EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 14 No. 1 (2022)

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci.* (F.Toxicology& Pest control) *Vol.14(1)pp159-178(2022)*
DOI: 10.21608/EAJBSF.2022.239448

Egypt. Acad. J. Biolog. Sci., 14(1):159-178(2022)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

The Efficiency of Chitosan & Silver and Their Nano-Particles on Histological and Enzymatic Activities of Land Snail, *Monacha obstructa* and Cutworm, *Agrotis ipsilon*

Amany R. Morsy, Ghada R. Mohamed and El- Shewy A.M.
Plant Protection Department, Faculty of Agriculture, Benha University, Egypt.
E-mail***** : **amani.alzoheri@fagr.bu.edu.eg**

ARTICLEINFO
Article History
Received: 3/3/2022
Accepted: 29/4/2022
Available:7/5/2022

***Keywords*:**
Agrotis ipsilon, *Monacha obstructa*, Toxicity, Nanoparticles, biochemical, histological effects

ABSTRACT

This study investigated the impact of chitosan & silver and their nanoparticles against the biochemical and histological aspects of the black cutworm, *Agrotis ipsilon* and the land snail *Monacha obstructa*. The activities of three vital enzymes [Aspartate amino transaminase (AST), Alanine amino transaminase (ALT) and acetyl cholinesterases (AChE)], total protein (TP) and total lipid (TL) were laboratory assayed. Results showed that chitosan and silver treatments increased the activity of AST, ALT and AChE in *A. ipsilon* and *M. obstructa*. On the contrary, chitosan and silver nanoparticles decreased the activity of these enzymes.

On the other hand, the levels of total protein and total lipid were decreased after treatment with all tested materials. In general, chitosan and silver nanoparticles affected the activities of enzymes, total protein and total lipid compared with control when applied against the tested cutworm larvae and snail. Meanwhile, many histological changes were observed in mid-gut of *A. ipsilon* larvae and the digestive gland of *M. obstructa* after treatments with LC_{50}'s of both nano chitosan and nanosilver.

Obtained data from this study suggest the possible use of chitosan and silver nanoparticles as alternatives to conventional pesticides and compatible with integrated pest management practices.

INTRODUCTION

The black cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) is a worldwide insect pest that comprises a great threat to the agriculture of many economic crop plants. Larvae cut the seedlings of cotton and many economic winter crops such as clover, wheat and bean (El-Kady *et al.*, 1990 and Abo El-Ghar *et al.*, 1994). *A. ipsilon* larvae always cause crop deficiency among open field crops by Cutting-off seedlings or tunneling into the bases of older plants and destroying their growing points, which can cause huge economic losses (Yu, *et al.*, 2012 and Du *et al.*, 2013).

Land snails are considered of the most serious pests of many crops and vegetables causing heavy economic damage as a result of feeding the plant leaves, roots and fruits (Hussein and Sabry, 2019) and contamination of agricultural products with their bodies, feces, or slime, leading to the deterioration of their qualities and financial loss (Ali, 2017). The glassy clover snail, *Monacha sp.* (Gastropoda: Helicidae) is considered the most predominant land snail in all localities in Sharkia Governorate attacking all plants (Mahrous, *et al.*, 2002).

Eobania vermiculata and *Monacha obstructa* are the important snail species in Egyptian governorates attacking various plantations (Miller, *et al.,* 1988 and Eshra, 2013).

Several attempts have been paid to control the dispersal of snails and insects by using nanocomposites. Nanotechnology has become one of the most promising new approaches for pest control (Owolade, *et al.,* 2008). In the past decade, nanomaterials (NMS) have provided a wide range of novel pesticide formulations, such as nano emulsion, nano capsules, nano suspension and metallic oxide NPS. These materials had higher efficacy in pest control and lesser harmful effects on the environment compared to the traditional ones (Buffle, 2006).

Silver nanoparticles (AgNPs) are used in pest management programs for Cowpea seed beetle, *callosobruchus maculates* (Rouhani, *et al.,* 2012). Also, silver nanomaterial over copper chlorophillin was most effective for controlling *Thrips tabaci* on onion fields (Merghany, *et al.,* 2019) and used to control *S. littoralis* in cotton fields (Ahmed, *et al.,* 2018).

Chitosan is derived by deacetylation of chitin, the second most abundant natural biopolymer isolated from crustaceans such as crab and shrimp (Kurita, *et al.,* 2000). Chitosan may serve as a good alternative because, it can be considered non-toxic to vertebrates and humans, biodegradable and may possess insecticidal properties (Robea, *et al.,* 2003 and Badawy, *et al.,* 2005). It has significant biological and chemical properties such as biodegradability minimizing the noxious effects of pesticides, biocompatibility which shield the toxic pesticide impact reducing the phytotoxicity and bioactivity, accordingly helping in leaching pesticide residues (Goodwin, *et al.,* 2007 and Sharma, *et al.,* 2019). At the same time, the silver nanoparticles (AgNPs) the most prevalent metallic nanoparticles in consumer products due to their effects on microbes (Schmid, *et al.,* 2003). The ultimate target of nano silver composition for necessary world applications is to obtain nanoparticles that are characterized by: (a) constant and lean size distribution (b) well-known shape (c) known chemical structure with no impurities and (d) no aggregation or clot (Sooresh, *et al.,* 2011).

The present study was planned to determine the biochemical effects of two nanoparticle materials namely, chitosan and silver nanoparticles on larvae of the black cutworm, *Agrotis ipsilon* and the land snail *Monacha obstructa* on the activities of enzymes such as Aspartate amino transaminase (AST), Alamine amino transaminase (ALT) and acetyl cholinesterases (AchE). Also, total proteins (TP) and total lipids (TL) to spot a light on the toxicity of such chemicals. Besides, the effect of half sublethal concentrations of the two nanoparticles on the histology of mid-gut of *Agrotis ipsilon* and the digestive gland for snail using light microscopy has been studied.

MATERIALS AND METHODS

Tested Materials:

1-Chitosan was obtained from Egyptian – Candian company for Humate Technology and Agricultural consultancy.

Chitosan bulk (CS Bulk) with a purity of 99.99%, degree of deacetylation (DDA 90%), chitosan nanoparticles Cs, NPs) with a purity of 99.99%, size of 100 nm, degree of deacetylation (DDA 89%).

2-Silver nanopowder 99.5% (CAS no 576832) was purchased from sigma-Aldrich, St. Louis, Mo, USA.

