Table (14) The LC_{50} and 1/2 the LC_{50} compound used with *Monacha cartusiana*

The compound	*Monacha cartusiana*	
	1/2 LC_{50}	LC_{50}
Newmyl	1.144	2.288
Amino	0.8295	1.659
Vertimec	1.00	2.00

a-Newmyl changes

Effects of 1/2 LC_{50} of Newmyl :

Following exposure of *M. cartusiana* snails to 1/2 the LC_{50} of Newmyl insecticide, the digestive gland showed mild degree of degenerations. Also, digestive cells were of abnormal appearance with more granulation of the cytoplasm. Excretory cells revealed larger

excretory vacuoles containing abundant excretory granules calcium cells were clearly vacuolated (Fig. 12).

Effects of LC_{50} of Newmyl :

The architecture of the digestive gland of *Monacha cartusiana* exposed to the high concentration of Newmyl (LC_{50}) was obviously affected. Degenerations were abundant and the cytoplasm of digestive cells was altered. Numerous excretory vacuoles were seen in the excretory cells. Calcium cells were granulated but vacuoles in the cytoplasm were dominated (Fig.13).

b-Amino changes

Effects of 1/2 LC_{50} of Amino:

If compared to the effects of Newmyl, Amino revealed less histopathological perturbations. A few degenerations were observed while digestive cells exhibited lesser affection. Excretory cells exhibited accumulation of large excretory granules. Vacuolation of calcium cells was also seen (Fig. 14).

Effects of LC_{50} of Amino:

Following exposure of *M. cartusiana* snails to the high concentration of Amino, degenerative changes were conspicuous and were of wide size. Digestive cells were more affected compared with the previous concentration. Excretory cells were seen containing larger excretory vacuoles with dark stained excretory granules of variable size and shape. Calcium cells architecture was altered (Fig. 15).

c-Vertimec changes

Effects of 1/2LC_{50} of Vertimec:

Exposure of *M. cartusiana* snails to 1/2 LC_{50} of Vertimec biocide led to pathological changes in the digestive gland. Degenerative regions were observed in cytoplasm of digestive cells were altered. Numerous dark excretory granules were abundant inside large excretory vacuoles filling

the excretory cells. Calcium cells were deteriorated by vacuolation (Fig. 16).

Effects of LC_{50} of Vertimec:

The whole picture of the digestive gland of *M. cartusiana* snails exposed to the LC_{50} of Vertimec was dramatically altered. Digestive cells lost their normal appearance with large cytoplamic vacuolar degenerations. The excretory vacuoles in the excretory cells were abundant and larger in size. Excretory vacuoles contained large excretory granules. Calcium cells acquired a perforated appearance (Fig. 17).

2.2. The Intestine

2.2.1. Intestine of control *E. vermiculata*

The intestine of *Eobania vermiculata* is a thin walled tube divided into pro-, mid- and post intestine. The lining epithelium in pro and mid intestine is ciliated, but in post intestine the cilia are restricted in folds. The epithelium is organized in large villi with a fan-like contours. The villi harboring a lacteal in its centre. The intestinal epithelium is composed of columnar high and narrow ciliated and un-ciliated cells with broad apices. Many mucous cells are scattered throughout the epithelium and basal cells. The latter are elongated to oval cells with their lower surfaces attached to the basement membrane. The deeply basophilic nuclei are elongated oval rich in chromatin granules and lie at various levels in the lower halves of the cells. (Figs.18 and 19).

2.2.2. Intestine of treated *E. vermiculata*

a-Newmyl changes

Effects of 1/2 LC_{50} of Newmyl :

Treatment with 1/2 LC_{50} of Newmyl caused reduction of cilia, fragmentation of muscle tissue and increase in mucus secretion. The columnar cells have become highly vacuolated and their nuclei were dilated or showed little hypertrophy. Parts of free mucosa exhibited

dissociation. Degenerative regions were also demonstrated in the submucosa (Fig. 20).

Effects LC_{50} of Newmyl :

When Newmyl LC_{50} was applied the cells become distended and deformed in shape with the increase of mucous droplet in vacuoles, lumen became narrow. Dissociation of parts of the free mucosa was also seen. Degenerations were detected in the submucosa in addition to presence of obvious oedema. Nuclei of the columnar cells were hypertrophy and appeared more elongated but deeply basophilic (Fig. 21).

b-Amino changes

Effects of 1/2 LC_{50} of Amino:

Treatment with Amino 1/2 LC_{50} made the cytoplasm highly eosinophilic. Degenerative changes were observed in the submucosa and at the basal regions of columnar cells. The free mucosal surface was intact and nuclei were strongly basophilic (Fig. 22).

Effects of LC_{50} of Amino:

Treatment with Amino LC_{50} induced drastic changes in the intestinal mucosa. Numerous degenerative regions and mucosal dissociations were seen. The basal halves of columnar epithelial cells were highly vacuolated producing clear gaps between the cells and the muscularis mucosa. Nuclei were pyknotic. The villi as a whole acquired a perforated architecture (Fig. 23).

c-Vertimec changes

Effects of 1/2 LC_{50} of Vertimec:

The columnar epithelial cells exhibited hypertrophy whereas their nuclei showed pyknosis. Degeneration was observed in the submucosa and superficial dissociation was detected in columnar cells. Oedematous regions were also seen in the submucosa (Fig. 24).

Effects of LC_{50} of Vertimec:

Treatment with the high dose of the biocide Vertimec showed obvious deleterious effects. Hypertrophy of intestinal cells and pyknosis of nuclei were occurred. Dissociation of the free mucosa and degenerations in the submucosa were also demonstrated. Degenerative cytoplasmic parts were detected at the bases of columnar cells. Nuclei were elongated and darkly stained. Oedoema was also detected in the submucosa (Fig. 25).

2.2.3. Intestine of control *M. cartusiana*

The intestine epithelium of non-treated *Monacha cartusiana* were snail composed of the same cells of *Eobania vermiculata* ciliated and unciliated columnar cells lie on the basement membrane with basal oval nucleus and mucous cells. The cytoplasm is deeply eosimophilic (Fig. 26).

2.2.4. Intestine of treated *M. cartusiana*

a-Newmyl changes

Effects of 1/2 LC_{50} of Newmyl :

When land snails were treated with 1/2 LC_{50} of Newmyl, the cytoplasm of columnar cells suffered from degradation and appearance of degeneration and the mucous secretion increased. sloughing of superficial parts of the mucosa in addition to submucosal degenerations were also demonstrated. The basal parts of the columnar cells were obviossly degenerated. Nuclei of the cells were deeply basophilic (Fig. 27)

Effects of LC_{50} of Newmyl

This concentration of Newmyl showed deeper dissociation in the mucosa in addition to conspicuous degeneration in the submucosa. The epithelial columnar cells were highly vacuolated especially at their bases. (Fig. 28)

b-Amino changes

Effects of 1/2 LC_{50} of Amino

After treatment with this low concentration little pathological effects were developed. These were represented by degenerative regions at the bases of the columnar cells. No superficial dissociations were observed. The columnar cells as a whole were highly vacuolated (Fig. 29).

Effects of LC_{50} of Amino:

Conspicuous oedoema was observed following treatment with the LC_{50} of Amino. Vacuoles in the cytoplasm of columnar cells together with a few dissociations in the free end of the mucosa were observed. Degenerations in the submucosa were also demonstrated and attached to the submucosal oedema (Fig. 30)

c-Vertimec changes

Effects of 1/2 LC_{50} of Vertimec:

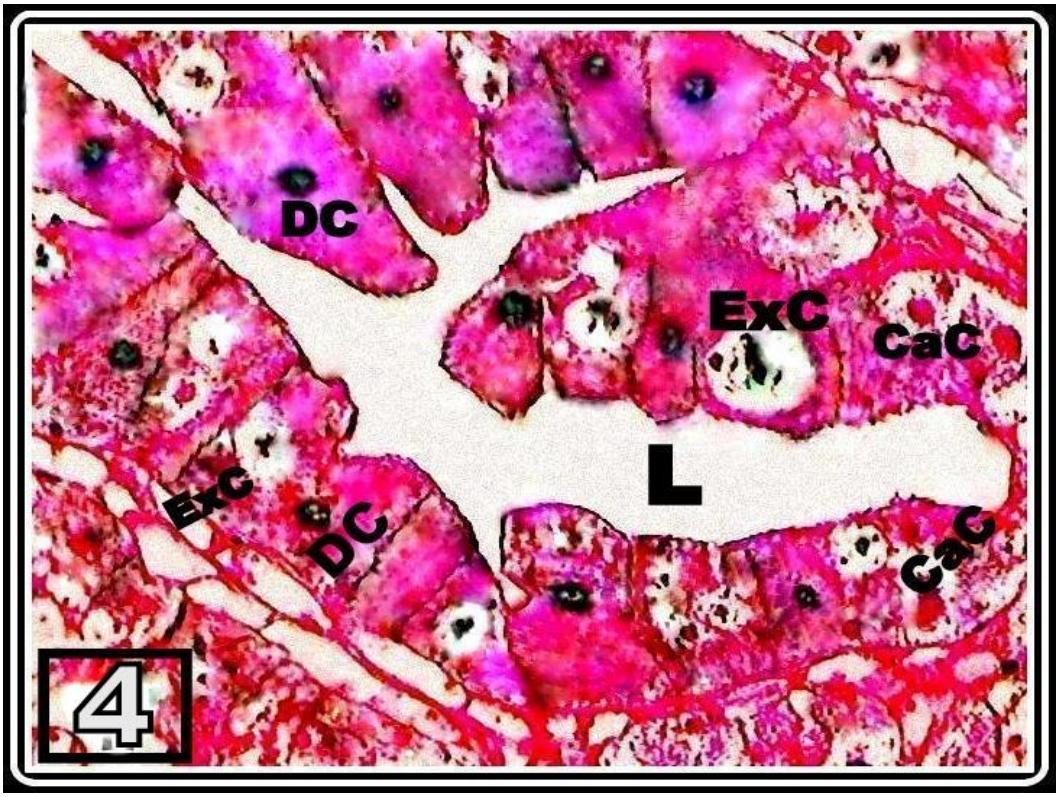
Treatment with the low concentration of Vertimec exhibited clear vacuolation in the cytoplasm of the columnar epithelial cells. Degenerative regions were detected in the submucosa as well as oedema. Vacuolation of the epithelia was obvious and gave the tissue a perforated architecture (Fig. 31).

Effects of LC_{50} of Vertimec:

The high concentration of the biocide Vertimec caused conspicuous degeneration in the submucosa. This was accompanied by submucosal oedema. Free parts of the superficial mucosa suffered from dissociation. The columnar cells were clearly vacuolated and suffered from cytoplasmic degenerations (Fig. 32)

Fig (4) Photomicrograph from a cross section passing through the digestive gland of control *E. vermiculata* showing lumen (L), calcium cells (CaC), digestive cells (DC), and excretory cells (ExC). X400, (Hx & E)

Fig (5) Photomicrograph of cross section passing through neighboring tubules of digestive gland of *E. vermiculat*a treated with 1/2 LC_{50} Newmyl showing calcium cells (CaC), digestive cells (DC), excretory cells (ExC) and the appearance of degenerations (Dg) . X100, (Hx & E)


DC
ExC
CaC
L
ExC
DC
CaC
4

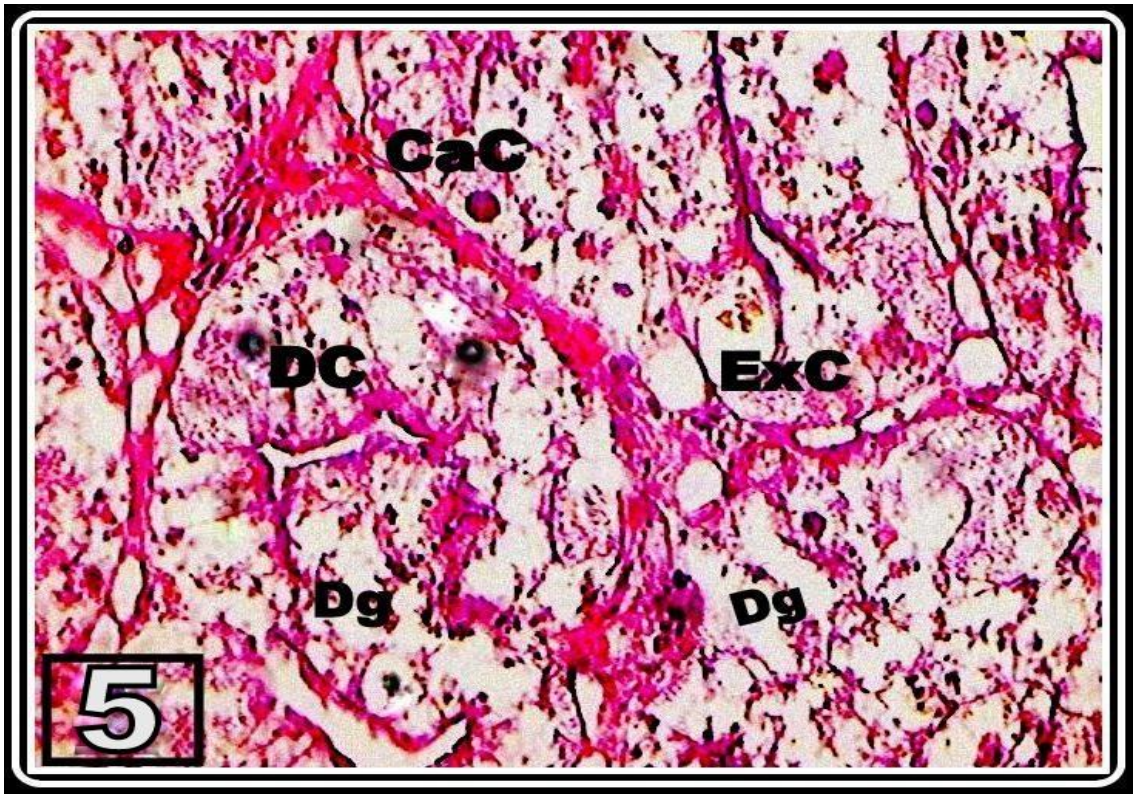
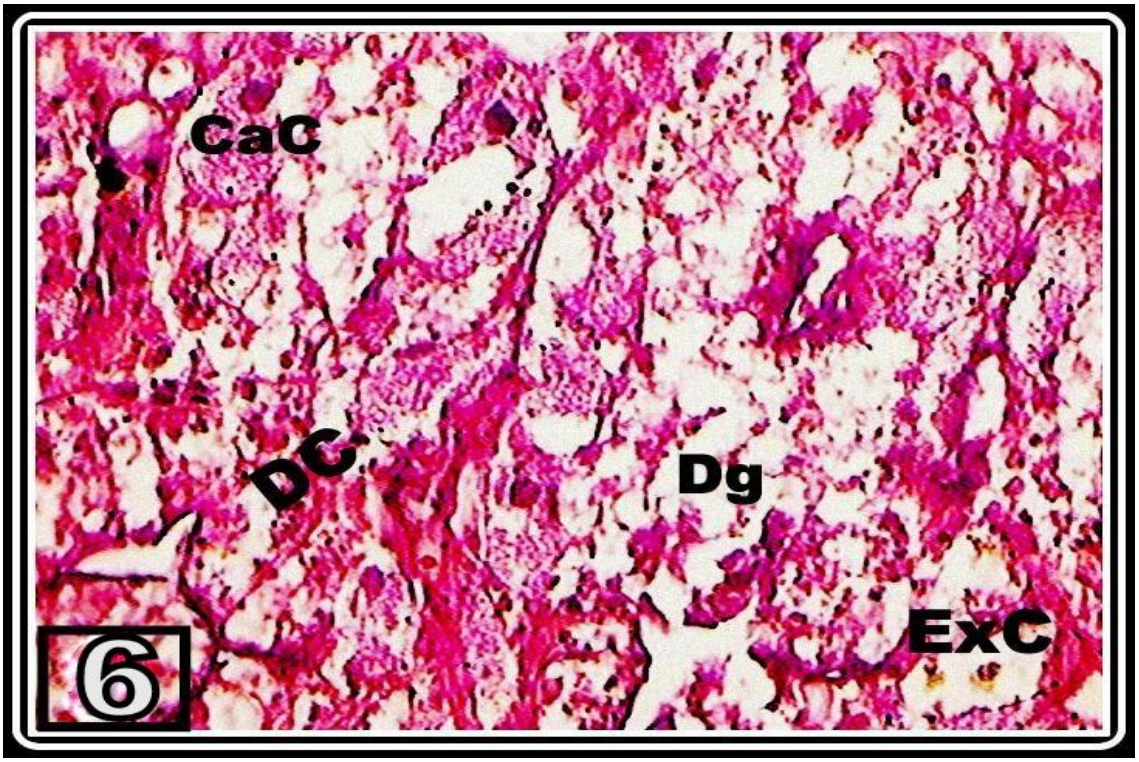

CaC
DC
ExC
Dg
Dg
5

Fig (6) Photomicrograph from a cross section passing through neighboring tubules of the digestive gland of *E. vermiculat*a treated with the LC_{50} of Newmyl showing calcium cells (CaC), digestive cells (DC), excretory cells (ExC) and increasing of degeneration (Dg). X100, (Hx & E)

Fig (7) A magnified photomicrograph from a cross section passing through the digestive gland of treated *E. vermiculata* with amino 1/2 LC_{50} showing lumen (L), calcium cells (Ca C), digestive cells (Dc), excretory cells (Ex C) and dissociation (Diss.). X400, (Hx & E)


CaC
DC
Dg
ExC
6

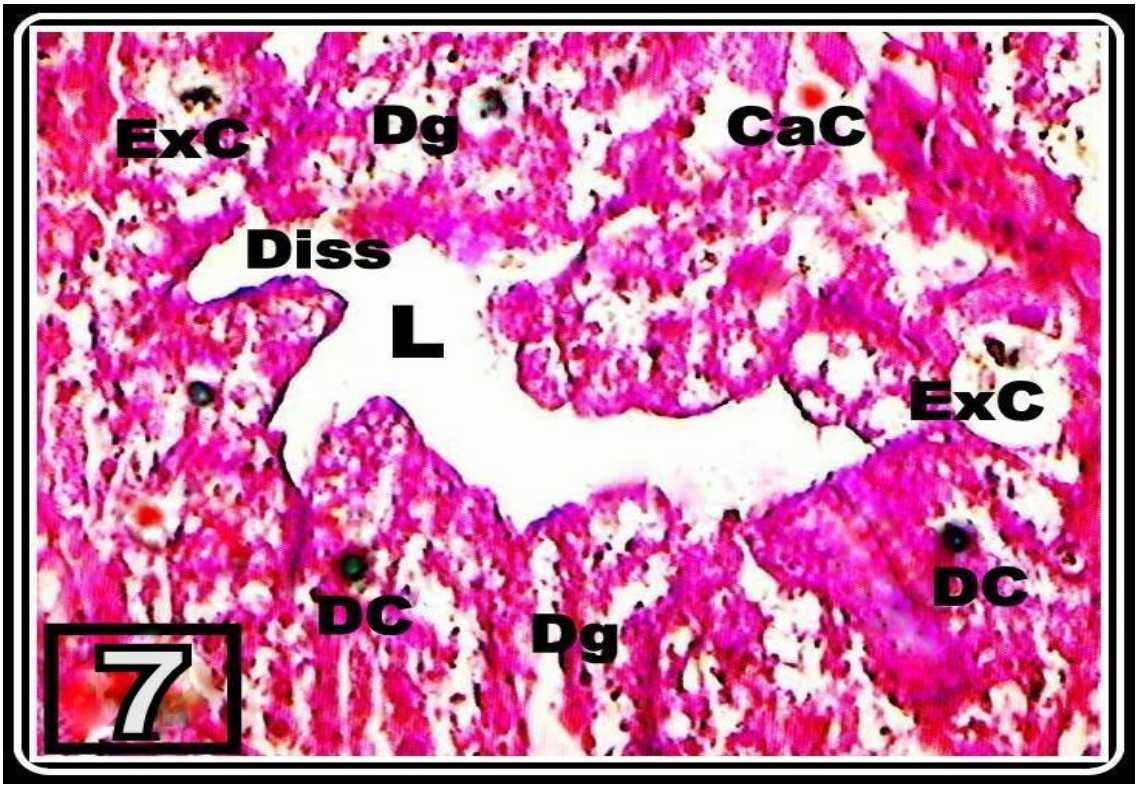
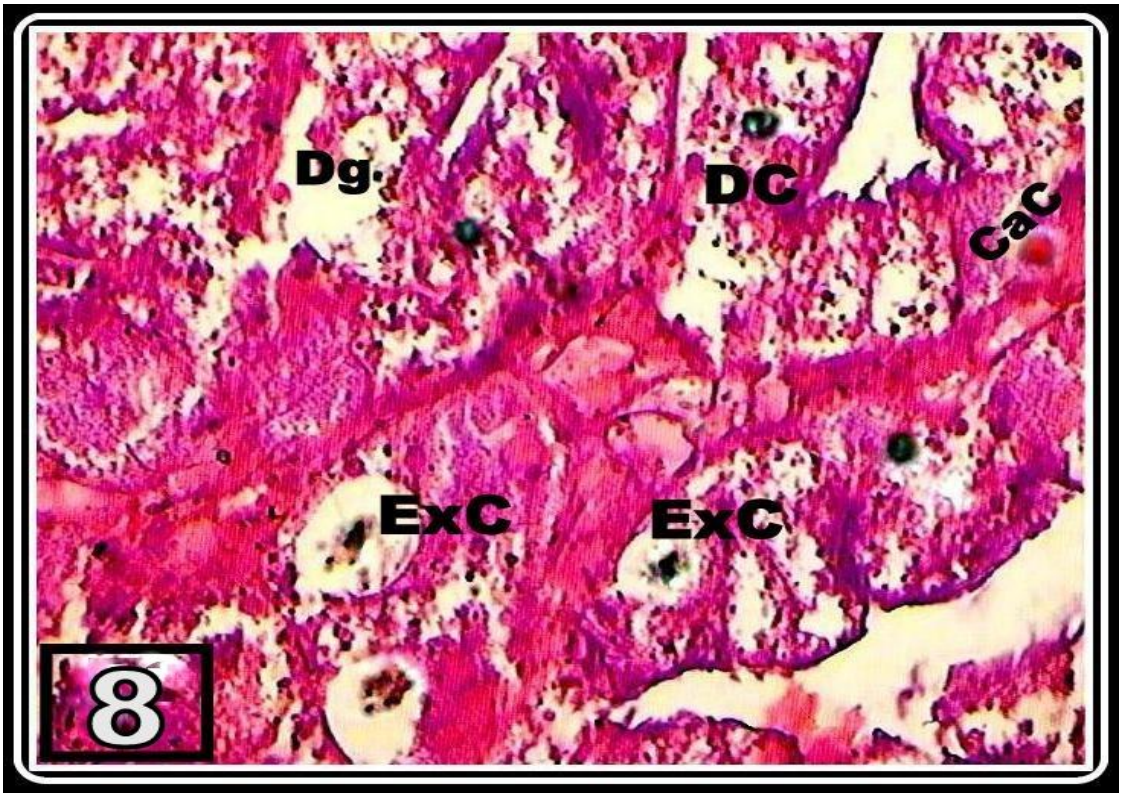

ExC
Dg
CaC
Diss
L
ExC
DC
DC
Dg
7

Fig (8) Photomicrograph form a cross section passing through the digestive gland of *E. vermiculata* treated with LC_{50} Amino showing calcium cells (CaC), digestive cells (DC), excretory cells (ExC), and increasing of degenerations (Dg). X100, (Hx & E)

Fig (9) Photomicrograph from a cross section passing through digestive gland of *E. vermiculata* treated with 1/2 LC_{50} Vertimec showing calcium cell (CaC), digestive cell (DC), excretory cell (ExC) and degeneration (Dg). X100, (Hx & E)


Dg.
DC
CaC
ExC
ExC
8

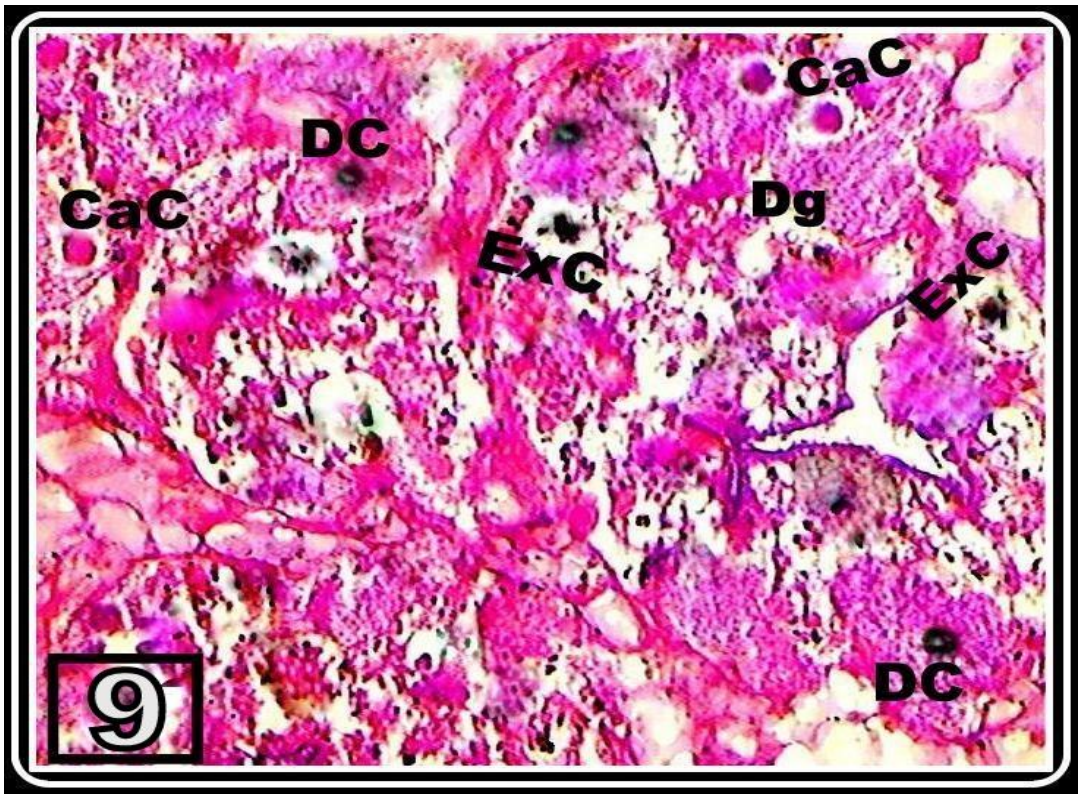
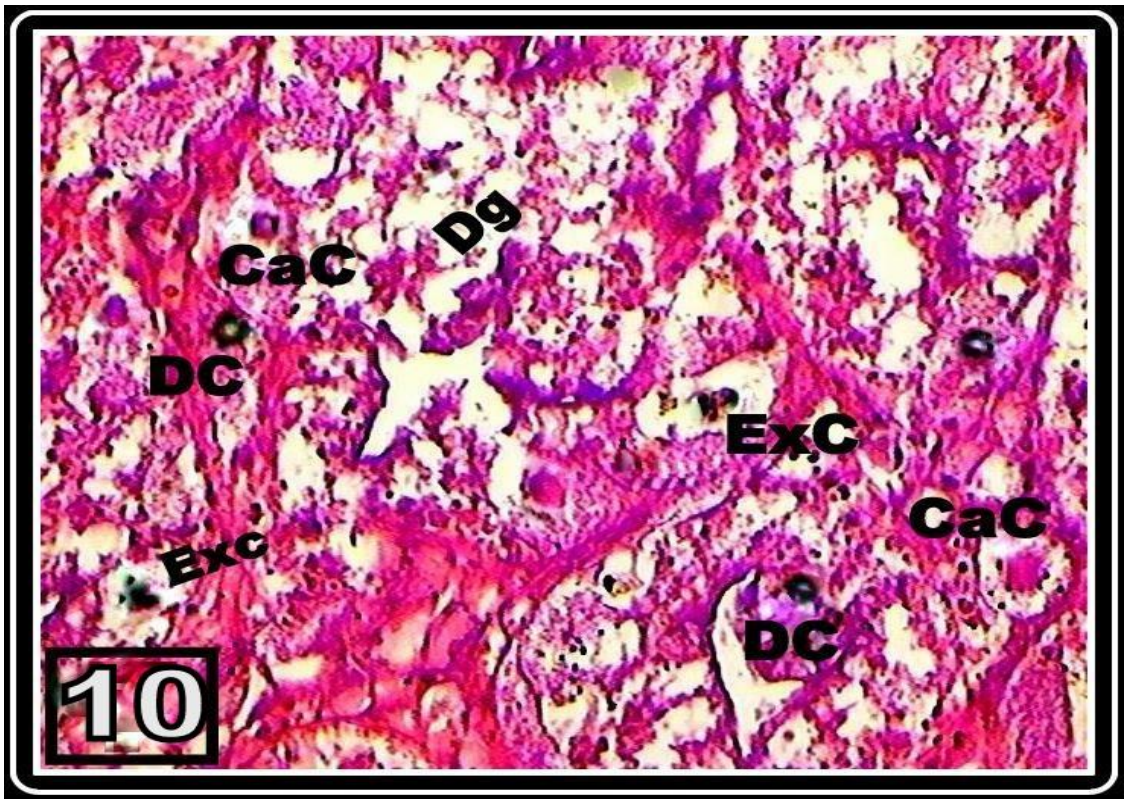

CaC
DC
CaC
Dg
ExC
ExC
DC
9

Fig (10) Photomicrograph from a cross section passing through the digestive gland of *E. vermiculata* treated with LC_{50} Vertimec. Note calcium cells (CaC), digestive cells (DC), excretory cells (ExC), and degeneration (Dg). X100, (Hx & E)

Fig (11) A magnified photomicrograph from a cross section passing through digestive gland of control *M. cartusiana* showing calcium cells (CaC), digestive cells (DC) and excretory cells (ExC).X 400, (Hx & E)


CaC
Dg
DC
ExC
CaC
ExC
DC
10

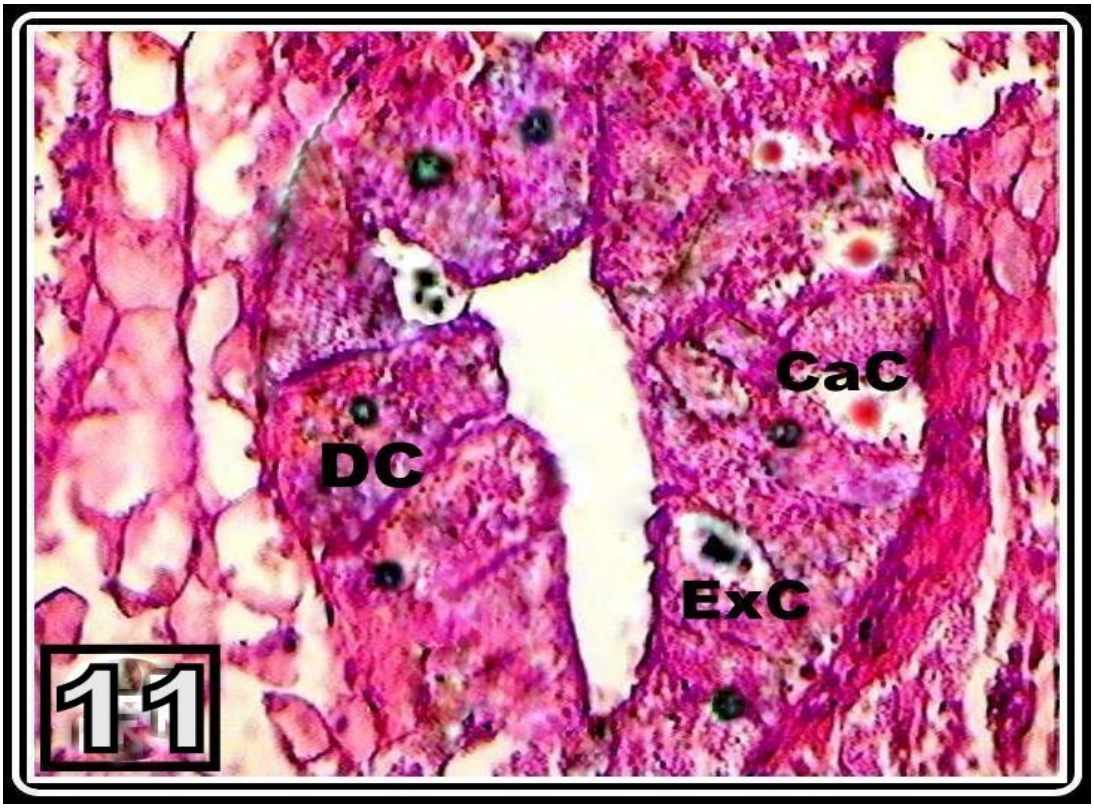
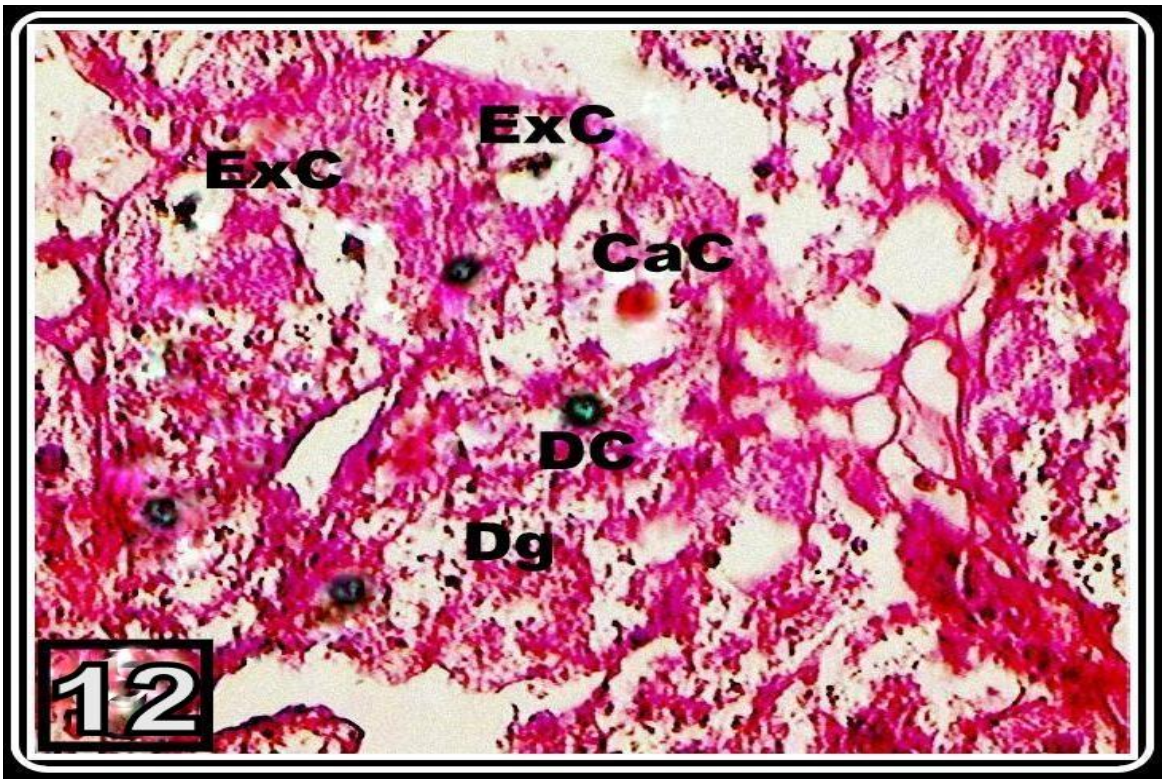

CaC
DC
ExC
11

Fig (12) Photomicrograph from a cross section passing through one crista of the digestive gland of *M. cartusiana* treated with 1/2 LC_{50} Newmyl showing calcium cells (CaC), digestive cells (DC), excretory cells (ExC), and degenerations ((Dg). X100, (Hx & E)

Fig (13) Photomicrograph from a cross section passing through the digestive gland of *M. cartusiana* treated with LC_{50} Newmyl showing calcium cells (CaC), digestive cells (DC), excretory cells (ExC) and degenerations (Dg) increase. X100, (Hx & E)


ExC
ExC
CaC
DC
Dg
12

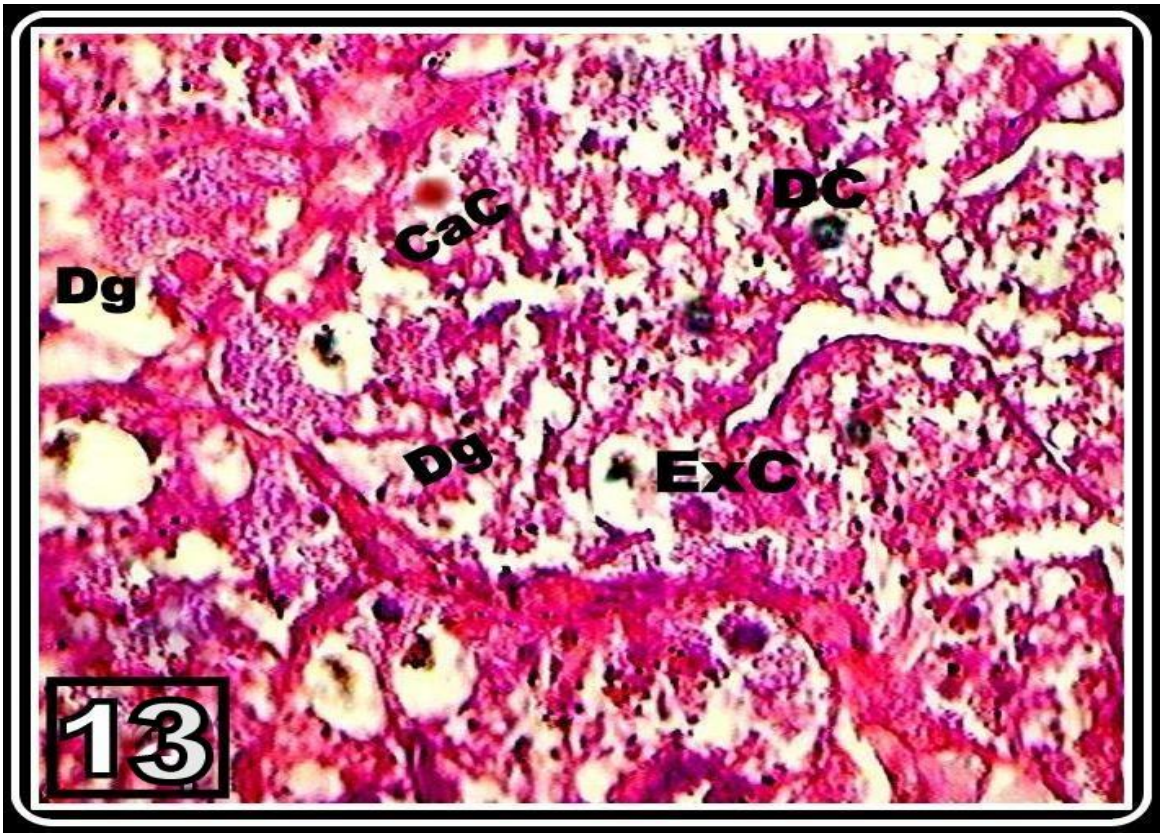
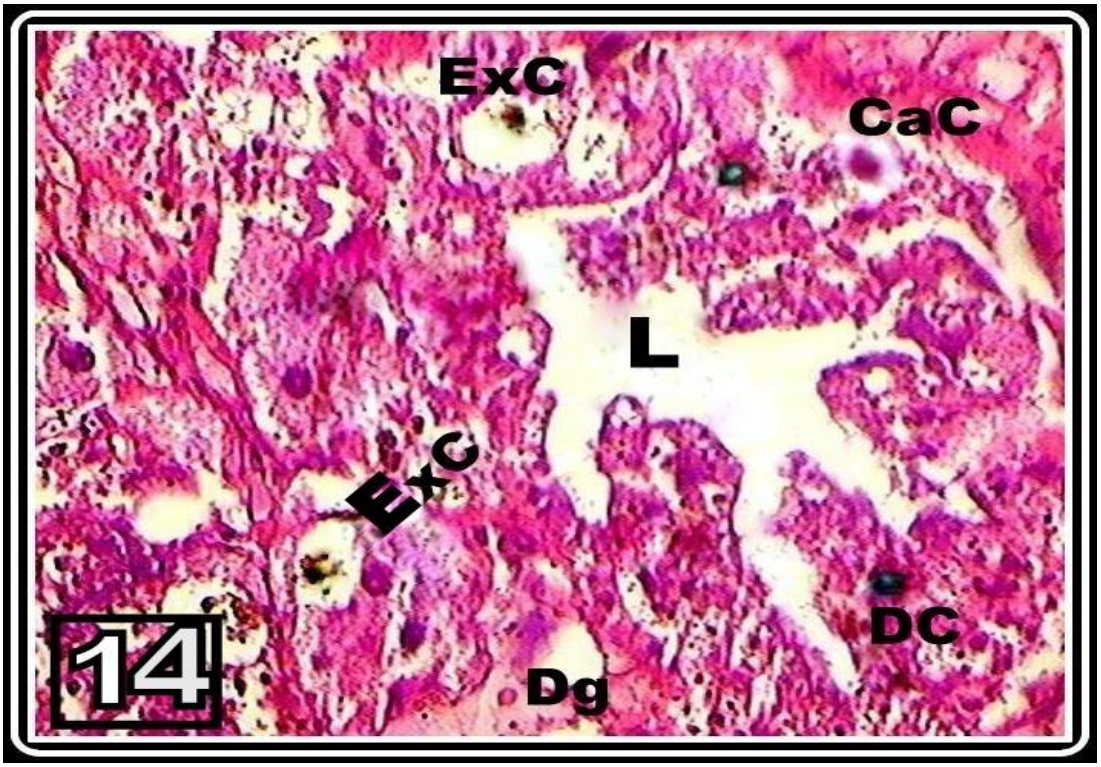

CaC
DC
Dg
Dg
ExC
13

Fig (14) Photomicrograph from a cross section passing through the digestive gland of *M. cartusiana* treated with 1/2 LC_{50} Amino showing branching lumen (L), calcium cells (CaC), digestive cells (DC), excretory cells (ExC) and degenerations (Dg). X100, (Hx & E)

Fig (15) Photomicrograph from a cross section passing through the digestive gland of *M. cartusiana* treated with LC_{50} Amino showing narrowing in the lumen (L), calcium cells (CaC), digestive cells (DC), excretory cells (ExC), degenerations (Dg). X100, (Hx & E)