All prepared samples were characterized by Transmission Electron Microscopy (TEM) as a base tool for scaling the particles' size and shape in the Nanotechnology and Advanced Material Central Lab, National Research Center (NRC). Gun type: LaB_6 Gun. (Figs.1&2)

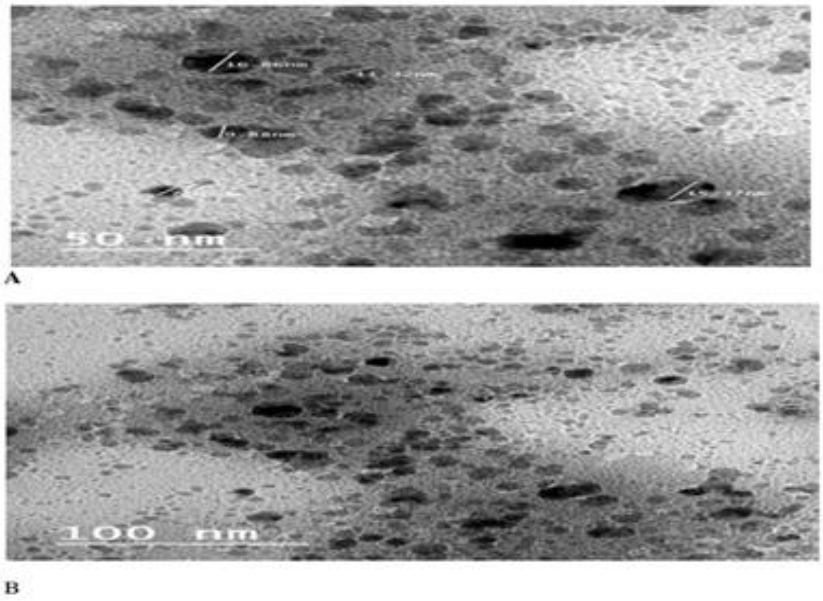

Fig. 1: Nano chitosan particles photographed under scanning electron microscopy (A and B) at 50 and 100 nanometers**.**

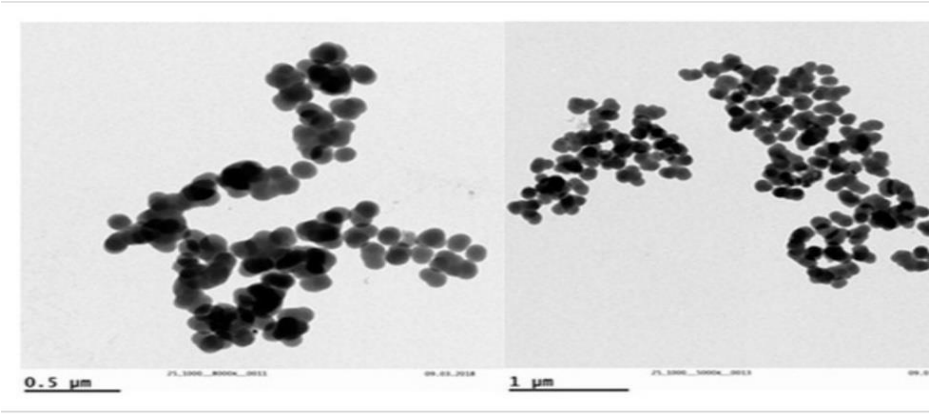

Fig. 2: Characterization of silver nanoparticles (AgNP) using transmission electron microscopy (TEM).

Preparing Tested Insect and Animal and Laboratory Treatments:

A- Black Cutworm (*Agrotis ipsilon*):

Larvae of the black cutworm, *A. ipsilon* was obtained from the Plant Protection Research Institute, Ministry of Agriculture Dokki, Giza, without any insecticidal contamination. The larvae were fed on castor bean leaves, *Ricinus communis* L. maintained at 22 ± 2°C and 65 ±5% relative humidity. The rearing technique was the same adopted by Abdin (1979).

For treatments serial concentrations of chitosan and silver were prepared; 6, 4, 2, 1 and 0.5%, while those were; 0.5, 0.2, 0.1, 0.06 and 0.03% for nano chitosan and silver. From the maintained insect culture, larvae of the 4th instar were the only ones used for experiments. Larvae were fed on castor leaf discs 2.5cm in diameter which were dipped in each concentration of tested compounds for two minutes and left to dry at room temperature. Three replicates of ten larvae/each were used for each concentration. Mortality counts were recorded after 24, 48, 72 and 96 hours. Mortality percentages were corrected according to Abbott's formula (1925). To estimate the LC_{50} values, the corrected mortality percentages were subjected to probit analysis as reported by Finny (1971).

B-Land Snail *(Monach obstructa)*:

Individual adults of land snail, *Monacha obstructa* were collected from the infested ornamental plants at Qalubia Governorate. Animal individuals were transferred to the laboratory, kept in glass boxes and fed on fresh lettuce leaves for two weeks for acclimatization before treatment (El-Okda, 1981). The glass boxes were covered with a light perforated cloth to help the animals breathe and prevent the individuals from escaping (Mohamed, 2018). For each treatment, thirty healthy animals were allocated and divided into three replicates (each of 10 individuals) for treatment (Ghamry *et al.,* 1993) and another for control. The animal adults were starved for a week before the start of the experiments.

For treatment serial concentrations of the tested compounds were prepared at 10, 8, 6, 4 and 2% for chitosan and silver while those were 0.8, 0.4, 0.2, 0.1 and 0.05% for nano chitosan and nanosilver. Three replicates (each of 10 adults) were used for each concentration. Fresh lettuce leaves were mixed with different concentrations for tested compounds and placed in the boxes with *M. obstructa* and covered to prevent escape. The killed animals were daily counted and removed. Mortalities were recorded after 1, 3, 5, 7 and 10 days of treatment. The recorded mortality percentages were corrected according to Abbott's formula (1925) and LC_{50} values were determined according to Finny (1971).

Biochemical Studies:

insects and animals were treated with LC_{50} of each compound to determine their effect on some biochemical parameters i.e., Alanine amino transaminase (ALT), Aspartate amino transaminase (AST) and Acetylcholinesterase (AChE) enzymes, in addition to total proteins (TP) and total lipids (TL). These parameters were measured at 3, 5 and 7days post-treatment.

Samples Preparation for Biochemical Tests:

One gram weight of snail or insect was used according to the method of Bergmeyer (1963). Shells of snails were removed and tissues of snails or insects were homogenized with 10 folds (w/v) of distilled water by using a glass homogenizer. Homogenates were centrifuged at 5000 rpm for 30 minutes using a cooling centrifuge at 4°C and the supernatant was used as a source of protein and enzyme assays.

Determination of Total Proteins and Lipids:

Total proteins were colorimetrically determined according to Gornall, *et al.*, (1986). While total lipids were assayed by the method of Zollner and Kirsch (1962).

The activity of AST and ALT was determined according to Farkas, *et al.,* (2004):

Determination of AChE:

Acetylcholinesterase (AChE) was determined according to Ellman *et al.,* (1961).

Histological Studies:

A- Black cutworm (*Agrotis ipsilon*):

All tested compounds at their LC_{50} values were applied to 4th instar larvae of (*A. ipsilon*). Larvae were, then, dissected in saline solution (0.75% sodium choloride in distilled water), and the mid-gut of the larvae was fixed in alcoholic Bouin's fluid for 24 hrs and then processed using the routine technique for paraffin embedding sections of 5µ thickness and stained with Haematoxylin and Eosin prepared for observation and photomicroscopy. The morphological alterations of the mid-gut tissue structure and organization of each specimen were analyzed by microscopic examination and compared to the tissue taken from the control group (Humason and Freeman, 1979).

B- Land Snail, (*M. obstructa*):

The animal was treated with LC_{50} of each tested compound for histological investigation, 5 snails were selected randomly from each experimental group. Shells were crushed and fragments were removed and the digestive gland was immediately excised and cut into small pieces. These specimens were fixed in an aqueous bouin's solution for 24 hrs.,

then kept in a mixture of 70% ethanol and glycerol (95:5). Then dehydration is achieved through an ascending series of ethanol followed by clearing in terpineol for three days, washed in benzene and embedded in paraffin wax. Sections of 6µ thick were prepared, mounted on clean glass slides and stained with Ehrlich's acid alum haematoxylin and counterstained by Eosin.

Finally, the slides were examined and photographed using a light microscope (Olympus CX31) connected with a digital camera at the central lab., Faculty of Education, Ain Shams University according to the method described by Romeis (1989):

Statistical Analysis:

All the experiments were carried out carefully and the data were recorded and analyzed statistically. Results were presented as mean ± standard error (SE). $P < 0.05$ was regarded as statistically significant (Sokal. and Rohilf. 1981).

RESULTS AND DISCUSSION

Toxicities of Chitosan & Silver and Their Nano-Particles on *A. ipsilon* and *M. Obstructa* under Laboratory Conditions:

The LC_{50} values for chitosan, silver and their nanoparticles after three- and five days post-treatment on *A. ipsilon* were (0.07%) for nano chitosan, while for chitosan was (1.10%) (Table, 1). Also, the LC_{50} values for silver were calculated as 1.02% and 0.11% for nanosilver. The correspondent values of LC_{90} were much lower in nanosilver treatment (1.07%) than that of natural silver (40.73%). The LC_{50} values for chitosan were calculated as (1.182%) for *M. obstructa* and (1.854 and 0.97%) for silver and nanosilver, respectively.

The correspondent values of LC_{90} were much lower in nano chitosan treatment (6.873%) than that of natural chitosan (13.826%). Also, the LC_{90} values were lower in nanosilver treatment (16.29%) compared with (22.939%) for natural silver. (Table, 2)

The obtained results were in agreement with Sabbour and Solieman (2016) who found that chitosan nanoparticles were more effective than chitosan in bulk for different instars. Also, Sahab *et al.,* (2015) proved that nano chitosan showed the highest effect against the three insets of soybean. At the same trend, Barrera-Necha *et al.,* (2018) confirmed that chitosan bulk (chitosan nanoparticles and chitosan-loaded botanical extracts against *Altermaria* spp. and *colletotrichum gloeosporioides* had a potent pesticidal activity. Kheiri *et al.,* (2017) demonstrated that chitosan nanoparticles at low molecules were more effective than at high molecules.

Also, Sayed *et al.,* (2014) declared that nano chitosan was active against the 4th *S. littoralis* larval instar when larval mortality increased with nano chitosan concentration, mortality percentages ranged between 27.08 and 92.19%.

Similarly, Emam Kamel Khidr (2018) stated that the LC_{50} values recorded 8.6% and 6.9% for *Eobania vermiculata* and *Monacha obstructa* treated with chitosan, respectively. On the other hand, snails treated with different concentrations of nano chitosan resulted in LC_{50} values of 1.4% and 0.16% for *E. vermiculata* and *M. obstructa,* respectively.

Safaa, *et al.,* (2015) found that the exposure of the snails and soil matrix to silver nanoparticles (AgNPs) in a laboratory experiment reduced the activity and the viability of the land snail (20% of AgNPs treated snails died). Moustafa *et al.,* (2018) proved that Ag and Ag NPS were effective in killing *Biomphalaria alexandrina* snails, with 30Mg/m/Ag and 160Mg/ml Ag causing 100% mortality. The LC_{50} of 9.68 Mg/ml for Ag NPS and 133.7 Mg/ml for (Au) gold NPs prevented snail infection with *S. mansoni miracidia*. Ag NPs at 50 mg/ml and Au NPs at 100 Mg/ml increased the morality of *S. mansoni cercariae* in a dose and time-dependent manner, reaching 100% mortality after 1h.

Table 1: LC_{50} and LC_{90} values of chitosan & silver and their nanoparticles on *A. ipsilon*.

Treatments	LC_{50} (Lower-upper)	LC_{90} (Lower-upper)	Slope ± SE	R
Chitosan	1.1 0.05-0.21	1.58 0.66-3.81	1.16± 0.07	0.97
Nano chitosan	0.07 0.02-0.20	1.37 0.68-2.79	0.96±0.01	0.992
Silver	1.02 0.53-1.94	40.73 3.55-467.11	0.80±0.008	0.993
Nano silver	0.11 0.06-0.20	1.07 0.59-1.85	1.34±0.12	0.964

Table 2: LC_{50} and LC_{90} values of chitosan & silver and their nanoparticles on *M. obstructa*.

Treatments	LC_{50} (Lower-upper)	LC_{90} (Lower-upper)	Slope ± SE	R
Chitosan	1.182 0.538-2.597	13.826 6.294-30.370	1.213± 0.174	0.979
Nano chitosan	0.391 0.21-0.634	6.873 2.65-92.61	1.029±0.256	0.966
Silver	1.854 1.08-2.82	22.939 10.59-143.97	1.173±0.26	0.959
Nano silver	0.97 0.60-1.58	16.29 3.57-74.23	1.04±0.04	0.977

Biochemical Studies:

The biochemical effects of chitosan & silver and their nanoparticles on the activity of AST, ALT, AChE enzymes, total proteins (TP) and total lipids (TL) on *A. ipsilon* were recorded in Tables (3 & 4).