ExC
CaC
L
ExC
DC
14
Dg

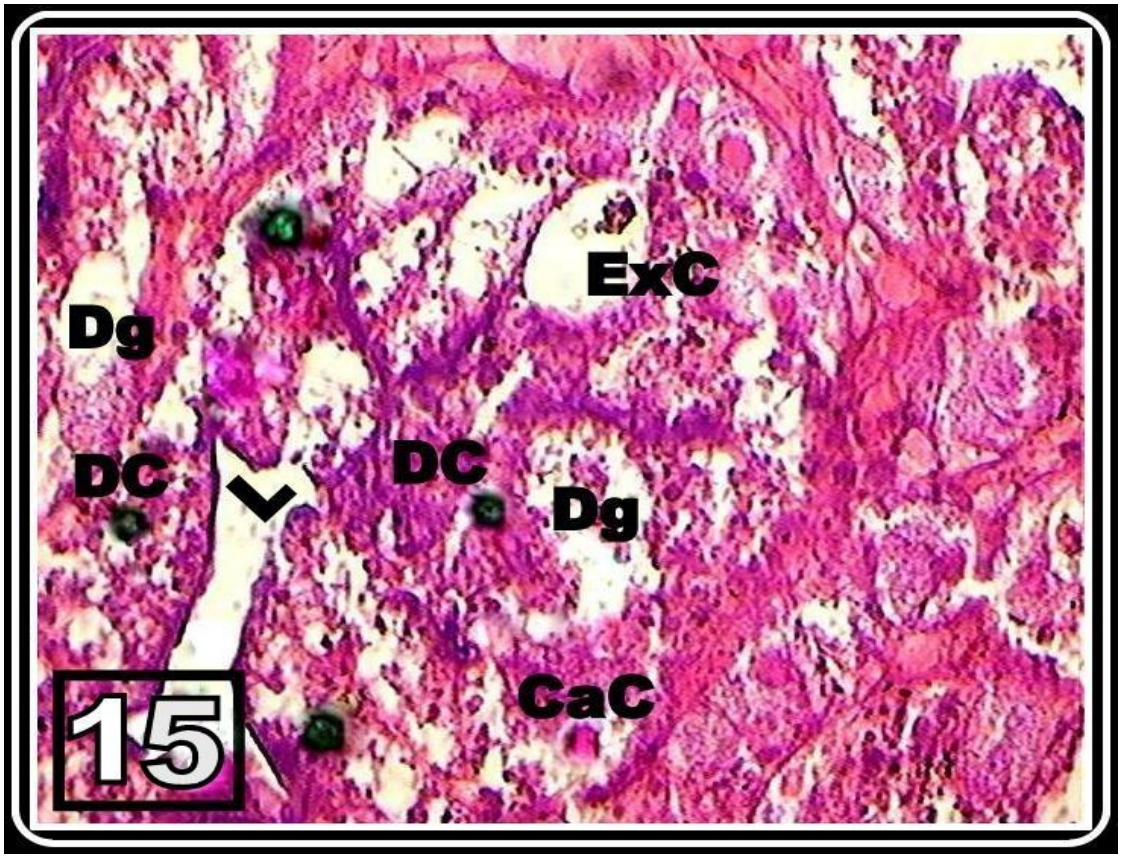
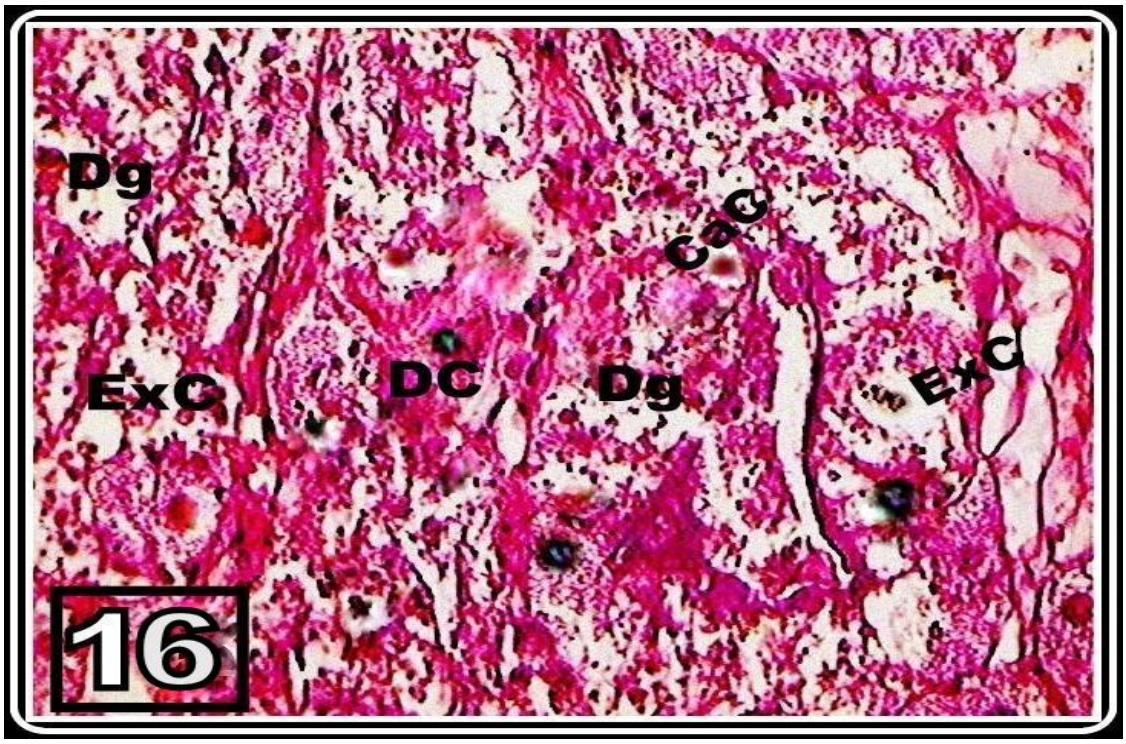

ExC
Dg
DC
DC
Dg
15
CaC

Fig (16) Photomicrograph from a cross section passing through the digestive gland of *M. cartusiana* treated with1/2 LC_{50} Vertimec showing calcium cells (CaC), digestive cells (DC), excretory cells (ExC) and some degenerations (Dg). X100, (Hx & E)

Fig (17) Photomicrograph from a cross section passing through the digestive gland of *M. cartusiana* treated with LC_{50} Vertimec showing calcium cells (CaC), digestive cells (DC), excretory cells (ExC). X100, (Hx & E)


Dg
CaC
ExC
DC
Dg
ExC
16

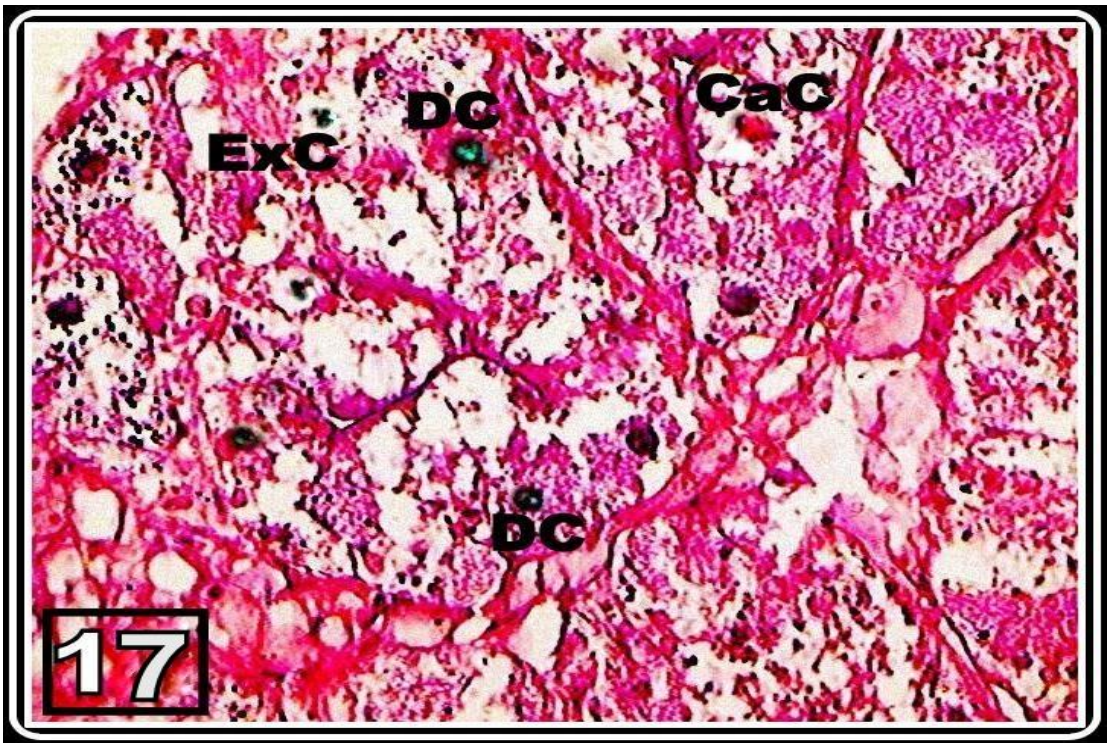
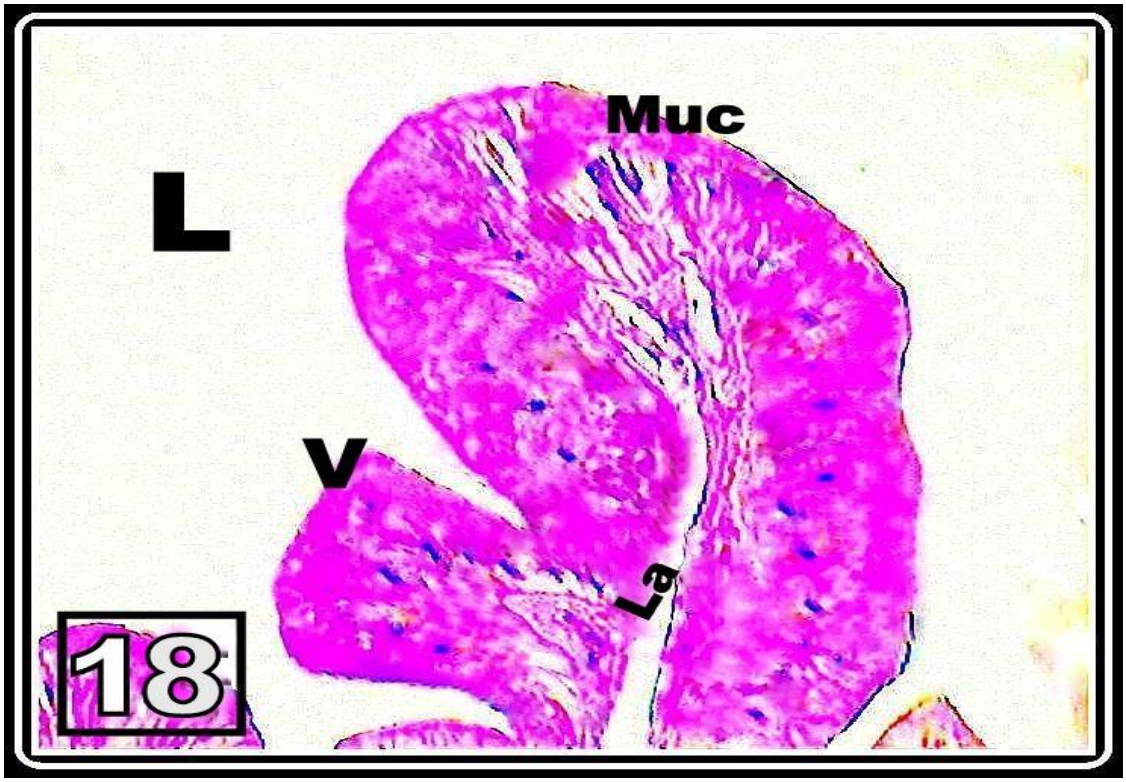

DC
CaC
ExC
DC
17

Fig (18) Photomicrograph from a cross section passing through one microvillus of intestine of control *E. vermiculata* showing lumen (L), mucosa (Muc), villus (V) and lacteal (La). X100, (Hx & E)

Fig (19) Photomicrograph from a cross section passing through the intestine of control *E. vermiculata* showing lumen (L), mucosa (Muc), villus (V). X100, (Hx & E)


Muc
L
V
La
18

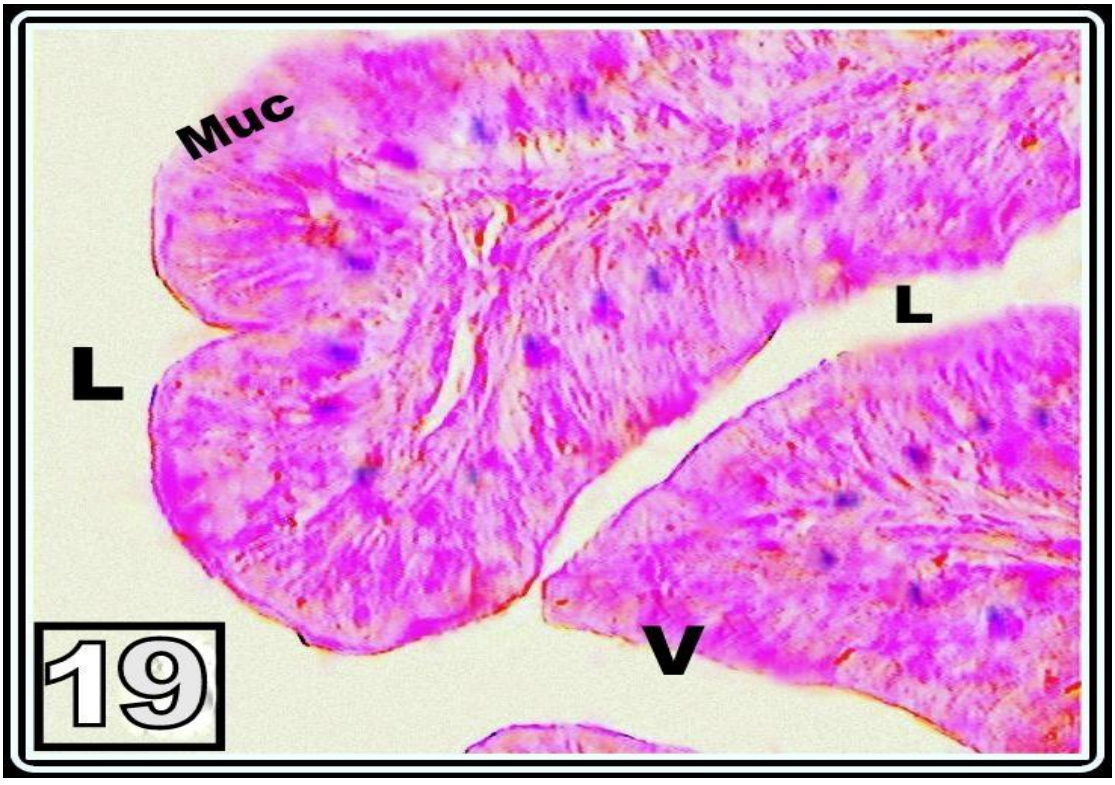
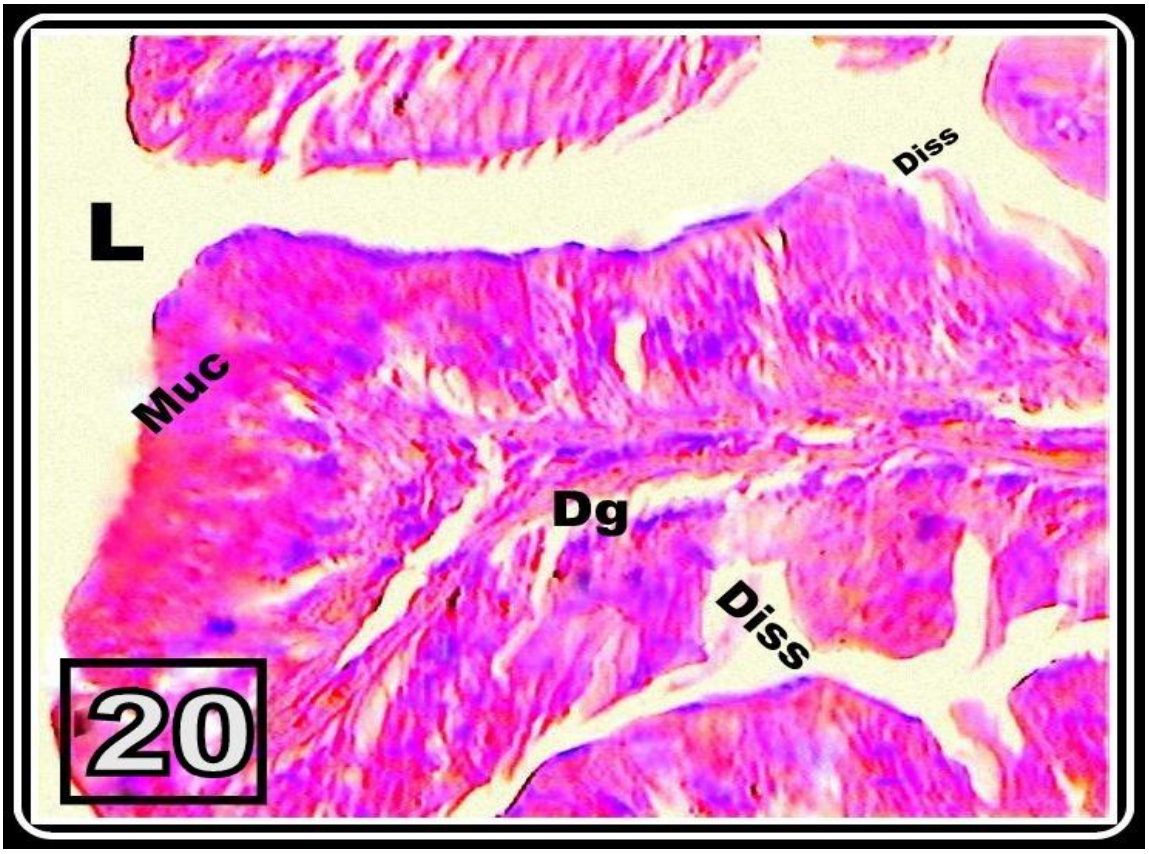

Muc
L
L
V
19

Fig (20) Photomicrograph from a cross section passing through the intestine of *E. vermiculata* treated with Newmyl 1/2LC_{50} showing lumen (L), mucosa (Muc) and degenerations (Dg) start to appear in the middle region together with dissociations (Diss) start marginally. X100, (Hx & E)

Fig (21) Photomicrograph from a cross section passing through the intestine of *E. vermiculata* treated with LC_{50} Newmyl showing lumen (L), submucosal oedema (Oed) degenerations (Dg) and dissociations (Diss)at the free mucosal surface. X100, (Hx & E)


Diss
L
Muc
Dg
Diss
20

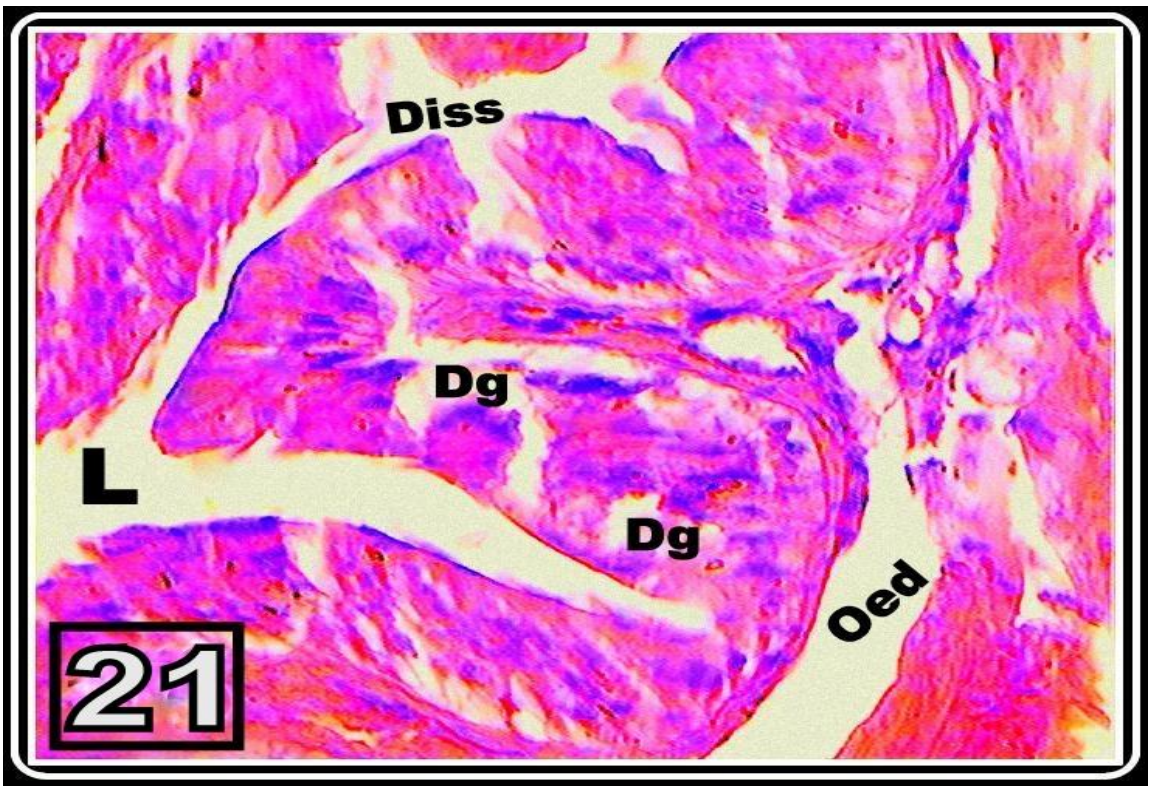
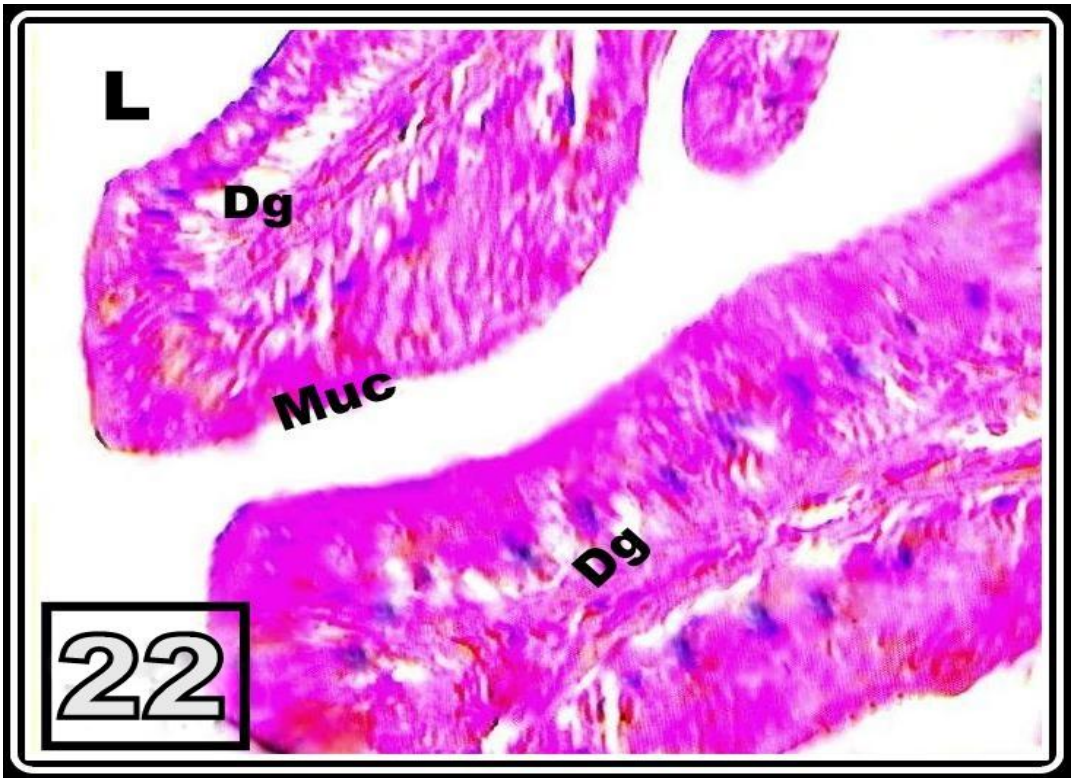

Diss
Dg
L
Dg
Oed
21

Fig (22) Photomicrograph from a cross section passing through the intestine of *E. vermiculata* treated with 1/2 LC_{50} Amino showing lumen (L), mucosa (Muc) and degenerations (Dg) partially started at the bases of columnar cells. X100, (Hx & E)

Fig (23) Photomicrograph from a cross section passing through the intestine of *E. vermiculata* treated with LC_{50} Amino showing lumen (L) and increased degenerations (Dg) increased with peripheral dissociations (Diss). X100, (Hx & E)


L
Dg
Muc
Dg
22

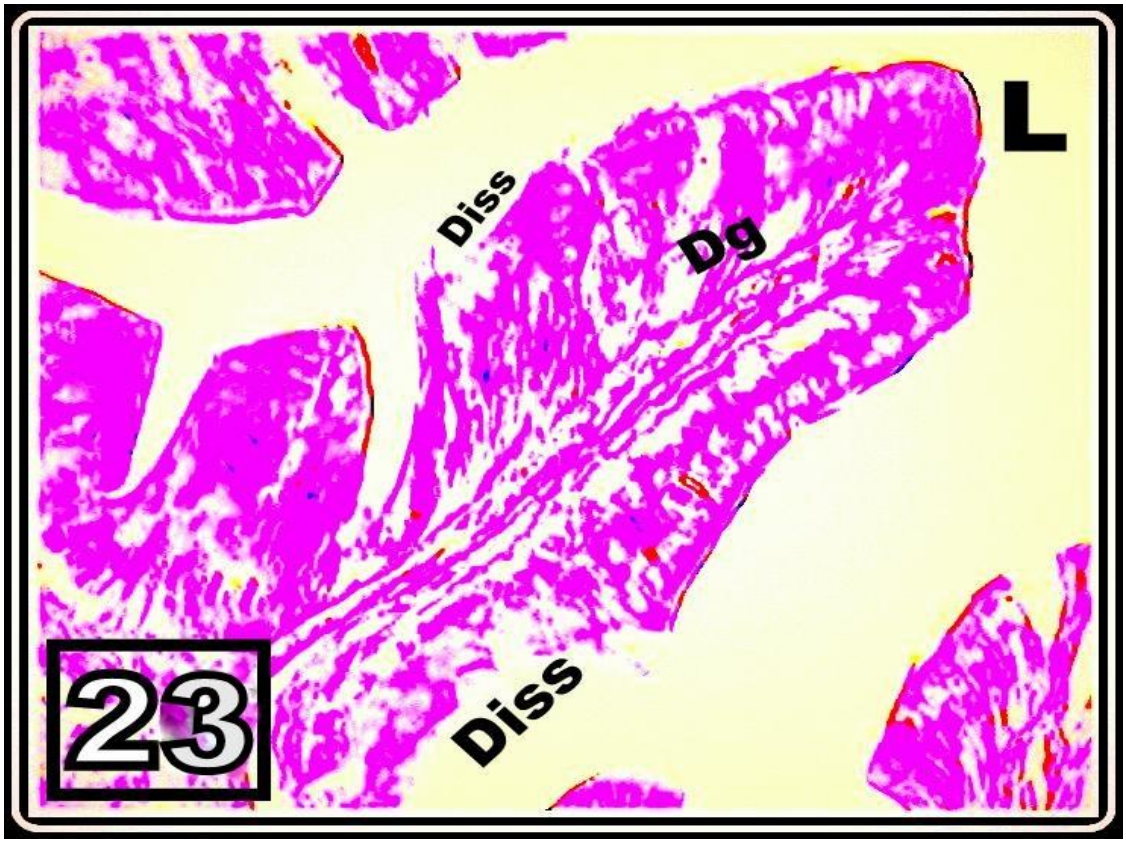
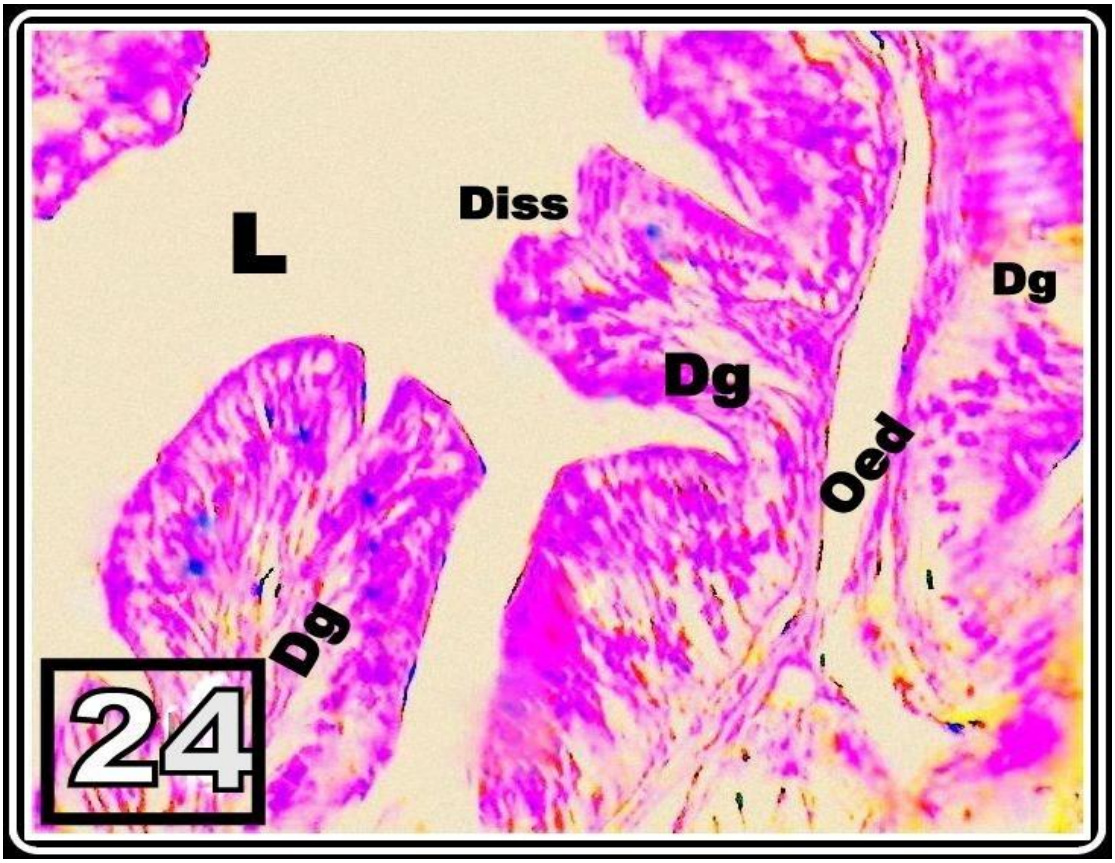

L
Diss
Dg
Diss
23

Fig (24) Photomicrograph from a cross section passing through the intestine of *E. vermiculata* treated with1/2 LC_{50} Vertimec showing lumen (L), degenerations (Dg), dissociations (Diss.) and oedema (Oed). X100, (Hx & E)

Fig (25) Photomicrograph from a cross section passing through the intestine of *E. vermiculata* treated with LC_{50} Vertimec showing lumen (L), degenerations (Dg), free mucosal dissociations (Diss) and oedema (Oed) strongly present. X100, (Hx & E)


L
Diss
Dg
Dg
Oed
Dg
24

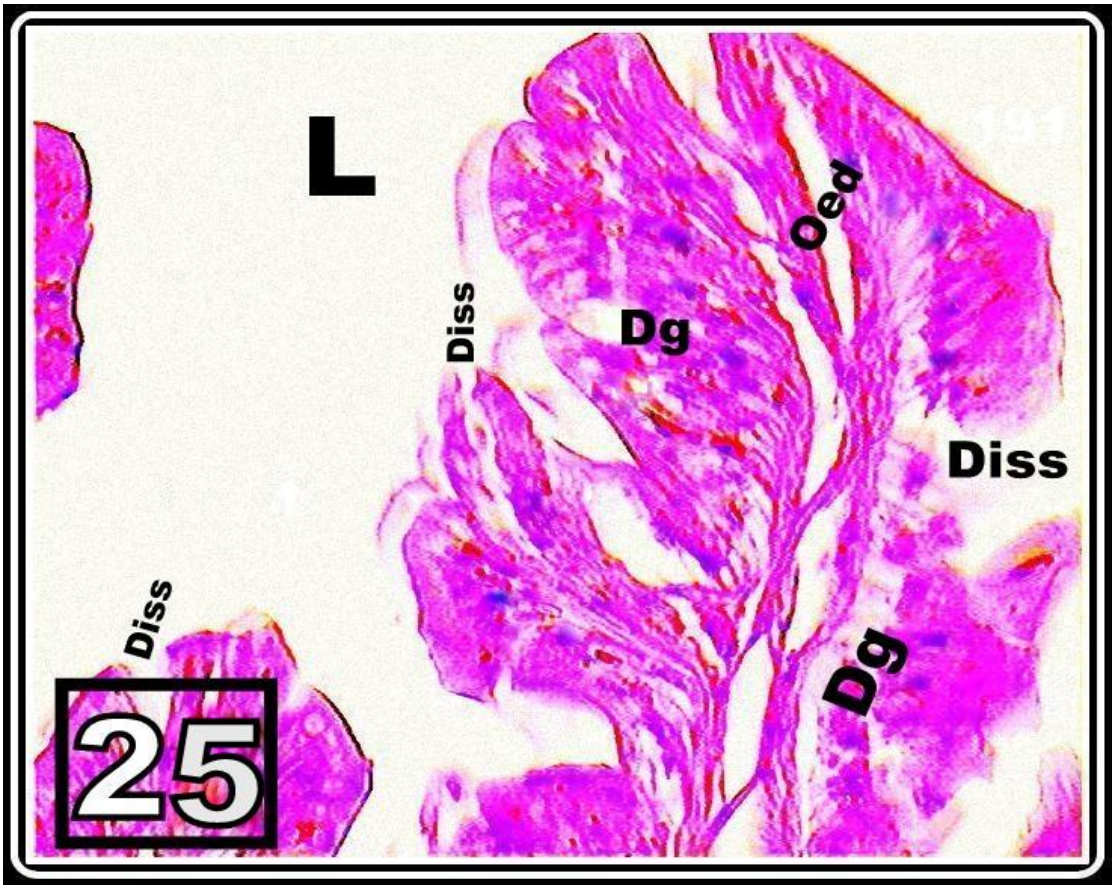
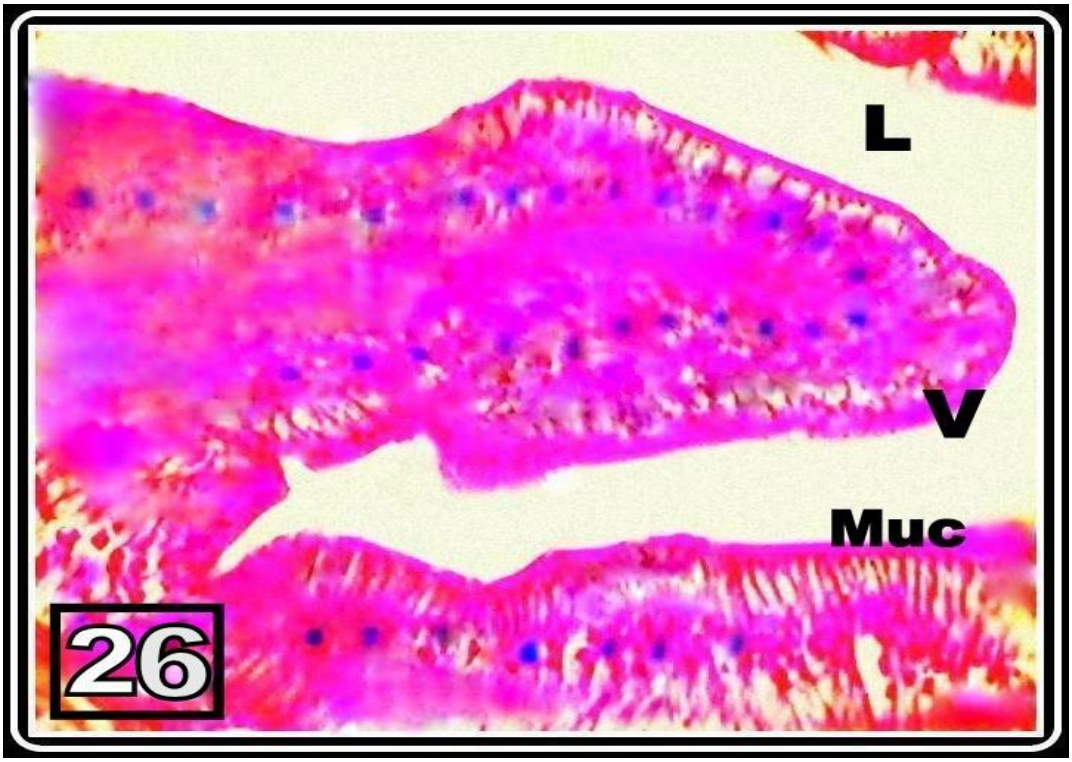

L
Oed
Diss
Dg
Diss
Diss
Dg
25

Fig (26) Photomicrograph from a cross section passing through one villus of intestine of control *M. cartusiana* showing lumen (L), mucosa (Muc), villus (V). X100, (Hx & E)

Fig (27) Photomicrograph from a cross section passing through the intestine of *Monacha cartusiana* treated with1/2 LC_{50} Newmyl showing lumen (L), degenerations (Dg) and dissociations (Diss). X100, (Hx & E)


L
V
Muc
26

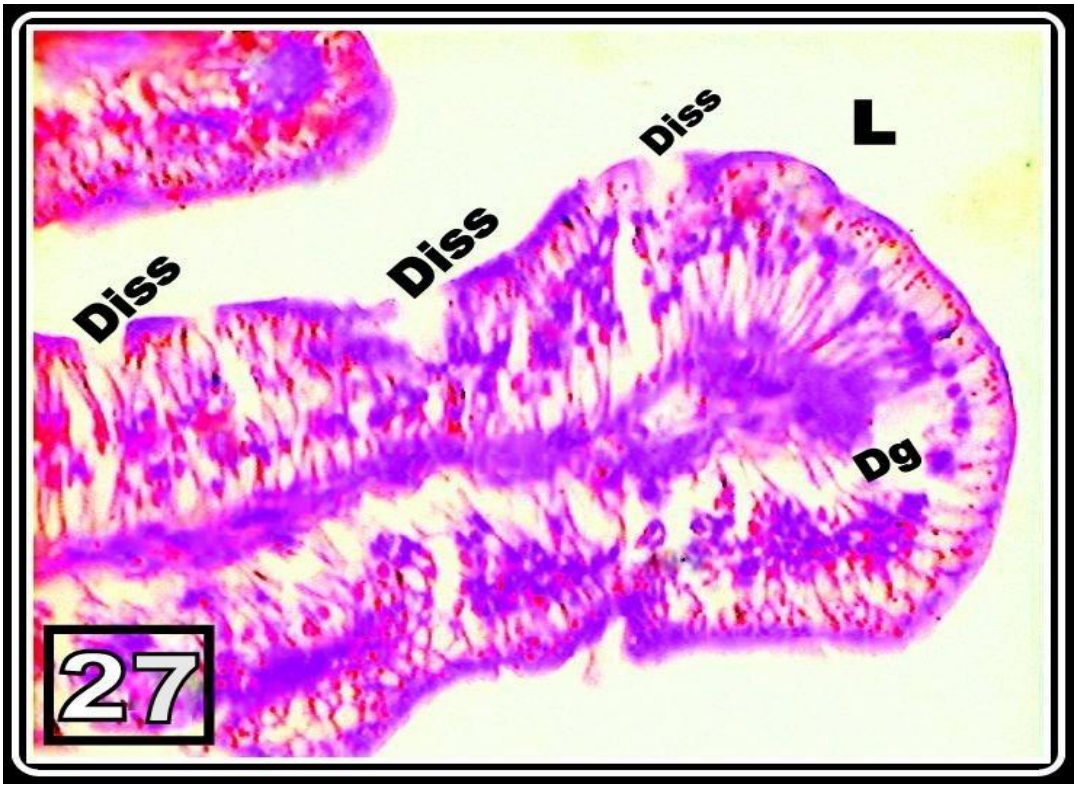
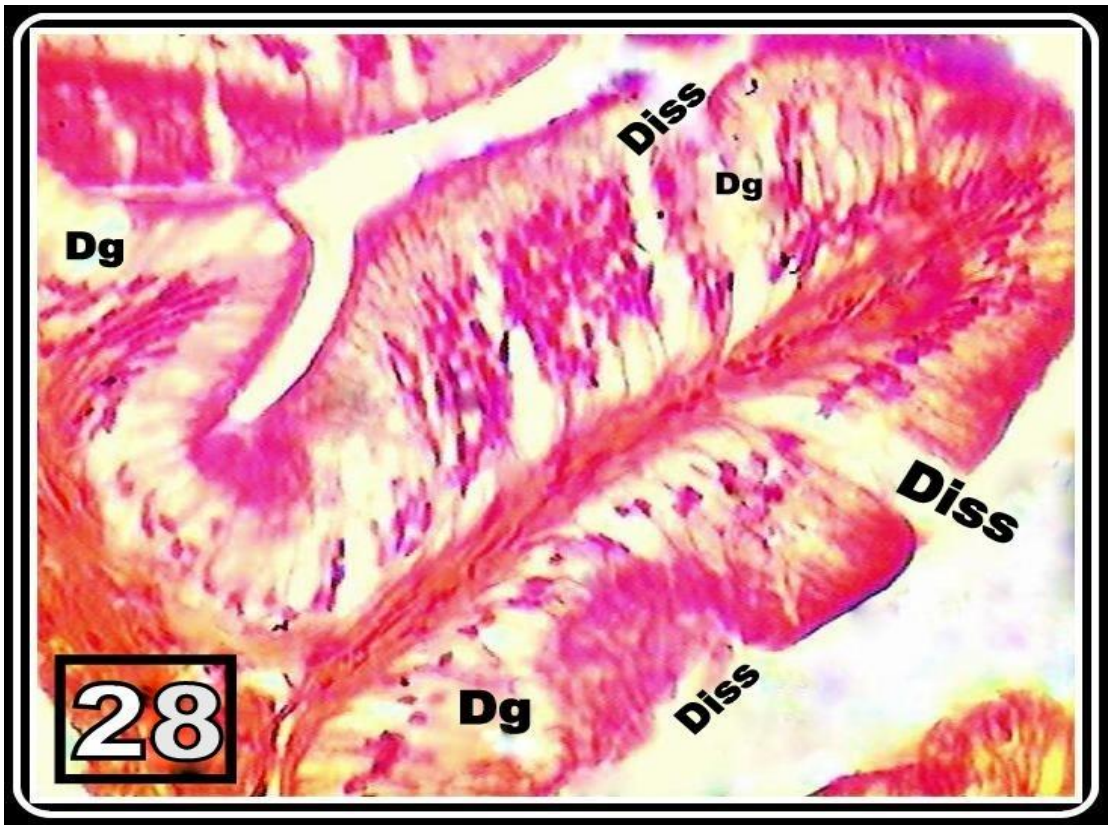

Diss
Diss
Diss
L
Dg
27

Fig (28) Photomicrograph from a cross section passing through a magnified villus of intestine of *M. cartusiana* treated with LC_{50} Newmyl showing nucleus precipitate downward where degenerations (Dg) and dissociations (Diss) increased. X400, (Hx & E)

Fig (29) Photomicrograph from a cross section passing through one villus of intestine of *M. cartusiana* treated with 1/2 LC_{50} Amino showing villus (V), lumen (L), mucosa (Muc), with degenerations (Dg) . X200, (Hx & E)