Activity of Aspartate Amino Transaminase (AST):

The optional data showed that chitosan increased the level of (AST) after 3, 5and 7 days post-treatment with (50, 61.05 and 90.82 u × 10^3/mg). on contrary, nano chitosan decreases the level of (AST) enzyme after 5 and 7days post-treatment with (51.73 and 50.20 u × 10^3/mg) than control (55.12 u × 10^3/mg).

Activity of Aspartate Amino Transaminase (ALT):

Chitosan increased the level of (ALT) enzyme after 5 and 7days post-treatment except at 3 days which decrease the level of ALT enzyme. On the other hand, nano chitosan began to increase the level of (ALT) enzyme after 5 and 7days post-treatment. While after 7 days decreased the level of ALT was then controlled.

The activity of Acetylcholinesterase (AChE):

The activity was increased in *A. ipsilon* during all different periods post-treatment. There were significant differences between all day's post-treatment and control causing the highest increase with (214.28 and 261.0 nol/min/mg), respectively. While nano chitosan caused the lowest increase in the level of this enzyme especially, after 7 days post-treatment (140.13 nol/min/mg) in comparison with control (143.82 nol/min/mg).

Total Proteins:

Chitosan and nano chitosan decreased the level of total proteins less than the control.

Total Lipids:

Data illustrated that there was a significant increase in the case of chitosan and nano chitosan at different periods post-treatment and control. Data showed that silver increased the level of (AST) enzyme after 3 and 5 days with (76.90 and 57.51 u × 10^3/mg) compared to control (47.44 u × 10^3/mg). On contrary, nanosilver began to reduce the activity of AST after 5- and 7-days post-treatment with (48.40 and 30.80 u × 10^3/mg), respectively. Data also revealed that silver increased the level of ALT after 3 days with (39.02 u × 10^3/mg). while after 5 and 7 days of reduced the activity of ALT similar results were observed in nanosilver, the ratio of ALT activity achieved a slight reduction from 3 to 7 days with (19.02, 19.90 and 17.46 u × 10^3/mg) compared with (20.90 u × 10^3/mg) for control. Data showed that silver caused significantly increased the activity of AChE after three periods post-treatments. But nanosilver caused decrease in AChE level after 5 and 7 days with (82.32 and 76.30 nol/min/mg), respectively compared with 109.43 nol/min/mg) for control. Data also indicated that silver and nanosilver reduced the levels of total protein and lipid after all tested periods with significantly different values and the highest reduced values were noticed after 7 days in the case of silver and their nanoparticles.

Table 3: Biochemical components (mean±SE) of *A.ipsilon* exposed to half sublethal concentrations of chitosan and nano chitosan at different periods.

Tested compound	Different periods post-treatment	AST (u×10^3/mg)	ALT (u×10^3/mg)	AChE (u×10^3/mg)	T. P (u×10^3/mg)	T. L (u×10^3/mg)
Chitosan	3	50.00 ± 1.00	5.66 ± 0.47	200.66 ± 12.17	6.92 ± 0.75	6.98 ± 0.34
	5	61.05 ± 1.39	16.06 ± 0.11	214.28 ± 26.50	12.26 ± 0.62	6.17 ± 0.53
	7	90.82 ± 3.16	15.70 ± 0.44	261.0 ± 1.40	9.81 ± 0.22	8.33 ± 0.73
Nano chitosan	3	60.20 ± 1.15	31.15 ± 1.33	243.40 ± 20.0	20.73 ± 1.17	9.06 ± 0.60
	5	51.073± 0.91	26.82 ± 0.71	189.70 ± 2.46	15.74 ± 1.32	8.09 ± 0.51
	7	50.20 ± 1.03	16.36 ± 0.60	140.13 ± 4.15	13.20 ± 0.33	6.49 ± 0.46
Control		55.12 ± 2.30	17.33 ± 1.25	143.82 ± 5.80	30.22 ± 1.88	9.00 ± 0.69

Table 4: Biochemical components (mean±SE) of *A.ipsilon* exposed to half sublethal concentrations of silver and nanosilver at different periods.

Tested compound	Different periods post treatment	AST (u×10^3/mg)	ALT (u×10^3/mg)	AChE (u×10^3/mg)	T. P (u×10^3/mg)	T. L (u×10^3/mg)
Silver	3	76.90 ± 2.26	39.02 ± 2.60	271.0 ± 20.3	12.41 ± 0.50	14.13 ± 1.50
	5	57.51 ± 0.93	19.50 ± 1.00	234.36 ± 10.5	12.55 ± 0.22	11.12 ± 0.80
	7	46.78 ± 0.93	17.52 ± 0.60	155.19 ± 9.2	10.00 ± 0.68	6.96 ± 1.14
Nano silver	3	58.33 ± 0.44	19.02 ± 0.13	100.89 ± 3.00	15.02 ± 1.80	9.11 ± 1.90
	5	48.40 ± 1.02	19.90 ± 1.73	82.32 ± 5.56	11.77 ± 0.14	7.22 ± 0.50
	7	30.80 ± 1.40	17.46 ± 0.45	76.30 ± 0.41	9.30 ± 0.88	5.20 ± 0.10
Control		47.44 ± 2.15	20.90 ± 0.78	107.43 ± 1.80	25.23 ± 0.68	12.99 ± 1.16

2. Effect of Chitosan & Silver and Their Nanoparticles on The Activity of AST ALT, AChE Enzymes, T.P and T.L Levels in *M. obstructa:*

The biochemical effects of chitosan & silver and their nanoparticles on the activity of AST, ALT, AChE enzymes, total proteins (TP) and total lipids (TL) on *M. obstructa* were recorded in Tables (5 & 6).

Effect of Aspartate Aminotransferase (AST):

Data showed that chitosan increased the activity of AST after 5 and 7 days with (55.5 and 79.72 u × 10^3/mg), respectively. On the contrary, nano chitosan began to reduce the level of the enzyme also, after 5- and 7-days post-treatment with (45.60 and 74.4 u × 10^3/mg), respectively compared with (50.15 u × 10^3/mg) for control.

Effect on Alanine Aminotransferase (ALT):

Data showed a significantly increased level of ALT after three periods post-treatments when applied with chitosan against the snail. Whereas nano chitosan reduced the activity of ALT, especially after 3 days compared with control.