Diss
Dg
Dg
Diss
28
Dg
Diss

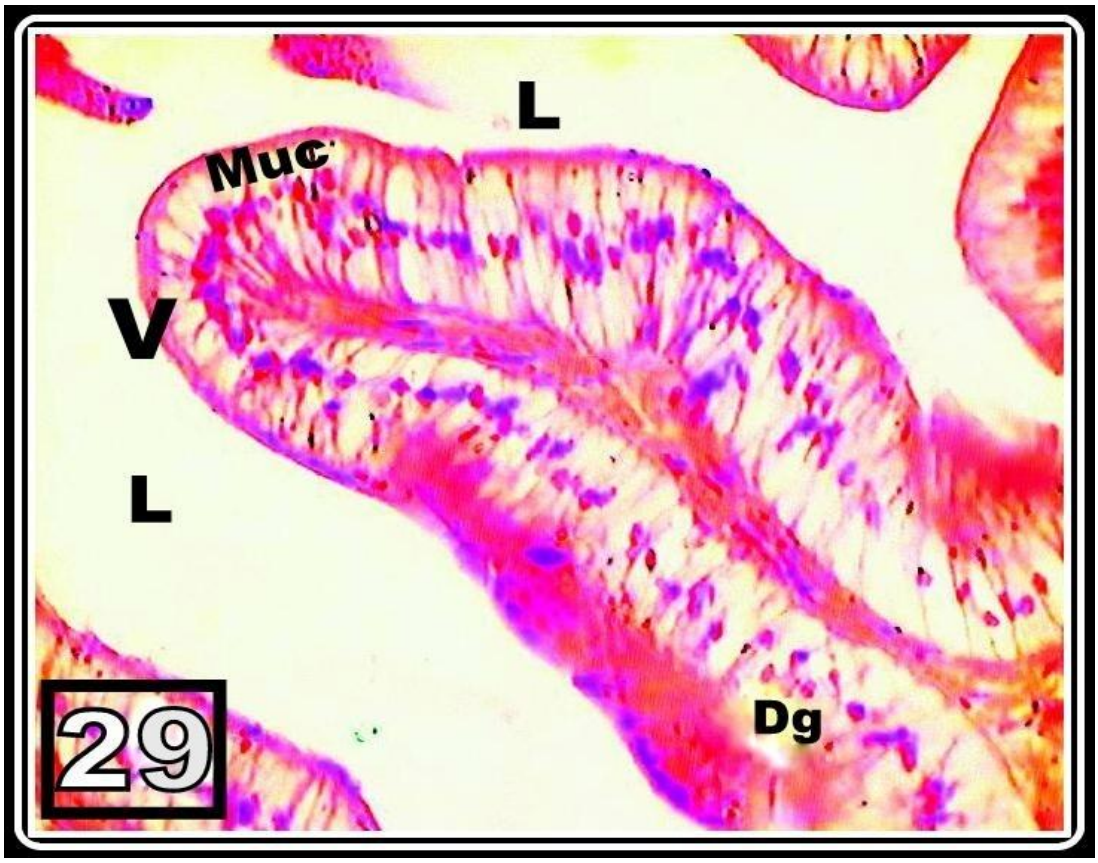
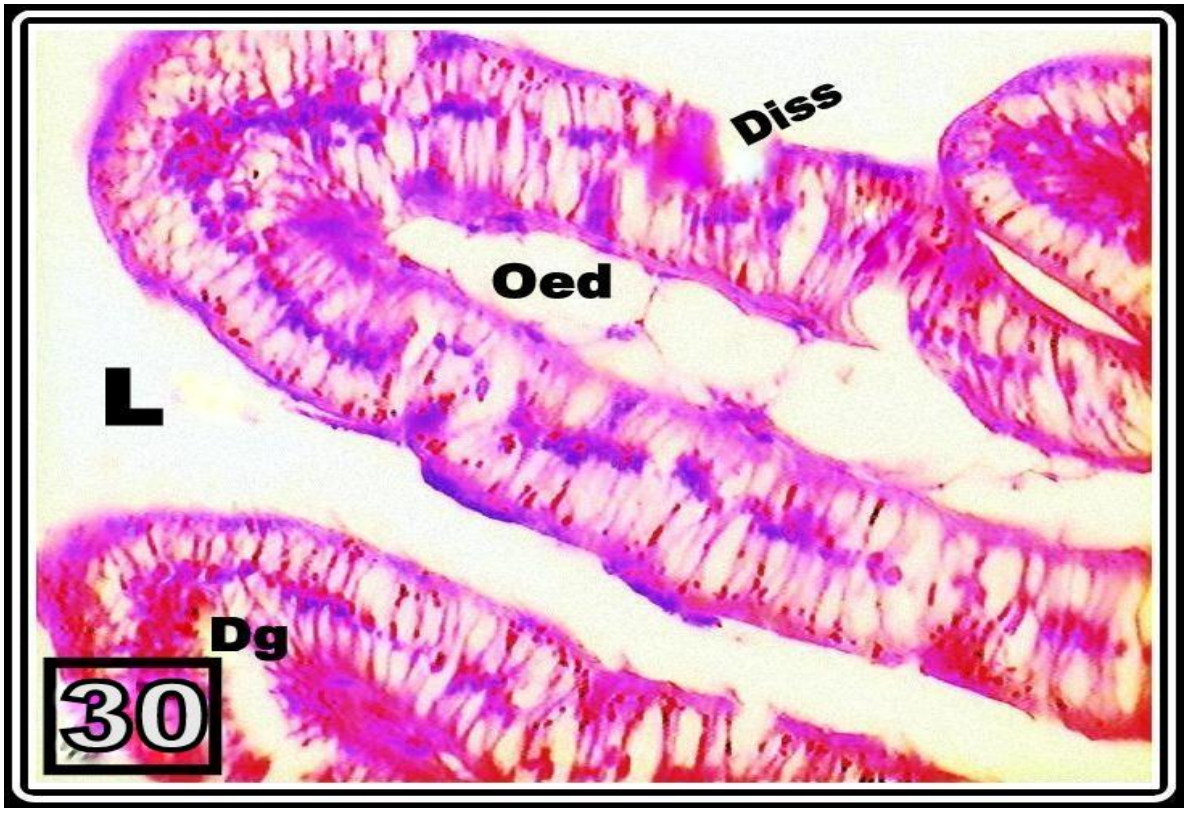

L
Muc
V
L
29
Dg

Fig (30) Photomicrograph from a cross section passing through one villus of intestine of *M. cartusiana* treated with LC_{50} Amino showing lumen (L), degeneration (Dg) dissociation (Diss) and submucosal oedema (Oed). X200 (Hx & E)

Fig (31) Photomicrograph from a cross section passing through villus of intestine of *M. cartusiana* treated with1/2 LC_{50} Vertimec showing lumen (L), mucosa (Muc) degenerations (Dg) and oedema (Oed). X200, (Hx & E)


Diss
Oed
L
Dg
30

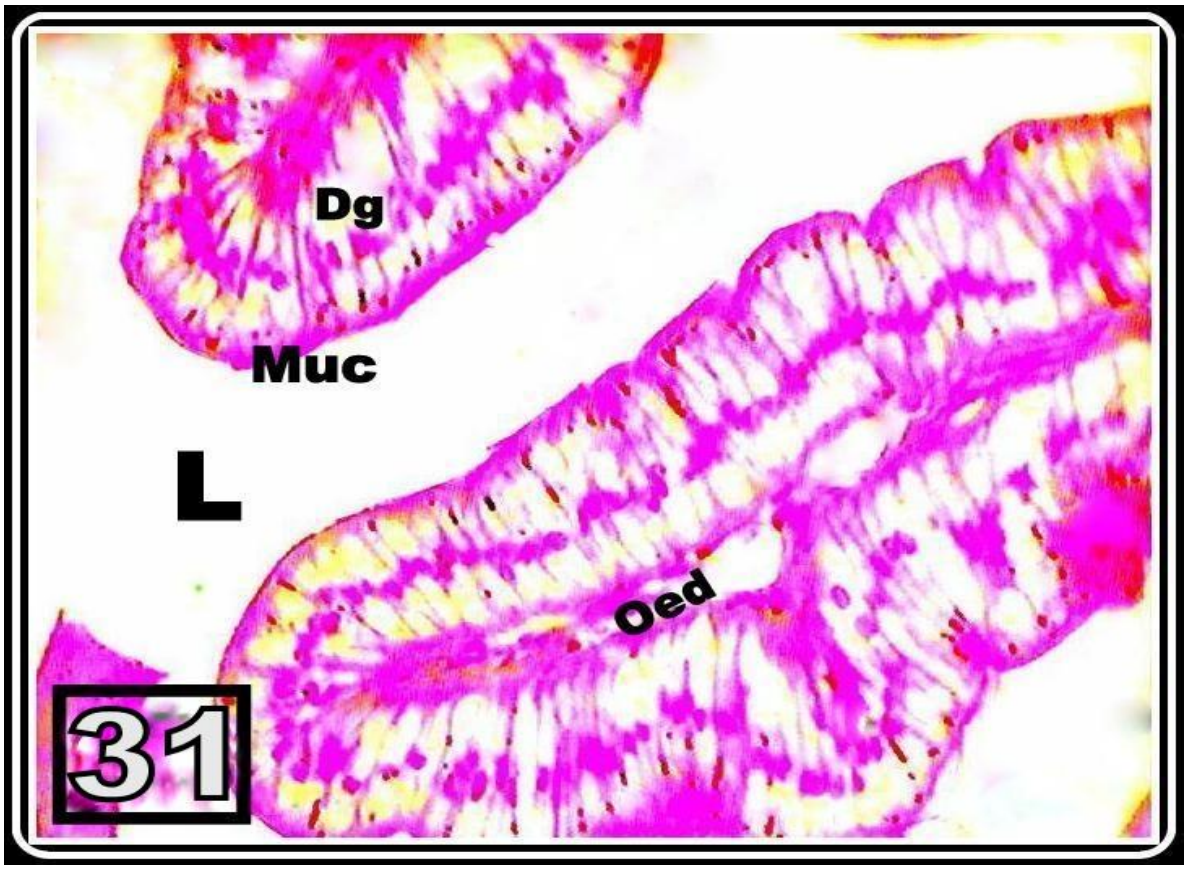
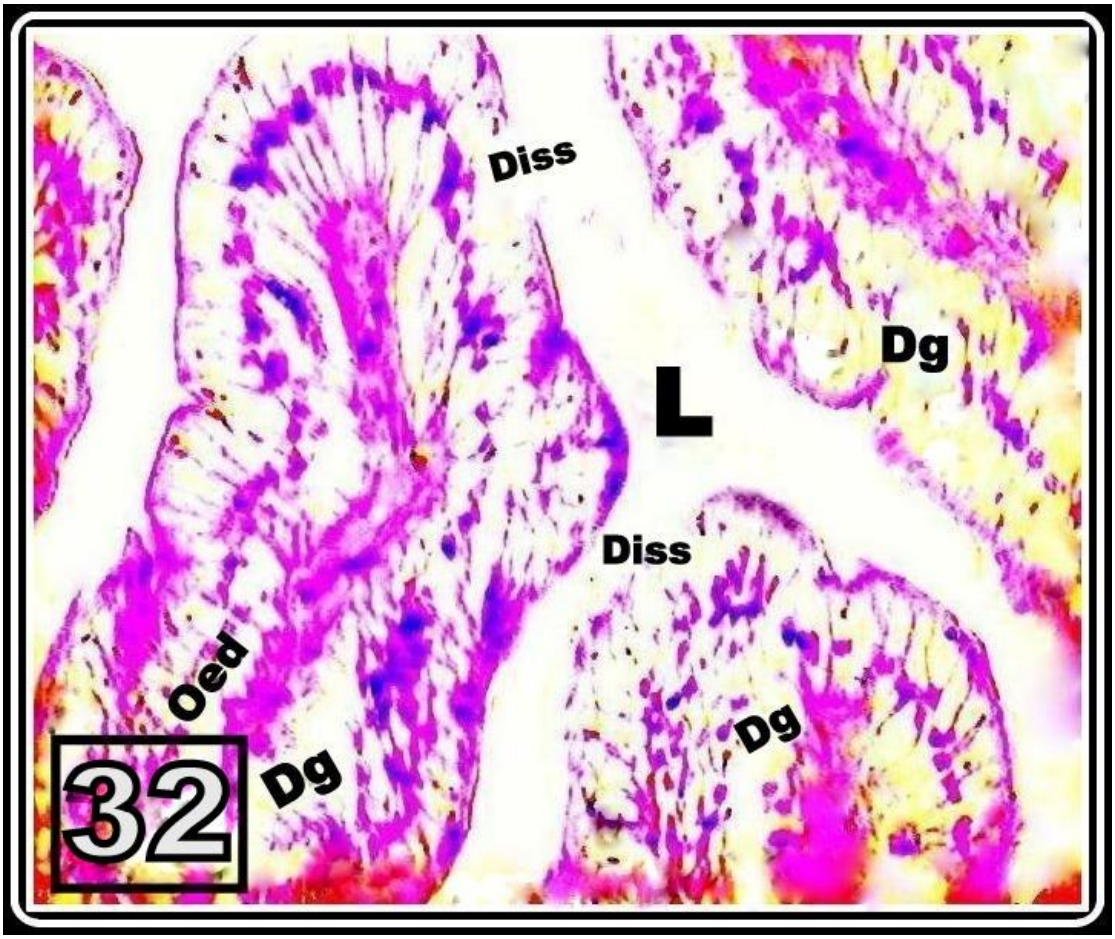

Dg
Muc
L
Oed
31

Fig (32) Photomicrograph from a cross section passing through villus of intestine of *M. cartusiana* treated with Vertimec LC_{50} showing lumen (L), degenerations (Dg), dissociations (Diss) and oedema (Oed). X100, (Hx & E)


Diss
Dg
L
Diss
Oed
Dg
Dg
32

III-Ultrastructural changes

3.1-Digestive gland of *E. vermiculata*:-

3.1.1 Digestive gland of control *E. vermiculata*

Investigation of electron micrograph obtained from the digestive gland of control *E. vermiculata* revealed the presence of 3 cell types:-

1-The digestive cells :-

These are long columnar cells appear in various shapes owing to the plane of sectioning. The cells are surrounded with clearly demarcated cell membranes. The free surface of these cells is ending with a huge number of microvilli. The cytoplasm is filled with a lot of rounded to oval mitochondria with cross cristae. The huge numbers of small mitochondria are scattered throughout the whole cytoplasm. These mitochondria may appear concentrated in the apical part of the cytoplasm (Fig. 33). Golgi apparatus and cisternae of the rough endoplasmic reticulum are hardly detected in the digestive cells. The nuclei of digestive cells are large spherical containing scattered euchromatin and clumps of deeply osmiophilic heterochromatin and large nucleoli (Figs 34, 35 and 36).

2- Excretory cells :-

These cells are fewer in number than the digestive cells. These cells are outlined by a distinct cell membrane and characterized by the presence of a large vacuole nearly occupied the whole cell and surrounded by a thin rim of cytoplasm. This layer of thin cytoplasm is pressed around the nucleus at one side of the cell. The nuclei of excretory cells are oval to elongated and contain moderately osmiophilic nucleoplasm in which clumps of highly osmiophilic heterochromation and conspicuous nucleoli are scattered (Figs. 36 and 37). Excretory cells have numerous excretory vacuoles filled with darkly osmiophilic excretory granules of variable shapes (Figs. 37 and 38).

3- Calcium cells:-

These are large spherical to oval cells of lowest distribution in the mucosa of the digestive gland. They have free cell membrane ending with brush border of microvilli (Fig. 39). The cytoplasm of calcium cells is filled with calcium spherules localized inside membrane bound vacuoles (Figs. 39 and 40). These spherules either completely darkly osmiophilic or interrupted with pale lucent vacuoles of variable size (Fig 39). The spherules are seen in different degrees of growth and numerous elongated mitochondria could be demonstrated in the cytoplasm (Fig. 40).

3.1.2 Digestive gland of treated *E. vermiculata*:

a-Newmyl changes

Effects of 1/2 LC_{50} of Newmyl

Application of 1/2 LC_{50} of Newmyl resulted in perturbations in the cellular organelles. Digestive cells suffered from cytoplasmic degeneration while the nucleoplasm was moderately osmiophilic and little heterochromatin clumps were seen. Mitochondria were pleomorphic and others were hypertrophied showing ballooned cristae. Cisternae of RER and Golgi saccules were undistinguishable (Figs. 41 and 42).

The excretory cells displayed large and numerous excretory vacuoles loaded with abundant deeply osmiophilic scattered or clumped excretory granules. Some cytoplasmic regions were widely suffering from degenerative changes (Fig. 42).

Effects of LC_{50} of Newmyl

This high concentration of Newmyl exhibited drastic cytoplasmic alterations. Digestive cells displayed wide degenerative regions and pleomerphic mitochondria. Less clumps of heterochromatin were detected in the nuclei and the nucleoplasm was more homogeneous. As for the excretory cells, they were obviously occupied with large excretory vacuoles containing variable shapes of granules. These were of different maturation stages and exhibited variable osmiophilia. Nuclei of some of

these cells showed some indentations where others were about to be pyknotic (Figs 43 and 44).

b-Amino changes

Effects of 1/2 LC_{50} of Amino

Treatment of *Eobania vermiculata* with Amino led to deteriorations in cells of the digestive gland. Digestive cells exhibited the appearance of cytoplasmic degenerations. These degenerative regions sometimes seen neighboring to the nucleus (Fig. 45). Mitochondria showed a little hypertrophy with ballooned cristae. The rough endoplasmic reticulum appeared normal while its cisternae are abundant and arranged in parallelism (Figs. 45 and 46). Nuclei of digestive cells revealed some enlargement with conspicuous nucleoli and patches of heterochromatin scattered in the nucleplasm (Fig. 45). The excretory cells displayed the presence of vacuoles filled with low to moderately osmiophilic excretory granules (Fig 46).

Effects of LC_{50} of Amino

Application of this high concentration of Amino resulted in occurrence of higher alterations. Degenerations in the cytoplasm of digestive cells were numerous and larger in size. Mitochondria showed undiscrimination of their cristae. Nuclei displayed hypertrophy and moderately osmiophilic nucleoplasm and some scattered clumps of heterochromatin (Figs. 47 and 48). The excretory cells were highly loaded with excretory vacuoles harboring abundant deeply osmiophilic excretory granules.

c-Vertimec changes:

Effects of 1/2 LC_{50} of Vertimec

Treatment of *Eobania vermiculata* with 1/2 LC_{50} of Vertimec biocide resulted in pathological alterations at the ultrastructural level. Digestive cells showed conspicuous degenerative changes in the cytoplasm.

Mitochondria were coalesced and pleomorphic. The matrix of mitochondria contained elongated darkly osmiophilic strands (Figs 49 and 50). The nuclei showed pale osmiophilic nucleoplasm with less clumped heterochromatin. Nuclei of other neighboring cells were deeply indented with obvious clefts and wavy contour. Such nuclei were characterized by darkly osmiophilic nucleoplasm and strongly osmiphilic heterochromatin clumps in addition to marginated aggregates of heterochromatin (Fig 50).

Excretory cells displayed the presence of large excretory vacuoles contained darkly osmiophilic excretory granules of variable shape and size (Fig. 51). Mitochondria of these cells were crowded coalesced and pleomorphic.

Effects of LC_{50} of Vertimec

Treatment with the high dose of Vertimec revealed the occurrence of advanced pathological deteriorations where the digestive cells showed wide and numerous cytoplasmic degenerative regions. The superficial microvilli were short and disorganized (Fig.53). Mitochondria were coalesced and obviously pleomorphic (Fig. 53). Nuclei were indented and the nucleaplasm was faintly to moderately osmiophilic. Nuclei of many cells acquired abnormal shape and the nucleolus had a bizarre architecture and was coalesced with heterochromatin (Figs 53 and 54).

Excretory cells suffered from degenerations and their mitochondria were pleomorphic and coalesced together (Figs 54 and 55). The excretory vacuoles increased in number and size and harbored strongly to moderately osmiophilic excretory granules of variable size and shape (Fig.55).

3.2. The digestive gland of *M. cartusiana*

3.2.1 Digestive gland of control *M. cartusiana*

Digestive cells

These are the most abundant cell type in the digestive tubules of the gland. Cells are barrel-shaped having a wide flat basal region and a tapering or dom-like apex. These cells have superficial microvilli projecting into the luminal cavity of the gland.

Present in the cytoplasm are abundant cisternae of rough endoplasmic reticulum distributed and scattered near the nucleus (Fig 57). The cytoplasm of the upper part of digestive cells is filled with spherical to oval mitochondria. These mitochondria have cross cristae. The electron micrograph seen on figure 57 showing that the plane of sectioning is passing through the apical parts of many cells which are obviously filled with a huge number of mitochondria (Fig. 57).

Nuclei of digestive cells are localized at the basal part of the cell or in middle part. These nuclei either spherical (Fig. 56) or acquire a tuber-like appearance (Fig.57).The nucleus is marginated with strongly osmiophilic heterochromatin. A few scattered clumps of heterochromatin together with the prominent nucleolus are bathed in the moderately osmiophilic nucleoplasm.

Excretory cells

These are large columnar to pyramidal cells localized mostly at bases of the digestive tubules. The cytoplasm of excretory cells contains many large excretory vacuoles filled with irregular excretory granules of variable shape and size (Figs. 58, 59 and 60). These granules displayed strongly osmiophilic reaction and some showed moderate osmiophilia. Some excretory granules revealed variable degrees of maturation as parts were deeply osmiophilic while other parts were moderately to faintly osmiophilic (Fig 59).

Nuclei of excretory cells were obviously revealing indentation (Fig. 58). While others were deeply clefted (Fig. 60). The nucleoplasm was moderately osmiophilic while clumps of heterochromatin were deeply osmiophilic

3.2.2. Digestive gland of treated *M. cartusiana*

a-Newmyl changes:

Effects of 1/2 LC_{50} of Newmyl

Treatment of *Monacha cartusiana* with 1/2 LC_{50} of Newmyl insecticide produced degenerative cytoplasmic sites free of any osmiophilic reaction. Mitochondria showed slight hypertrophy and were pleomorphic. Nuclei revealed indentation in the nuclear membrane. The nucleoplasm was moderately osmiophilic while the heterochromatin aggregations scattered in the nucleoplasm were deeply osmiophilic together with those marginating the inner surface of the nuclear envelope (Fig. 61).

The excretory cells revealed some cytoplasmic degenerations, while the excretory vacuoles were crowded and occupied with excretory granules. These excretory granules showed variable size, shape as well as degrees of osmiophilia ranging from faint to strong (Fig. 61).

Effects of LC_{50} of Newmyl

This high concentration of the insecticide increased the pathological cellular alterations in the digestive gland of the snail. Digestive cells exhibited numerous and wide degenerative regions (Figs. 62 and 63).

Mitochondria of digestive cells showed obvious pleomorphic architecture. Their interiors appeared homogenously moderately osmiophilic. Their internal cristae were hardly discriminated (Figs 62 and 63). Excretory cells displayed degenerations and their mitochondria were pleomorphic. Excretory vacuoles were occupied with excretory granules with variable size and shape (Fig. 62).

b-Amino changes

Effects of 1/2 LC_{50} of Amino

Following treatment with 1/2 LC_{50} of Amino changes in the digestive cells were represented by clear cytoplasmic degenerations. Mitochondria exhibited pleomorphism and faint osmiophilia. Rough endoplasmic reticulum showed dissociation. The nucleus was elongated and appeared tuber-shaped and with faint to moderate osmiophilia (Figs 64 and 65). Excretory cells showed degenerative sites and large excretory vacuoles harboring excretory granules of variable size and osmiophilic. Other excretory vacuoles representing advanced degrees of maturation and appeared nearly empty of granules (Fig. 65). Some excretory vacuoles still harboring strongly osmiphilic granules (Figs 65 and 66).

Effects of LC_{50} of Amino

Treatment with the high concentration of Amino produced higher cytological perturbations. Digestive cells displayed wide cytoplasmic degenerations. Mitochondria were pleomorphic and of homogeneous moderate osmiophilia without clear cristae (Figs 63 and 64). The superficial microvilli were disarranged. The excretory cells revealed wide degenerations and many of the excretory vacuoles appeared empty and faintly osmiophilic (Fig. 66). Other excretory vacuoles were seen harboring remnants of excretory granules. These exhibited a moderate osmiophilia (Fig. 67).

c-Vertimec changes

Effects of 1/2 LC_{50} of Vertimec

Vertimec biocide treatment resulted in conspicuous cytological changes. In the digestive cells most of the cytosol was degenerated and appeared perforated and the cell as a whole looked-like a sieve (Fig. 68). Mitochondria appeared tappering and were pleomorphic and moderately

osmiophilic. The nuclear membrane was thinned out and showed scarcity of heterochromatin on its inner surface (Fig. 68).

Effects of LC_{50} of Vertimec

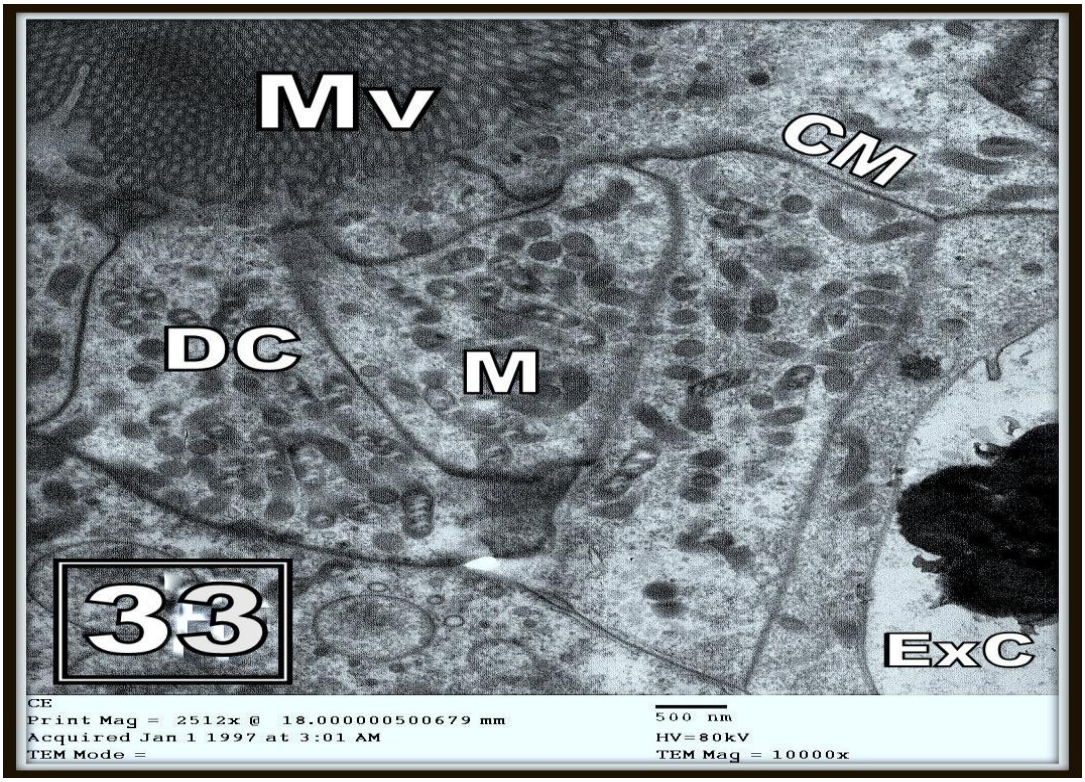
Increasing the concentration of the Vertimec produced conspicuous cytological perturbations. Digestive cells displayed highly vacuolated cytoplasm due to advanced degrees of degenerations. The cytoplasm appeared nearly empty except the presence of a few pleomorphic mitochondria (Figs. 69 and 70). The nucleoplasm of digestive cells nuclei reflected faint osmiophilia. The marginal heterochromatin lining the nuclear membrane was greatly reduced (Fig. 70).

The excretory cells showed cytoplasmic degenerations and large excretory vacuoles depleted of their granules. Other excretory vacuoles harbored large compact strongly osmiophilic granules interrupted by variable-size regions of negative osmiophilia (Fig. 69).

Figs. (33-57) TEM micrographs showing the ultrastructures of the digestive gland of the control and treated *E. vermiculata*

Fig.(33) Cross section in the digestive gland of control *E. vermiculata* showing microvilli (MV), digestive cell (DC), mitochondria (M) cell membrane (CM) and excretory cell (ExC). X10000

Fig. (34) Cross section in the digestive gland of control *E. vermiculata* showing mitochondria (M) , excretory cell (ExC), excretory granule (ExG), excretory vacuole (ExV), nucleus(N), nucleolus (nu) and cell membrane(CM).X 6000


Mv
CM
DC
M
33
ExC
CE
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HV=80kV
TEM Mag = 10000x

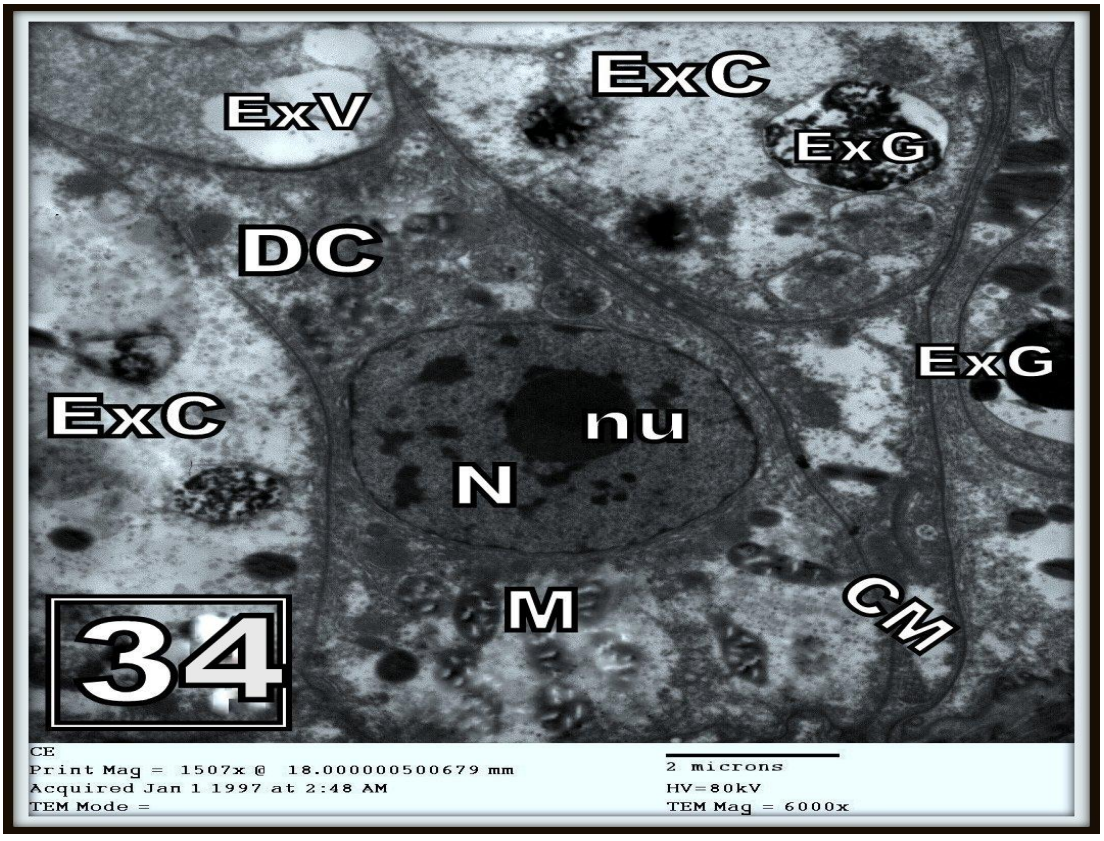
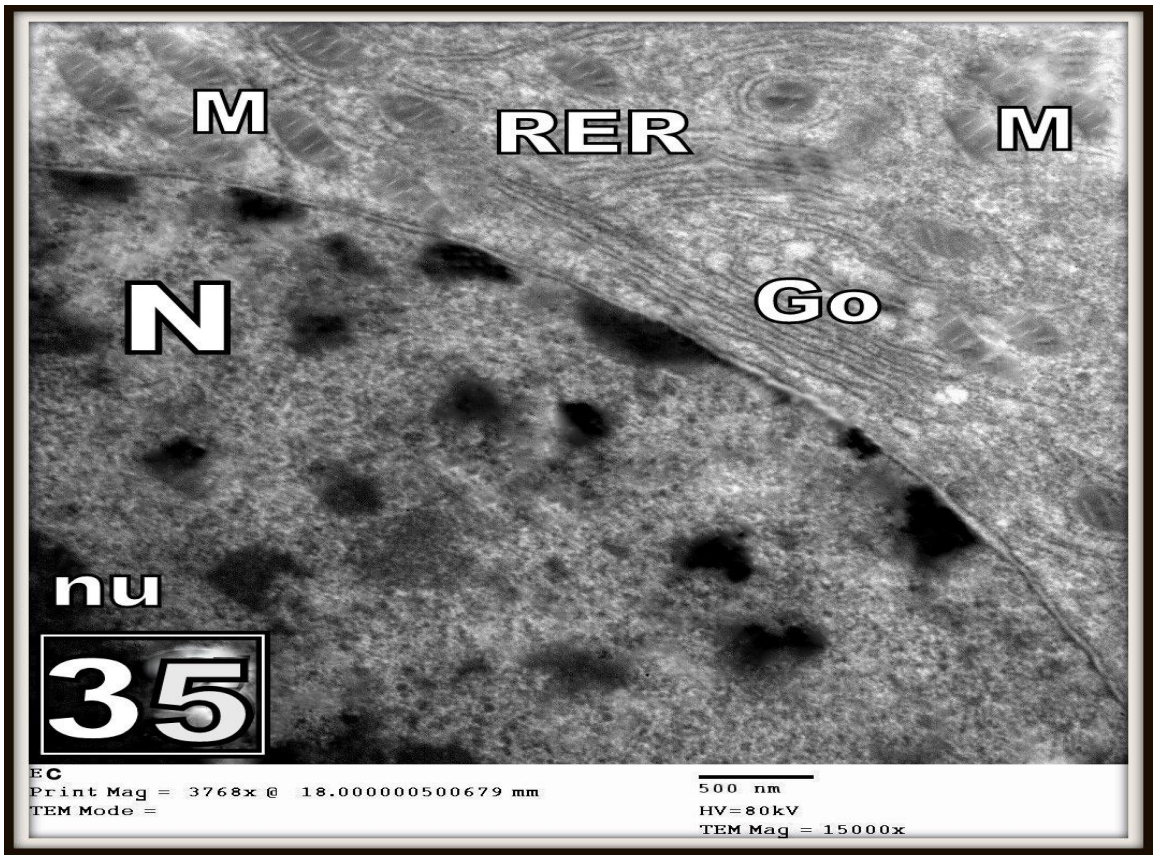

ExC
ExV
ExG
DC
ExG
ExC
nu
N
M
CM
34
CE
Print Mag = 1507x @ 18.000000500679 mm
Acquired Jan 1 1997 at 2:48 AM
TEM Mode =
2 microns
HV=80kV
TEM Mag = 6000x

Fig. (35) Highly magnified cross section in one digestive cell of the digestive gland in control *E. vermiculata* showing nucleus(N) , nucleolus (nu), rough endoplasmic reticulum (RER), Golgi apparatus (Go), and (M) mitochondria. X15000

Fig. (36) Cross section showing the excretory cell of the digestive gland in control *E. vermiculata* show nucleus (N) , nucleolus (nu), excretory cell (ExC), excretory granules (ExG), rough endoplasmic reticulum (RER), Golgi apparatus (Go), and (M) mitochondria, lysosomes (Ly). X10000


M
RER
M
N
Go
nu
35
EC
Print Mag = 3768x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 15000x

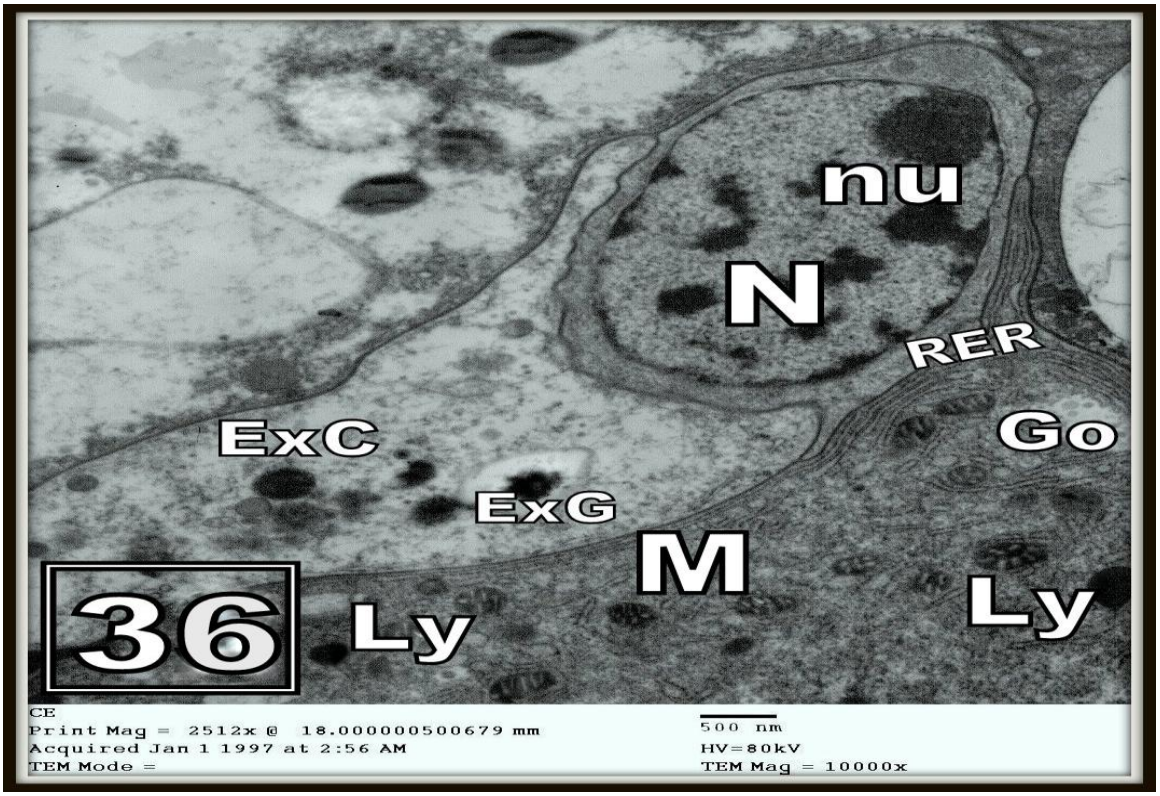
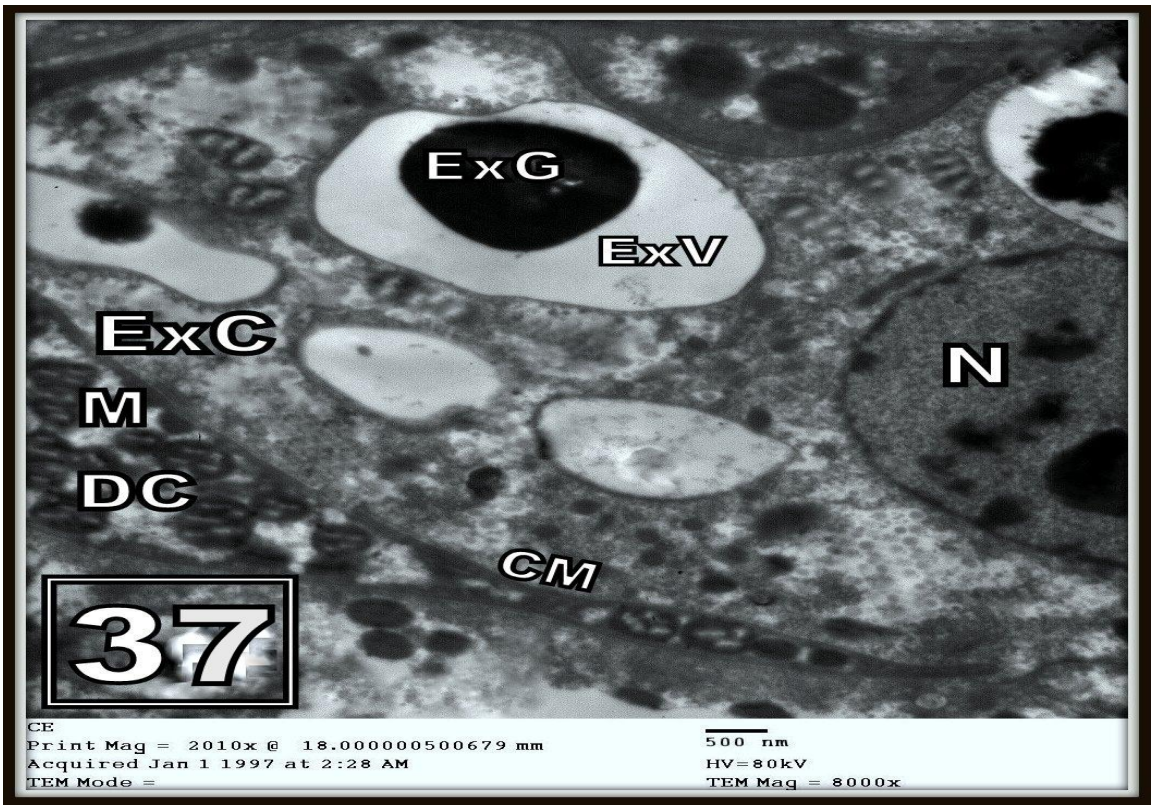

nu
N
RER
ExC
Go
ExG
M
36
Ly
Ly
CE
Print Mag = 2512x @ 18.000000500679 mm
Acquired Jan 1 1997 at 2:56 AM
TEM Mode =
500 nm
HV=80kV
TEM Mag = 10000x

Fig. (37) Magnified cross section through an excretory cell of digestive gland in control *E. vermiculata* showing excretory cell (ExC), excretory granules (ExG), excretory vacuole (ExV), nucleus (N), digestive cell (DC) and cell membrane(CM). X8000

Fig. (38) Cross section through a digestive cell and excretory cell of the digestive gland in control *Eobania vermiculata* showing excretory cell (ExC), excretory granule (ExG), mitochondria (M), digestive cell (DC), cell membrane (CM) and microvilli (MV). X8000


ExG
ExV
ExC
N
M
DC
CM
37
CE
Print Mag = 2010x @ 18.000000500679 mm
Acquired Jan 1 1997 at 2:28 AM
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

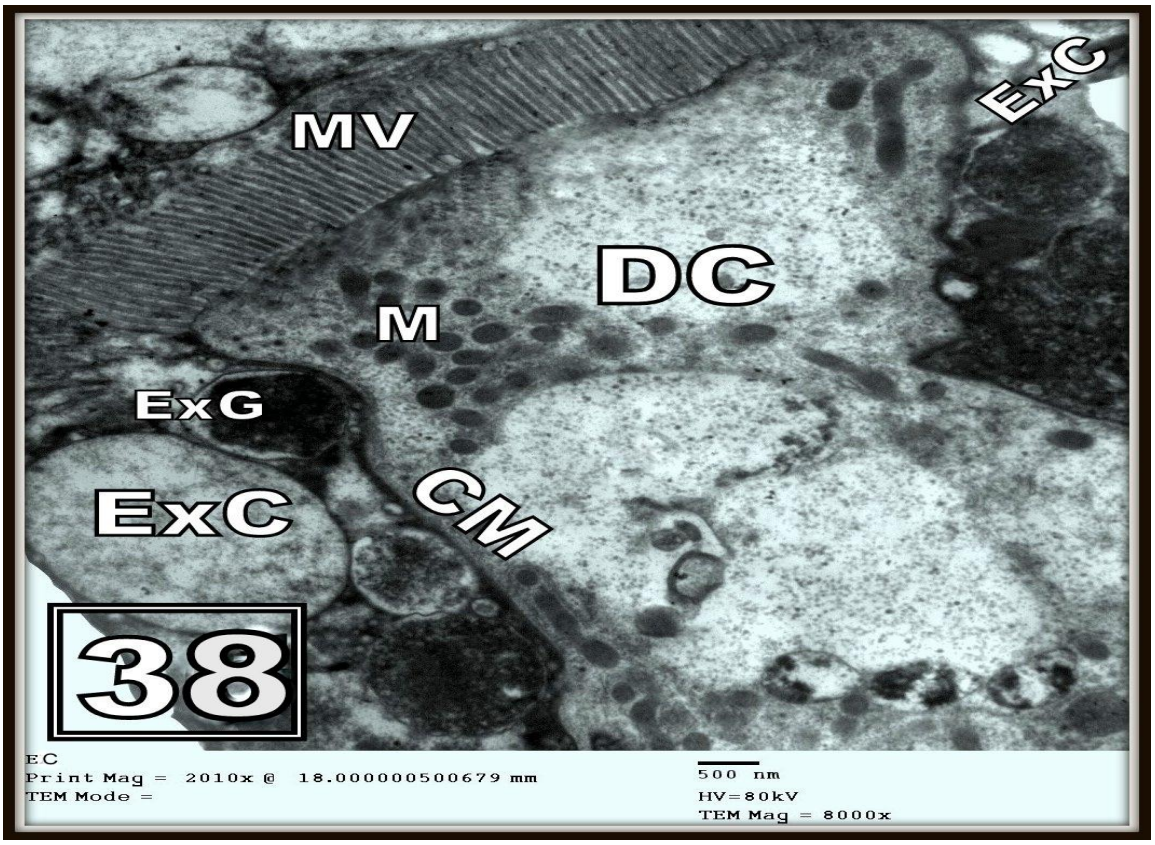
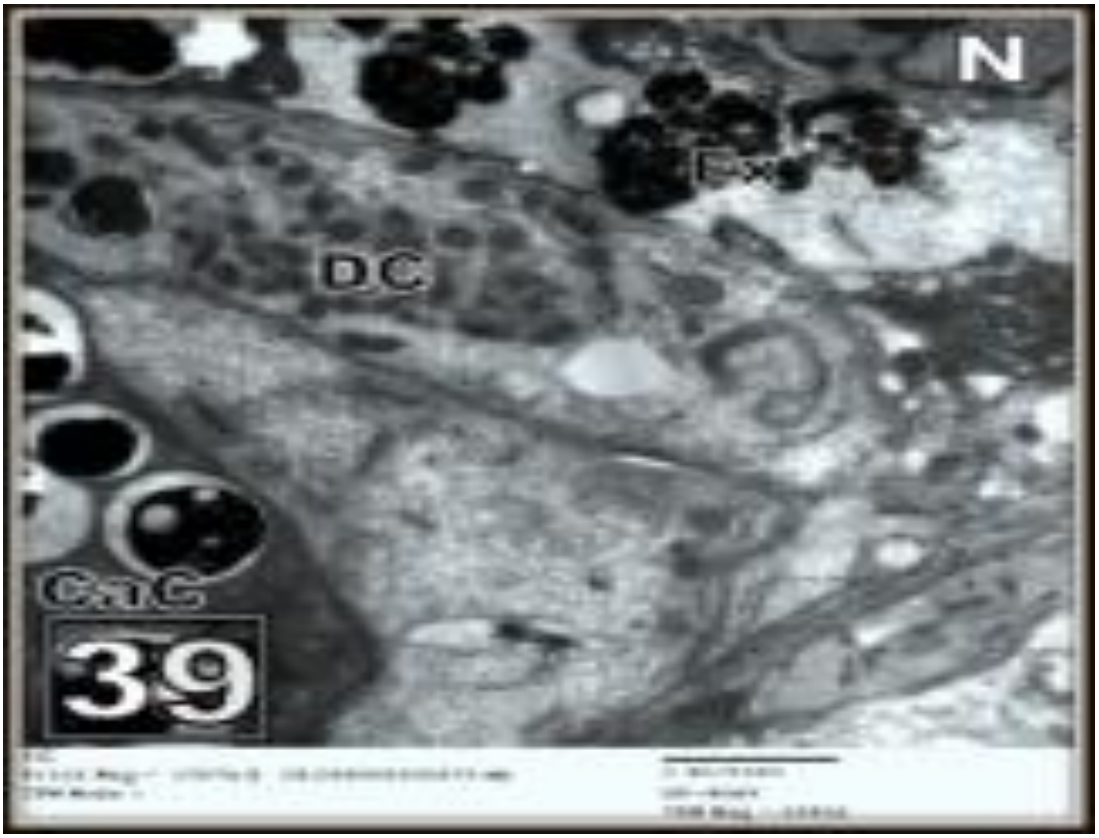

MV
ExC
DC
M
ExG
ExC
CM
38
EC
Print Mag = 2010x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

Fig. (39) Cross section through the digestive gland of control *E. vermiculata* showing excretory cell (ExC), digestive cell (DC), calcium cell (Ca C) and nucleus (N). X6000

Fig. (40) Cross section through a calcium cell of the digestive gland in control *E. vermiculata* showing digestive cell (DC), cell membrane (CM), calcium cell (Ca C), and microvelli (MV). X10000


N
Ex
DC
CaC
39

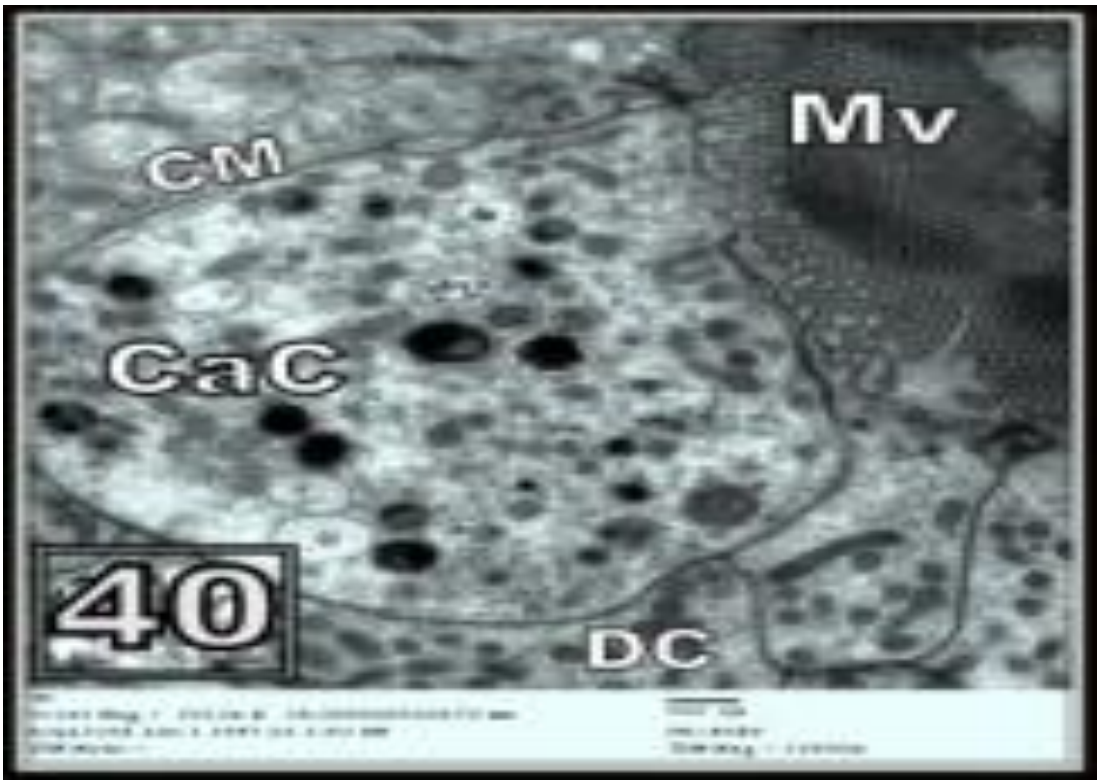
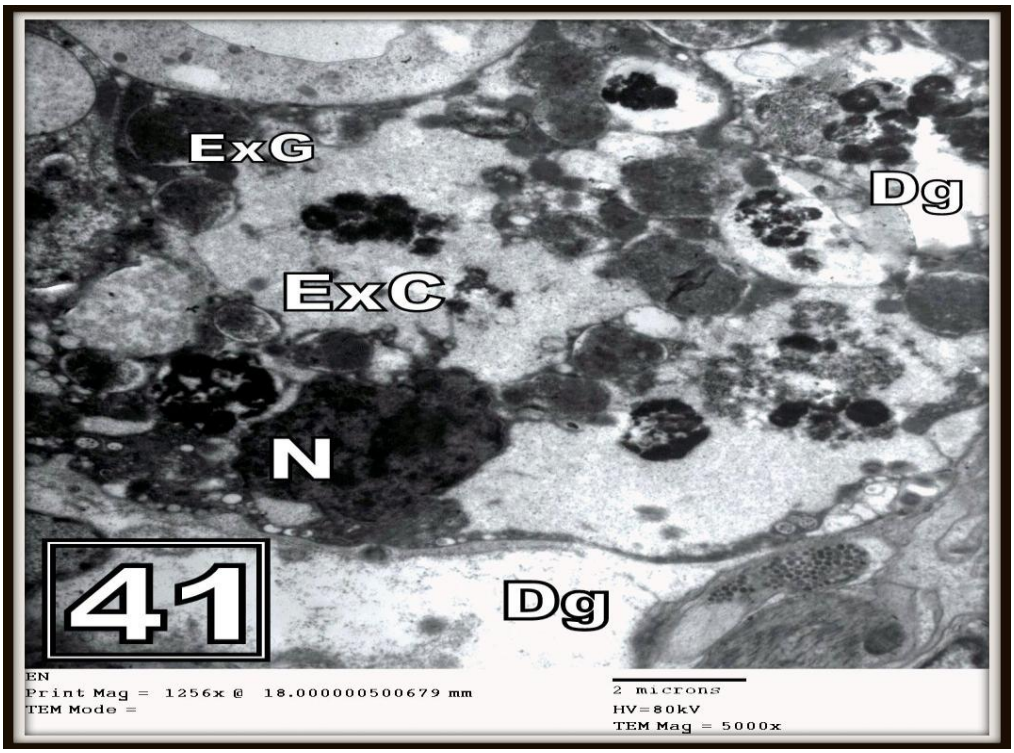

Mv
CM
CaC
40
DC

Fig. (41) Cross section through the digestive gland of *E. vermiculata* treated with 1/2 LC_{50} Newmyl showing excretory cell (ExC), excretory granule (ExG), nucleus (N) and degeneration (Dg). X5000

Fig. (42) Cross section through the digestive gland of *E. vermiculata* treated with Newmyl 1/2 LC_{50} showing excretory cell (Ex C), excretory granule (ExG), nucleus (N), nucleolus (nu), degenerations (Dg), excretory vacuole(EXV) and numerous mitochondria (M). X8000


ExG
Dg
ExC
N
41
Dg
EN
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TEM Mode =
2 microns
HV=80kV
TEM Mag = 5000x

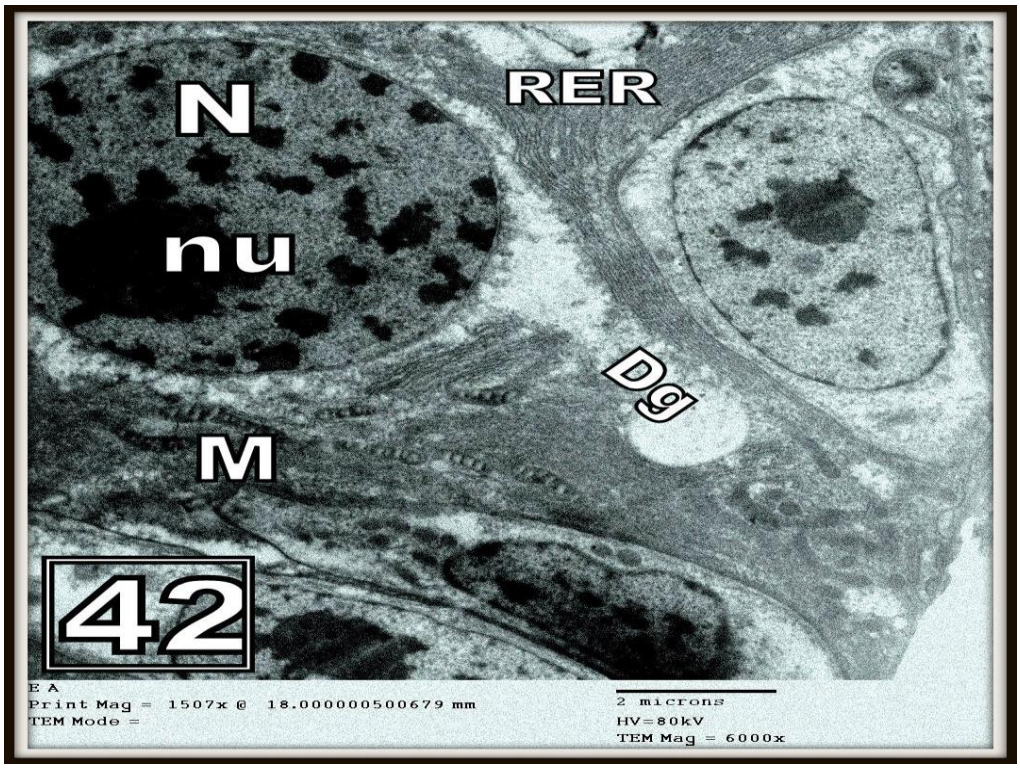
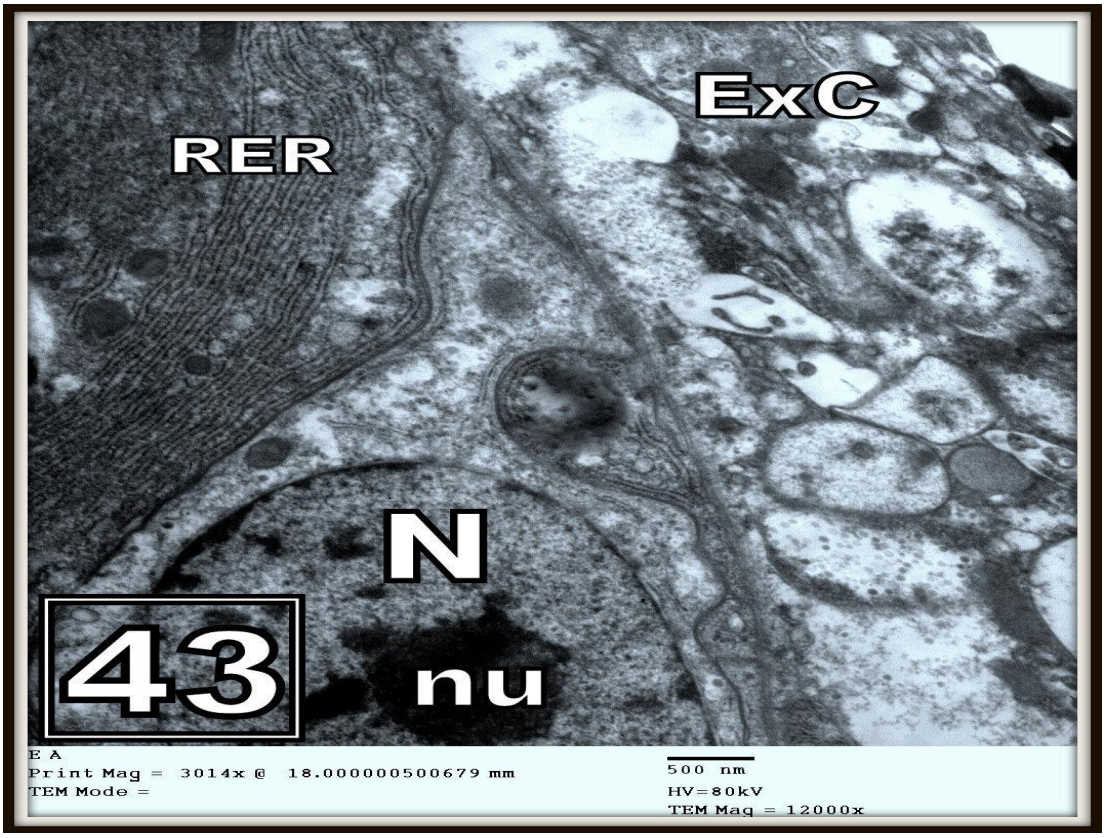

N
RER
nu
Dg
M
42
E A
Print Mag = 1507x @ 18.000000500679 mm
TEM Mode =
2 microns
HV=80kV
TEM Mag = 6000x

Fig. (43) Cross section through the digestive gland of *E. vermiculata* treated with LC_{50} Newmyl showing excretory cell (ExC), excretory granule (ExG), nucleus (N) and very large excretory vacuole (EXV). X5000

Fig. (44) Cross section through the digestive gland of *E. vermiculata* treated with LC_{50} Newmyl showing excretory cell (ExC), excretory granule (ExG), nucleus (N) and degeneration (Dg) start to appear. X5000


ExC
RER
N
43
nu
E A
Print Mag = 3014x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 12000x

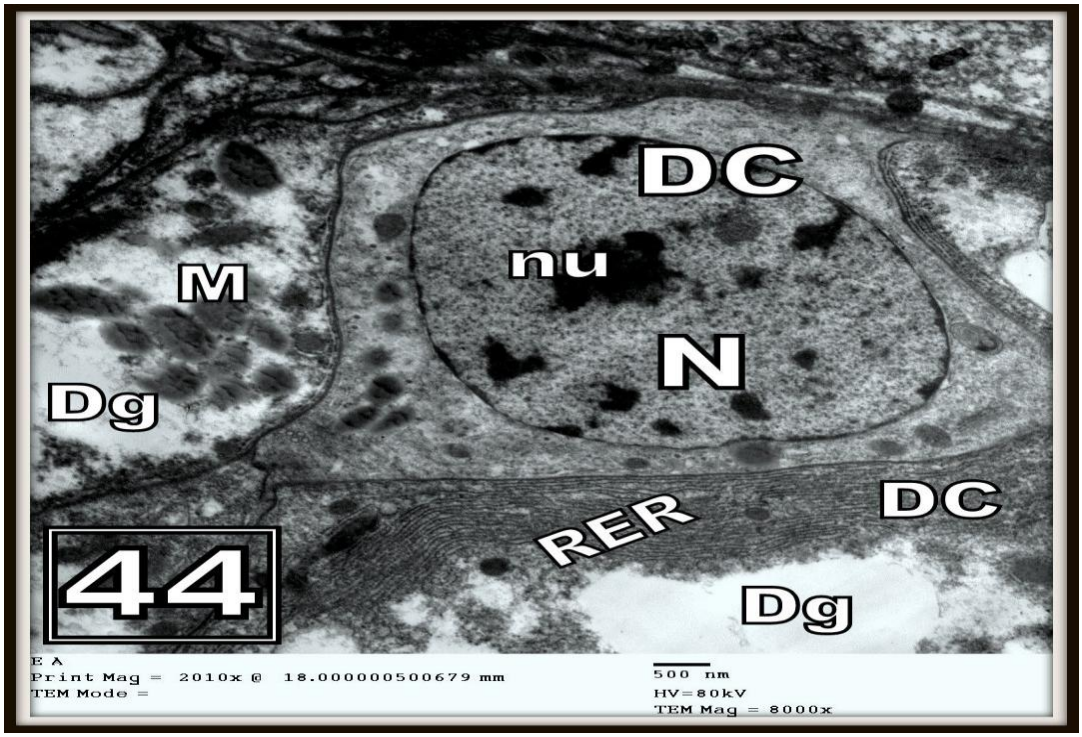
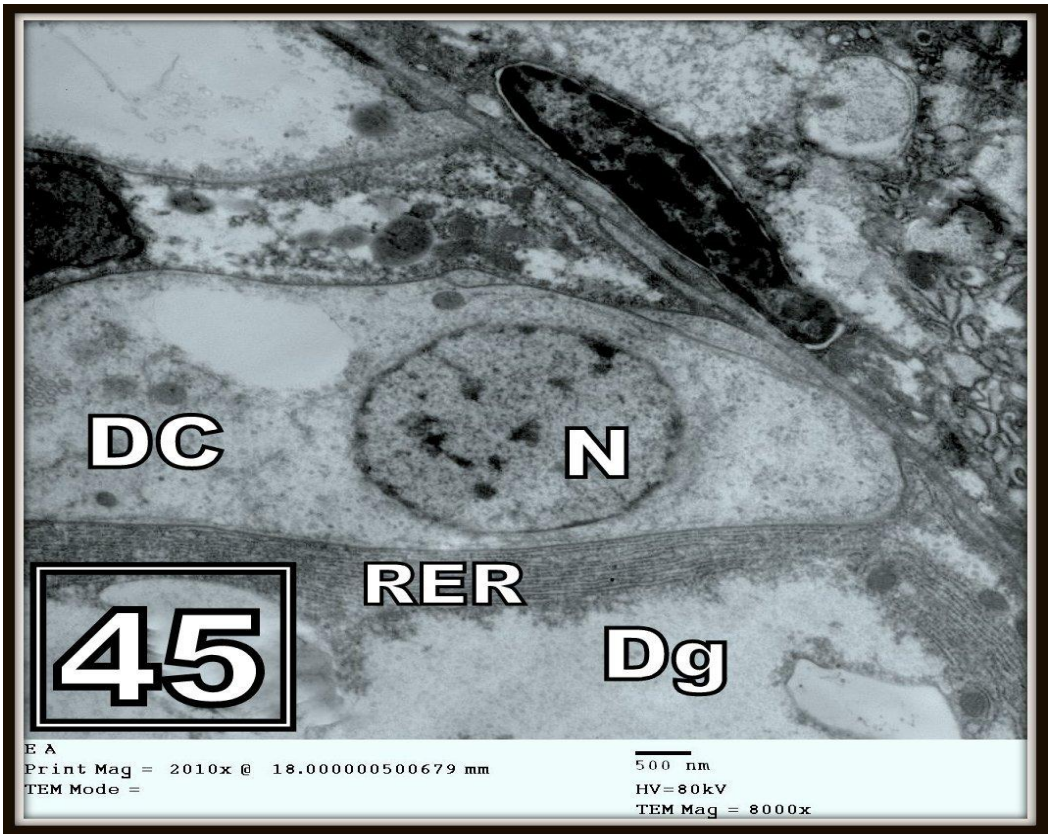

DC
nu
M
N
Dg
RER
DC
44
Dg
E A
Print Mag = 2010x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

Fig. (45) Cross section through the digestive gland of *E. vermiculata* treated with amino 1/2 LC_{50} showing mitochondria (M), enlarged nucleus (N), nucleolus (nu), clearly rough endoplasmic reticulum (RER) and degenerations (Dg). X6000

Fig. (46) Enlarged Cross section through the digestive gland of *E. vermiculata* treated with amino 1/2 LC_{50} showing nucleus (N), nucleolus (nu), rough endoplasmic reticulum (RER), excretory cell (ExC). X12000


DC
N
RER
45
Dg
E A
Print Mag = 2010x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

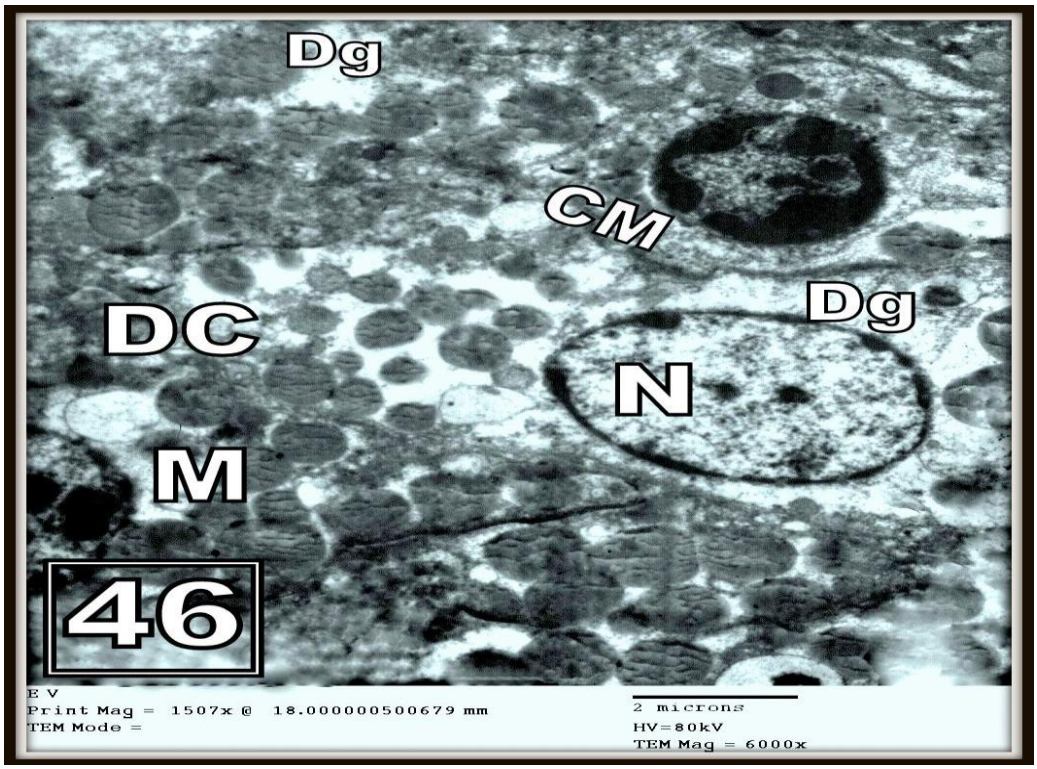
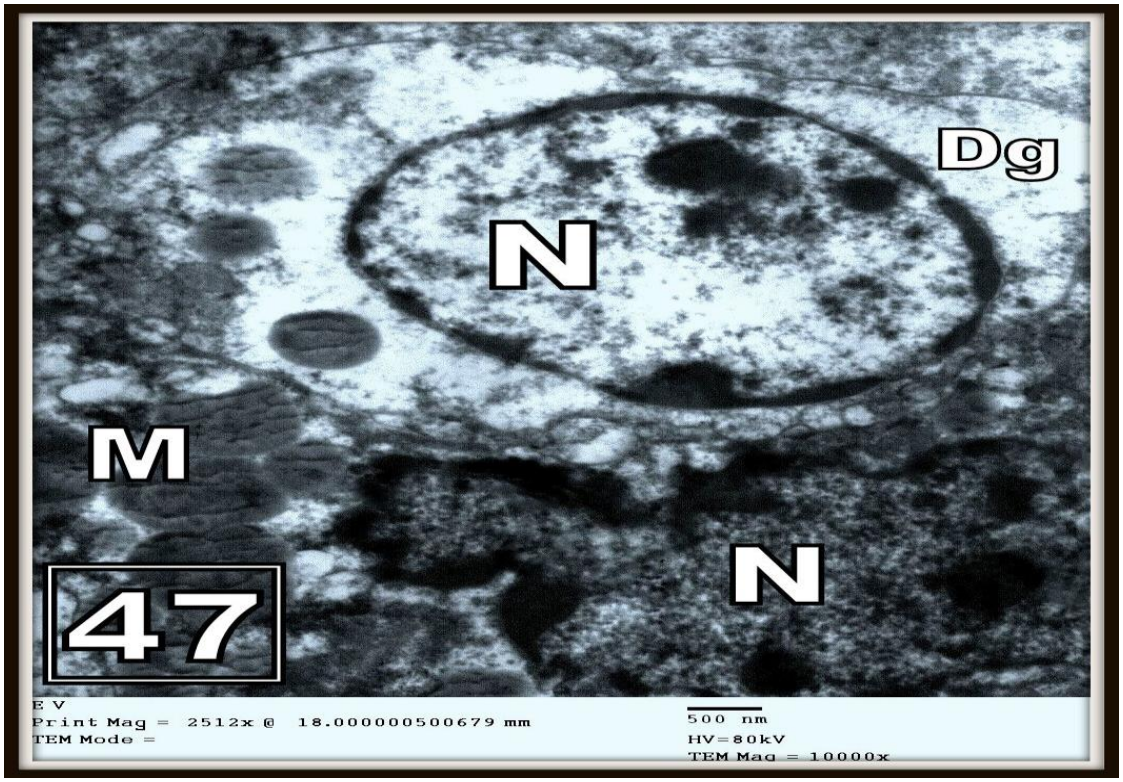

Dg
CM
Dg
DC
N
M
46
E V
Print Mag = 1507x @ 18.000000500679 mm
TEM Mode =
2 microns
HV=80kV
TEM Mag = 6000x

Fig. (47) Cross section through the digestive gland of *E. vermiculata* treated with Amino LC_{50} showing nucleus (N), nucleolus (nu), rough endoplasmic reticulum (RER), mitochondria (M), digestive cell (DC) and degenerations (Dg). X8000

Fig. (48) Cross section through the digestive gland of *E. vermiculata* treated with Amino LC_{50} showing nucleus (N), rough endoplasmic reticulum (RER), digestive cell (DC) and degenerations (Dg). X8000


Dg
N
M
N
47
E V
Print Mag = 2512x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 10000x

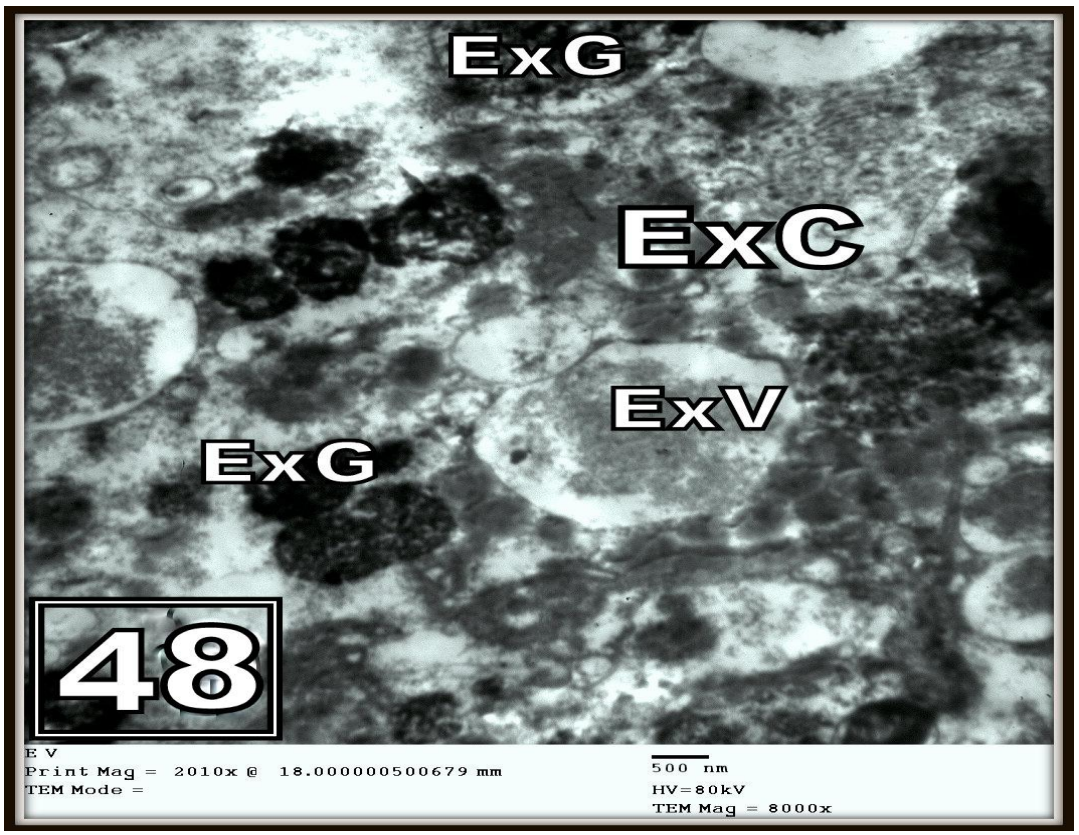
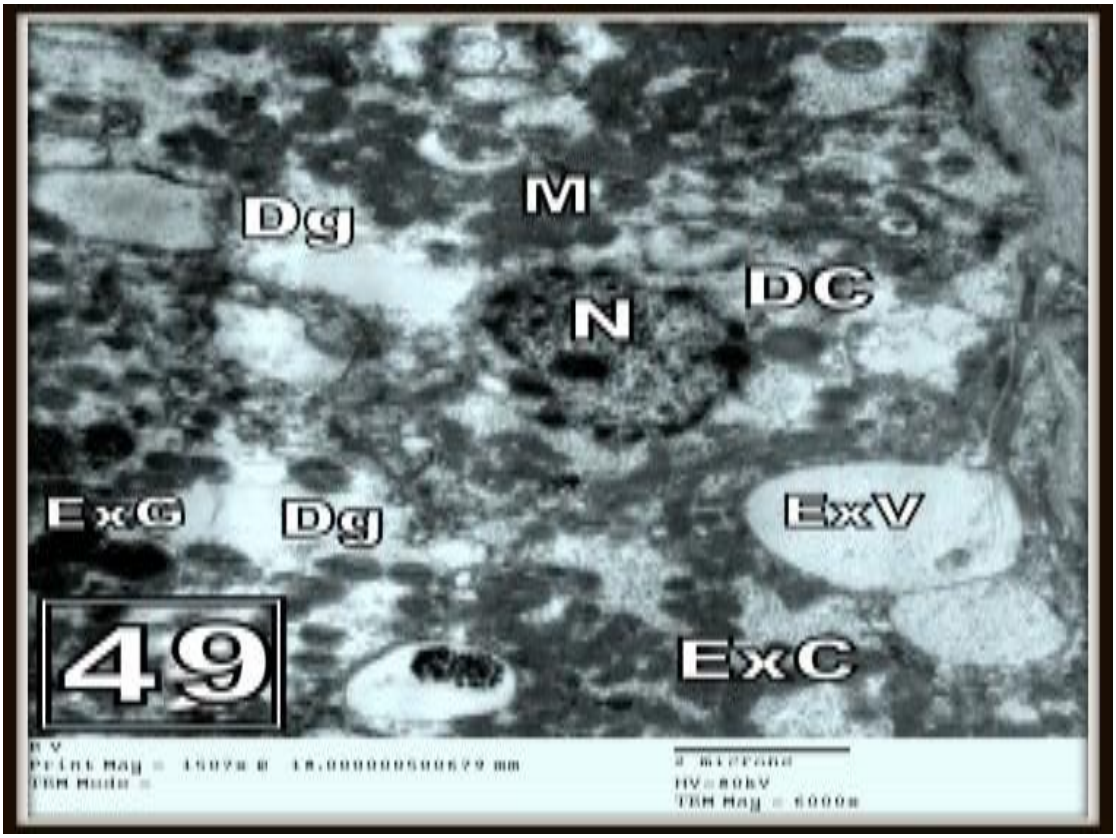

ExG
ExC
ExV
ExG
48
E V
Print Mag = 2010x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

Fig. (49) Cross section through the digestive gland of *E. vermiculata* treated with Vertimec 1/2 LC_{50} showing nucleus (N), digestive cell (DC), mitochondria (M), cell membrane (CM) and degenerations (Dg). X6000

Fig. (50) Cross section through the digestive gland of *E. vermiculata* treated with Vertimec 1/2 LC_{50} showing indented nucleus (N), mitochondria (M) and degenerations (Dg). X10000


Dg
M
DC
N
ExG
Dg
ExV
49
ExC
Print Mag = 1005x @ 18.000000500679 mm
TEM Mode =
2 microns
HV=80kV
TEM Mag = 4000x

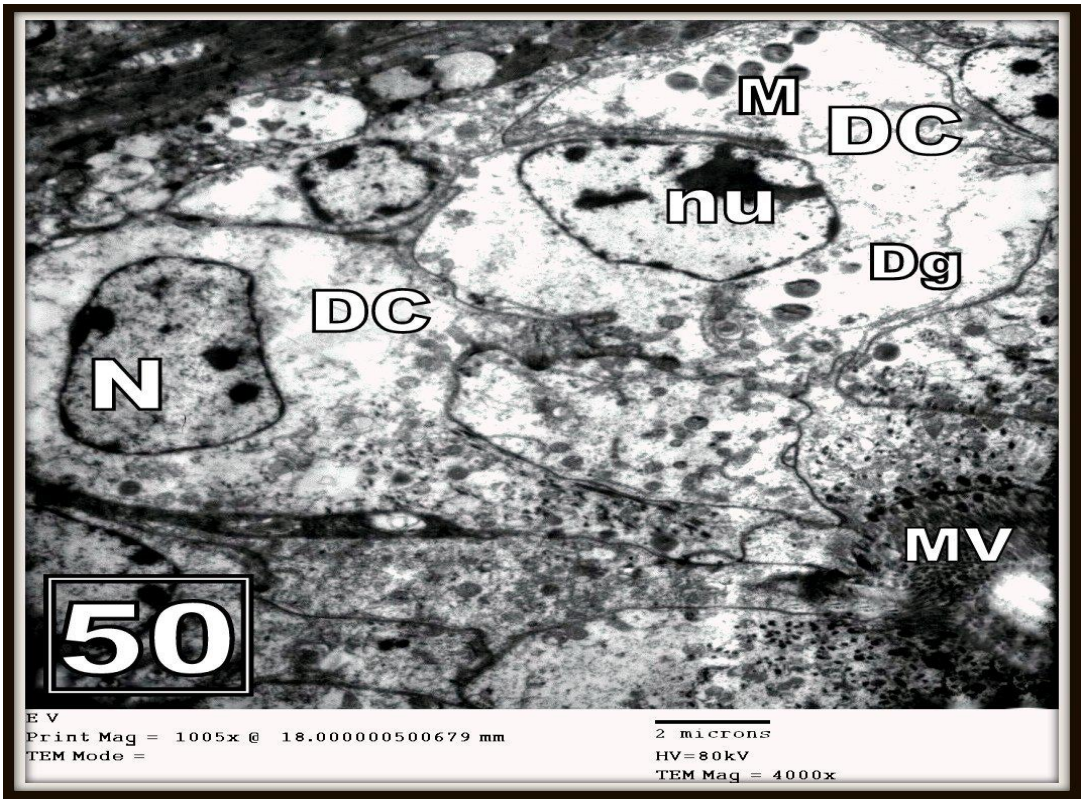
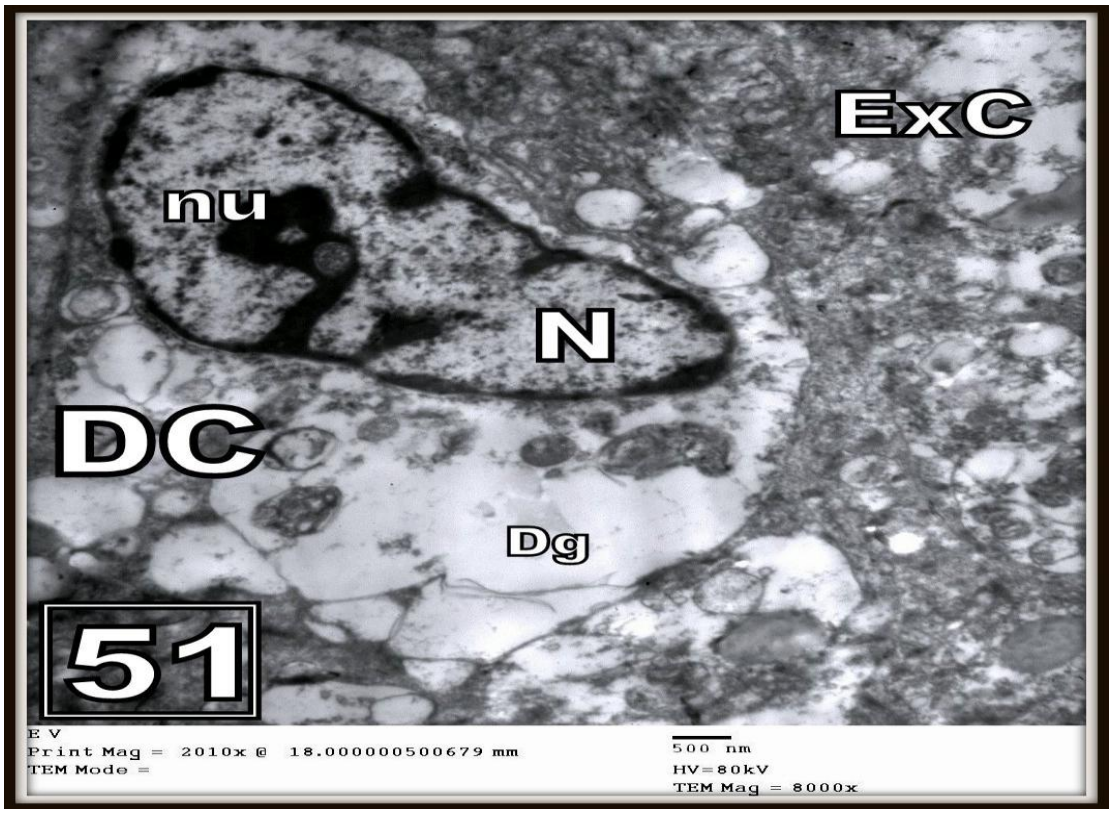

M
DC
nu
Dg
DC
N
MV
50
E V
Print Mag = 1005x @ 18.000000500679 mm
TEM Mode =
2 microns
HV=80kV
TEM Mag = 4000x

Fig. (51) Cross section through the digestive gland of *E. vermiculata* treated with Vertimec 1/2 LC_{50} showing excretory cell (ExC), fragmented excretory granule (ExG) and excretory vacuole (ExV). X 8000

Fig. (52) Cross section through the digestive gland of *E. vermiculata* treated with Vertimec 1/2 LC_{50} showing nucleus (N) with heterochromatin, mitochondria (M), digestive cell (DC), excretory cell (ExC), excretory granule (ExG), excretory vacuole (ExV) and degenerations (Dg). X6000


ExC
nu
N
DC
Dg
51
E V
Print Mag = 2010x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

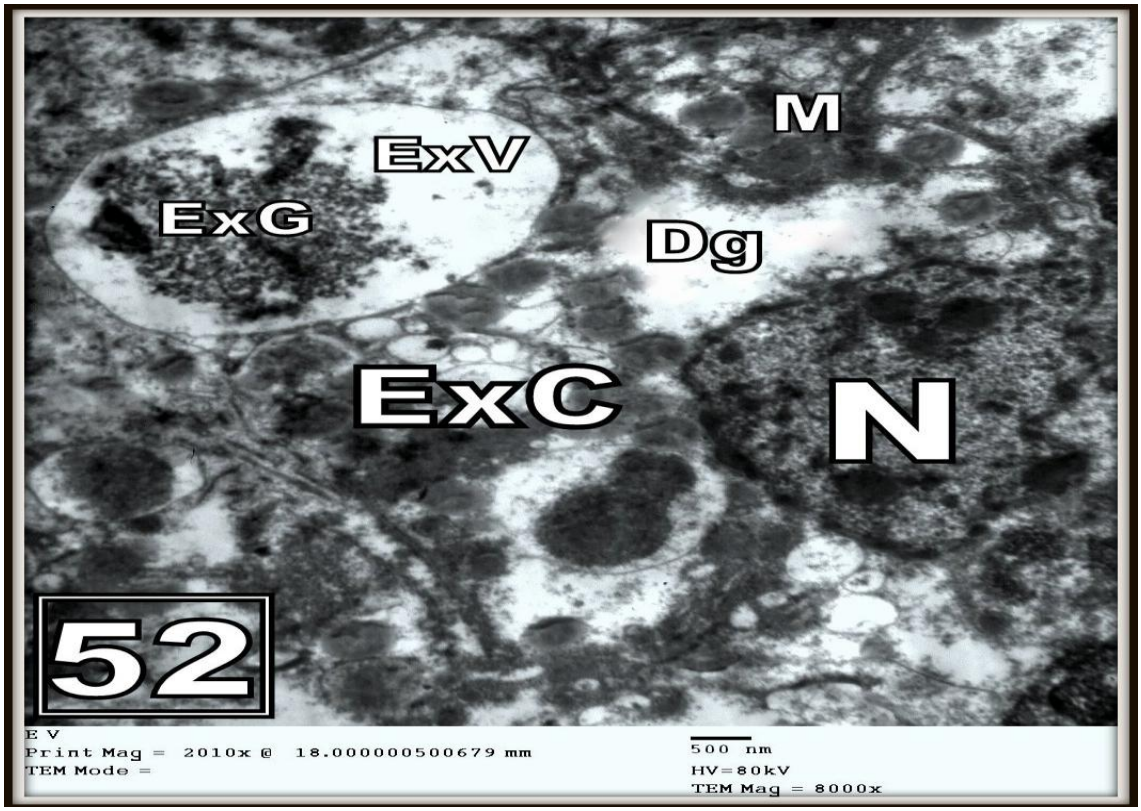
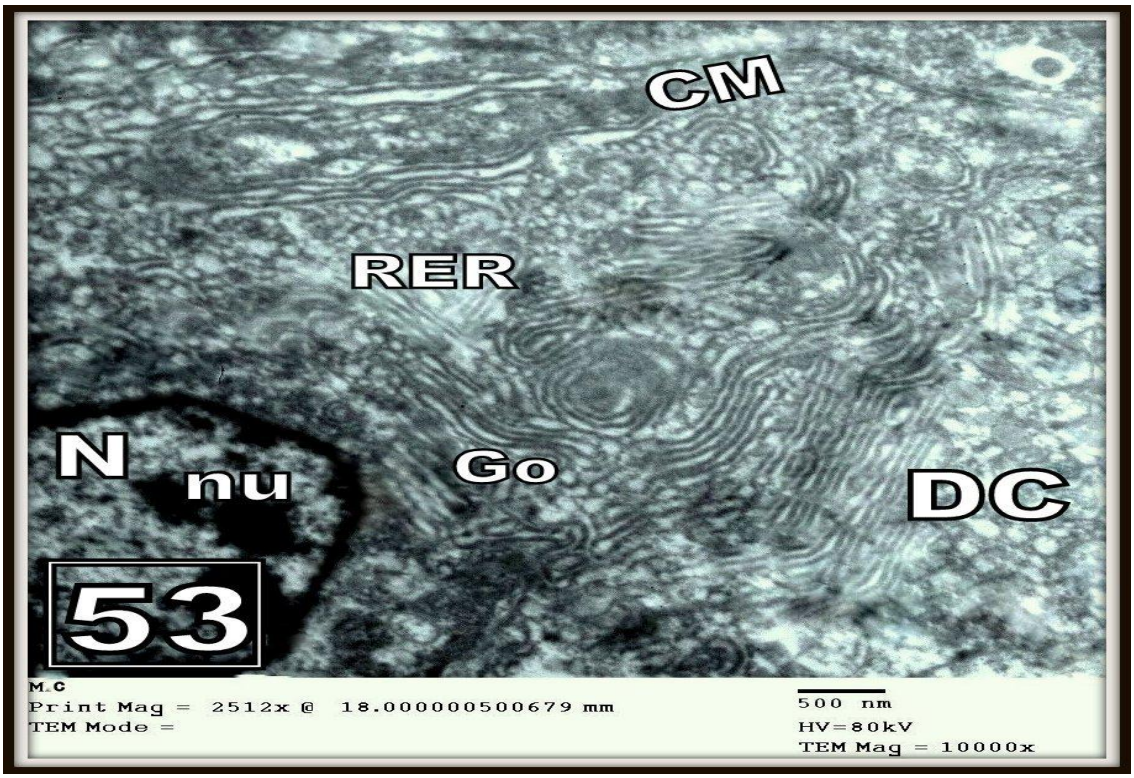