Effect on AChE:

AChE activity was increased in *M. obstructa* after treatment with chitosan after 5- and 7-days post-treatment (200.25 and 255.0 u × 10^3/mg) more than in control (140.73 u × 10^3/mg). On the other hand, nano chitosan caused the lowest increase in the level of this enzyme after 5- and 7-days post-treatment (185.66 and 136.12 nol/min/mg).

Total protein and lipid:

Chitosan and nano chitosan decrease the level of total protein and lipid less than control when applied against *M. obstructa* after all three periods post-treatments.

Data also illustrates that silver gradually reduced the mean of AST from 77.10 u × 10^3/mg after 3 days to 68.88 u × 10^3/mg after 7 days. Also, nanosilver showed the highest decrease in AST enzyme, especially after 7 days of pot-treatment compared with (99.67 u × 10^3/mg) for control.

Effect on ALT:

The obtained data showed that silver and nanosilver increased the level of (ALT) after 3- and 5-days post-treatment, that reaches (26.1 and 22.1 u × 10^3/mg), respectively in silver. But nanosilver reaches (5.33 and 4.8 u × 10^3/mg) more than the control (3.1 u × 10^3/mg).

Effect on AChE:

Data revealed that the two tested compounds increased the level of (AChE), especially after 5 days post-treatments with (30.8 u nol/min/mg) in silver. While in nanosilver (25.6 nol/min/mg) comparing with (16.4 nol/min/mg) for control.

Effect on Total Protein and Lipid:

Data indicated that silver caused an increased level of total lipid and protein, especially after 3 days post-treatment. While nanosilver gradually reduced the level of total protein and lipid from 3 days to 7 days post-treatment and the 7th day of exposure to nanosilver showed the highest decrease in total protein and lipid compared with control.

Table 5: Biochemical components (mean±SE) of *M.obstructa* exposed to half sublethal concentrations of chitosan and nano chitosan at different periods.

Tested compound	Different periods post treatment	AST (u×10^3/mg)	ALT (u×10^3/mg)	AChE (u×10^3/mg)	T. P (u×10^3/mg)	T. L (u×10^3/mg)
Chitosan	3	46.6 ± 0.09	27.12 ± 1.22	195.55 ± 11.11	4.83 ± 0.66	5.90 ± 0.30
	5	55.5 ± 1.20	23.72 ± 0.61	200.25 ± 20.30	10.22 ± 0.53	4.16 ± 0.50
	7	79.72 ± 1.15	19.2 ± 0.50	255.0 ± 1.30	7.70 ± 0.11	6.30 ± 0.71
Nano chitosan	3	51.10 ± 1.10	13.03 ± 0.12	240.33 ± 10.0	16.65 ± 1.16	7.03 ± 0.50
	5	45.60 ± 0.41	12.60 ± 0.33	185.66 ± 2.00	11.70 ± 1.24	6.06 ± 0.42
	7	47.4 ± 1.05	2.22 ± 0.26	136.12 ± 3.00	9.22 ± 0.22	4.40 ± 0.43
Control		50.15 ± 1.50	15.22 ± 1.15	140.73 ± 4.10	20.11 ± 1.70	8.11 ± 0.56

Table 6: Biochemical components (mean±SE) of *M.obstructa* exposed to half sublethal concentrations of silver and nanosilver at different periods.

Tested compound	Different periods post treatment	AST ($u\times10^3$/mg)	ALT ($u\times10^3$/mg)	AChE ($u\times10^3$/mg)	T. P ($u\times10^3$/mg)	T. L ($u\times10^3$/mg)
Silver	3	77.10 ± 1.83	26.1 ± 2.2	24.24 ± 1.6	2.8 ± 0.44	3.60 ± 1.62
	5	90.44 ± 3.90	22.1 ± 1.1	30.8 ± 2.66	1.8 ± 0.66	3.10 ± 0.58
	7	64.88 ± 1.44	18.3 ± 1.4	17.4 ± 1.22	1.4 ± 0.66	2.20 ± 1.23
Nano silver	3	81.33 ± 1.55	5.33 ± 0.25	21.4 ± 2.22	0.84 ± 2.21	0.88 ± 0.66
	5	72.92 ± 1.60	4.8 ± 0.24	25.6 ± 2.20	0.76 ± 1.11	0.68 ± 0.54
	7	64.57 ± 1.66	4.0 ± 0.62	18.2 ± 1.4	0.72 ± 3.10	0.42 ± 0.40
Control		99.67 ± 1.41	3.1 ± 0.50	16.4 ± 1.23	0.92 ± 1.80	1.10 ± 0.41

These results agree with Khater *et al.,* (1990) and Mobarak (2014) found that the increase in the total protein of *M. obstructa* and *E. vermiculata* could be attributed to the increased biosynthesis process that occurred to high enzyme stress. Also, Kaandil *et al.,* (2014) reported that acetylsalicylic acid exhibited the highest effect on total protein.

Saxena *et al.,* (1989), Kandil *et al.,* (2014) found that acetylsalicylic acid exhibited the highest effect on total lipid which is important for the synthesis of shell and mucus.

Eman Kamel Khidr (2018) total protein activities showed a significant increase compared to control for *E.vermiculata.* the same compounds showed a significant decrease in the level of total protein for *M. obstructa*. Natural chitosan increased the level of total lipid from 35.5 to 52.5 g/dl after treatment. While nano chitosan increased up to 78.33 g/dl for *E. vermiculata*. Hanan Alfy *et al.,* (2020) found that chitosan nanoparticles had a very large effect in increasing the enzymatic activity of both the enzyme chitinase and the protease but the decrease in the enzymatic activity of the enzyme alkaline phosphatase of *spodoptera littoralis* and *locusta migratoria.* Osman *et al.,* (2015) reported a decrease in the total protein of *S. littoralis* after treatment with silica nanoparticles.Galal and El-Samahy (2012) reported a successive increase in total protein. He owes this increase to over expressions of some proteins as the nanoparticles interact with cellular proteins. Radwa and Shireen (2020) found that total protein contents were 28.88, 28.65, 28.2 and 30.23 mg/ml after treatment with mesoporous silica nanoparticles (MSNS), silica nanoparticles (CESN), cinnamon oil and silica gel, respectively, compared to 32.56 mg/ml in the untreated control against rice moth, *Corcyra cephalonica* (Lepidoptera: pyralidae). Khaled Yassin, *et al.,* (2021) found that vertimec 1.8% Ec, fast max super 8.4% Sc and nano-derived form of abamectin (1% nano-emulsion) significantly decreased AchE activity, GST, ACP and ALP activities. However, AST, ALT activities exhibited an increase greater than the untreated group in haemolymph and digestive gland against land snail, *Helix aspersa*. Jyothsna and Pathipati (2015) reported that silver nanoparticular (AgNPs) showed changes in the antioxidative and detoxifying enzymes of the treated larva of *spodoptera litura* and *Achaea janata L*. Also, showed differences in the activities of detoxifying enzymes, carboxylest ereases (care), glucosidases (Glu) and glutathione –s- transferases (GST) in the larval gut, suggesting that exposure of larvae to nanoparticles induces oxidative stress, which is countered by antioxidant enzymes.Memarizadeh *et al.,* (2014) studied the effect of Tio_2- nanoparticles (Tio_2-NPs) on the activity of general estorases (EST), peroxidase (POD) and glutathione-S-transferase (GST) of the g*lyphodes pyloalis* walker. The activity of EST and GST significantly decreases compared to control, after 24 hours of treatments. By increasing exposure time, the expression of EST and GST was significantly increased. Sharaf *et al.,* (2016) investigated the impact of two pesticides namely: Methiocarb and chlorpyrifos against the biochemical activities of three enzymes. These enzymes were Aspartate amino transaminase (AST),