M
ExV
ExG
Dg
ExC
N
52
E V
Print Mag = 2010x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

Fig.(53) Cross section through the digestive gland of *E. vermiculata* treated with Vertimec LC_{50} showing nucleus (N), mitochondria (M), digestive cell (DC), microvilli (MV), nucleolus (Nu) and degenerations (Dg). X4000

Fig. (54) Cross section through the digestive gland of *E. vermiculata* treated with Vertimec LC_{50} showing a bizarre nucleus (N), excretory cell (ExC), digestive cell (DC), nucleolus (nu) coalesced with heterochromatin and obvious degenerations (Dg). X 8000


CM
RER
N
nu
Go
DC
53
M.C
Print Mag = 2512x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 10000x

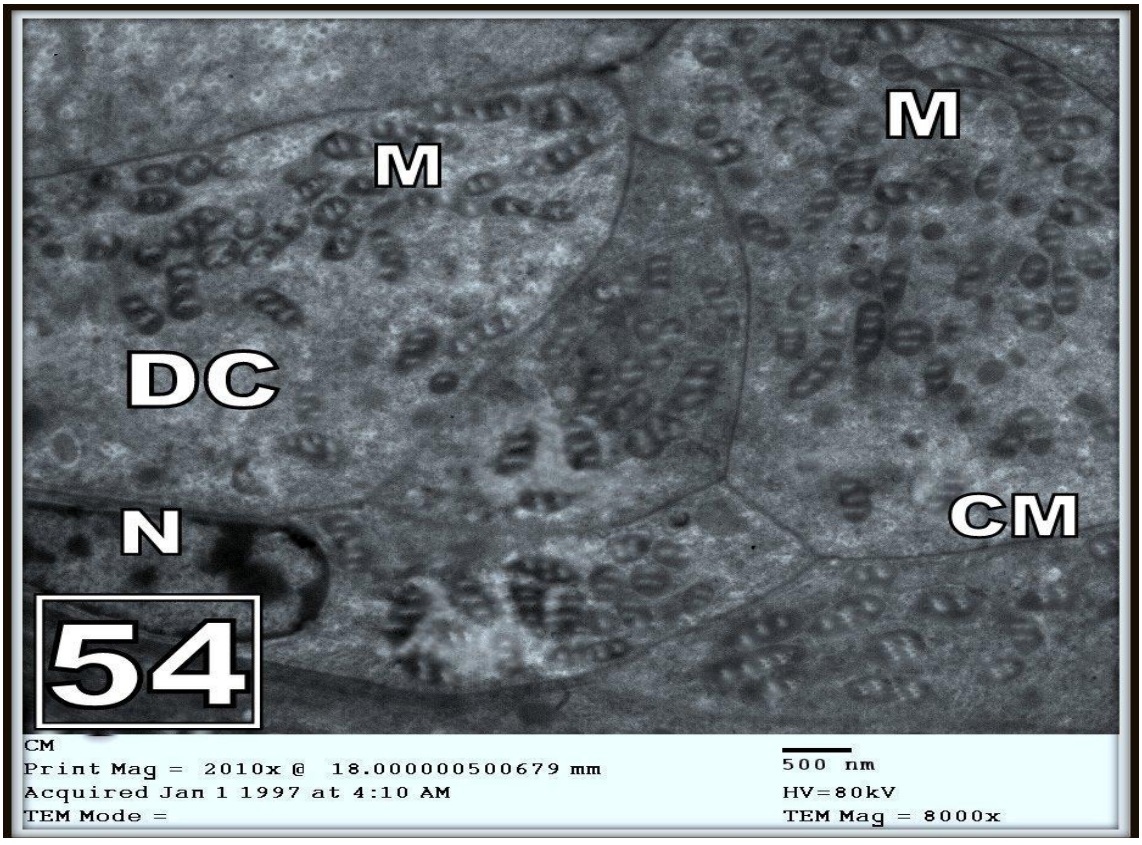
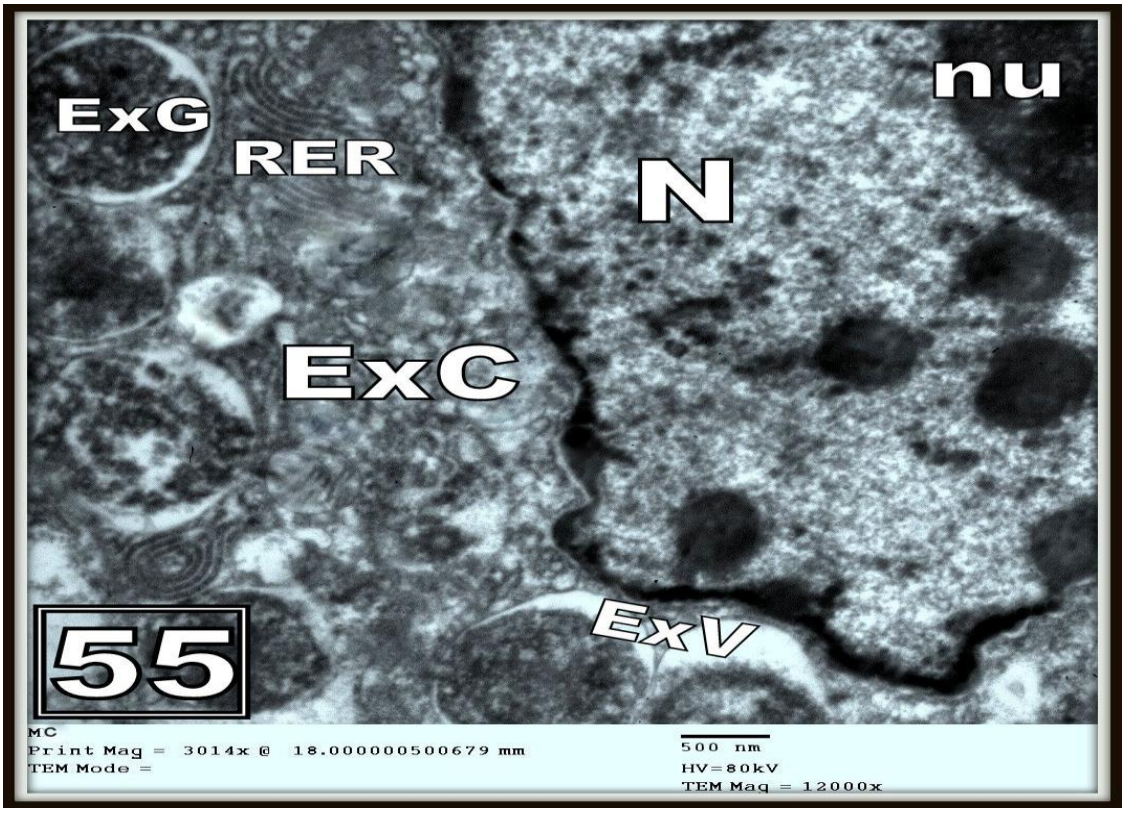

M
M
DC
N
CM
54
CM
Print Mag = 2010x @ 18.000000500679 mm
Acquired Jan 1 1997 at 4:10 AM
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

Fig. (55) Cross section through the digestive gland of *E. vermiculata* treated with Vertimec LC_{50} showing indented nucleus (N), excretory cell (ExC), excretory granule (ExG), excretory vacuole (ExV), mitochondria (M) and degenerations (Dg). X8000

Figs. (56-70) TEM micrographs showing the ultrastructures of the digestive gland of the control and treated *M. cartusiana*

Fig. (56) Enlarged Cross section through the digestive gland of control *M. cartusiana* showing nucleus (N), digestive cell (DC), nucleolus (nu), rough endoplasmic reticulum (RER), cell membrane (CM) and Golgi apparatus (Go). X10000


ExG
RER
nu
N
ExC
ExV
55
MC
Print Mag = 3014x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 12000x

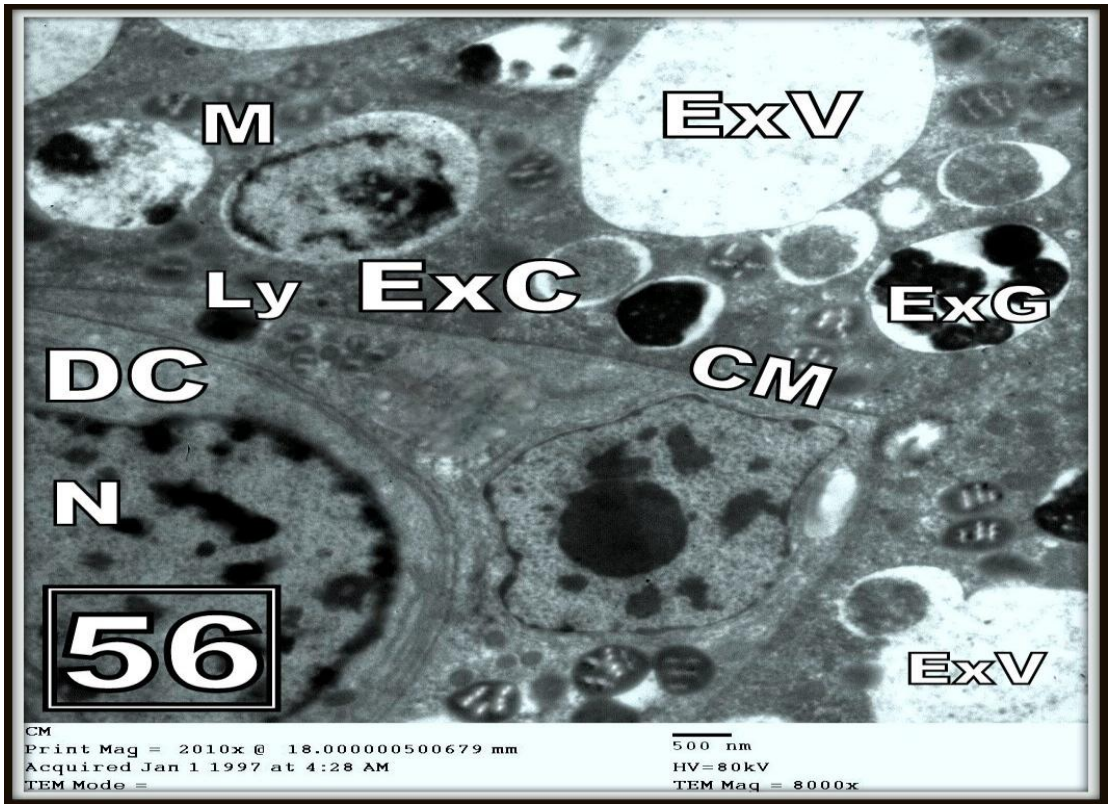
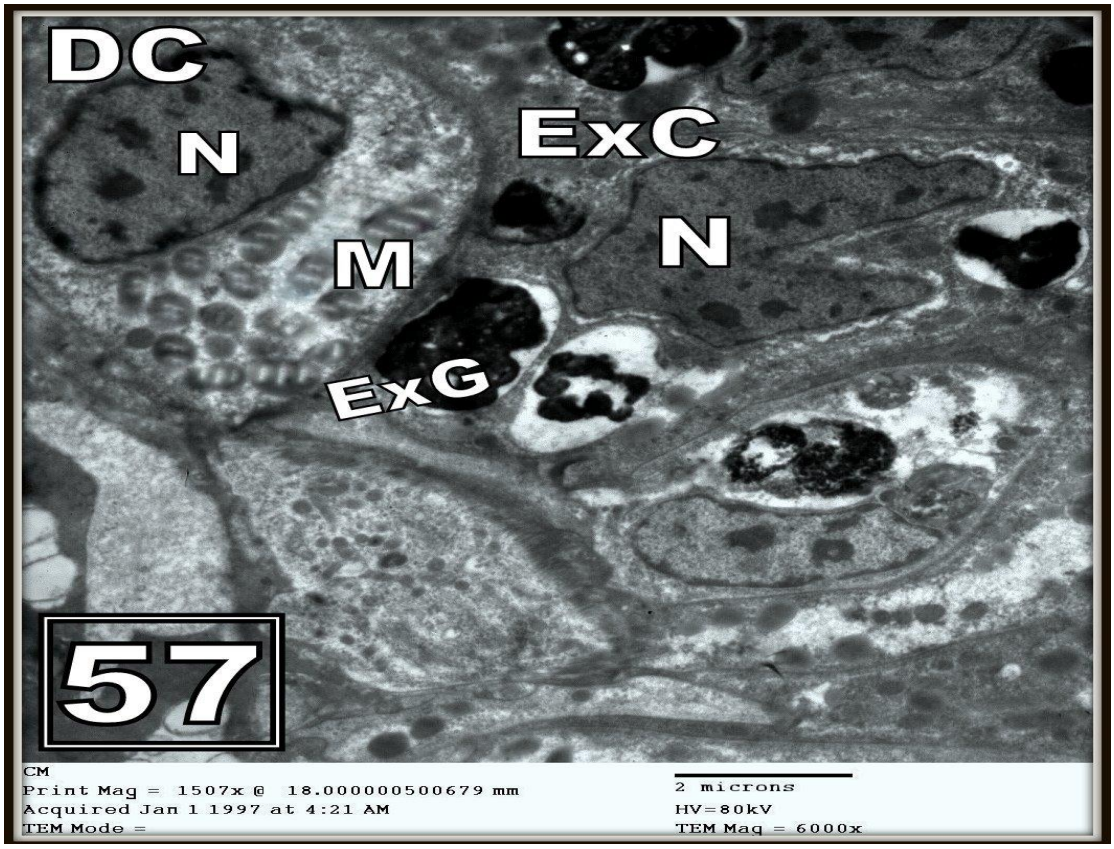

M
ExV
Ly
ExC
ExG
DC
CM
N
56
ExV
CM
Print Mag = 2010x @ 18.000000500679 mm
Acquired Jan 1 1997 at 4:28 AM
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

Fig. (57) Cross section through the digestive gland of control *M. cartusiana* showing mitochondria (M), digestive cell (DC), nucleus (N) and cell membrane (CM). X8000

Fig. (58) Enlarged Cross section through the excretory cell from the digestive gland of control *M. cartusiana* showing excretory cell (ExC), excretory granule (ExG), nucleus (N), nucleolus (nu) and rough endoplasmic reticulum (RER). X 12000


DC
N
ExC
M
N
ExG
57
CM
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TEM Mode =
2 microns
HV=80kV
TEM Mag = 6000x

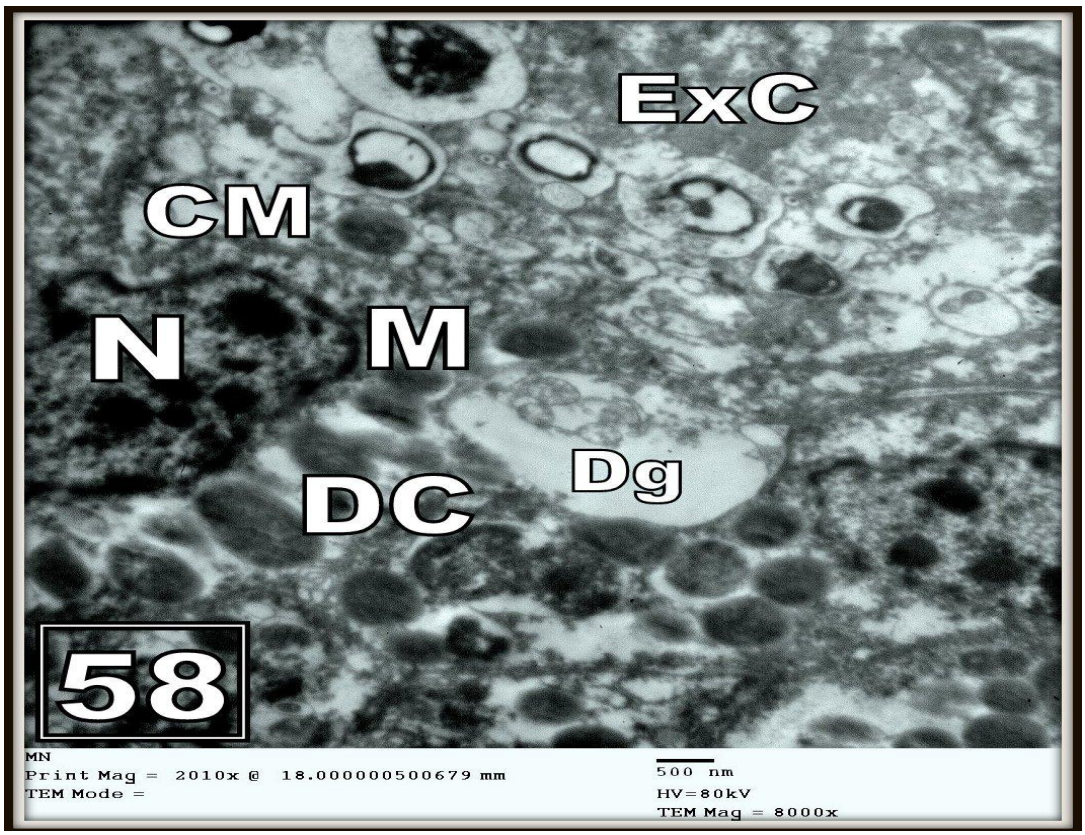
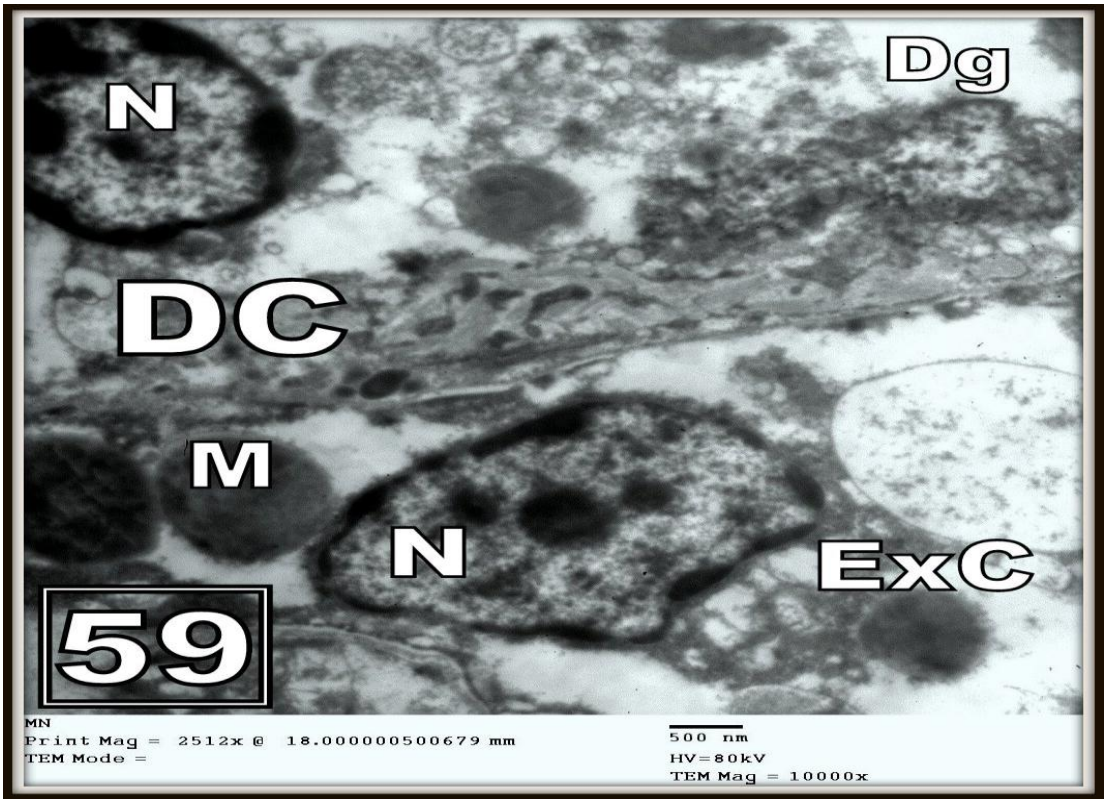

ExC
CM
N
M
Dg
DC
58
MN
Print Mag = 2010x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x

Fig. (59) Cross section through the digestive gland of control *M. cartusiana* showing excretory cell (ExC), excretory granule (ExG), large excretory vacuole (ExV), nucleus (N), mitochondria (M), digestive cell (DC), lysosomes (Ly), cell membrane (CM). X8000

Fig. (60) Cross section through the digestive gland of control *M. cartusiana* showing excretory cell (ExC), excretory granule (ExG), nucleus (N), mitochondria (M) and digestive cell (DC). X6000


Dg
N
DC
M
N
ExC
59
MN
Print Mag = 2512x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 10000x

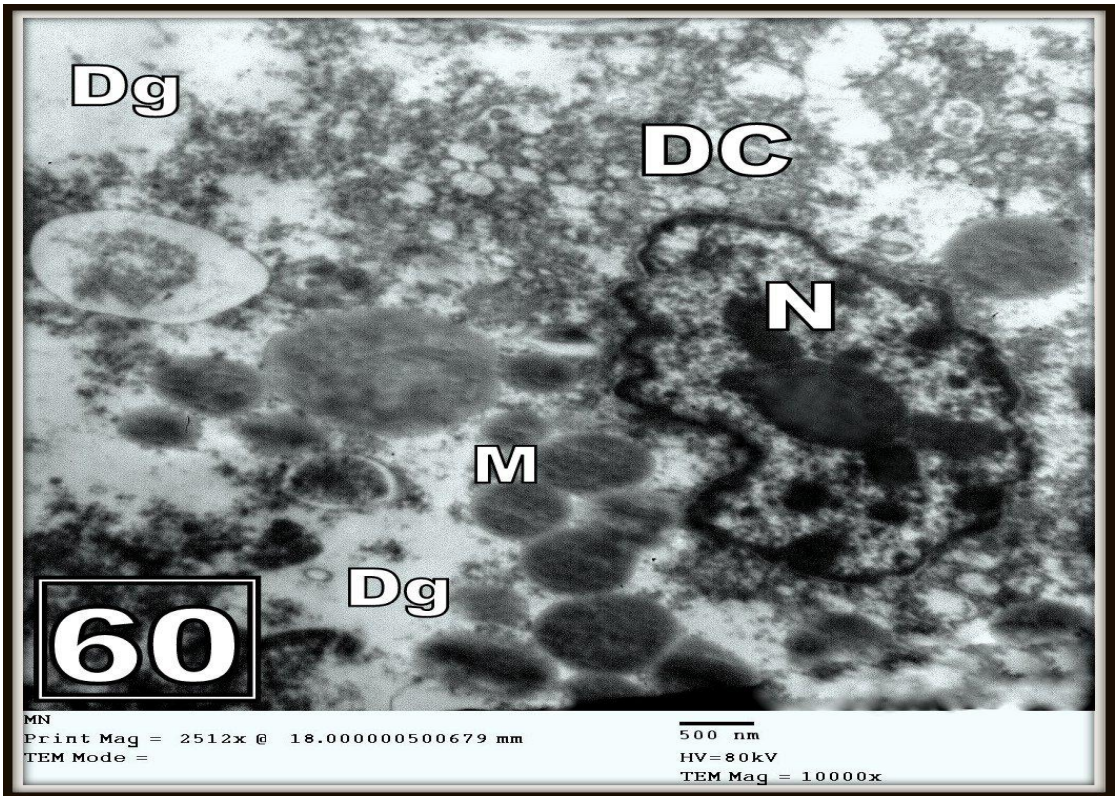
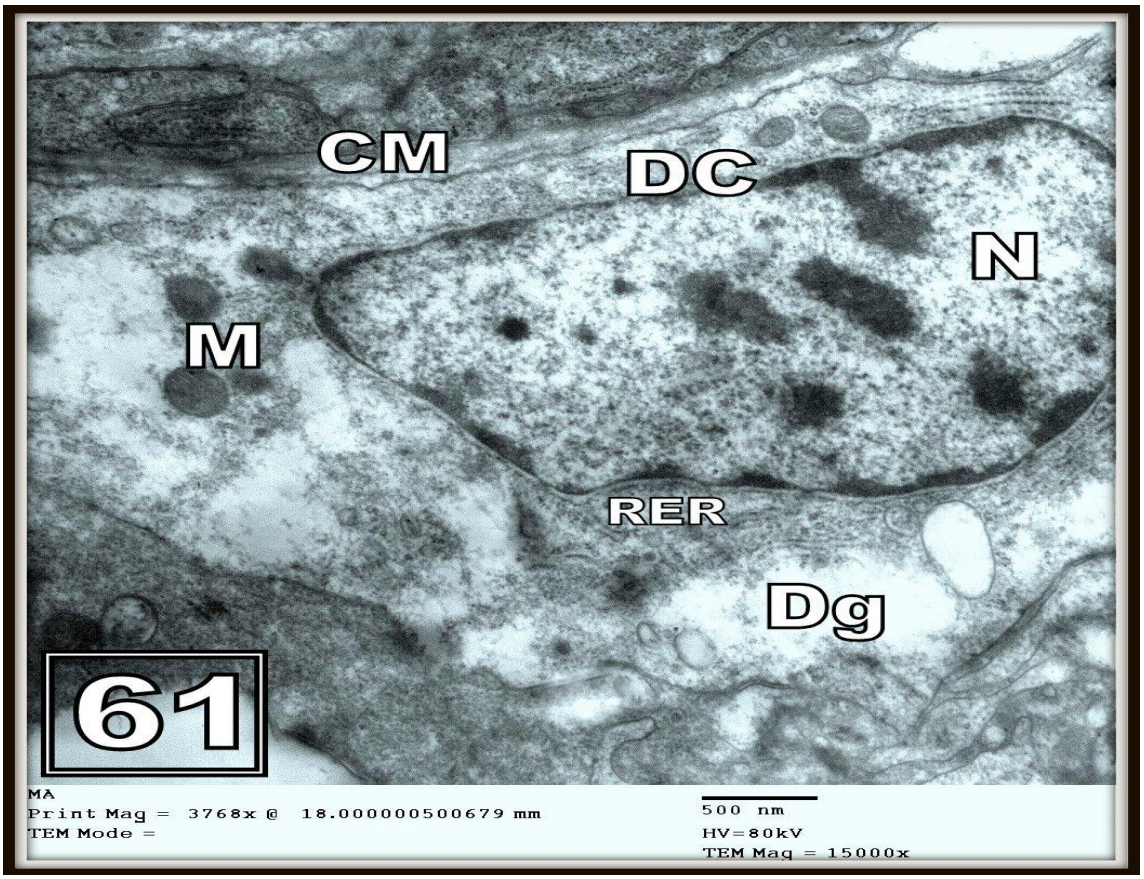

Dg
DC
N
M
Dg
60
MN
Print Mag = 2512x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 10000x

Fig. (61) Cross section through the digestive gland of *M. cartusiana* treated with 1/2 LC_{50} Newmyl showing calcium cell (CaC), nucleus (N), mitochondria (M), digestive cell (DC), cell membrane (CM) and degeneration (Dg) X8000

Fig. (62) Cross section through the digestive gland of *M. cartusiana* treated with LC_{50} Newmyl showing excretory cell (ExC), nucleus (N), mitochondria (M), digestive cell (DC) and degenerations (Dg). X10000


CM
DC
N
M
RER
Dg
61
MA
Print Mag = 3768x @ 18.000000500679 mm
TEM Mode =
500 nm
HV=80kV
TEM Mag = 15000x

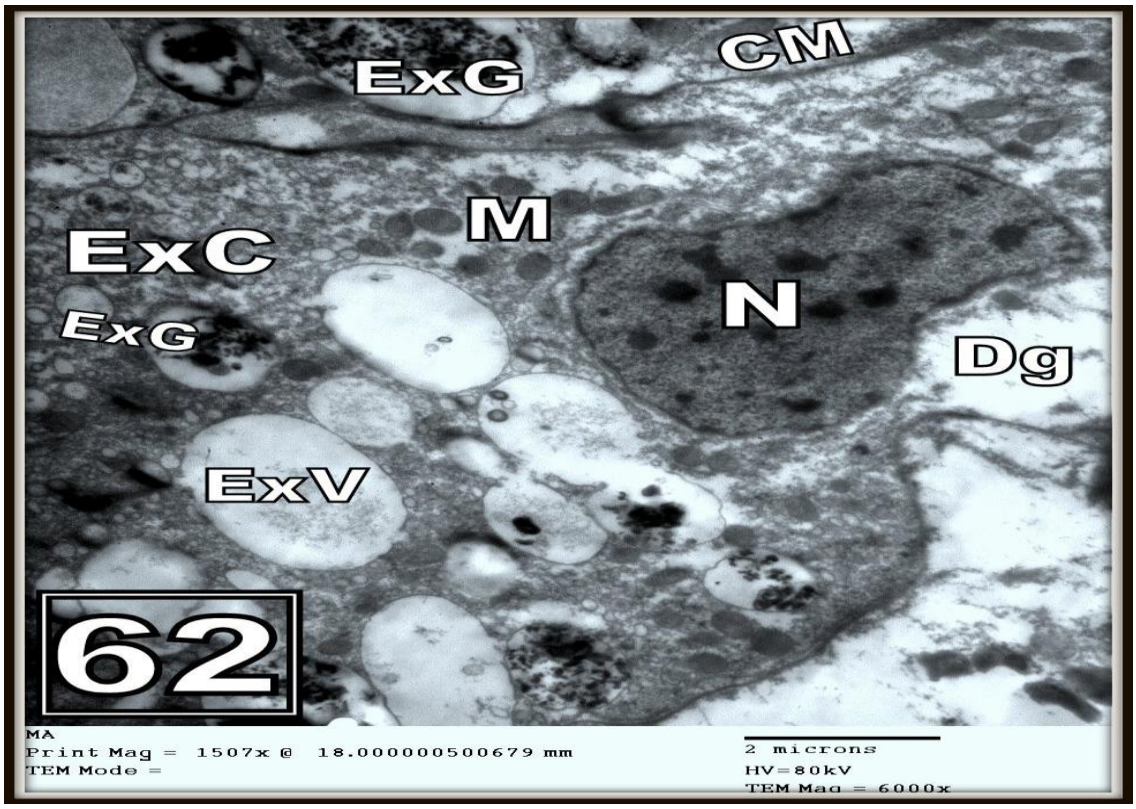
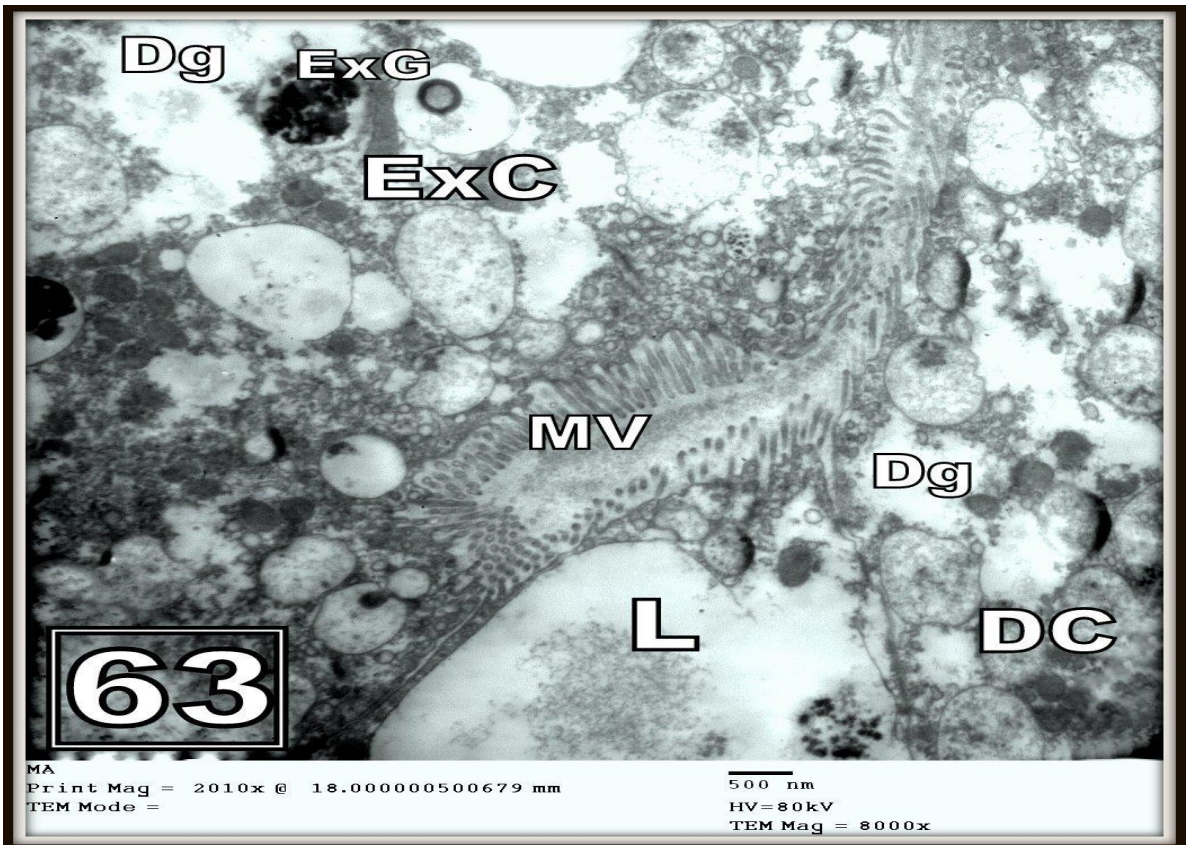

ExG
CM
M
ExC
ExG
N
Dg
ExV
62
MA
Print Mag = 1507x @ 18.000000500679 mm
TEM Mode =
2 microns
HV=80kV
TEM Mag = 6000x

Fig. (63) Cross section through the digestive gland of *M. cartusiana* treated with LC_{50} Newmyl showing nucleus (N), mitochondria (M), digestive cell (DC) and degenerations (Dg). X10000

Fig. (64) Enlarged cross section through the digestive gland of *M. cartusiana* treated with1/2 LC_{50} Amino showing nucleus (N), mitochondria (M), digestive cell (DC), cell membrane (CM), rough endoplasmic reticulum (RER) and degenerations (Dg). X15000


Dg
ExG
ExC
MV
Dg
L
DC
63
MA
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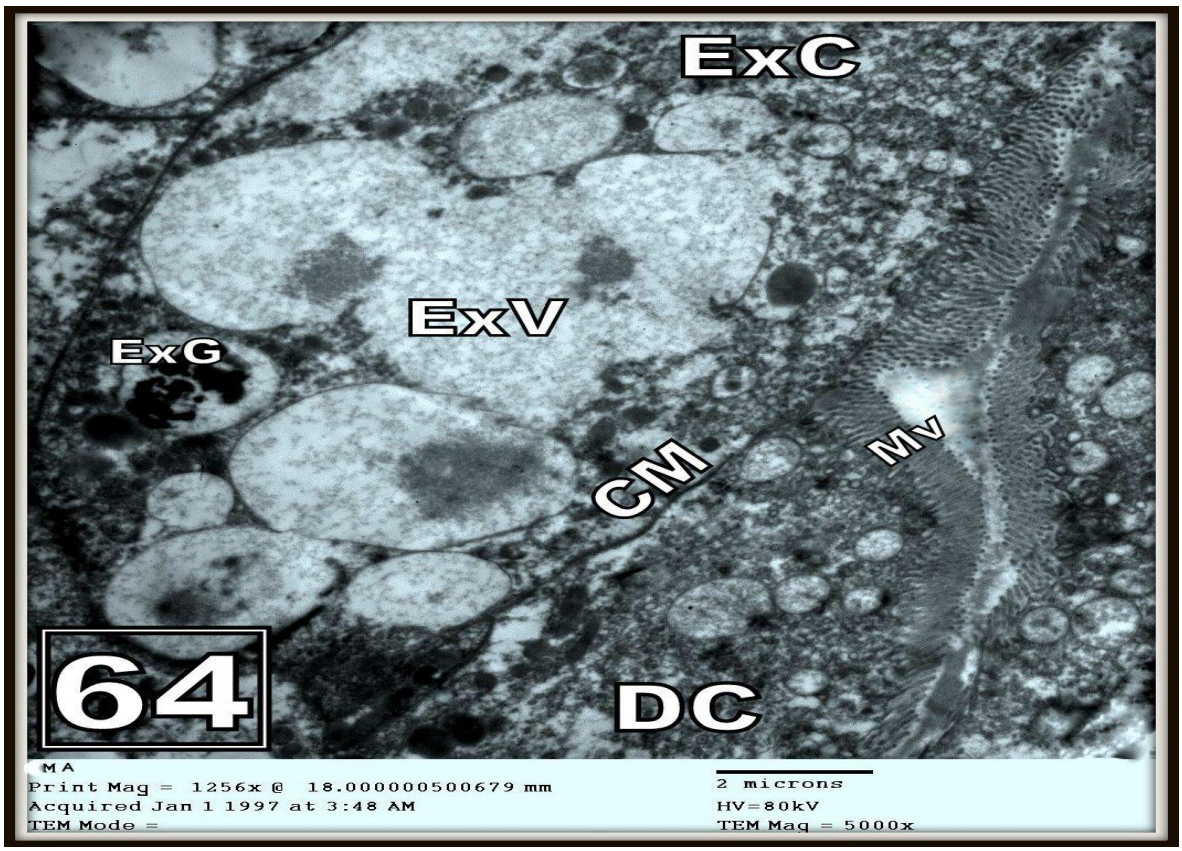
TEM Mag = 8000x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ExV
ExG
CM
Mv
64
DC
MA
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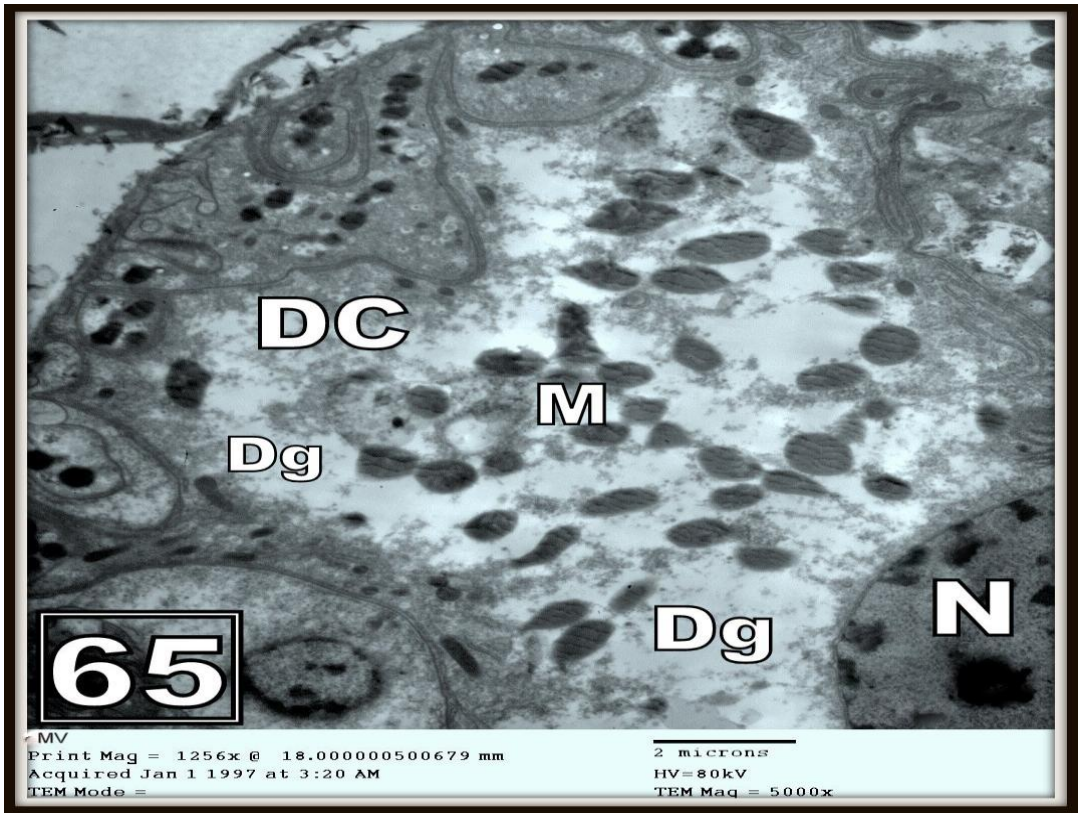
HV=80kV
TEM Mag = 5000x

Fig. (65) Cross section through the digestive gland of *M. cartusiana* treated with 1/2 LC_{50} Amino showing nucleus (N), mitochondria (M), excretory cell (ExC), excretory granule (ExG), excretory vacuole (ExV), cell membrane (CM) and degenerations (Dg). X6000

Fig. (66) Cross section through the digestive gland of *M. cartusiana* treated with LC_{50} Amino showing excretory cell (ExC), excretory granule (ExG), cell membrane (CM), lysosomes (Ly), microvilli (MV), digestive cell (DC) and abundant degenerations (Dg). X8000


DC
M
Dg
Dg
N
65
MV
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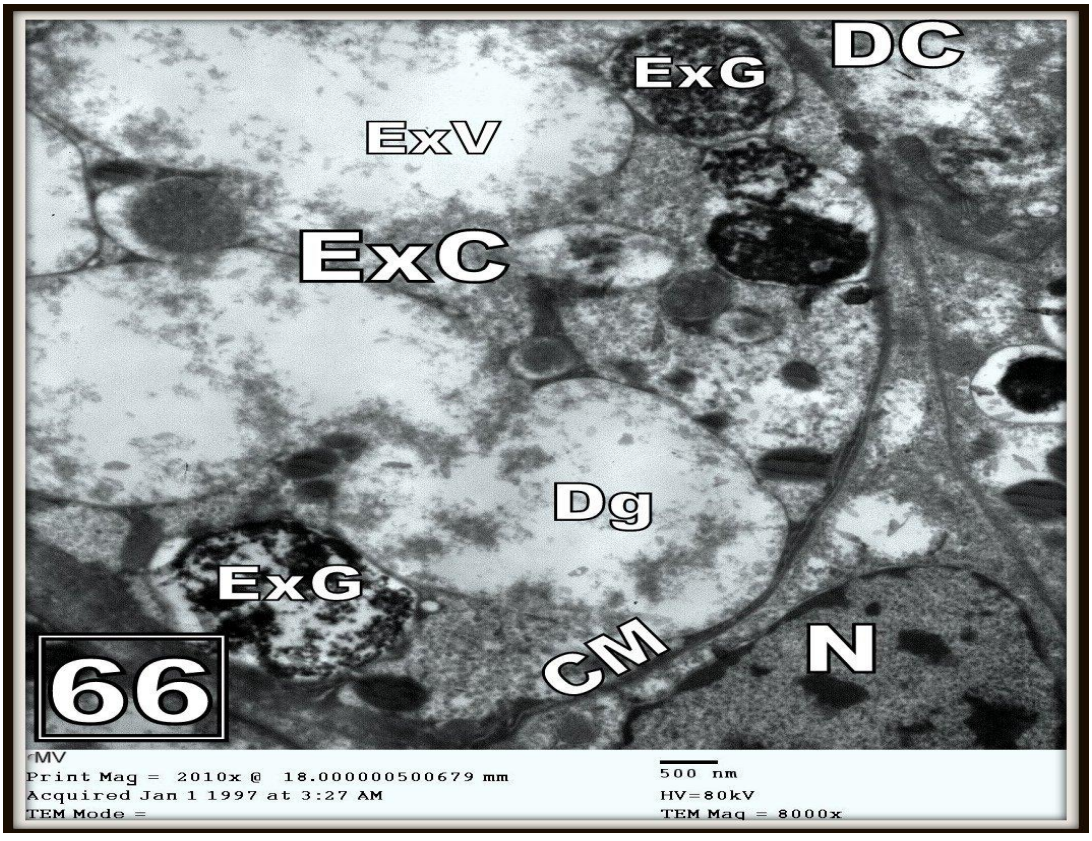
DC
ExG
ExV
ExC
Dg
ExG
CM
N
66
MV
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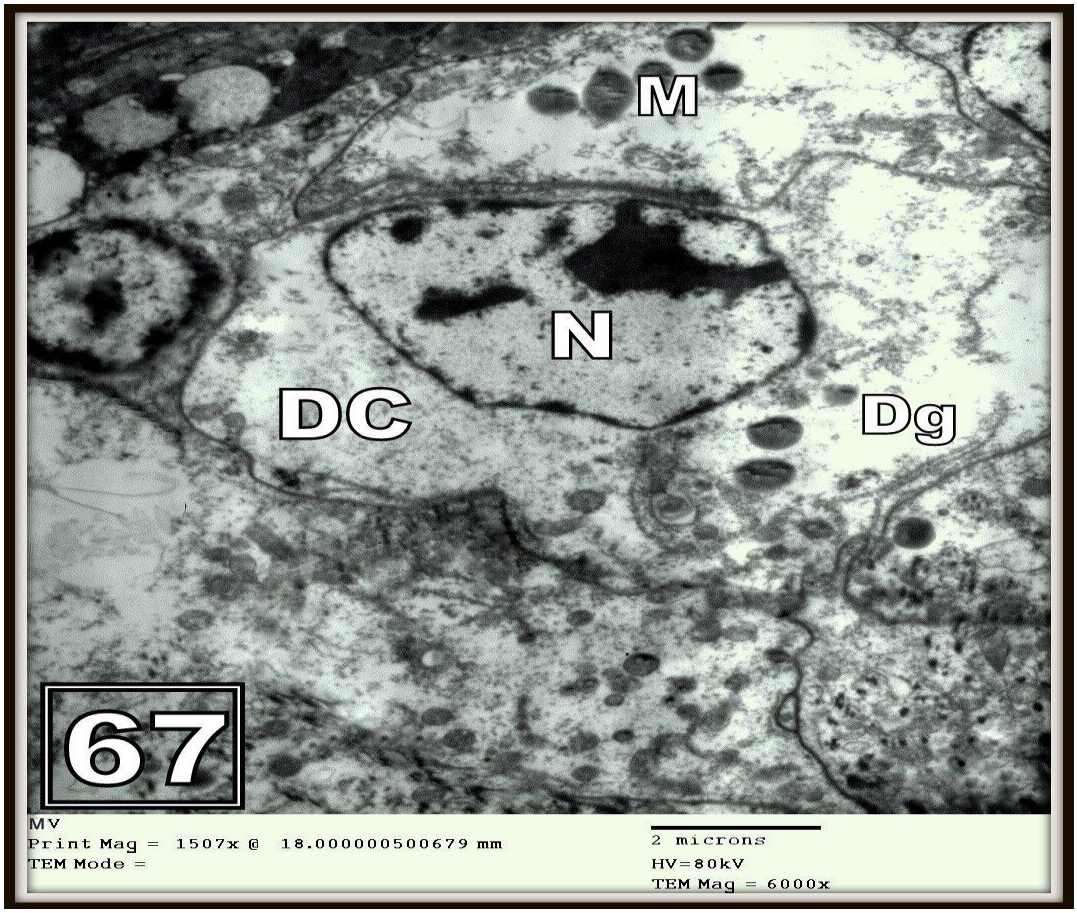
500 nm
HV=80kV
TEM Mag = 8000x

Fig. (67) Cross section through the digestive gland of *M. cartusiana* treated with LC_{50} Amino showing excretory cell (ExC), excretory granule (ExG), very wide excretory vacuole (ExV), cell membrane (CM), microvilli (MV) and digestive cell (DC). X5000

Fig. (68) Cross section through the digestive gland of *M. cartusiana* treated with 1/2 LC_{50} Vertimec showing digestive cell (DC), nucleus (N), numerous mitochondria (M) and degenerations (Dg). X5000


M
N
DC
Dg
67
MV
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TEM Mode =
2 microns
HV=80kV
TEM Mag = 6000x

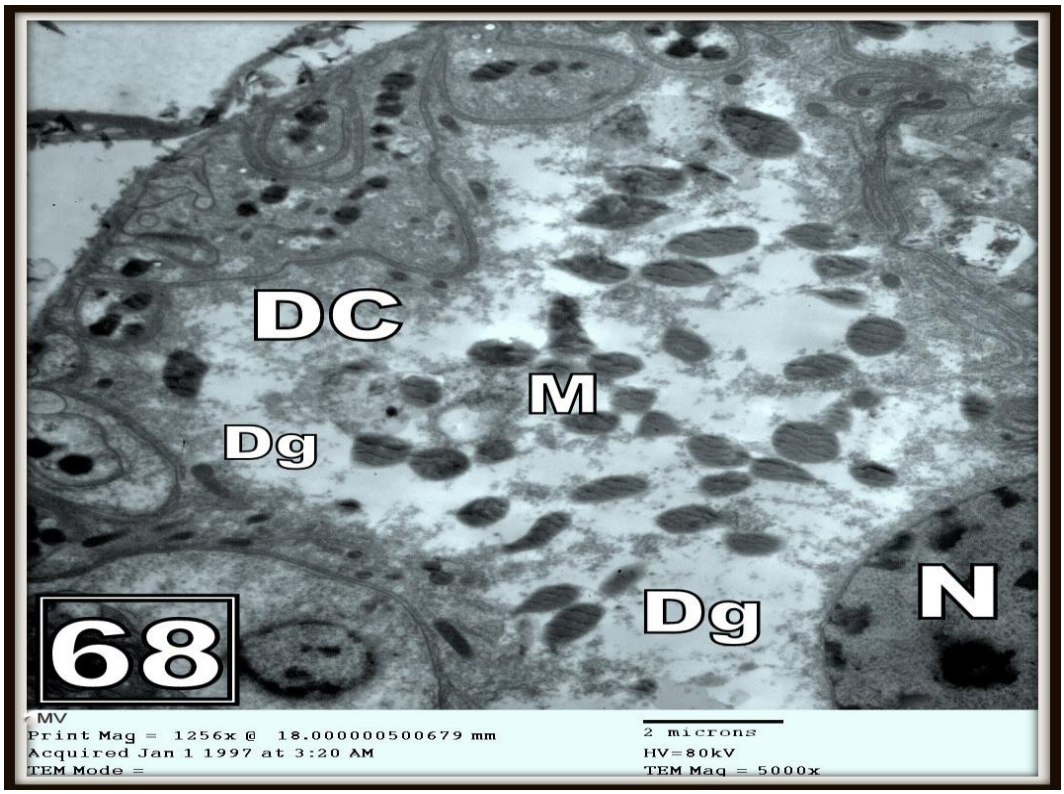
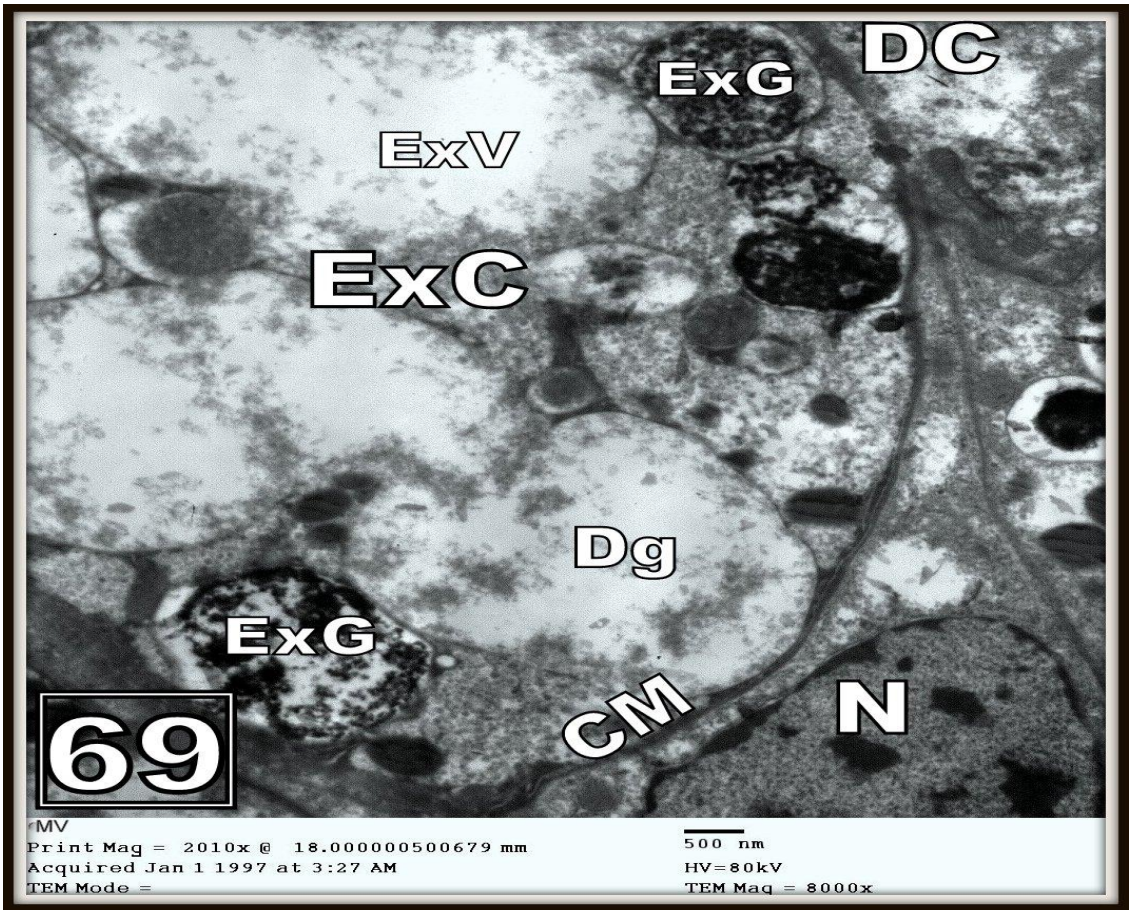
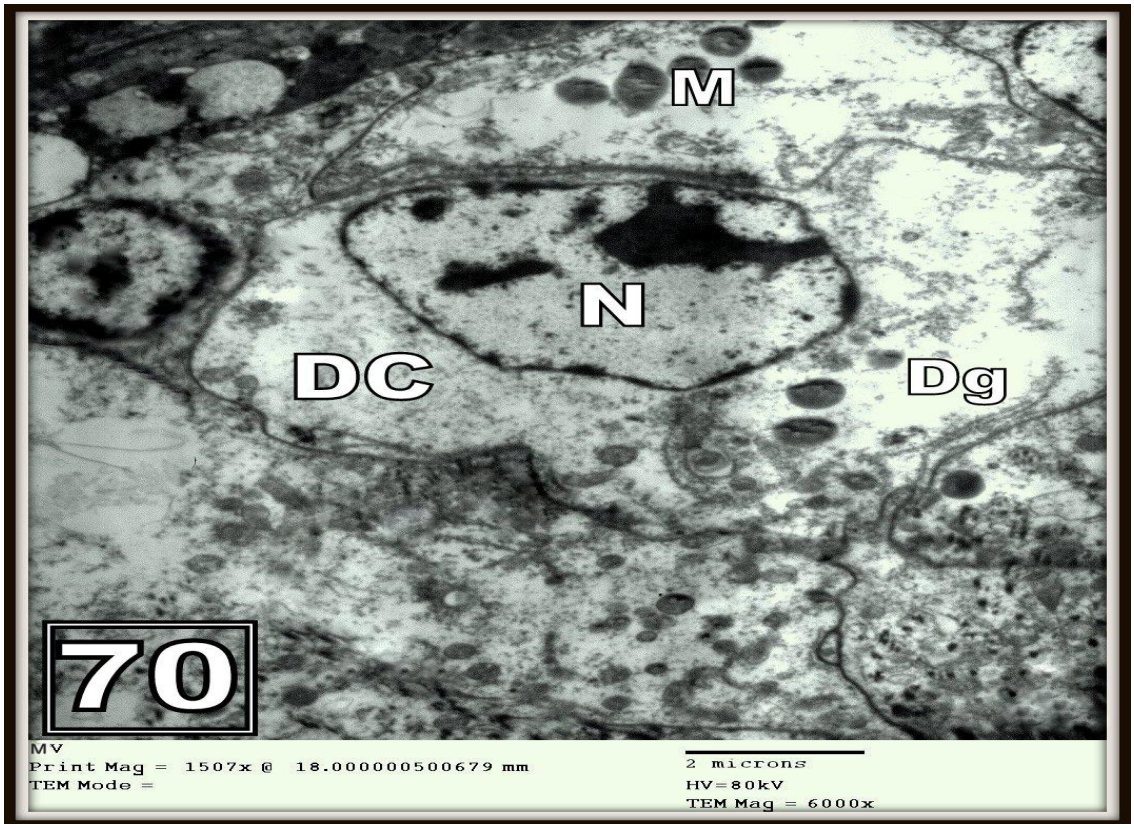

DC
M
Dg
Dg
N
68
MV
Print Mag = 1256x @ 18.000000500679 mm
Acquired Jan 1 1997 at 3:20 AM
TEM Mode =
2 microns
HV=80kV
TEM Mag = 5000x

Fig. (69) Cross section through the digestive gland of *M. cartusiana* treated with LC_{50} Vertimec showing digestive cell (DC), nucleus (N), excretory cell (ExC), excretory granule (ExG), excretory vacuole (ExV), cell membrane (CM) and degenerations (Dg). X8000

Fig. (70) Cross section through the digestive gland of *M. cartusiana* treated with LC_{50} Vertimec showing digestive cell (DC), nucleus (N), mitochondria (M) and degenerations (Dg). X6000


DC
ExG
ExV
ExC
Dg
ExG
CM
N
69
MV
Print Mag = 2010x @ 18.000000500679 mm
Acquired Jan 1 1997 at 3:27 AM
TEM Mode =
500 nm
HV=80kV
TEM Mag = 8000x


M
N
DC
Dg
70
MV
Print Mag = 1507x @ 18.000000500679 mm
TEM Mode =
2 microns
HV=80kV
TEM Mag = 6000x

IV- Evaluation of the biochemical parameters

In this section the changes in the total protein, albumin, urea, triglycerides and cholesterol values of the two tested land snails after treatment by Amino (chemical compound) and Vertimec (biocide) that have highest toxicity compared with those by Newmyl well be considered.

4.1. *E. vermiculata*

As seen in table (15) the biochemical values for control were as follow: glucose : 20.8 mg/dl, albumin 0.179 g/dl, total proteins 2.90 g/dl, cholesterol 32.27 mg/dl, triglycerides 75 mg/dl and urea 103.57 mg/dl.

Table (15) Biochemical parameters of *E. vermiculata* after treatment with 1/2 LC_{50} and LC_{50} of Newmyl, Amino and Vertimec

	Conc.	Glucose	Albumin	Total proteins	Cholesterol	Triglycerides	Urea
Control		**20.8**	**0.17**	**2.90**	**32.27**	**75**	**103.57**
Pesticide							
Newmyl	1/2 LC_{50}	37.5	1.02	2.18	58.06	125	133.93
	LC_{50}	145.8	2.55	8.36	200	316.67	121/24
Amino	1/2 LC_{50}	37.5	0.26	2.55	64.52	83.33	77.68
	LC_{50}	70.8	0.68	5.09	109.68	91.67	106.25
Vertimec	1/2 LC_{50}	58.3	1.79	4.55	96.77	150	106.25
	LC_{50}	150	2.38	4.73	135.48	175	112.5
LSD = 0.001		140.2082 *	2.7537 **	7.0675 Ns	148.0313*	186.0835**	31.0720 ***

***= very highly significant, **= highly significant, * = significant, ns =non- significant

Treatment of *E. vermiculata* snails with 1/2 LC_{50} of Newmyl resulted in a significant increase ($P \leq 0.001$) in the value of glucose and cholesterol. There was also a highly significant increase in the values of albumin, triglycerides and urea. This applied concentration of the

insecticide decreased the value of the total proteins to 2.18 mg/dl (control =2.90 mg/dl). Application of Newmyl LC_{50} after 72 hr led to a very high significant increase in glucose and cholesterol. Significant increase in albumin, triglycerides and urea values, was also recorded, whereas the total protein increased non- significantly.

When the snail was treated with 1/2 LC_{50} of Amino glucose and cholesterol were significantly increased, while albumin, triglycerides were highly significantly increased. As for urea it was highly decreased and the total proteins also decreased non- significantly. Treatment with the LC_{50} of Amino chemical compound after 72 hr. showed that glucose and cholesterol were significantly increased. While albumin and triglycerides were highly significantly increased but the total proteins and urea increased non-significantly.

After application of 1/2 LC_{50} and the LC_{50} of the biocide Vertimec the tested parameters glucose and cholesterol were significantly increased and the albumin as well as the triglycerides were highly significantly increased.

Likewise, the total protein and urea recorded an increase but was non-significantly comparing with control.

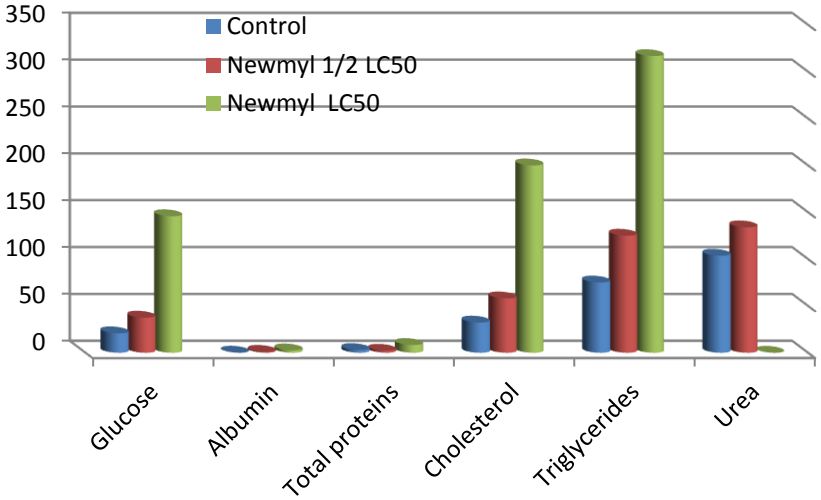


Fig. (71)Histogram illustrating the biochemical parameters of *E. vermiculata* after treatment with Newmyl compared with control

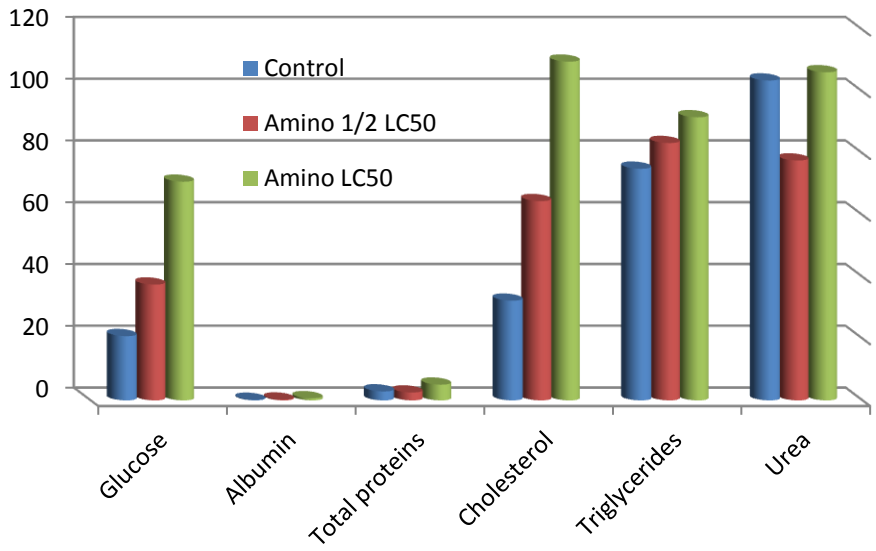


Fig. (72) Histogram illustrating the biochemical parameters of *E. vermiculata* after treatment with Amino compared with control .

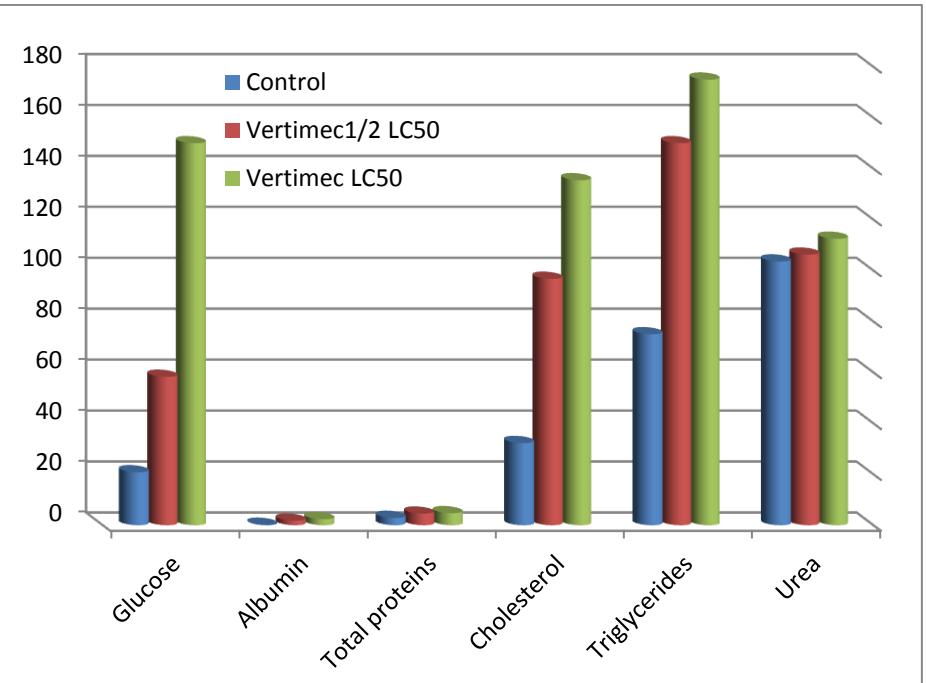


Fig. (73) Histogram illustrating the biochemical parameters of *E. vermiculata* after treatment with Vertimec compared with control.

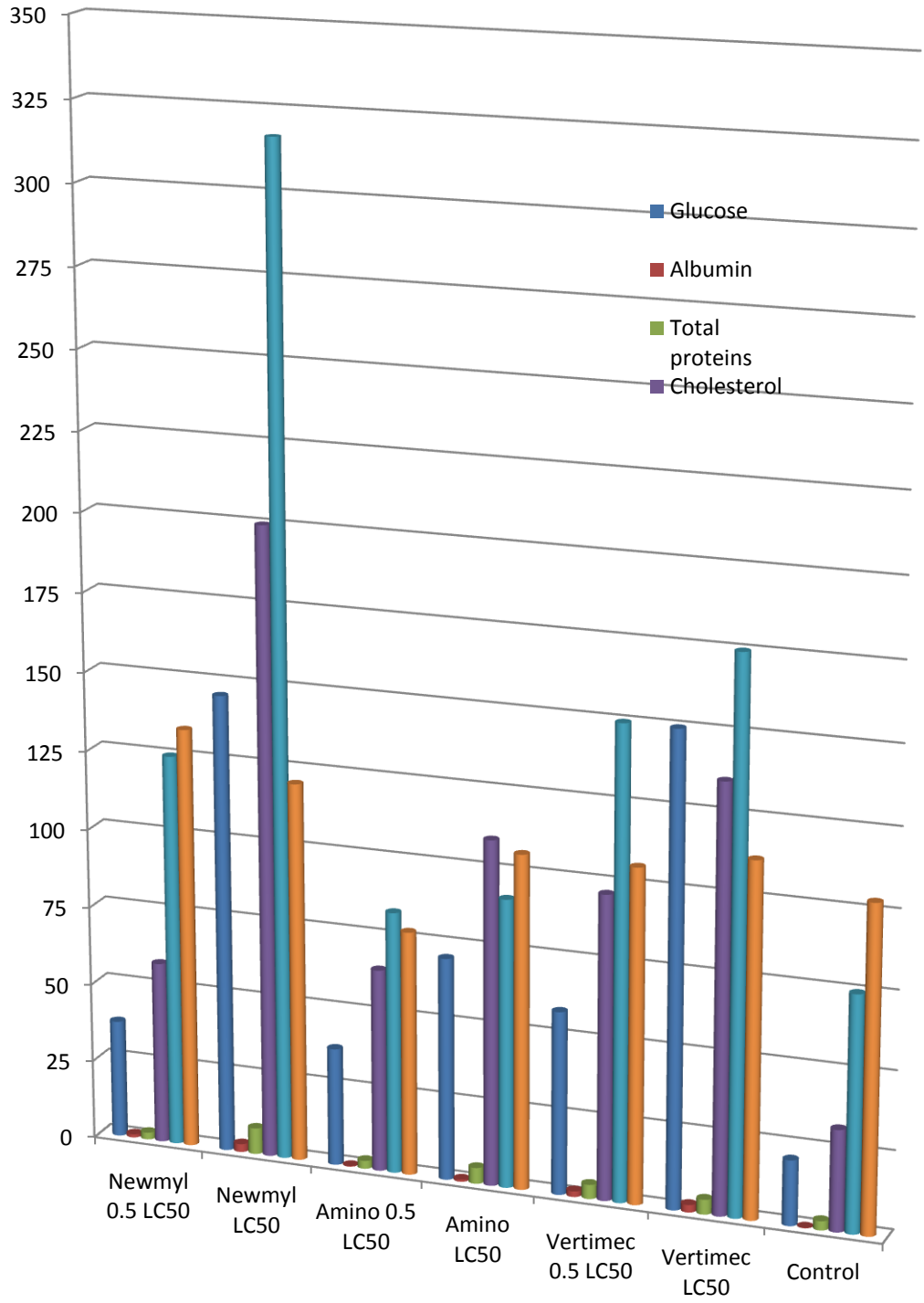


Fig. (74) Histogram illustrating the biochemical parameters of *E. vermiculata* after treatment with Newmyl, Amino and Vertemic compared with control .

4.2 *M. cartusiana*

As seen in table (16) the biochemical values for control samples were as follows: glucose 133.33 mg/dl, albumin 2.47 g/dl, total portions 4.91 g/dl, cholesterol 103.23 mg/dl, triglycerides 250 mg/dl and urea 83.04 mg/dl.

Table (16) Biochemical parameters of *M. cartusiana* after treatment with LC_{50} and 1/2 LC_{50} of Newmyl, Amino and Vertimec

	Conc.	Glucose	Albumin	Total proteins	Cholesterol	Triglycerides	Urea
Control		**133.33**	**2.47**	**4.91**	**103.23**	**250**	**83.04**
The pesticide							
Newmyl	1/2 LC_{50}	45.83	2.13	7.09	109.68	125	129..46
	LC_{50}	75	3.45	9.45	167.74	283	141.96
Amino	1/2 LC_{50}	25	1.45	4.73	70.97	100	96.43
	LC_{50}	66.67	2.98	8.00	83.87	125	107.14
Vertimec	1/2 LC_{50}	33.33	0.77	3.45	70.97	91.67	26.79
	LC_{50}	66.67	2.38	4.91	200	175	111.61
LSD = 0.001		3.3807 ***	3.2157 Ns	3.3807 ***	3.3807 ***	3.3807 ***	3.3977 ***

*** = highly significant, ns =non- significant

Treatment of *Monacha cartusiana* with low concentration of Newmyl (1/2 LC_{50}) showed that the biochemical parameters of glucose and triglycerides were highly significantly ($p \leq 0.001$) decreased comparing with the control values (45.83 mg/dl, and 125 mg/dl, respectively). As for albumin, it was decreased non-significantly, whereas the total proteins, cholesterol and urea were highly significantly increased. Treatment of snails with Newmyl LC_{50} showed that most tested parameters were highly significantly increased but albumin increased non-significantly. Glucose was the exception because it was highly significantly decreased (75 mg/dl) comparing with control.

The application of Amino 1/2 LC_{50} led to highly significant decrease in all biochemical parameters and non-significant decrease in albumin comparing with control. Urea was the single exception as it revealed significant increased giving value of 96.43 mg/dl. Application of Amino

LC_{50} caused a highly significant decrease in glucose, triglycerides and cholesterol comparing with control. Total proteins and urea were highly significantly increased (8.09 g/dl and 107.14 mg/dl, respectively). The albumin parameter was decreased non- significantly comparing with control.

When 1/2 LC_{50} of Vertimec was applied to *M. cartusiana* all parameters were highly significantly decreased and albumin was also decreased but non-significantly comparing with control. When Vertimec LC_{50} was applied after 72 hrs glucose, triglycerides and total proteins were highly significantly decreased and albumin was decreased but non-significantly comparing with control. The values of cholesterol and urea were highly significantly increased (200 mg/dl and 111.61 mg/dl, respectively).

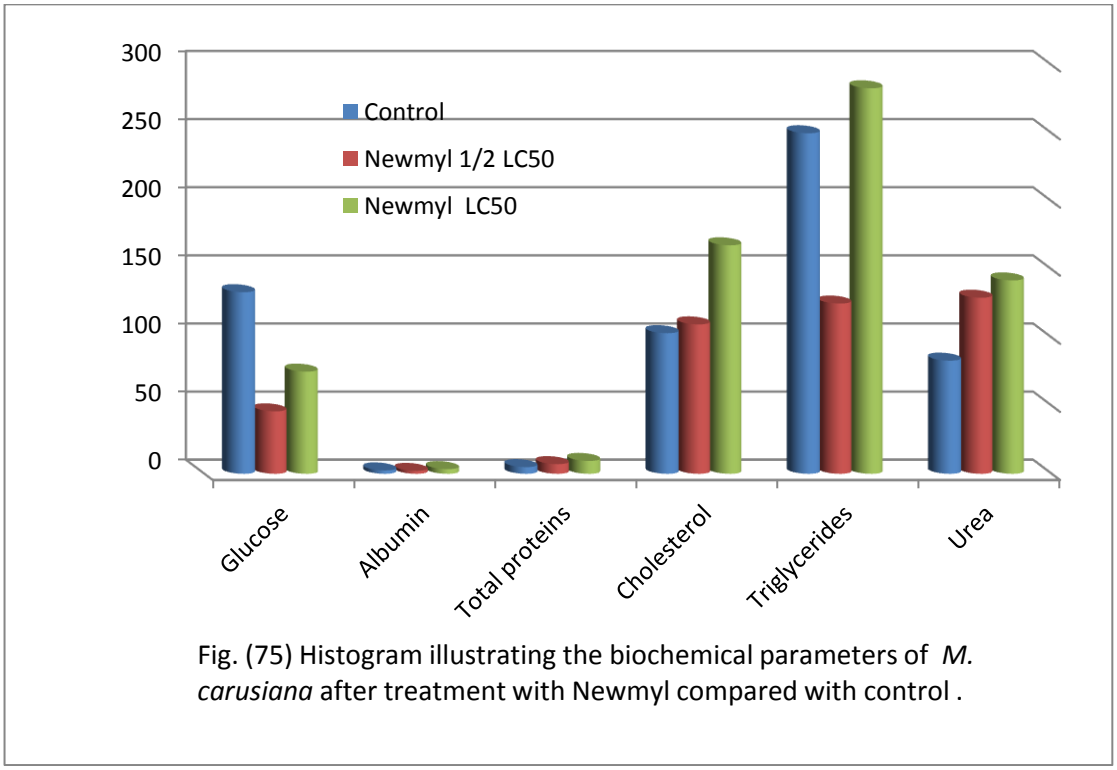


Fig. (75) Histogram illustrating the biochemical parameters of *M. carusiana* after treatment with Newmyl compared with control .

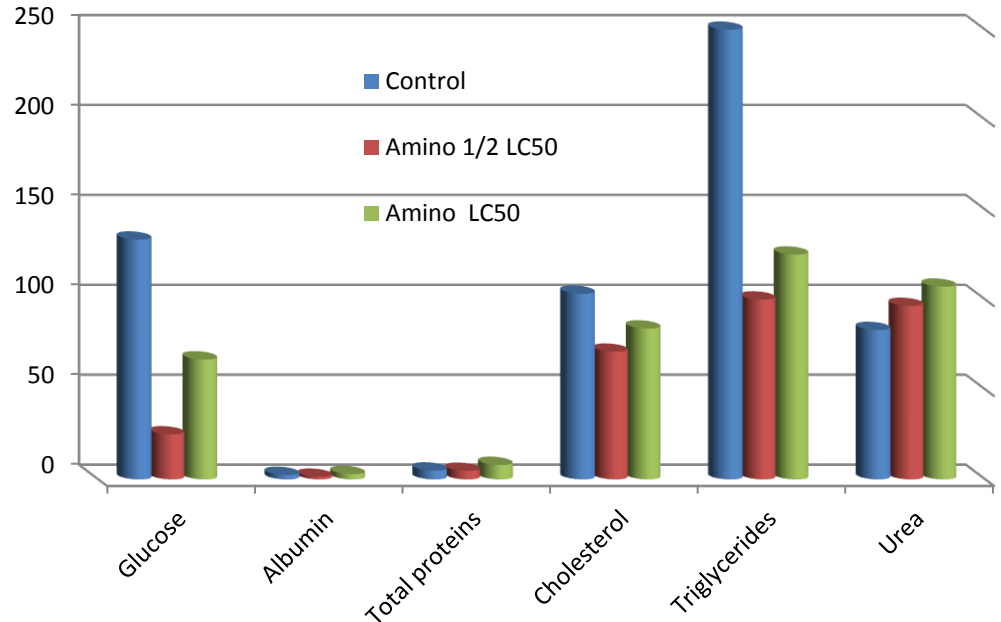


Fig. (76) Histogram illustrating the biochemical parameters of *M. carusiana* after treatment with Amino compared with control .

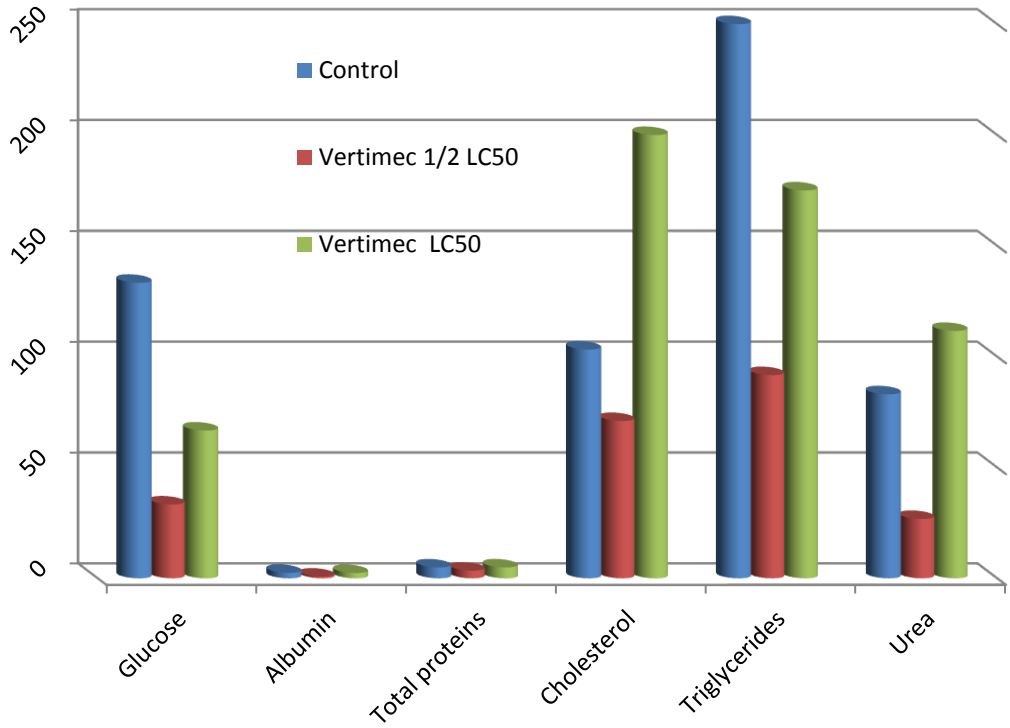


Fig. (77) Histogram illustrating the biochemical parameters of *M. carusiana* after treatment with Vertimec compared with control .

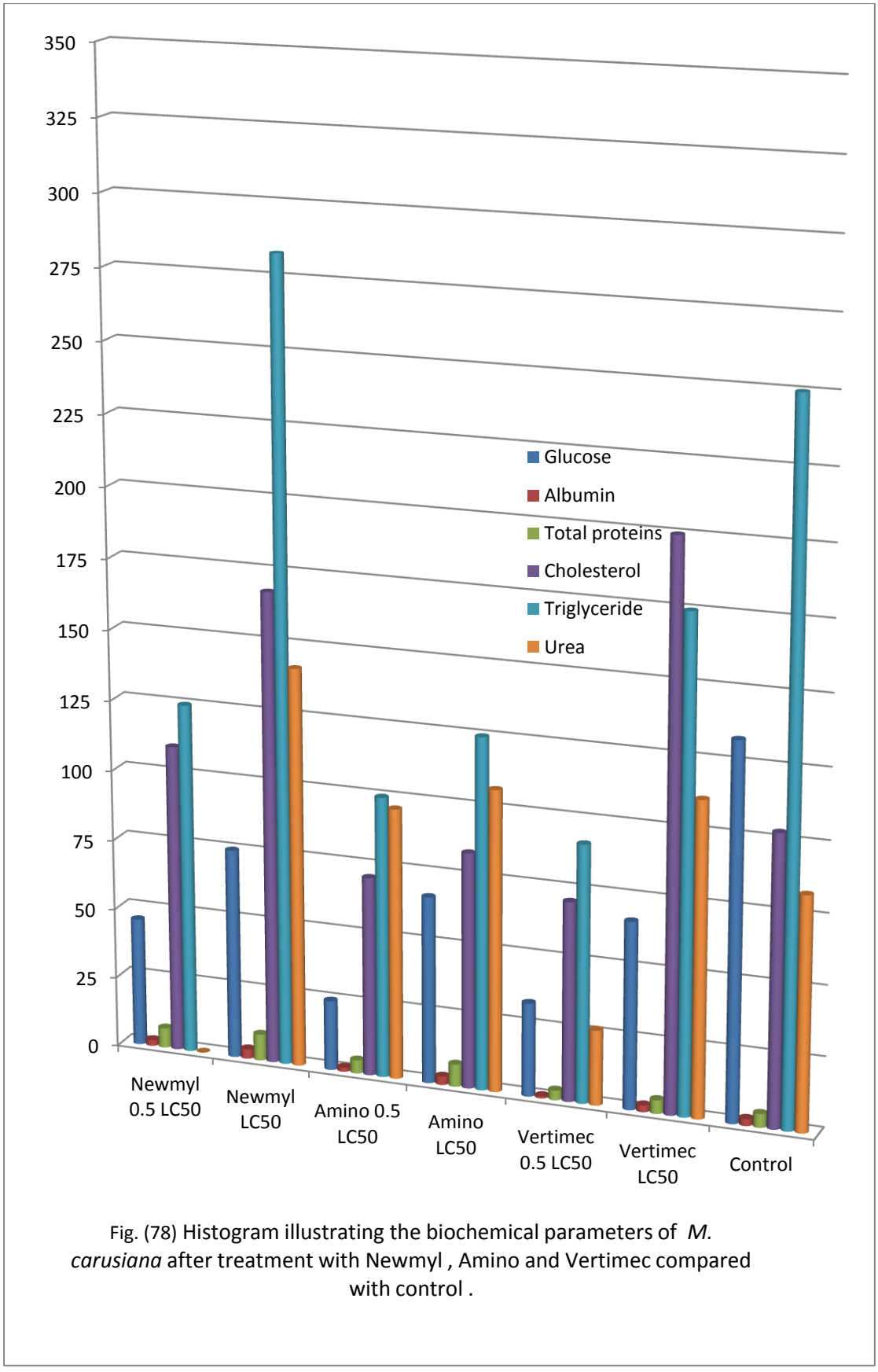


Fig. (78) Histogram illustrating the biochemical parameters of *M. carusiana* after treatment with Newmyl , Amino and Vertimec compared with control .

V-Field experiment

The field experiment was applied in Egyptian clover field highly infested with *M. cartusiana* due to snail wide dispersion in Sharkia Governorate. In this experiment only the highest concentrations of each compound was applied against *Monacha cartusiana*.

5.1- Reduction percentages of chemical compounds

Table (17) shows the reduction percentages in *M. cartusiana* following exposure to insecticides (Newmyl, k-othrine and cyflu-magic) and fertilizers (Amino, humic acid, combining Zn, micronized sulfur, urea and potassium thiosulfate) and the herbicide (fusillade).

Table (17) The reduction percentages of chemical compounds as poisonous baits against the land snail *M. cartusiana* under field conditions.

Chemical compounds	Conc. %	Reduction percentages						
		1day	3day	Initial effect	7 day	14 day	21 day	Residual effect
Newmyl	4	76.1	85.2	80.65	93.2	94.4	98.1	95.23
Amino	4	45.7	63.9	54.8	80.1	45.7	74.2	66.67
Humic acid	4	58.3	62.5	60.4	84.9	53.2	70	69.37
Cyflu-magic	2	65.3	68.8	67.05	72.9	83.3	86.8	81
Fusilade	8	40.3	33.6	36.95	40.3	78.3	79.8	66.13
Micronized sulfur	2	75.6	59.5	67.55	77.6	59.8	78.7	72.03
K-othrine	4	47.9	53.2	51.25	38.4	73.4	96.4	69.4
Potassium thiosulfate	5	24.5	57.4	40.95	37.4	63.8	84.3	61.83
Combining Zn	4	16.1	17.8	16.95	5.5	84.3	91	60.27
Urea	2.4	74.9	80.7	41.8	91.5	96.4	97.8	85.27
LSD =1/2	-	0.17031 ***	0.1645 ***	-	0.1703 ***	1.2099 ***	0.1703 ***	-

*** = very highly significant

The table (17) showed the reduction percentages and initial effect as well as the residual effect produced after application of tested chemical compounds after 1, 3, 7, 14 and 21 days. Newmyl recorded reduction percentages of 76.1, 85.2, 93.2, 94.4 and 98 %. The initial effect of Newmyl was 80.65 and residual effect was 95.23, respectively. Whereas Amino reduction percentages recorded 45.7, 63.9, 80.1, 45.7 and 74.2 % with initial effect of 54.8 and residual effect of 66.67, respectively. Then, humic acid reduction percentages were 58.3, 62.5, 84.9, 53.2 and 70 % with an initial effect of 60.4 and residual effect of 69.37, respectively. Cyflu-magic was 65.3, 68.8, 72.9, 83.3 and 86.8 % with its initial was effect 67.05 and its residual effect was 81, respectively. Then, fusillade reached 40.3, 33.6, 40.3, 78.3 and 79.8 % with initial effect of 36.95 residual effect of 66.13.

Micronized sulfur recorded reduction percentages of 75.6, 59.5, 77.6, 59.8 and 78.7 % with an initial effect of 16.95 and residual effect of 60.27, respectively. Then, k-othrine recorded reduction percentages of 47.9, 53.2, 38.4, 73.4 and 96.4% with an initial effect of 51.55 while its residual effect was 69.4, respectively. Potassium thiosulfate reduction percentages reached 24.5, 57.4, 37.4, 63.8 and 84.3 % reduction percentages with an initial effect of 40.95 and residual effect of 61.83. The combining Zn reduction percentages were 16.1, 17.8, 5.5, 84.3 and 91 % with its initial effect was 16.95 and the residual effect 60.27, respectively. Urea reduction percentages were 74.9, 80.7, 91.5, 96.4 and 97.8 % while its initial effect was 41.8 and residual effect was 85.27, respectively.

5.2- Reduction percentages of biocides

Table (18) shows the reduction percentages of the biocides (Vertimec, Protecto, Bioranza and Biover) as poisonous baits against the land snail *M. cartusiana* comparing with Newmyl.

Table (18) The reduction percentages of biocides as poisonous baits against the land snail *M. cartusiana* under field conditions.