Alanine amino transaminase (ALT), Alkaline phosphatase (ALP), total protein (TP) and total lipid (TL). Results showed that all tested pesticides lead to an increase in the activity of AST, ALT and ALP in the tissue homogenate of the digestive gland of the land snail *Helicella vestalis*. Also, the levels of total protein and total lipid were increased after treatment with all tested pesticides. Abdel Rahman *et al.,* (2019) studied the effect of both neem and peppermint oils as bulk, nano and loaded nano- emulsions on enzymatic activities for the 2nd and 4th larval instars of *Agrotis ipsilon* (Hufn) in the laboratory. Results show marked effects of the three formulations of either neem or peppermint oil; significant inhibitions were recorded for amylase, invertase, trehalas, protease and alkaline phosphatase.

Awad *et al.,* (2022) studied the effects of lethal and sublethal concentrations of chlorantransiliprole, this cyclam and their nano-forms on oxidative stress enzyme activity in the black cutworm, *Agrotis ipsilon*. Results showed significant decreases of oxidative stress enzymes (SOD, CAT) glutathione reductase and lipid peroxidase activities of 2nd instar larvae of *A. ipsilon.*

Histological Changes in The Mid Gut of the 4th Instar Larvae of *A. ipsilon* Treated with Nano Chitosan and NanoSilver:

The mid-gut of normal larvae of *A. ipsilon* consists of an epithelium cells layer surrounded by the basement membrane, possesses oval nuclei nearly central in position, scattered between them are small goblet cells shown with reduced granular cytoplasm and spherical nuclei. The epithelial cells show brush borders which in fact, are the microvilli of columnar cells extending from their free ends. The wall of the gut contains two distinct layers of muscle fibers, longitudinal muscle fibers to the outside and circular muscle fibers to the inside. The space between the different gut wall layers is filled with connective tissue. (Fig,3).

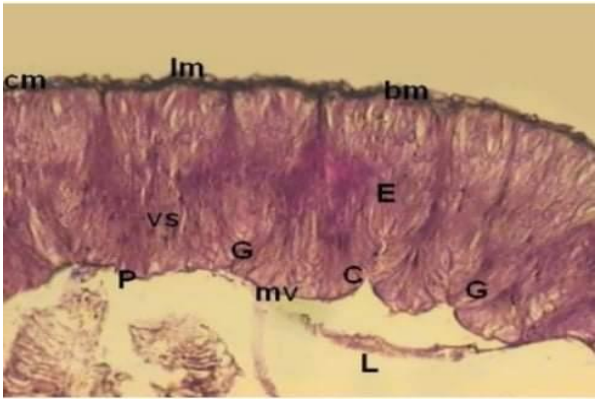

Fig. 3: Cross-section in the mid-gut of normal 4th larval instar of *A. ipsilon*
Bm: basement membrane; E: Epithelial cell; VS: vesicles; P: peritrophic membrane; L: lumen; G: Goblet cell; C: columnar cell; mv: microvilli; cm: circular cell; IM: longitudinal muscles.

Effect of Nano Chitosan:

Figure (4) shows the disruption of epithelial cells which lost their close association with the basement membrane. Those appeared destroyed and lost their columnar structure at some points. Disorganization of the peritrophic membrane was clear and, in some cases, disappeared. Also, the cytoplasm lost its granular appearance, the columnar cells appeared highly vacuolated, liquefied and most of it seemed to be necrotic.

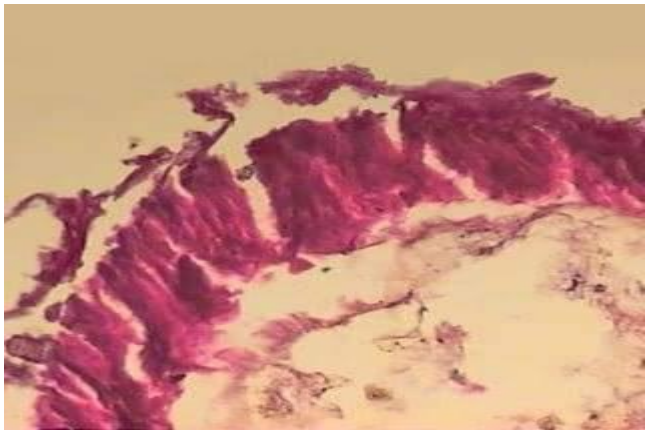

Fig. 4: Cross-section in the mid-gut of *A. ipsilon* larva treated with LC_{50} of nano chitosan

Effect of NanoSilver:

Many abnormalities changes appeared in *A. ipsilon* mid-gut. Those within Fig. (5) included epithelium cells detached from the basement membrane in many areas and the thickness of these epithelial cells. Deformations in larval mid-gut included, also some broken cells which became emptied from their cytoplasmic contents in the space between the epithelial cells and peritrophic membrane.

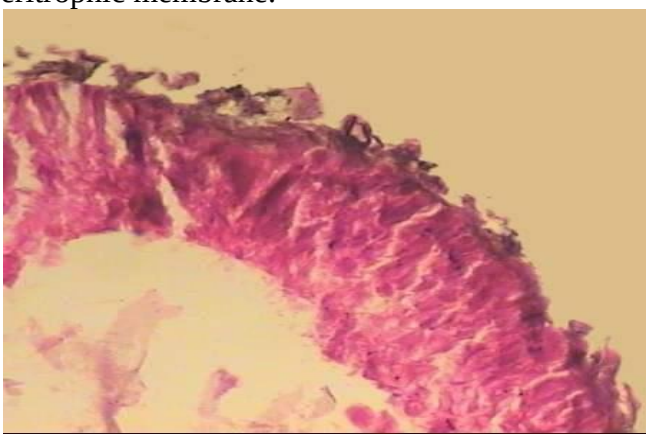

Fig. 5: Cross-section in the mid-gut of *A. ipsilon* larva treated with LC_{50} of nanosilver.

These results agree with Khaled *et al.,* (2019) who studied silver nanoparticles (AgNPs) as a pesticide carrier by loading the pyrethroid pesticide lambda-cyhalothrin (L-CYN) in mid-gut tissues of 6th instar larvae of *Spodoptera littoralis*.Their results showed thickness and deformation of mid-gut epithelial cells in treated larvae. Also, the basement membrane, some columnar cells, microcells and peritrophic membrane were destroyed. Saadiya, *et al.,* (2011) revealed that chitosan has some histopathological effects on the mid-gut of 3rd instar larvae of *Galleria mellonella*. The cells seemed to become thick; the epithelium cells were to some extent elongated, disorganized and disintegrated. Mishra, *et al.,* (2015) studied the impact of *Thevetia neriifolia* methanol stem extract against early fourth instar larvae of *Helicoverpa armigera*. Results showed epithelial cell increased disruption with augmented vacuolization. Detachment of the epithelial layer from the basement membrane resulted in the appearance of a large space detached area.

Manimegalai, *et al.,* (2020) showed that silver nanoparticles (AgNPs) caused severe tissue damage in the epithelial and goblet cells in the larval mid-gut region of *spodoptera litura* and *Helicoverpa armigera*.

Histopathological Effects of Nano Chitosan and Silver on Digestive Gland of *M. obstructa*:

Histology of Untreated Digestive Gland:

The digestive gland of *M. obstructa* Fig. (6) consists mainly of digestive tubules (DT) separated by intertubular connective tissue (CT) containing hemolymphatic sinues and hemocytes (HE). Each tubule is surrounded by a circular muscle layer (ML). three different cell types are observed in the epithelium lining the digestive gland tubules (Fig, 3), the cells are differentiated into, digestive cells (DC), Calcium cells (CC) and excretory cells (EC).

Digestive Cell:

Digestive cells constitute the most abundant cellular component of the digestive gland's tubular epithelium.

Digestive Cells Are Simple Columnar Epithelium:

The basally located nuclei (N) of digestive cells are rounded or oval.

Calcium Cells:

Fewer than digestive cells occur singly or in groups in the corners of tubules. They have a pyramidal shape with a pointed distal end. Calcium cells possess apical excretory granules (EC) and large rounded nuclei).

Excretory Cells:

Excretory cells have a rounded shape. They are characterized by the presence of a single large vacuole (v).

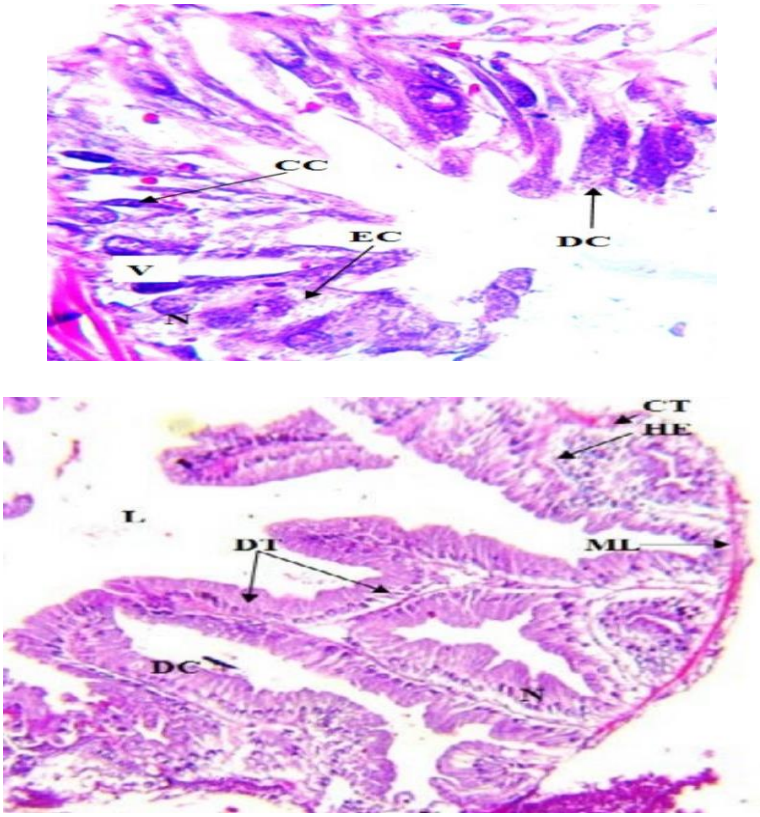

Fig. 6: Photomicrographs showing cross sections of digestive gland untreated *M. obstructa* DC: Digestive cell; DT: Digestive tubules; CT: Calcium cell; HE: Hemocytes; L: Lumen; ML: Muscle layer; N: Nucleus.

1. After Treatment with LC_{50} of Nano Chitosan:

Few tubules with severe atrophy were mingled in some places. The basement membrane of tubules appeared ruptured. The digestive cells show accumulation of large numbers of dark granules (DG) and appear to undergo extensive breakdown into membrane-bound vesicles. Calcium cells packed with enlarged calcium spherules and exhibited pyknotic nuclei (PN). The cytoplasm was replaced by large vacuoles containing darkly stained granules. Excretory cells increased the number of excretory granules with cellular debris. (Fig. 7).