Biocides	Conc. %	Reduction percentages						
		1day	3day	Initial effect	7 day	14 day	21 day	Residual effect
Newmyl	4	76.1	85.2	80.65	93.2	94.4	98.1	95.23
Vertimec	4	69.3	76.4	72.85	79.1	77.2	84.7	80.33
Protecto	6	53.2	69.3	61.25	84.9	74.7	83.1	80.90
Bioranza	4	69.9	73	71.45	84.4	96	96	92.13
Biover	4	58.3	65.9	62.1	91.9	91.2	99.1	94.07
LSD =1/2	-	0.1882 ***	0.1882 ***	-	0.1882 ***	0.1882 ***	0.1882 ***	-

*** = very highly significant

The table (18) showed the reduction percentages and initial effect as well as the residual effect produced after application of the tested biocides after 1, 3, 7, 14 and 21 days. The biocides concentrations used were Vertimec 4 %, Bioranza 4 %, Biover 4 %, and Protecto 6 %. Vertimec reduction percentages were 69.3, 76.4, 79.1, 77.2 and 84.7 % , respectively. The initial effect of Vertimec was 72.85 and the residual effect was 80.33. Protecto reduction percentages were 53.2, 69.3, 84.9, 74.7and 83.1 %, respectively. Its initial effect was 61.25 and its residual effect was 80.90. Where Bioranza reduction percentages were 69.9, 73, 84.4, 96 and 96 %, respectively. The initial effect of Bioranza was 71.45 and its residual effect was 92.13. Biover reduction percentages were 58.3, 65.9, 91.9, 91.2 and 99.1 %, respectively. Its initial effect was 62.2 and the residual effect was 94.07.

Chapter VI

Discussion

I-Toxicity test

Many investigators evaluated the toxicities of insecticides against *Eobania vermiculata* and / or *Monacha sp.* Under laboratory or filed conditions (**Ghamry *et al*., 1994; Awad, 1994; Okka *et al*., 1996; Ismail, 1997; Hanafy *et al*., 1998; Aioub *et al*., 2000; Abd El-Aal, 2001; Heiba *et al*., 2002; Mobarak, 2003; Abd El- Wahab, 2004; Abdel Nabey and Shaban, 2006; Hamed *et al*., 2007; Ismail and Mohamed, 2009**).

In the present toxicity test, Newmyl was used as a reference insecticide to compare its toxicity with those of the other applied compounds. The toxicity of Newmyl was high either with *E. vermiculata or M. cartusiana*. The reference insecticide caused 100 % mortalities to both snails after 2 weeks with the low and median expertized concentrations (1 and 2 %). The highest concentration (4 %) of Newmyl produced the highest mortality rate of *E. vermiculata* after 2 weeks but this percentage of mortality was attained after only one week in case of *M. cartusiana*. This result reflects that *M. cartusiana* was more susceptible to Newmyl than *E. vermiculata*.

Nearly, the toxicity action of Amino is similar to that of Newmyl. One hundred percent of mortality was reached in case of *E. vermiculata* after 2 weeks with all concentrations used (1, 2 and 4 %). In case of *M. cartusiana* 100 % mortality was attained after 2 weeks in the low and median concentrations (1 and 2 %), whereas this percent occurred after 1 week in case of 4 % Amino.

Abd El-Wahab (2004) found that Vertimec came secondly to methomyl in toxicity to *Monacha cartusiana* and *Eobania vermiculata*

when used as baits. **Helmy *et al.* (2006)** in a laboratory experiment showed that Newmyl exhibited the highest toxic effect against *M. cartusiana* followed by Vertimec biocide.

As for the toxicity of Vertimec biocide, it showed the highest toxic action on both snail types in comparison with the other applied biocides (Protecto, Bioranza and Biover). The highest mortality percent (100 %) was recorded in case of *E. vermiculata* after 2 weeks in the low and median concentrations (1 and 2 %). This percent of complete mortality (100 %) was attained after one week in case of the highest concentration of Vertimec (4 %). This result is similar to that obtained with the reference insecticide Newmyl. Results of the toxicity test revealed that *M. cartusiana* was highly sensitive to Vertimec biocide. One hundred percent mortality occurred after one week with the applied concentrations (1, 2 and 4 %).

Thus, Vertimec was more toxic to *M. cartusiana* than Newmyl. Vertimec with higher concentrations (2, 4 and 8 %), i.e. double the present concentrations, caused 100 % mortality for *M. cartusiana* and *E. vermiculata* after 9 days of baiting **(Yousef, 2001).**

Newmyl which was used in the present study as a reference insecticide showed the highest toxic action on both studied snails (*E. vermiculata* and *M. cartusiana*). It was more toxic than Vertimec biocide. **Azzam** in **2005** found that Newmyl with a concentration of 90 % displayed the highest toxic effects on *Monacha cartusiana* comparing with other 5 pesticides. However, 20 % of Newmyl and 1.8 % of Vertimec exhibited the least action against the same snail.

The present toxicity findings are in accordance with those of **Helmy *et al.* (2006)** with the same two land snails and using the poisonous bait technique. Newmyl applied in the laboratory experiment exhibited the highest toxicity action followed by Vertimec. This was also the case

obtained by **El-Sayed (2010)** where there were no snails (*E. vermiculata* and *M. cartusiana*) survivals recorded with the highest concentration of Newmyl (90 %) after only 24- hrs post treatment.

On the other hand, **Radwan *et al.* (2008)** recorded higher mortality rate in *E. vermiculata* by using the topical application technique with methomyl than in case of the poisonous bait technique. Nevertheless, **Samy *et al.* (2015)** showed that Newmyl when applied according to the leaf dipping technique in lettuce and cabbage infested by *Monacha* sp. displayed high toxicity action. It seems likely that the poisonous baits technique is an effective one in controlling the terrestrial or land snails. **Ismail *et al*. (2014)** proved that the poisonous baits application method was more effective than the spraying method when evaluated the efficacy of methomyl against *Monacha cartusiana*. However, **Kandil *et al*. (2014)** found that the combination of methomyl and acetylsalicylic acid enhanced the molluscicidal activity of spraying method against *E. vermiculata* and *M. cartusiana*.

Earlier, it has been pointed out **(El-Sebae, *et al.* 1982)** that when 0.15 %. methylene blue was added to the insecticide baits, the toxicity potency to *E. vermiculata* was increased.

It seems likely that Vertimec biocide toxicity to the 2 land snails under investigation (*E. vermiculata* and *M. cartusiana*) was higher than classical insecticides (curacron, dursban and Marshal) but methomyl was more toxic than Vertimec **(Abd El-Wahab, 2004)**.

In accordance to the above finding, Vertimec applied in the present study showed higher toxicity than Newmyl because the highest concentration (4 %) killed all *E. vermiculata* snails after one week. The same concentration (4 %) and the lower concentrations (1 and 2 %) killed all the individuals of *M. cartusiana* within one week. It has also been shown that toxicity of insecticides, fertilizers and biocides is dose and

time dependent. Results of the laboratory experiment reflect this concept. Newmyl which is considered as the reference insecticide showed 100 % mortality against *M. cartusiana* after 2 weeks when the concentration was 1 or 2 %. In case of the highest concentration (4 %) the 100 % mortality occurred after one week. With the same snail, Amino (fertilizer) exhibited the same mortality percent (100 %) after the same periods (2 weeks with concentrations 1 % and 2 % and one week with concentration of 4 %). In case of *E. vermiculate* the 3 concentrations (1, 2 and 4 %) from either Newmyl or Amino caused 100 % mortalities after 2 weeks of treatment.

Lokma (1998) documented that the snail (*M. cartusiana*) mortality increased with increasing the concentration and exposure period.

Concerning toxicity of Vertimec biocide, 100 % mortality occurred in *E. vermiculata* after 2 weeks with the low and intermediate concentrations (1 and 2%) the highest concentration (4 %) produced the 100 % mortality after only one week. Vertimec with *M. cartusiana* was highly effective as it caused 100 % mortality after one week with the 3 applied concentrations (1, 2 and 4 %).

This finding proved that *M. cartusiana* was more sensitive to Vertimec than *E. vermiculata*. In this concern, **Arafa (2006)** reported that the mortality percentage of biocides increased by increasing concentrations and exposure period.

In their experimental study, **Helmy *et al.* (2006)** illustrated that Vertimec exhibited the highest toxic action against *E. vermiculata* snails followed by Newmyl. The present investigation is confirmatory to that finding because 4 % of Vertimec caused 100 % mortality of *E. vermiculata* snails after only one week, whereas the same concentration (4 %) of Newmyl caused 100 % mortality after 2 weeks of treatment.

As for Protecto toxicity, results of the laboratory experiment displayed that *E. vermiculata* was more sensitive to this biocide than *M.*

cartusiana. The lowest concentration of Protecto (1.5 %) killed all snails within 3 weeks. The same concentration when applied against *M. cartusiana* did not produce 100 % mortality till the end of 4 weeks which is the longest application period. However, the highest concentration (6 %) killed all snails from both species.

El-Said (2009) evaluating the efficacy of Protecto and Biover comparing with Newmyl against *M. cartusiana* put Protecto (67 % mortality) secondly to Newmyl (100 % mortality) after 3 weeks of baiting. Biover was the least one (60 % mortality). However, Protecto was found to be the least effective compound, between 5 molluscicides, against *M. cartusiana* **(Lokma, 1998)**.

2-Histological and histopathological observations:-

As for the histological pattern of the digestive gland, 3 cell types were illustrated in glands of both studied snails. These cells were differentiated into digestive cells, excretory cells and calcium cells. This is different from the results of **El-Mahrouki (1991)** who described only two types of cells; digestive and excretory cells in the digestive gland of *Eobania vermiculata*. To the contrary, **Dimitreiadis *et al.* (1992)** recorded 3 cell types**;** digestive, excretory and calcium cells in the digestive gland epithelium of *Helix sp.* Likewise, **Besnaci *et al.* (2016)** described 3 types of cells including digestive, excretory and calcium cells. **Zaldibar *et al.* (2007)** pointed out that digestive cells constitute the most abundant cellular component of the digestive gland but the calcium and excretory cells are fewer. In *E. vermiculata* we noticed these 3 cell types and this is in accordance with the description of other scientists in the same snail specie **(Mersal, 1990; Aioub *et al.*, 2000; Heiba *et al.*, 2002; Hemmaid and Mohammadein, 2003; Abo Bakr, 2011)**.

However, there was a little agreement and high controversy about number, nomenclature and functions of cell types constituting the epithelium of the digestive gland of gastropods. Many investigators described two cell types namely: digestive and basophilic cells in digestive gland of *E. vermiculata* **(Saad and Farag, 1988)** and *Littorina littered* **(Zaldibar *et al*., 2007).**

Al-Zahaby *et al.* (1993) also described 2 cell types; digestive and excretory, in the digestive gland of *E. vermiculata*. However, most studies were in agreement with findings of the present study that epithelium of the digestive gland is composed of 3 cell types: digestive cells, excretory cells and calcium cells **(Dimitriadis, 2001; Chabicovsky *et al.*, 2004; Snyman *et al.*, 2005; Abo Bakr, 2011; Besnaci *et al.*, 2016). Heiba *et al.* (2002)** reported the presence of digestive, excretory and secretory cells in the digestive gland of *E. vermiculata*.

Other studies added a fourth cell type which was named as the thin cells in the gland of some gastropods **(Brooks and White, 1995; Beshr, 2000; Hamed *et al.*, 2007).**

Thus, concerning the types of the snail's digestive gland cells there was a controversy present in different investigations. However, due to present investigation we are in complete believe with the side of occurrence 3 cell types.

In the present study, the two snail species were treated with Newmyl, Vertimec and Amino. These treatments led to an increase in the number of excretory granules. These granules were also larger in size and darker than those of control cells. In the digestive gland of *E. vermiculata*, **Aioub *et al.* (2000)** found that oxamyl insecticide caused loss of the excretory substance from excretory cells. They pointed out that many of these cells appeared empty of any excretory material. A clear increase in the size of excretory vacuoles of the excretory cells was

recorded in the gland of *E. vermiculata* due to treatment with the carbamate insecticide oxamyl.

Digestive cells investigated form glands of treated snails showed cytoplasmic degenerations. These were wide and conspicuous and the whole tissue due to plenty of the digestive cells was apparently perforated. In the insecticidal experiment by lannate carried out by **Heiba *et al.* (2002)** digestive cells of *E. vermiculata* and *M. cartusiana* showed vacuolation and they were swollen due to the presence of numerous yellowish brown granules (residual bodies). Hypertrophied cells were also observed by **Hemmaid and Mohammadein (2003)** in the digestive gland of *E. vermiculata* treated with oxamyl. The authors also recorded degenerative cytoplasmic regions. Likewise, **Abo Bakr (2011)** observed an extensive vacuolation in the cytoplasm of digestive cell of *E. vermiculata* due to baiting by metaldehyde molluscicide. Such vaculoation was accompanied by degeneration and necrosis in the lining epithelium of the digestive tubules.

Exposure of snails under study to the reference insecticide Newmyl, Vertimec biocide and Amino fertilizer showed obvious alterations. The excretory cells of the digestive gland of both *E. vermiculata* and *M. cartusiana* displayed accumulation of a huge number of the excretory granules. Residual bodies inside the excretory cells were larger in size and darker in staining. Likewise, **Hamed *et al.* (2007)** recorded an aberrant increase in the number of excretory cells in the digestive gland of *E. vermiculata* exposed to methomyl and methiocarb. In this concern, **Radwan *et al.* (2008)** investigating effects of these 2 insecticides on the same snail pointed out that methomyl revealed greater efficacy than methiocarb after topical or baiting techniques.

Moreover, damaged and atrophied glandular acini of *M. cartusiana* were observed by **El-Said (2009)** due to Protecto and Biover treatment.

Such pathological alterations were accompanied with disappearance of glandular and calcium cells.

The recorded histopathlogical qualitative changes in the digestive gland at the cellular level surely alter the functions of such cells. Treatment with the chemical compounds cause very significant cytological alterations in the gland. The hepatopancreas plays a crucial role in the detoxification of pollutants (**Frias-Esperi-cueta *et al.*, 2008**). The deterioration of digestive cells entail altering the digestive process. This could be the first biological response due to the presence of xenobiotics (**Chabicovsky *et al*., 2004; Boucenna *et al*., 2015**).

Radwan *et al*. (2008) pointed out that the digestive gland represents the main target organ for molluscicide impact. Hence, the reactions following application of these compounds should be investigated.

Under the effect of treatment with the present compounds (Newmyl and the other comparable biocides and fertilizers) nuclei of cells of the digestive gland of both snails were suffered from pyknosis. Similar nuclear changes were observed by **Hamed *et al.* (2007)** in nuclei of cells of the digestive gland of *E. vermiculata* following methomyl and mithocarb treatment. They recorded the presence of bizarre nuclei exhibiting ranged degenerative changes from karyolysis to severe karyorrhexis and complete pyknosis. Also, **Sharaf *et al.* (2013)** illustrated pyknosis of glandular cells nuclei of the land snails due to pesticicdal exposure.

As for the intestine results showed that the mucosa of both snails responded to various molluscicidal treatment by similar pathological changes. Columnar cells of the intestine of the treated snails were vacuolated and parts of the free surface were dissociated. Degenerations and oedem were detected in the submucosa. To a large extent these deteriorations were illustrated under the effect of the reference insecticide

Newmyl and Vertimec biocide and Amino fertilizer. In spite of the absence of studies concerned with pathological changes in the intestinal mucosa of land snails, a few studies were related to the normal intestineal mucosa **(Lobo and Batista, 2005; Lobo *et al*., 2011)**.

Following Niclosamide treatment the intestine of the snail *Biomphlaria glabrata* showed histological changes represented by the appearance of gaps between the epithelial cells and the connective tissue **(Zhou *et al*., 1993)**. The formation of these gaps resulted in the derangement of the ciliated cells. **Mane *et al*. (1979)** pointed out that pesticides led to the loss of the connection between mucous as well as ciliated cells from one side and the muscle fibers of the intestine.

3-Ultrastructural changes in the digestive gland

At the ultrastructural level, the digestive glands of the present two terrestrial snails were harboring 3 types of cells. Investigation of the glands of both species revealed the presence of digestive cells, excretory cells and calcium cells. On the other hand, **Arrighetti *et al*. (2015)** described the presence of only two types of cells subjected to cyclical changes in the digestive gland of the giant predator snails. The authors called these cell types as digestive and vacuolated cells (sometimes called basophilic or cryptic cells). **Costa (1994)** illustrated three cell types: digestive, basal and excretory cells in the digestive gland of *Sepia officinalis*.

Hemmaid and Mohammadein (2003) found also three cell types in the digestive gland of *E. vermiculata.* The authors called these cells as digestive, excretory and calcium cells. In the digestive gland of gastropods some investigators have reported the presence of more cell types (**Taieb and Vicentea, 1999; Dimitriadis and Andrews, 2000; Taieb, 2001; Gros *et al*., 2009**).

The ultrastructure of cells in the digestive glands of gastropod mollusks were studied **(Walker, 1970; Franchini and Oltaviani, 1993; Rebecchi *et al*., 1996; Taieb and Vicentea, 1999; Lobo-da-Cunha, 2000; Chaki and Misra, 2004; Arrighetti *et al*., 2015; Nath *et al*., 2015; Ojeda *et al*., 2015).**

The ultrastructural characteristics of the digestive cells of *E. vermiculata* are nearly similar to those of other gastropods. Earlier, **Boghen and Farley (1974)** described similar fine structure in cells of *Littorina saxatilis.* Such similarity was also reported by many investigators in *Helix sp*. **(Dimitriadis and Hondros, 1992),** *Planorbis corneus* **(Franchini and Oltaviani, 1993),** *Aplysia depilans* **(Lobo-da-Cunha, 2000),** *Achatima fulica* **(Chaki and Misra, 2004)** and *Laevicaulis alte* **(Nath *et al*., 2015).**

Recently, **Arrighetti *et al*. (2015)** and **Ojeda *et al*. (2015)** suggested multiple roles for digestive cells of snails and pointed out that data on digestive cells functions are incomplete.

Under the effects of any of the applied compounds (Newmyl, Vertimec and Amino) the cytoplasm of the digestive cells responded by degenerations. Such degenerative reaction was variable according to the applied dose (1/2 LC_{50} or LC_{50}) and the compound expertized.

Application of the LC_{50} exhibited wider cytoplasmic degenerations than in case of 1/2 LC_{50}. The most drastic cytoplasmic degenerations were observed in case of Newmyl followed by Vertimec and lastly came Amino. Severe cytoplasmic vacuolization were observed in digestive cells of *E. vermiculata* after 14 days of topical application of methomyl **(Hamed *et al*., 2007).**

Oxford and Fish (1979) considered that the release of hydrolytic enzymes from the cytoplasm of digestive cells was related to an increase in the rate of cell autolysis.

The digestive cells of *E. vermiculata* contained a huge number of mitochondria distributed throughout the cytoplasm and many mitochondria were located in the apical part of the cytoplasm. **Nath *et al*. (2015)** postulated that the number and position of mitochondria may reflect the activity of the cell.

In many studies on digestive cells of gastropods, mitochondria were localized around the basal nucleus **(Walker, 1970; Boghen and Farley, 1974; Dimitriadis and Hondros, 1992).** Mitochondria were also demonstrated in the apical portion of digestive cells **(Chaki and Misra, 2004; Nath *et al*., 2015)**.

Mitochondria of the digestive cells revealed coalescence and acquired pleaomorphism without discrimination of their cristae following treatment with the chemical compounds under experimentation. However, **Hamed *et al*., (2007)** demonstrated hyperdenisty of the mitochondrial matrix (mitochondria pyknosis) and the appearance of ballooned cristae in digestive cells of *E. vermiculata* due to methomyl treatment. **Goel and Dhawan (2001)** attributed such alterations to decrease in the mitochondrial energy production in addition to interaction of the toxin with membrane components. This interaction may lead to changes in ion transport along the mitochondrial membrane.

After molluscicidal treatment with the present compounds, there were changes in nuclei of digestive cells. The electron micrographs investigated from the digestive cells of *E. vermiculata* snails treated with Newmyl, Amino or Vertimec showed nuclear perturbations. Some nuclei displayed enlargement or hypertrophy accompanied with the appearance of weak to moderately osmiophilic nucleoplasm. Nuclei of some other cells were indented by deep clefts acquiring a wavy contour.

In the digestive cells of *M. cartusiana* snail treated with the same chemical compounds, Vertimec biocide or Amino fertilizer, the nuclei

were also altered. The nucleoplasm revealed moderate osmiophilia and deep osmiophilic heterochromatin. Indentation of the nuclei and thinning of the marginated heterochromatin were sometimes illustrated. In the digestive cells of *E. vermiculata* treated with methomyl, **Hamed *et al.*, (2007)** demonstrated compaction and margination of nuclear chromatin. The nuclear envelope displayed dilation and presence of nuclear clefts. Such deteriorations in the irregularly-shaped nuclei were accompanied with lightening of the karyoplasms and reduction of the heterochromatin. The authors also described gradual degenerative changes in the nuclei of digestive cells ranged from karyolysis to severe kryorrhexis and complete pyknosis. **Bayne *et al*. (1985)** considered karyolysis as a late reaction to intoxication in both vertebrates and invertebrates. At the same time, **Tribskorn (1989)** pointed out that the reaction of nuclei is a primary cell response that leads to cell death. This was also confirmed in many studies **(Triebskorn and Kunast, 1990; Tribskorn *et al*. 1996).**

As for the excretory cells of the digestive gland the action occurring following the present baiting treatment was the appearance of large excretory vacuoles filled with abundant deeply osmiophilic excretory granules. The excretory vacuoles number and size were dose-dependent as these were larger when the LC_{50} was applied than after 1/2 LC_{50}. Due to application of the present chemical molluscicides, excretory cells in the digestive glands of the experimental large excretory vacuoles filled with strongly osmiophilic granules of variable shape and size. Other excretory vacuoles were depleted of their granules and appear nearly empty of granules except some remnants of these granules with moderate osmiophilia.

However, the contained granules clumped inside such vacuoles were variable in size and osmiophilia in different treatment chemical compounds (Newmyl, Vertimec and Amino). In *E. vermiculata* exposed

to carbamate pesticide oxamyl, the excretory cells showed obvious increase in the size of their excretory vacuoles **(Hemmaid and Mohammadein, 2003)**. The increase in the number and size of the excretory vacuoles as well as the increase in the contained granules reflects the role of these cells in detoxification process. Similar increase in the number of excretory granules was also illustrated in methomyl-treated *E. vermiculata* **(Hamed *et al.*, 2007).** Methomyl application topically or by toxic baiting technique to *E. vermiculata* resulted in the enlargement of yellow granules or residual bodies **(Hamed *et al.*, 2007).** These authors pointed out that the apparent rise in the number of residual bodies suggests accumulation of the tested molluscicide in these granules due to intoxication. Various studies were achieved for demonstrating the effects of insecticides or molluscicides on the digestive gland of snails **(Miller *et al.*, 1988; Radwan *et al.*, 1992; Schuytema *et al.*, 1994; Aioub *et al.*, 2000**). Toxicological effects on digestive cells of *E. vermiculata* had been declared by the electron microscope under the effect of oxamyl pesticide (**Hemmade and Mohammadein, 2003**) and methomyl as well as methiocarb (**Hamed *et al.*, 2007**).

The ultrastructural changes recorded here in cells of the digestive gland of *E. vermiculata* and *M. cartusiana* following treatment with either of the three chemical compounds were mostly similar and of nearly identical appearances. Digestive cells suffered from cytoplasmic degenerations leading to aberrant vacuolization. Such vacuolization may render the cells to appear perforated and had leached appearance devoiding of a lot of its ground cytoplasm. The vacuolated digestive cells were believed to be primary originated from the digestion of food substances absorbed by these cells from the lumen of the gland **(Reader, 1976).**

Digestive cells of gastropods seem to be responsible for nutrient absorption via endocytosis during the absorption phase (**Henry *et al.*, 1991; Dimitriadis and Andrews, 2000; Arrighetti *et al.*, 2015**).

4-Changes in biochemical parameters

Concerning the effects of the applied compounds on the biochemical parameters of the experimental snails, variable and fluctuated results were recorded. In case of Newmyl as a reference insecticide it caused changes in the values of the studied parameters ranged between highly significant to significant or sometimes very highly significant. All the studied parameters recorded degrees of increase when 1/2 LC_{50} or the LC_{50} concentrations were applied. In case of the LC_{50} albumen, glucose and cholesterol showed the highly significant increase where they recorded fifteenth, seventh and sixth folds increase, respectively. Cholesterol in *E. vermiculata* and *M. cartusiana* treated with methomyl was reduced after 1 and 8 days and increased after 7 days. The total proteins were decreased after application of 1/2 LC_{50} of Newmyl (2.18 mg/dl) but its value was increased after treatment with the LC_{50} (8.36 mg/dl) which equal nearly to3 folds increase comparing with the control (2.90 mg/dl). The protein contents were significantly decreased in *E. vermiculata* treated with the carbamate pesticides methomyl and methiocarb (**Radwan *et al*., 2008**). The decrease was more obvious after topical application than after using baiting technique. **Kandil *et al*. (2009)** recorded increase in total protein of *E. vermiculata* after 3 and 7 days of methomyl treatment. In case of *M. obstructa* adverse effects were detected. Significant increase in total protein was observed after 1 and 3 days of methomyl treatment then it significantly decreased after 7 days. However, in case of *Monacha obstructa* and *E. vermiculata* treated with abamectin (its commercial name is Vertimec which was used in the present study) the total protein content was increased gradually post 1, 3 and 7 days of treatment. The authors attributed the fluctuation in the level of total protein to the imbalance between the rate of degradation and synthesis. In this concern, **Khater *et al*. (1990)** attributed the increase in the total protein to the

increased biosynthesis process occurred by high enzyme stress. **Ismail *et al*. (2013)** recorded decrease in glucose and total protein during aestivation of *M. cartusiana*. Also, level of glucose and total protein were decreased in *M. cartusiana* due to starvation **(Shahawy, 2005)**. The values of increase in case of triglycerides were variable and the highest increase was recorded after application of the LC_{50} of Newmyl (316.67 mg/dl) comparing with the control (75 mg/dl).

As for urea in *E. vermiculata* all concentrations of Newmyl, Amino and Vertimec recorded an increase in its value except in case of 1/2 LC_{50} of Amino which showed decrease (77.68 mg/dl) in comparison with the control condition (103.57 mg/dl). Urea values were increasing during aestivation of M. cartusiana. Urea was also increased in starvation of *M. cartusiana* **(Shahwy, 2005).**

5- Field experiment

As for the field experiment, it was applied in clover field at Sharkia Governorate infested with the glassy clover snail *Monacha cartusiana*. The reduction percentage was high in case of urea (74.9 % and 80.77 % after 1 and 3 days, respectively) comparing with the reference insecticide Newmyl (76 % and 85.2 % after 1and 3 days, respectively). Cyflu-magic came secondly to urea in the percentage of reduction (65.3 % and 68.8 % after 1 and 3 days, respectively). The least reduction percentage was recorded after treatment with combining Zn (16.1 % and 17.8 % after 1 and 3 days, respectively). The reduction percentage recorded a high value in case of treatment with micronized sulfer (75.6 %) after one day but after 3 days the reduction percentage was lowered (59.5 %). **Ismail *et al*. (2010)** reported that under field conditions, methomyl induced a higher effect on the reduction percentage (73.19 %) than metaldehyd against *M. cartusiana*. Concerning the residual effect the highest value in case of the reference insecticide Newmyl was 995.23 %. Urea came secondly to

Newmyl with a value of 85.27 %, whereas cyfly-magic followed urea with a residual effect value of 81 %.

As for the biocides applied in the present field experiment, the reduction percentage in case of treatment with Vertimec was 69.3 % after one day and 76.4 % after 3 days. Bioranza also registered a high reduction percentage (69.9 % after one day and 73 % after 3 days). According to these reduction percentages value it could be arranged in the second order following the reference insecticide Newmyl (76.1 % and 85.2 % after 1 and 3 days, respectively).

In addition, the residual effect values in case of these biocides were very high in case of Biover (94.07 %) followed by Bioranza (92.13 %). These values nearly around the residual effect value of the reference insecticide Newmyl (95.23 %). The highest toxic action against *M. cartusiana* snails was recorded by Newmyl followed by Vertimec **(Daoud, 2004).**

However, under field conditions, **Ismail (2009)** found that metaldehyde was more effective than methomyl in controlling *M. cartusiana*. Under field conditions it was the highest effective molluscicide against *M. cartusiana*, where the mean reduction percentage was 53.59 % **(Ghaly *et al.*, 2009).** In the present field experiment the molluscicides under experiment were offered as baits on plastic sheets. In this concern, **Ismail *et al.* (2014)** reported that when poisonous baits were put as piles on plastic pieces this was the best technique to reduce numbers of *M.* .*cartusiana* in Egyptian clover field.

Finally, it could be concluded from results of the present work that the digestive gland of gastropods is of prime importance for the snail and is a target organ for toxicants or any xenobioticl entering the body.

Dimitriadis and Konstantinidou (2002) pointed out that the digestive gland of gastropods is concerning with production of digestive enzymes, absorption of nutrients, endocytosis of food substances, food storage and excretion.

Thus, this gland is considered as a multifunctional organ in such terrestrial snails and the deteriorations occurring in it during molluscicidal control programs is responsible for death of the snails. The toxicity produced in snails alters the functions of this important gland and finally leading to their death.

Due to the explosion in using the chemical insecticides for combating and controlling pests, including snails, infesting and the eating the sustainable agriculture and vegetation, risks and hazards are stressing on the ecosystem and environment. Moreover, control programs of land snails with high concentrations of chemical insecticides or molluscicides present bad adverse effects to non-target organisms such as birds, mammals and farm animals. Therefore, there was a need to find and use non-classical compounds such as biocides or fertilizers instead of the dangerous insecticides.

In the current study, biocides represented by Vertimec, Protocto, Bioranza and Biover as well as fertilizer (Amino, humic acid, urea and potassium thiosulphate) were used to evaluate their efficiency against *E. vermiculata* and *M. cartusiana* in comparison with Newmyl insecticide. Nearly, Vertimec biocide and Amino fertilizer were found to be as efficient as Newmyl and surely of little risks on the ecosystem.

The present study clearly revealed the pathological, biochemical and ultrastructural changes in the digestive gland of *E. vermiculata* and

M. cartusiana in response to the applied chemical compounds. The present findings may help in changing the view of those who work in the field of terrestrial gastropods management hoping in using biocides or fertilizers in control programs instead of insecticides. Such trend may help in saving non-target organisms and preserve soil and environment from hazards of chemical insecticides and conserving biodiversity.

Chapter VII

Summary

1-Toxicity test

Controlling the land snails *E. vermiculata* and *M. cartusiana* with using ten chemical compounds (Amino, humic acid, cyflu-magic, micronized sulfur, fusillade, k-othrine WG250, potassium thiosulfate, urea and combining Zn comparing) and four biocides (Vertimec, Protecto, Biover and Bioranza) as poisonous baits under laboratory conditions are investigated. The results showed that, the Amino as chemical compound was the highest toxic compound against *E. vermiculata* while combining Zn was the lowest toxic. As for biocides, Vertimec was the highest toxic biocide and Bioranza were the lowest against the same snail comparing with the reference insecticide Newmyl.

In case of *M. cartusiana* the highest toxic compound was Amino and the lowest toxic one was K-othrine WG250. As for biocides, Vertimec was the highest toxic biocide and Biover was the lowest one comparing with Newmyl.

2-Histopathological observations:-

2.1. The digestive gland

The digestive gland in both tested land snails were a bilobed tubulo-acinar gland located in the dorsal portion of the animal and it was surrounded by a thin membrane composed of a single layer of short columnar cells resting on basal membrane underlied with circular muscle fibers. The digestive glands tissue consists mainly of three cells types (a) digestive cells, (b) excretory cells and (c) calcium cells.

(a) Digestive cells**:**

The major cellular population columnar with flattened or slightly rounded apical surfaces bearing well-developed brush border. Nuclei of digestive cells

are basally-located rounded or oval in outline with condensed chromatin and have a single nucleolus.

(b)Excretory cells:

These cells Characterized by the presence of a single large vacuole filling nearly the whole volume of the cell. The excretory products are accumulated in the vacuoles often in the form of a large brown body (excretory granules).The nucleus was small and usually pressed flat against the cell base.

(c) Calcium cells**:**

Fewer than digestive cells they have pyramidal shape with narrow distal end and a marked broad base. Calcium spherules are round. These cells possess apical secretary granules and large rounded nuclei.That nuclei are rich in heterochromatin and possess nucleolus and usually located near the center or in the basal half of the cell

The hisopathlogical observations of treated snails digestive glands were investigated after the application of half the LC_{50} and the LC_{50} of the compounds (Amino and Vertimec) that had been proven to be the highly toxic compounds after the toxicity test comparing with Newmyl the reference compound. In case of the LC_{50} the effects were more expressed.

Newmyl changes:- The digestive cells showed accumulation of large number of granules. Excretory cells showed increased number of excretory granules. The cytoplasmic degeneration was increased. Calcium cells were packed with enlarged calcium spherules and they exhibited pyknotic nuclei. The cytoplasm of most calcium cells was replaced by large vacuoles containing granules.

Amino changes:- Wide cytoplasm degenerative regions were demonstrated in digestive cells. Excretory granules exhibited increased size and number. There was a decrease in the spherules of calcium cells.

Vertimec changes:- Cytoplasm of digestive cells was highly degenerated.The number of excretory vacuoles increased and the excretory granules were seen larger in size and darkly stained.

2.2. The Intestine

The intestinal epithelium of the tested land snails was composed of columnar high and narrow ciliated and un-ciliated cells with broad apices. Many mucous cells are scattered throughout the epithelium and basal cells. The latter are elongated to oval cells with their lower surfaces attached to the basement membrane. The deeply basophilic nuclei are elongated oval rich in chromatin granules and lie at various levels in the lower half of the cells.

The hisopathlogical observations of treated snails intestines were investigated after the application of half the LC_{50} and the LC_{50} of the compounds (Amino and Vertimec) that had been proven to be the highly toxic compounds after the toxicity test comparing with Newmyl the reference compound. In case of the LC_{50} the effects were more expressed.

Newmyl changes:- The columnar cells have become highly vacuolated and the nuclei were hypertrophy and appeared more elongated and deeply basophilic. The mucous cells become distended and deformed in shape with the increase of mucous droplet in vacuoles, lumen became narrow. Dissociation of parts of the free mucosa was also seen. Degenerations were detected in the submucosa in addition to presence of obvious oedema.

Amino changes:- Produced drastic changes in the intestinal mucosa. Numerous cytoplasmic degenerative regions and mucosal dissociations were seen. The basal halves of columnar epithelial cells were highly vacuolated producing clear gaps between the cells and the muscularis mucosa. Nuclei were enlarged and pyknotic. The villi as a whole acquired a perforated architecture.

Vertimec changes:- Hypertrophy of columnar cells and nuclei were occurred. Dissociation of the free mucosa and degenerations in the submucosa were also demonstrated. Degenerative cytoplasmic parts were detected at the bases of

columnar cells. Nuclei were elongated and darkly stained. Oedoema was also detected in the submucosa .

3-Ultrastructural observations of the digestive glands

Using the transmission electron microscope (TEM) on the digestive glands of the control two land snails of the present study revealed their ultrastructure as follows:

(a)The digestive cells:-

The cytoplasm is filled with a lot of rounded to oval mitochondria with transverse cristae. Golgi apparatus and cisternae of the rough endoplasmic reticulum are detected. The nuclei are large spherical containing scattered euchromatin and clumps of deeply osmiophilic heterochromatin and large nucleoli

(b) Excretory cells:-

The nuclei was oval to elongated and contain moderately osmiophilic nucleoplasm in which clumps of highly osmiophilic heterochromation and conspicuous nucleoli are scattered. Excretory vacuoles filled with darkly osmiophilic granules of variable shapes

(c) Calcium cells:-

The cytoplasm of calcium cells was filled with calcium spherules localized inside membrane bound vacuoles. Numerous elongated mitochondria also demonstrated in the cytoplasm.

With respect of the ultrastructure, changes on the digestive glands of the present snails after treatment with half the LC_{50} and the LC_{50} of the compounds (Amino and Vertimec) that had been proven to be the highly toxic compounds after the toxicity test comparing with Newmyl the reference compound, these changes were as follows: (In case of the LC_{50} the effects were more expressed)

Newmyl changes :- Digestive cells displayed wide cytoplasmic degenerative regions and pleomerphic mitochondria. Less clumps of heterochromatin were detected in the nuclei. Nuclei were about to be pyknotic. The excretory cells

displayed large and numerous excretory vacuoles loaded with abundant deeply osmiophilic scattered or clumped excretory granules.

Amino changes:- In the digestive cells mitochondria showed hypertrophy with ballooned cristae. The rough endoplasmic reticulum cisternae are abundant and arranged in parallelism in the cytoplasm. Nuclei displayed hypertrophy and moderately osmiophilic nucleoplasm and some scattered clumps of heterochromatin. The excretory cells were highly loaded with excretory vacuoles harboring abundant deeply osmiophilic excretory granules.

Vertimec changes:- Nuclei of digestive cells acquired abnormal shape and the nucleolus had a bizarre architecture and was coalesced with heterochromatin. Excretory cells had cytoplasmic degenerations and their mitochondria were pleomorphic and coalesced together. The excretory vacuoles increased in number and size and harbored strongly osmiophilic excretory granules of variable size and shape**.**

4- Biochemical parameters changes

The effect of the applied compounds on some biochemical parameters of the experimental snails, variable and fluctuated results were recorded. Treatment of *Eobania vermiculata* snails with 1/2 LC_{50} of Newmyl resulted in a significant increase in the values of glucose and cholesterol. There was also a highly significant increase in the values of albumin, triglycerides and urea and decreased the total proteins value. Application of Newmyl LC_{50} led to a very high significant increase in glucose and cholesterol. Significant increase in albumin, triglycerides and urea values, whereas the total protein increased non-significantly.

When the snails were treated with 1/2 LC_{50} of Amino glucose and cholesterol were significantly increased, while albumin, triglycerides were highly significantly increased. But urea showed highly significant decreased and the total proteins also decreased but non- significantly. Treatment with the LC_{50} of Amino showed that glucose and cholesterol values were significantly

increased. While albumin and triglycerides values were highly significantly increased. The total proteins and urea increased non- significantly.

After application of 1/2 LC_{50} and the LC_{50} of the biocide Vertimec the tested parameters glucose and cholesterol were significantly increased and the albumin as same as the triglycerides were highly significantly increased. Likewise, the total protein and urea recorded an increase but was non-significantly comparing with control.

Treatment of *Monacha cartusiana* with low concentration of Newmyl (1/2 LC_{50}) showed that glucose and triglycerides were highly significantly decreased. As for albumin, it was decreased non-significantly, whereas the total proteins, cholesterol and urea were highly significantly increased. Treatment of snails with Newmyl LC_{50} showed that the most tested parameters were highly significantly increased but albumin increased non-significantly. Glucose was the exception because it was highly significantly decreased comparing with control.

The application of Amino 1/2 LC_{50} led to highly significant decrease in most biochemical parameters and non-significant decrease in albumin comparing with control. Urea was the exception as it revealed significant increased. Application of Amino LC_{50} caused a highly significant decrease in glucose, triglycerides and cholesterol. Total proteins and urea were highly significantly increased. The albumin parameter was decreased non- significantly comparing with control.

When 1/2 LC_{50} of Vertimec was applied to *M. cartusiana* some parameters were highly significantly decreased but albumin was decreased non-significantly. When Vertimec LC_{50} was applied glucose, triglycerides and total proteins were highly significantly decreased and albumin was decreased but non-significantly. The values of cholesterol and urea were highly significantly increased comparing with control.

5- Field experiment

The field experiment was applied in Egyptian clover field highly infested with *Monacha cartusiana* due to snail wide dispersion in Sharkia Governorate. In this experiment, only the highest concentrations of each compound were applied against *Monacha cartusiana*. The results showed that the micronized sulfur revealed the highest initial effect (the average of reduction percentages after 1and 3 days) while combining Zn showed the lowest initial effect comparing with Newmyl. As for biocides application in the field, Vertimec showed the highest initial effect while Protecto the lowest effective one comparing with Newmyl. The residual effect (the average of reduction percentages after 7, 14 and 21 days) when chemical compound applied urea, the fertilizer was the highest one, while combining Zn still the lowest effective one comparing with Newmyl. As for biocides Biover was the highest one where Vertimec was the lowest.

Finally, control programs of land snails with high concentrations of chemical insecticides or molluscicides present bad adverse effects to non-target organisms such as birds, mammals and farm animals. Therefore, there was a need to find and use non-classical compounds such as biocides or fertilizers instead of the dangerous molluscicides.

In the current study, Vertimec biocide and Amino fertilizer were found to be as efficient as Newmyl and surely of little risks on the ecosystem. The present findings may help in changing the vied of those who work in the field of terrestrial gastropods management hoping in using biocides or fertilizers in control programs instead of insecticides. Such trend may help in saving non-target organisms and preserve soil and environment from hazards of chemical insecticides and conserving biodiversity.

Chapter VIII

References

Abd-Allah, E. A. M; El-Wakil, H. B.; Kassem, F. A.; El-Agamy, E. I. and Abo-Bakr, Y. (1998): Impact of aldicarb and metaldehyde exposure on different molluscan enzyme activities and stress protein response. Ann. Agric. Sci. Cairo., Vol. (3): 1103-1117.

Abd-Allah, M. H. A. (1994): Studies on Agricultural Molluscs at Dumyat Governorate. M.Sc. Thesis, fac. Agric., Mansoura Univ. 153pp.

Abd El-Aal, E. M. (2001): Studies on Certain Land Snails at Sharkia Governorate. M.Sc. Thesis, Agric. Zagazig Univ. 137 pp.

Abd El-Aal, E. M. (2007): Ecological, Biological and Control Studies on Certain Land Snail Species in Sharkia Governorate. Ph. D. Thesis, Fac. Agric. Zagazig Univ. 188 pp.

Abd Elgaleil, S. A. M. and Badawy, M. E. I. (2006): Acaricidal and molluscicidal potential of three essential oils isolated from Egyptian plants. J. Pest Control Environ. Sci., 14: 35-46.

Abd El-Haleim, K. Y.; Abou-El Khear, R. K. and Hussein, A. A. (2006): Molluscicidal efficacy and toxicity of some pesticides under laboratory and field conditions. Arab Univ. J. Agric. Sci., 14 (2): 861 – 870.

Abd El-Karim, N. (2000): Ecological and Biological Studies on Some Terrestrial African Agriculture Snail Pests. M. Sc. Thesis, Inst. Afric. Res. stud., Cairo Univ. 141 pp.

Abd ElNabey, S. Y. and Shaban, M. A. (2006): Residual effect of certain pesticides against two land snails species, *Monacha cantiana* and *Eobania vermiculata* under laboratory and field conditions. Envir. Encyclopedia Ass. Univ.,99-100

Abd El-Wahab, M. I. (2004): Ecological and Morphological Studies on Some Terrestrial Snails in Dakahlia Governorate. M. Sc. Thesis, Fac. Agric. Al-Azhar Univ. Egypt.

Abo Bakr, Y. (1997): Toxicological and Environmental Studies on Some Terrestrial Gastropods. M. Sc. Thesis, Faculty of Agric., Alex. Univ., Egypt.

Abo Bakr, Y. (2011): Histopathological changes induced by metaldehyde in *Eobania vermiculata* (Müller 1774). Alexandria Sci. Exch. J., Vol. (32) 3: 300-309.

Abo-Elnaser H. A. K. (2013): Ecological and Biological Control Studies on Some Terrestrial Snails and Their Associated Arthropods in Assiut Governorate. M. Sc. Thesis, Faculty of Agriculture, Assiut University, Egypt, Pp.108.

Abolins, K. A. (1965): Electron microscope observations studies of intracellular origin and formation of calcifying granules and calcium spherites in the hepatopancreas of the snail *Helix pomatia* (L). Zell Forsch, 108:501-515.

Abolins, K. A. (1970): Ultrastructural study of the shell-repair membrane in the snail, *Helix pomatia* (L.). Tissue and Cell, 172: 455-476.

Aioub, A. A.; Ismail, Sh. A. and Mohammdein, A. A. (2000): Toxicological and histologicals studies on some pesticides-treated land snails. The International Conference on Biological Sciences., 1 (2): 19 – 38.

Almendros, A. and Porcel, D. (1992): Phosphatase activity in the hepatopancreas of *Helix aspersa*. Comp. Biochem. Physiol. Comp. Physiol., 103(3): 455-60.

Al-Zahaby, A. S.; Abdel Rehim, A. H. and Al-Mahrouki, A. A. (1993): Histochemical features of the digestive gland of banded and unbanded phenotypes of the polymorphic land snail, *Eobania vermiculata*. J. Egypt. Ger. Soc. Zool., (12): 291-310.

Amaral, A. F.; Anselmo, H.; Pires, R. M. and Rodrigues, A. S. (2004): The connective tissue index of *Helix aspersa* as a metal biomarker. Bio. Metals, 17: 625–629.

Arafa, A. A. I. (2006): Studies on Terrestrial Molluscs in Some Delta Governorates. Ph.D. Thesis, Fac. of Agric., Al-Azhar Univ. Egypt.167pp

Arrighetti, F.; Teso, V. and Penchaszadeh, P.E. (2015): Ultrastructure and histochemistry of the digestive gland of the giant predator snail *Adelomelon*

beckii (Caenogastropoda: Volutidae) from the SW Atlantic. Tissue and Cell, 47 (2): 171-177.

Aukkanimart, R.; Thidarut,B.; Somchai, P.; Smarn, T.; Surasit, A.; Chantana, B.; Pranee, S.; Porntip, L. and Wiyada, P. (2013): Histopathological changes in tissues of *Bithynia siamensis* goniomphalos incubated in crude extracts of camellia seed and mangosteen pericarp. Korean J. Para., Vol. 51 (5): 537-544.

Awad, M. H. (1994): Studies on Agriculture Molluscs at Domyat Governorate. M.Sc. Thesis, Fac. Agric. Mansoura. Univ. 153 pp.

Awad, M. H. (2013): Logical control and population density studies on land snails in south district of Port Saied, Port Saied Governorate. Egypt. Acad. J. Biolog. Sci., 5(2): 47- 63.

Azzam, K. M. (2005): Molluscicidal activity of certain compounds against *Monacha cartusiana* land snails under laboratory and field conditions. Egypt J. Agric. Sci. Mansoura. Univ.,(30): 8147-8151.

Barker, G.M. (2002): Molluscs as crop pests, 1st edition, CAB International.

Bayne, B. L.; Brown, D. A.; Burns, K.; Dixon, D. R.; Ivanovici, A.; Livingstone, D. R.; Lowe, D. M.; Moore, M. N.; Stebbing, A. R. D. and Widdows, J. (1985): The Effects of Stress and Pollution on Marine Animals. Praegez Publshers, New York.

Beeby, A. and Richmond, L. (1988): Calcium metabolism in two populations of the snail *Helix aspersa* on a high lead diet. Arch. Environ. Conta. Toxic., 17:507-511.

Beshr, S. M. (2000): Ecotoxicological Studies on Two Species of Snails and Associated Insects Infesting Fruit Trees in Three Egyptian Governorates. M. Sc. Thesis, Alex. Univ. 161pp

Beltagi, S. M.; El-Shennawy, M. S.; Elkatan, N. A. and Yousef, H. N. (2011): Physiological changes in the brown garden snail, *Eobania vermiculata*

induced by sublethal doses of two botanical molluscicides. J. Egypt. Ger. Soc. Zool. (63A): Comparative Physiology, 375- 397.

Besnaci, S.; Bensoltane, S.; Zerari, L.; Chrairia, S.; Hamlet, A. and Berrebbah, H. (2016): Impact of nanometric iron oxide in the hepatopancreas of terrestrial gastropod *Helix aspersa*: Histological changes and biochemical parameters. Int. J. Pharm. Sci. Rev. Res., 36(2) 37: 234-241.

Bezerra, C. B.; Kemper, A. and Becker, W. (1999): Profile of organic acid concentrations in the digestive gland and hemolymph of *Biomphalaria glabrata* under aestivation. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 94(6): 779-784.

Birgul, O.; Elif, I. C.; Zeki, Y.; Ozkan, U. and Erhan, U. (2004): The effects of endosulfan on the great ramshorn snail *Planorbarius corneus* (Gastropoda, Pulmonata): a histopathological study. Chemosphere, 56:707–716.

Boer, H. H. and Kits, K. S. (1999): Histochemical and ultrastructuralstudy of the alimentary tract of the fresh water snail *Lymnaea stagnalis*. J. of Morphology, 205: 97–111.

Boghen, A. and Farley, J. (1974): Phasic activity in the digestive gland cells of the intertidal prosobranch, *Littorina saxatilis* (Olivi) and its relations to the tidal cycle. Proceedings of the Malacological Society of London, 41: 41–56.

Bolognani, F.; Bolognani, L.; Ottaviani, E. and Franchini, A. (1987): Histochemical research on metabolic pathways of glucose in some species of mollusca gastropoda. Acta. Histochem, 81 (2): 155-62.

Boucenna, M.; Berrebbah, H.; Atailia, A.; Grara, N and Reda, M. D. (2015): Effects of metal dust on functional markers and histology of gland digestive and kidney of the land snails (*Helix aspersa*) in the North East of Algeria. Global Veterinaria 14 (2): 189-198

Bourne, N. B.; Jones, G. W. and Bowen, I. D. (1991): Endocytosis in the crop of the slug *Derocerase reticulatum* (Muller) and the effects of the ingested molluscicides metaldehyde and methiocarp. J. Moll. Stud., (57) : 71-80.

Brackenbury, T.D. (1999): Gross histopathological effects of an extract of *Agave attenuata* on the epithelium of the digestive tract of *Bulinus africanus*. Ann. Trop. Med. Parasit., 93 (5): 519-26.

Brooks, **A. W. and White, K. N. (1995):** The localization of aluminium in the digestive gland of the terrestrial snail *Helix aspersa*. Tissue and Cell, Vol. (27) : 61–72.

Burtis,A. (1999): Tietz Textbook of Clinical Chemistry, 3rd ed AACC.

Chabicovsky, M.; Klepal, W. and Dallinger, R. (2004): Mechanisms of cadmium toxicity in terrestrial pulmonates: Programmed cell death and metallothionein overload. Environ. Toxicol. Chem., 23: 648-655.

Chaki, K. K. and Misra, K. K., (2004): Further studies on the digestive gland cells of *Achatina fulica* (Pulmonata: Gastropoda) Proc. Zool. Soc. Calcutta, 57:95-104.

Chaudhari, R.D. and Kulkarni, A.B. (1994): Aspects of lipid metabolism in the terrestrial snail, *Zootecus insularis* due to monocrotophos intoxication. J. Eco. and Envir. Monit., 4(2): 121-126.

Chaudhari, T.R.; Patil, P. N.; Rao, K.R.; Deshmukh, S.B. and Diwate, S.G. (1999): Effect of pesticide Rogor on some biochemical constituents in fresh water snail, *Thiara lineata*. Envir. Eco., 17 (1): 146-148.

Christiane, T. and Plawen, L.S. (2005): The digestive tract of *Helicoradomenia juani* (Solenogastres, Mollusca), aplacophoran molluscs from the hydrothermal vents of the East Pacific Rise. Inver. Biol., 124 (3): 230–253.

Costa, A. F. (1994): Farmacognosia. Lispoa: Calouste Gulbenkian.

COSTAT program (1986): Co-Hort softweare, P.a. bax.1149, Brerkely Ca 94701. U.S.A.

Coupland, J.B., (1996): The efficacy of metaldehyde formulations against helicid snails: the effect of concentration, formulation and species. BCPC Symp. Proc., 66: 65-72.

Daoud, M. I. A. (2004): Ecological and morphological studies on some terrestrial snails in Dakahlia Governorate. M.Sc. thesis Fac.Agric. AlAzhar Univ., 177pp.

Dawidar, A. M.; Ayyad, S. N.; Mortada, M. M. and Raghib, H. M. (2012): Molluscicidal activity of *Balanites aegyptiaca* against *Monacha cartusiana.* Pharm. Biol. 50 (10): 1326-1329.

Dimitriadis, V. K. and Andrews, E. B. (2000): Ultrastructural and cytochemical study of the digestive gland cells of the marine prosobranch mollusk *Nucella Lapillus* (L.) in relation to function. Malacologia., 42(1-2): 103 – 112.

Dimitraidis, V.K.; Dimiyris S. H. and Pirpasoploulon A. (1992): Crop epithelium of normal feed starved and hibernated snails *Helix lucorum* a fine structural cytochemical study. Malacologia. 34:343-354.

Dimitriadis, V. K. and Domoucht, S. G. (1995): Carbohydrate cytochemistry of the intestine and salivary glands of the snail *Helix lucorum*: Effects of starvation and hibernation. J. Mollu. Stud., 61: 215 – 224.

Dimitriadis, V. K. and Hondros, S. D. (1992): Effect of starvation and hibernation on the fine structural morphology of digestive gland cells of the snail *Helix Lucorum.* Malacologia., (34) : 63–73.

Dimitriadis, V. K. and Konstantinidou, V. (2002): Origin of the excretory cells in the digestive gland of the land snail, *Helix lucorum*. Malacologia., 44: 145-151.

Doumas, B.T.; Bayse, D. D. and Carter, R. J. (1981): Candidate reference method for determination of total protein in serum. I. Development and validation, II. Tests for transferability. Clin. Chem., 27: 1642-1654.

Drury, R. A. and Wallington, E. A. (1980): Carlton's histochemical technique 5th edition Oxford Univ. press**.**

Ebenso, I. E.; Umoren, E. P.; Binang, W.; Edet, G.; Izah, M.; Udo, I. O.; Ibanga, G. and Ukpong, E. E. (2005): Effect of carbamate molluscicide on

african giant land snail *Limicolaria aurora*. J. Appl. Sci. Environ. Mgt., Vol. 9 (1): 99 – 102.

El-Akhrasy, F. I. (2010): Histopathological and Histochemical Studies on The Effect of Molluscicide Metaldehyde on The Land Snail *Monacha cartusiana*. M.Sc. Thesis, Zagazig Univ. Fac. Sci. Zoology Dep. 110 pp.

El-Banhawy, M. A.; Al Zahaby, A. S.; Abd-Rehim, A. H. and Al- Mahrouki, A. (1991): Comparative histology of the digestive gland in two morphs of the polymorphi land snail *Eobania vermiculata*. Bull. Fac. Sci. Zagazig Univ.,13 (1): 452 – 467.

El-Deeb, H. I.; Ewies, E. A.; Kandil, M. A.; Gabr, W. M. and Mobarak, S. A. (2003a): Toxicity and biochemical studies of methomyl and diazinon on different ages of the land gastropod species Monacha obstructa. J. of Agricultural Science Mansoura University 28 :(9) 7011-7023.

El-Deeb, H. I., Zedan, H. A.; Abd-All, S. M. and Mohamed, H. L. (1999b): Toxicity and biochemical studies on the terrestrial snail *Monacha cantiana* treated with some natural products and pesticides. 2nd Int. Conf. of Pest Control, Mansoura, Egypt Sept.

El-Deeb, H. I.; Zidan, Z. H.; Fouad, M. M. and Asran, F. D. (2003b): Survey of terrestrial snails and their malacophagous insects at three Governorates in Egypt. Egyptian J. of Applied Science 18: 355-361.

El-Emam, M.A. and Ebeid, F.A. (1989): Effect of *Schistosoma mansoni* infection, starvation and molluscicides on acid phosphatase, aminotransferases and total protein in tissues and hemolymph of the snail *Biomphalaria alexandrina*. Helminthological, 26:155-161.

El-Gendy, K.S.; Radwan, M.A. and Gad A.F. (2009): *In vivo* evaluation of oxidative stress biomarkers in the land snail, *Theba pisana* exposed to copper-based pesticides. Chemosphere, 77:339–344.

El–Gohary, L. R. and Genena A. M. (2011): Biochemical effect of molluscicide baits against the two land snails, *Monacha cantiana* and *Eobania vermiculata* (gastropoda: Helicidae). Inter. J. Agric. Res., 1816-4897.

El-Khodary, A. S.; Sharshir, F. A.; Helal, R. M. and Shahawy, W. A. (2001): Evaluation of some control methods against the land snail *Monacha cantiana* (Montagu) at Kafr El sheikh Governorate**.** Egypt J. agric. Res., Tanta Univ., 27(2): 290-300.

El-Mahrouki, A. (1991): Morphological, Cytochemical and Cytological Studies on Two Morphs of The Polymorphic Land Snail *Eobania vermiculata* from Zagazig and Surrounding Localities. Thesis, Fac. Sci. Zagazig Univ. 158 pp.

El-Masry, S. A. (1997): Studies on The Control of Some Land Snails Infesting Certain Fruit Trees. Ph.D. Thesis, Fac. Agric. Zagazig Univ. 158 pp.

El-Okda, M. (1979): Land snails of economic importance at Alexandria region with some notes on the morphological features, classification, economic damage and population on the ornamental plants. Agric. Res. Rev. 57: 125-31.

El-Okda, M. M. (1980): Land snails of economic importance on vegetable crops at Alexandria and neighboring regions. Agric. Res. Rev., 58 (1): 79-85.

El-Okda, M. M. (1984): Land mollusca infestation and chemical control in El-Ismaelia Governorate. Agric. Res. Rev., 62(1): 87-92.

El-Said, A. M. (2009): Biological Control of Terrestrial Molluscs by Using Some Microorganisms. M. Sc. Thesis, Fac. of Science, Zagazig Univ. Egypt.

El-Sarar, A.; Hamdy, H.; Yasser, A. and Bayoumi, A. (2012): Molluscicidal activity of methomyl and cardenolide extracts from *Calotropis procera* and *Adenium arabicum* against the land snail *Monacha cantiana*. Molecules, Vol. (17): 5310-5318.

EL-Sayed, A. H. (2010): Mollusicidal effects on some chemical compounds against *Monacha cartusiana* (Muller) and *Eobania vermiculata* (Muller) land snails under laboratory and field conditions. Egypt. J. Agric. Res., 88 (4): 1197-1207.

El-Sayed, A.M. (2014): Inhibition of terrestrial snails' glutamate decaboxylase (GAD) by abamectin and emamectin benzoate. 3rd International Summit on Toxicology & Applied Pharmacology Chicago, USA.

El-Sayed, H. E.; Yasser, A. and El-Shahaat, M. S. (2015): Seasonal incidence and chemical control of land snails in pear orchards at Alexandria Governorate. J. Advanc. Agric. Res., 20(1): 128-136.

El-Sebae, A. H.; El-Okda, M. M. and Marei, A. S. (1982): Local formulation of three carbamoylated oximes as terrestrial molluscicides. Agric. Res. Rev., 60 (1): 85 – 92.

El-Shafey, S. N.; Eitta, A. M.; Sitohy, M. Z. and El-Sheikh, A. A. (2010): Evaluation of molluscicidal activity of *Jatropha curcas* L. seed extracts against the glassy clover snail, *Monacha cartusiana* (Muller). Egyptian J. of App. Sci., 25 (8 B): 249 – 256.

El-Shahaat, M. S.; Abo Bakr, Y. and Eshra, E. H. (2007): Persistence of methomyl and aldicarb as molluscicides in different baits under laboratory and field conditions. J. Adv. Agric. Res., 12: 295-302.

El-Shahaat, M. S.; Eshra, E. H. and Abo Bakr, Y. (2009): Evaluation of urea fertilizer in comparison with some conventional pesticides against terrestrial snails in fruit orchards. J. Pest Cont. Environ. Sci. 17: 19-30**.**

El–Wakil, H.B.; Kassem, F.A. ; Abdallah, E.A.M. and Abo Bakr, Y. (2000): Ecological and biological studies on some terrestrial gastropod species in Alexandria and El Beheira, Egypt. Alex. J. Agric. Res. 45: 207- 24.

El-Wakil, H. B. and Radwan, M. A. (1991): Biochemical studies on the terrestrial snail *Eobania vermiculata* (Muller) treated with some pesticides. J. Environ. Sci. and Health. Part B, Pesticides Food Contaminates and Agricultural Wastes., 26 (5 - 6): 479 – 489.

Eshra, E.H., (2004): Studies on terrestrial mollusks at some governorates of West Delta with special reference to its integrated management. Ph.D. Thesis, Faculty of Agric., Al-Azhar Univ., Egypt

Eshra, E. H. (2014): Toxicity of methomyl, copper hydroxide and urea fertilizer on some land snails. Ann. Agri. Sci., 59(2), 281–284.

Farag, M. F. (2012): Efficiency of Some Natural Products and Pesticides on Controlling of The Glassy Clover Snail, *Monacha cartusiana* (Muller) and Brown Garden Snail, *Eobania vermiculata* (Muller) at Sharkia Governorate. D. Ph. Thesis, Fac. Agric. Tanta Univ. Egypt., p163.

Fearon, W. R. (1939): Biochem. J. 331:902.

Flari, V. and Charrier, M. (1992): Contribution to the study of carbonydrases in the digestive tract of the edible snail *Helix lucorum* (Gastropoda: Pulmonata: Stylomanatophora) in relation to its physiology state. Comp. Bioch. Phys., 102A: 363 – 372.

Fouad, M. M. (2005): Histological changes induced in the mucus glands of brown garden snail, *Eobania vermiculata* treated with malathion and methomyl pesticides. The third international conference on IPM role in integrated crop management and impacts on environment and agricultural products. 26-29. Giza, Egypt.

Fouad, M. M.; Khider, I. K. and Soliman, A. M. (2004): Laboratory studies on the molluscicides effect of Sumithion, Bindiocarb and Macher pesticides against three land snail species. J. agric. sci. Mansoura Univ. 29(1): 451-455.

Franchini, A. and Ottaviani, E. (1993): Histochemical and ultrastructural study of the digestive gland of the freshwater snail *Planorbarius corneus* (L.) (Gastropoda, Pulmonata). Anim. Biol., 2: 192-198.

Frias-Espericueta, M.G.; Abad-Rosales, S.; Aidée C.N., Isidro-Osuna, L; Pàez-Osuna, F; Olvera, R.L. and Voltolina, D. (2008): Histological effects of a combination of heavy metals on pacific white shrimp Litopenaeusvannamei juveniles. Aquat. Toxicol. 89:152-157.

Gabr, W. M.; Khidr, K. F. and Hussien, S. S. (2007): Molluscicidal activity of some pesticides against glassy clover *Monacha obstructa*. Egypt J. Agric. Res., 8566: 2017-2025.

Gabr, W. M.; Khidr, F. K. and Youssef, A. S. (2006a): Effect of spinosad bioinsecticide as a bait and contact applications against three land snail species. Egypt J. Agric. Res., 84 (5): 1403- 1409.

Gabr, W. M.; Youssef, A. S. and Khidr, F. K. (2006b): Molluscicidal effect of certain compounds against two land snail species *Monacha obstructa* and *Eobania vermiculata* under laboratory and field conditions. Egyptian J. Agric. Res., 84 (1): 43 – 50.

Genena, M. A. and Mostafa, F. A. (2008): Molluscicidal activity of six pesticides against the two land snails, *Monacha cantiana* and *Eobania vermiculata* (gastropoda: helicidae) under laboratory conditions. J. agric. sci. Mansoura Univ., 33 (7): 5307 – 5315.

Gerard, C. and Theron, A. (1996): Altered nutrition and assimilation of the snail *Biomphalaria glabrata* as a consequence of the parasitic spatial constraint *Schistosoma mansoni*. Acta. Tropica., 61(1): 51-55.

Ghaly, M. F.; Ismail, S. A. and El-Said, A. M. (2009): Microbial control of land snail *Monacha cartusiana* (Muller) under laboratory and field conditions. Bull. Fac. Sci. Zagazig Univ., 31: 1 – 19.

Ghamry, E.M.; EL-Deeb, H.I. and Abd El-All, S.M. (1993): Efficacy of certain pesticides against some land snails under field conditions of Sharkia Governorate. Egypt. J. App. Sce., 8(6): 213-225.

Ghamry, E.M.; Kokab, Y. A. and Wilson, B. M. (1994): Screening test of some insecticides against two land snails *Monacha cantiana* and *Eobania vermiculata* in Sharkia Governorate Zagazeg. J. agric. Res., 21(5): 1539-1545.

Ghamry, E. M.; El-Massry, S. A.; Ismail, Sh. A. A. and Khattab, M. M. (2000): Effect of certain local surfactants on the land snail *Monacha cartusiana* (mollusca; Helicidae). Egypt. J. Appl. Sci., 15(8)239-245.

Godan, D. (1983): Pest slugs and snails biology and control springer-verlag. Berlin.

Goel, A. and Dhawan, D. K. (2001): Zinc supplementation prevents liver injury in chlorpyrifos treated rats. Biol. Trace Element Res., 82: 185-200.

Gros, O.; Frenkiel, L. and Aranda, D. (2009): Structural analysis of the digestive gland of the queen conch *strombus gigas linnaeus*, 1758 and its intracellular parasites. J. Moll. Stud., 75: 59–68.

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

Hamlet, S.; Bensoltane, S.; Mohamed, D.; Fatiha, Y. and Houria, B. (2012): Histological changes and biochemical parameters in the hepatopancreas of terrestrial gastropod *Helix aspersa* as biomarkers of neonicotinoid insecticide exposure. Afric. J. Biote., Vol. 11(96): 16277-16283.

Hanafy, A. A; Mohamed, A. M. and Youssef, M. H. (2003): Comparing the efficiency of two methomyl baits against the land snails in some vegetation. Egypt**.** J. appl. Sci., (18):418-428**.**

Hanafy, A. A.; Youssef, H. M. and El-Shahaat, M. S. (1998): Preparation of methomyl baits and efficacy against certain land mollusca in different vegetations. Adv. Agric. Res., 3:435-441.

Hasheesh, W. S.; Mohamed, R. T. and Abd El-Monem, S. (2011): Biological and physiological parameters of *Bulinus truncatus* snails exposed to methanol extract of the plant *Sesbania sesban*. Adv. Biol. Chem., (1): 65-73.

Hegab, A. M. (2003): Efficacy of certain pesticides against *Monacha cartusiana* (Müller) snails under laboratory and field conditions in Sharkia Governorate Zagazig. J. Agric. Res., 30 (5): 2009-2020.

Heiba, F. N.; Al-Sharkawy, I. M. and Al-Batal, A. A. (2002): Effects of the insecticide lannate, on the land snails, *Eobania vermiculata* and *Monacha cantiana*, under laboratory conditions. Online J. Biol. Sci., 2: 8-13.

Helmy, A. Z.; Shoeib, A. A. and El-khamesy, M. M. (2006): Assessment of molluscicidal activity of certain pesticides against two land snails under laboratory and field circumstances at Dakahlia Governorate. Mansoura Univ. J. Agric. Sci. Vol. 31(6):3957-3962.

Hemmaid, K. Z. and Mohammadein, A. A. (2003): Ultrastructural alterations in the digestive gland cells of the snail *Eobania vermiculata* (Muller) as environmental monitors of insecticidal pollution. J. Egypt Soc. Biotech. Environ. Sci., Vol. 1(A) 211-238.

Henderson, G. F. and Tillton, E. W. (1955): Test with acaricides against the brown wheat mite. J. Econ. Entomol., 48:157-161.

Henry, M.; Boucaud-Camou, E. and Lefort, Y. (1991): Functional microanatomy of the digestive gland of the scallop *Pecten maximus* (L.). Aqua. Living Res., 4: 191-202.

Hesham, M. S. (2009): Histochemical changes of carbohydrate and protein contents in the digestive gland cells of the land snail *Monacha cartusiana* following starvation. Saudi J. Biolo. Scies., Vol. 16(1): 51-55.

Hussein, H. I.; Al-Rajhi, D. H.; El-Shahawi, F. I. and Hashem, S. M. (1999): Molluscicidal activity of *Pergularia tomentosa* (L.), methomyl and methiocarb against land snails. Int. J. Pest Manag., 45 (3): 211-213.

Hussein, H.I.; Karmel, A.; Abuu-Zeid, M.; El-Sabae, A.K.H. And Saleh, Uscharin, M.A. (1994): The most potent molluscicidal compound tested against land snails. J. Chemical Ecology, 201: 135-140.

Hussien, S. S. (2006): Molluscicidal effect of caffeine, theophylline and tea leaf extract against three land snail species. Egypt, J. of App. Sci., 21(12A): 266-274.

Hussien, S. S. and Gabr, W. M. (2006): Biochemical effects of two natural pesticides on the brown garden snail *Eobania vermiculata* (Muller). Egypt J. Agric. Res., 84 (3) 713-723.

Ismail, Sh. A. (1997): Ecology Biology and Control of Certain Terrestrial Snails Infesting Some Vegetables and Field Crops in Sharkia Governorate. Ph.D. Thesis, Fac. Agric., Zagazig Univ. 130 pp.

Ismail, Sh. A. (2009): Effect of baits color, carrier and attractive materials on the efficiency of methomyl against two land snail species under field conditions. Al-Azhar J. agric. Sci. Sector Res., Vol. 6: (245-254).

Ismail, Sh. A.; Abd-Allah, A. A.; El-massry S. A. and Hegab A. M. (2005): Evaluation of certain chemicals and insecticides against *Monacha cartusiana* snails infesting some vegetable crops at Sharkia Governorate. J. Agric. Sci., Mansoura Univ., 30 (10): 6283-6291.

Ismail, Sh. A. and AbdEl-Kader, S. M. (2011): clove: is it has a molluscicidal activity against land snails (*Monacha cartusiana*). J. Plant Prot. And Pathology, Mansoura Univ., Vol. 2 (5): 561 – 569.

Ismail, Sh. A. and Hegab, A. M. (2006): Response of juveniles and adults of the brown garden snail, *Eobania vermiculate* (Muller) to certain chemicals. Egypt. J. Appl. Sci., 21 (8): 227-236.

Ismail, Sh. A. and Mohammed, O.M. (2009): Persistence of fresh prepared baits of certain pesticides tested at different intervals against *Monacha cartusiana* snails under laboratory conditions. Egypt. J. Appl. Sci., 24(1): 274-280.

Ismail, SH. A., Rashed, A. A.; Abou-Senna F. M. and Abed M. (2013): Physiological and histochemical studies on the land snail, *Monacha cartusiana* in Sharkia Governorate. Egypt. J. Agric. Res., 91 (1): 207-216.

Ismail, Sh. A.; **Shetaia, S. Z. and Abdel Kader, S. M. (2010):** Effect of neem extract (neemazal T.S) on two land snail species under laboratory conditions. J. Plant Pro. And path. Mansoura Univ., Vol. 1 (10)799-806.

Ismail, Sh. A.; Shetaia, S. Z. Arafa, A.A.I. and Khattab, M. M. (2014): Field trials on the bait attractive distances and evaluation the efficacy of methomyl using different control application methods against the gastropod pest *Monacha cartusiana* (müller) infesting clover fields. J. Plant Prot. and Path., Mansoura Univ., Vol. 5 (6): 697 - 703.

Kandil, M. A.; El- Deeb, H. I.; Eweis, E. A.; Gabr, W. M. and Mobarak S. A. (2014): Effect of acetylsalicylic acid on the physiological role of mucus gland of land snail species. Egypt. J. Agric. Res., 92 (1): 53-71**.**

Kandil, M. A.; El-Deeb, H. I.; Mobarak, S. A. and Eweis, E. A. (2009): Biochemical impacts of methomyl and abamectin and their binary mixtures with acetylsalicylic acid against the land snails *Eobania vermiculata* and *Monacha obstructa*. The Conference on Recent Technologies in Agriculture, 138.

Kaplan, A. (1984): Triglycerides Clin. Chem. the C.V. Mosby Co. St Louis. Toronto. Princeton 437 and Lipids, 1194-1206.

Keiichiro, Y.; Naomi, S. and Emiko, F. (2000): Histochemical and ultrastructural analyses of the epithelial cells of the body surface skin from the terrestrial slug, *Incilaria fruhstorferi*. Zoological Science, 17(8): 1137-1146.

Keilin, D. and Hartree, E. F. (1948): Properties of glucose oxidase (notatin): Addendum. Sedimentation and diffusion of glucose oxidase (notatin). Biochem. J. 42:250.

Kemajl, B.; Avni, B.; Ilir, M.; Agim, G. and Sali, A. (2015): Effect of industrial pollution in some biochemical parameters of the garden snail *Helix Pomatia* (L.) in the region of "Trepça" smelter in Mitrovica, Kosovo. 5th International Conference on Environment Science and Engineeringn, Vol. 83:28-32.

Khater, A. A.; El-Sheakh, A. A.; El-Sheamy, M. K. and Hussein, M. Z. (1990): Biochemical effects of lannate and larvin on Tilapia nilotica fingerlings. Egypt. J. Appl. Sci., 5(8): 227-235.

Kumari, P.G.; Doss, P.J.; Ramani, K. S. A.; Vijaya, P. and Rao, M. R. (1999): Studies on some tissue lipid metabolism profiles of the snail *Pila globosa* under phenthoate toxic stress. J. Eco. Phys., 2: 25-30**.**

Lobo-da-Cunha, A. (2000): The digestive cells of the hepatopancreas in *Aplysia depilans* (Mollusca, Opisthobranchia): Ultrastructural and cytochemical study. Tissue and Cell, (32): 49-57.

Lobo-da-Cunha, A. and Batista-Pinto, C. (2005): Light and electron microscopy studies of the oesophagus and crop epithelium in *Aplysia depilans* (Mollusca, Opisthobranchia) . Tissue and Cell,37 (6): 447-456.

Lobo-da-Cunha, A.; Malheiro, A. R.; Alves, A.; Oliveira, E.; Coelho, R. and Calado, G. (2011): Histological and ultrastructural characterisation of the stomach and intestine of the *Opisthobranch bullastriata* (heterobranchia: cephalaspidea). Thalassas, 27 (2): 61-75.

Lokma, H. E., (1998): Efficacy of some pesticides against two species of land snails *Monacha cartusiana* (Müller) and *Rumina decollato* (Linne) *Zagazig.* J. Agric. Res., 26 (2): 421 – 427.

Lokma, H. E. and Al Harby, F. N. (1999a): Effect of *Bacillus thuringiensis* on two land snails *Monacha cartusiana* (Müller) and *Rumina decollata* (Linne). Zagazig. Agric. Res., 26 (2): 429 – 435.

Lokma, H. E. and Al Harby, F. N. (1999b): Molluscicidal effect of some pesticides on two land snails under field conditions at Riyadh area in Saudi Arabia. Zagazig. Agric. Res., 26 (2): 437 – 444.

Lokma, M. H. (2007): Studies on Some Terrestrial Gastropods Injurious to Field Crops at Sharkia Governorate. M.Sc. Thesis, plant protection Dept. Fac. Agric., Zagazig Univ. 147 pp**.**

Lokma, M. H. (2013): Studies on Some Terrestrial Molluscs Injurious to Vegetables and Field Crops in East Delta Locality (Sharkia and Ismailia). Ph.D. Plant Protection Dep. Fac. Agric., Moshtohor. Benha Univ.

Lopes, M.; Rodrigijes, A. and Marigomez, I. (2001): Morphology and histology of the digestive gland of *Oxycliilus atlarzticus* (Gastropoda: Pulmonata). Arquipélago, Life and Marine Sciences. Supplement, 2 (Part B): 7 1-76.

Mane, U.H; Kachole, M. S. and Pawar, S. S. (1979): effect of pesticides and narcotants on bivalve mollusks. Malacologia., 18:347-360.

Mersal, H. T. (1990): Changes Induced in One Land Snail of Agriculture Importance in Egypt. M.Sc. Thesis, Fac. Girls, Ain Shams Univ.

Miller, E.; Swails, S.; Swails, D.; Olson, F.O. and Staten, R.T. (1988): White garden snail *Theba pisana* (müller) efficacy of selected bait and sprayable molluscicides. J. Agric. Entomology. 5(30):189-197.

Mobarak, S. A. (2003): Molluscicidal Activity of Methomyl and Diazinon Against Land Snails *Monacha obstructa* and *Eobania vermiculata*. M. Sc. Thesis, Fac. of Agric., Cairo Univ. Egypt.

Mohamed, A. M.; El-Fiki, S.; El-Sawy, M. F. and El-Sawy, M. F. (1981): Effect of prolonged exposure of *Biomphalaria alexandrina* snails to low concentrations of some molluscicides. II. on total tissue proteins, carbohydrates and lipids. Egypt J. Soc. Paras., 11(2): 459-68.

Moran, S.; Gotib, Y. and Yaakov, B. (2004): Management of land snail in cut green ornamentals by copper hydroxide formulations. J. crop protection, 23(4): 647-650.

Mounir, B.; Houria, B.; Amira, A.; Nedjoud, G. and Mohamed, R. D. (2015): Effects of metal dust on functional markers and histology of gland digestive and kidney of the land snails (*Helix aspersa*) in the North East of Algeria. Global Veterinaria, 14 (2): 189-198.

Mourad, A. P. (2014): Molluscicidal effect of some plant extracts against two land snail species, *Monacha obstructa* and *Eobania vermiculata*. Egypt. Acad. J. Biolog. Sci., 6(1): 11–16

Nath, T. K.; Sao, S. and Misra K. K. (2015): Ultrastructure of digestive gland cells of an estuarine slug, *Laevicaulis alte* from Sundarban coast of India International J. Res. Zoology, 5(2): 21-28.

Nedjoud, G.; Amira, A.; Mounir, B.; Fadila, K.; Houria, B. and Mohamed, R. D. (2012): Effects of heavy metals on the snails *Helix aspersa* bioindicators of the environment pollution for human health. International conference on applied life sciences, Turkey. 241-246

Ojeda, M.; Arrighetti, F. and Giménez, J. (2015): Morphology and cyclic activity of the digestive gland of *Zidona dufresnei* (Caenogastropoda: Volutidae). Malacologia, 58(1-2):157-165.

Okka, M. A.; Ahmed, F. A. and Sharashir, F. A. (1996): Efficacy of certain pesticides against the land snail *Monacha cantiana* (Montagu) found on some orchards under laboratory conditions. 4th Arabic. Conf. Hortic. Crops El-Minia, Egypt 903-910.

Oxford, G. S. and Fish, L. J. (1979): Ultrastructural localization of esterase and acid phosphatase in digestive gland cells of fed and starved *Cepaea nemoralis* (L.). Protoplasma, 101: 186-196.

Radwan, M. A.; El-Wakil, H. B. and Osman, K. A. (1992): Toxicity and biochemical impact of certain oxime carbamate pesticides against terrestrial snail, *Theba pisana* (Muller). J. Environ. Sci. and Health, Part B Pesticides, Food Contaminates and Agricultural., 27 (6): 759 – 773.

Radwan, M. A.; Essawy, A. E.; Abdelmeguied, N. E.; Hamed, S. S. and Ahmed, A. E. (2008): Biochemical and histochemical studies on the digestive gland of *Eobania vermiculata* snails treated with carbamate pesticides. Pesticide Biochemistry and Physiology, Vol. 90(3): 154-167.

Rajalakshmi, B. R.; Shyamasundari, K. and Hanumantha, R. K. (1982): Histological and histochemical studies on the albumen gland and capsular gland of *Thais bufo* (Mollusca: Gastropoda)., Vol. 91(5): 407-415.

Reader, T.A.(1976): Studies on the ultrastructure, histochemistry and cytochemistry of the uninfected digestive gland of *Bithynia tentaculata* (Moiiusca: Gastropoda) and on the ultrastructure of this host organ in snails infected with larval digeneans. Z. parasitenk.,50(1):11-30

Rebecchi, B.; Franchini, A. and Bolognani Fantin, A. M. (1996): The digestive gland of *Viviparus alter* (Mollusca, Gastropoda, Prosobranchia): an ultrastructural and histochemical study. Tissue and Cell, 28: 731–739.

Reinhold, J. G. (1953): Standard methods of clinical chemistry. Academic Press, New York. Res., 88 (4): 1185- 1195.

Reynolds, E. S., (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol., 172: 208-208.

Saad, A. (1988): Comparative Histochemical and Physiological Studies on Some Gastropods. Ph. D. Thesis, Ain shams Univ.

Saad, A. and Farag, E. A. (1988): Morphological studies on the digestive system of the land snail, *Eobania vermiculata* (Muller) (Gastropoda: Stylomatophora). Alex. J. Agric. Res., 33: 311-326.

Saad, A. and Mohammed, Sh. (1989): Histological and histochemical studies on the digestive gland of *Bulinus truncatus* infected with *Shistosoma heamatobium*. J. Egypt soc. Parasitology, 19(2): 671-629.

Sabha, M. E.; Adayel, S. A.; El-Masry, S. A. and Alazazy, H. M.(2013): *Bacillus thuringiensis* (Bt) toxin for the control of citrus trees snails. Researcher 5 (7):26-32.

Salama, A. K.; Osman, K. A.; Saber, N. A. and Soliman, S. A. (2005): Oxidative stress induced by different pesticides in the land snails, *Helix aspersa*. Pakistan J. Biological Sciences, 8 (1): 92 – 96.

Salem, A. A.; Mahrous, M. E.; Mervat, H. I. and Abd El-Aal, E. M. (2007): Different control measures for controlling certain land snail species in Sharkia Governorate, Egypt. Zagazig J. Agric. Res., 34(21): 291-305.

Samy, M. A.; El-Fakharany, K. M. and Hendawy, A. S. (2015): Population fluctuation and host preference of land snail *Monacha* Spp. And its control of biocides compared with Newmyl. Egypt. J. Agric. Res., 93(1). p93

Schuytema, G.S.; Nebeker, A.V. and Griffis, W.L. (1994): Effects of dietary exposure to forest pesticides on the brown garden snail, Helix aspersa (Muller). Arch. Environ. Contam. Toxicol. 26: p23-28.

Shaaban, M. A. (2005): Laboratory studies on the molluscicidal toxicity against some land snails species: Assiut J. Agric. Sci., Vol. 36: 1.

Shahawy, W. A. (1998): Ecological studies and some control methods for land snail *Monacha cantiana* (Montagu) at Kafr El-Sheikh governorate. M. Sc. thesis, fac. agric. Tanta univ. 86pp.

Shahawy, W. A. (2005): Biological and Histological Studies on The Land Snail *Monacha cantiana* (Montagu) and Its Control at Kafr El-Sheikh Region. Ph.D. Thesis, Fac. of Agric. Tanta Univ. Egypt. 129pp

Sharaf, H. M.; Salama, M. A. and Abd El-Atti, M. S. (2013): Biochemical and histological alterations in the digestive gland of the land snail *Helicella vestalis* (locard, 1882) exposed to methiocarb and chlorpyrifos in the laboratory. International J. Science and research, Val. 6(14): 334-343.

Shetaia, S. Z. (2005): Integrated Control of Land Snail Pests in The Fields of Sharkia Governorate. Ph.D. Thesis, Fac. Agric., Al-Azhar Univ. pp. 150.

Shetaia, S. Z.; Arafa A. A. and Abd-El-Atty S. F. (2013): Efficacy of certain compounds against the glassy clover snail, *Monacha cartusiana* (Müller) at Sharkia Governorate. J. Plant Protection and Pathology, Mansoura Univ., Vol. 4 (1): 67 – 73.

Synman, R. G.; Reinecke A. J. and Reinecke, S. A. (2005): Quantitative changes in the digestive gland cells of the snail *Helix aspersa* after exposure to the fungicide copper oxychloride. Ecoto. Environ. Safety, 60: 47-52.

Taieb, N. (2001): Distribution of digestive tubules and fine structure of digestive cells of *Aplysia punctata* (Cuvier, 1803). J. Moll. Stud., 67: 169-182.

Taieb, N. and Vicentea, N. (1999): Histochemistry and ultrastructure of the crypt cells in the digestive gland of *Aplysia punctata* (Cuvier, 1803). J. Moll. Stud., 65: 385-398.

Trevor, A. R. (1976): Studies on the ultrastructure, histochemistry and cytochemistry of the uninfected digestive gland of *Bithynia tentaculata* (Mollusca: Gastropoda) and on the ultrastructure of this host organ. Parasitenk, Vol. 50(1): 11-30.

Triebskorn, R. (1989): Ultrastructural changes in the digestive tract of *Deroceras reticulatum* (Muller) induced by a carbamate molluscicide and by metaldehyde. Malacologia, 31(1): 141-156.

Triebskorn, R. (1991a): The impact of molluscicides on enzyme activities in the hepatopancreas of *Deroceras reticulatum* (Muller). Malacologia, 33(1-2): 255-272.

Treibskorn (1991b): Cytological changes in the digestive system of slugs induced by molluscicides. J. Med. Appl. Malacol. 3: 113-123.

Triebskorn, R.; Christensen, K. and Heim, I. (1998): Effects of orally and dermally applied metaldehyde on mucus cells of slugs (*Deroceras reticulatum*) depending on temperature and duration of exposure. J. Moll. Stud., 64(4): 467-487.

Triebskorn, R.; Henderson, I. F.; Martin, A. and Kohler, H. R. (1996): Slugs as target or non-target organisms for environmental chemicals. BCPC Symposium Proceedings No. 66: Slug and snail pests in agriculture.

Triebskorn, R. and Kunast, C. (1990): Ultrastructural changes in the digestive system of *Deroceras reticulatum* (Mollusca; Gastropoda) induced by lethal and sublethal concentrations of the carbamate molluscicide cloethocarb. Malacologia, 32: 89-106.

Triebskorn, R.; Künastf, H. and Bremcf, R. (1989): The tracing of a C^{14} labeled carbamate mollnscicide through the digestive system of *Deroceras reticulatum*. Pesticide science, 28:321–330.

Ulrich, B. (1991): Histological observed of gonads and digestive gland in starving *Dreissena polymorpha*. Malacologia, 33(1-2): 31-42.

Walker, G., (1970): The cytology, histochemistry and ultrastructure of the cell types found in the digestive gland of the slug, Agriolimax reticulatus (Muller). Protoplasma, 71: 9-1109.

WHO (1965). Molluscicidal screening and evaluation. Bull., 38: 507-581.

Yasser, A.; El-Sayed H. E. and Hamdy, I. H. (2007): *Calotropis procera* glycosides are more effective on *Eobania vermiculata* (Müller) than methomyl and other plant glycosides. J. Agri. Sci., Mansoura Univ., 32(12): 10519-10527.

Youssef, A. E. (2001): Efficacy of certain pesticides against land snails *Monacha cantiana* and *Eobania vermiculate* under laboratory conditions. J. agric. Sci, .Mnasoura Univ., 26(2): 1161-1167.

Zaldibar, B.; Cancio, I. and Marigomez, I. (2007): Reversible alterations in epithelial cell turnover in digestive gland of winkles (*Littorina littorea*) exposed to cadmium and their implications for biomarker measurements. Aquat. Toxicol., 81: 183-196.

Zedan, H. A.; Saleh, A. A. and Ali, S. M. (1999): Bactericidal activity of *Bacillus thuringiensis* against snails toxicological and histological studies. The 2nd Int. Conf. of Pest Control, Mansoura, Egypt. 489 – 497.

Zhou, X.; Upatham, E.S.; Kruatrache, M. and Sretarugsa, P. (1993): Effects of Niclosamid and *Eucalyptus camaldulensis* on *Biomphalaria glabrata*, the snail intermediate host of *schistosoma mansoni*. J. sci. soc., Thailand 19: 117-128.

Chapter XIII: Arabic Summary

جامعة الزقازيق
كلية العلوم
قسم علم الحيوان

كفاءه بعض الكيماويات والمبيدات الحيوية على أنسجة وخلايا بعض القواقع الأرضية التي تصيب محاصيل الخضر في محافظه الشرقية

للحصول على درجة

دكتوراه الفلسفه فى العلوم

(علم الحيوان)

رسالة مقدمة من

فاطمة ابراهيم حسن عبدالله الأخرسي

باحث مساعد بمعهد بحوث وقاية النباتات مركز البحوث الزراعية

بكالوريوس علوم علم الحيوان - كلية العلوم- جامعة الزقازيق-٢٠٠٠

ماجستيرفي العلوم- كلية العلوم - جامعة الزقازيق- ٢٠١٠

تحت إشراف

الأستاذ الدكتور / كامل زكى حميد
أستاذ الخلية و الانسجة – قسم علم الحيوان
كليه العلوم - جامعه الزقازيق

الاستاذ الدكتور / سالم عبد الفتاح أحمد
استاذ المبيدات بمعهد بحوث وقاية النباتات
فرع الشرقيه - مركزالبحوث الزراعية

قسم علم الحيوان

كلية العلوم

جامعة الزقازيق

٢٠١٧

جامعة الزقازيق
كلية العلوم
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كفاءه بعض الكيماويات والمبيدات الحيوية على أنسجة وخلايا بعض القواقع الأرضية التي تصيب محاصيل الخضر في محافظه الشرقية

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فاطمة ابراهيم حسن عبدالله الأخرسي

باحث مساعد بمعهد بحوث وقاية النباتات مركز البحوث الزراعية

بكالوريوس علوم علم الحيوان - كلية العلوم- جامعة الزقازيق-٢٠٠٠

ماجستيرفي العلوم- كلية العلوم - جامعة الزقازيق- ٢٠١٠

قسم علم الحيوان

كلية العلوم

جامعة الزقازيق

٢٠١٧