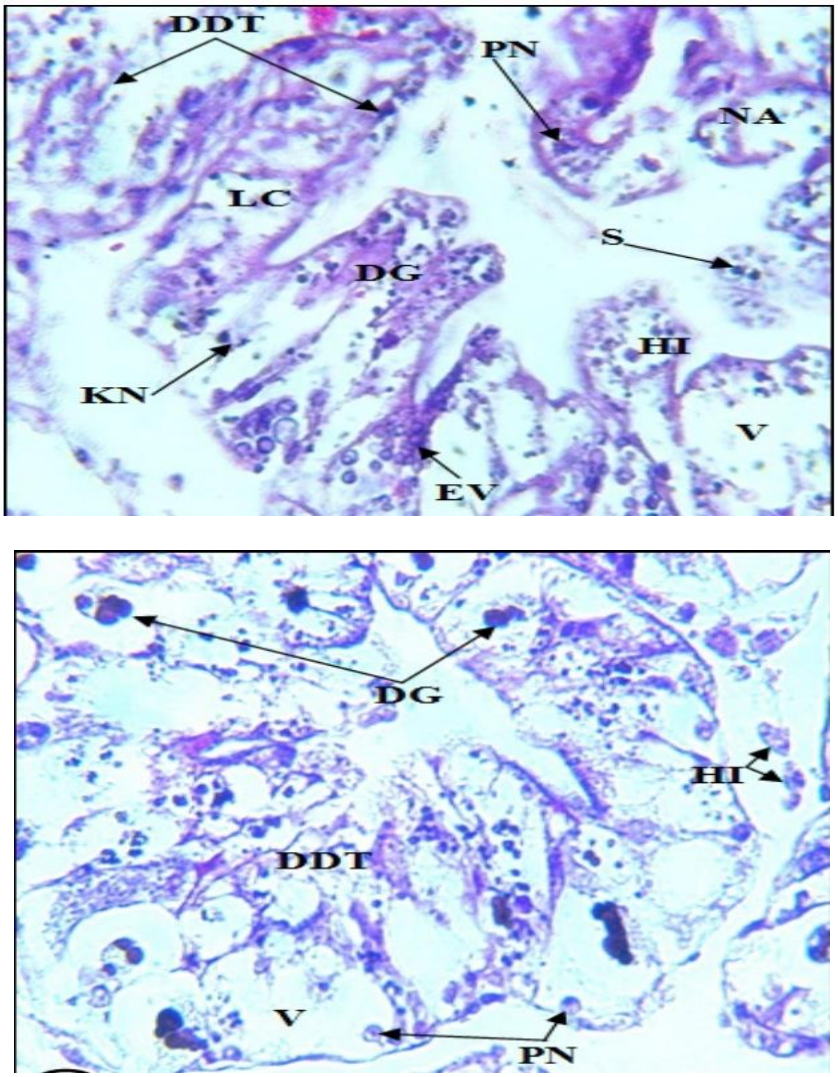

Fig.7: Photomicrographs showing cross sections of digestive gland treated with LC_{50} of nano chitosan on *M. obstructa*

DDT: Destructed digestive tubules; DG: Dark granules; HI: Hemocyte infiltration; KN: Karyolitic nuclei; V: Vacuolation; PN: Pyknotic nuclei; LC: Lytic cells; S: Secretions; EV: Excretory vesicles; NA: Necrotic areas

2. After Treatment with LC_{50} of Nanosilver:

Light microscopic examination after silver treatment revealed various tubular deteriorations, inflammatory hemocytic infiltrations, tissue exudates and excessive luminal secretions. The epithelial lining undergoes vacuolization, and the basement membranes and muscular layers surrounding tubules were lacerated. The digestive cells have accumulations

of large numbers of darkly stained granules and pyknotic nuclei. Excretory cells showed a decreased amount of dark brown granules (Fig 8).

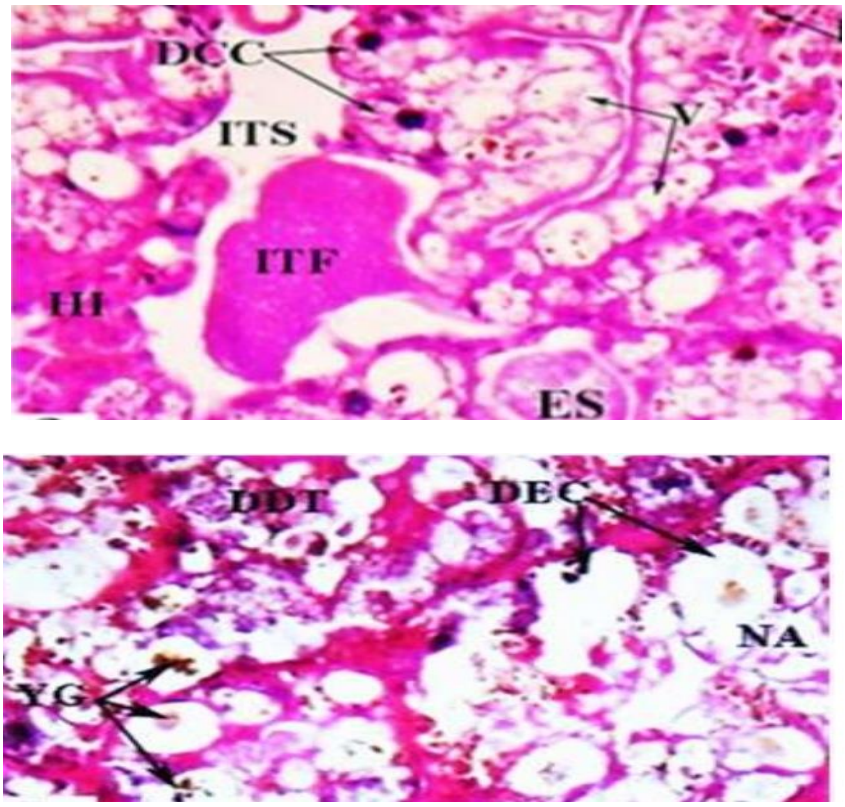

Fig. (8): Photomicrographs showing cross sections of digestive gland treated with LC_{50} of nanosilver on *M. obstructa*
DCC: Deformed calcium cells; DEC: Deformed excretory cells; DDT: Deformed digestive tubules; ES: Excessive secretions; ITF: Intertubular filtrate; HI: Hemocytic infiltration; NA: Necrotic area; V: Vacuoles; YG: Yellowish-brown granules.

Histological inspection showed that the normal digestive gland consists of three types of cells; digestive, calcium and excretory cells as indicated by (Lopes, *et al.*, 2001) in the land snail *Oxychilus attanticus*, Sharaf, *et al.*, (2015) in land snail *Helicella vestalis* and Mustafa and Awad (2018) in the slug *Lehmannia marginata*. Moreover, four cell types: digestive, calcium, excretory and thin cells were found in *E. vermiculata* by Hamed, *et al.*, (2007).

Saral, *et al.*, (2015) observed histological changes in the digestive gland of treated land snail *H. vestalis* with methiocarb included: severe tubular disruption, nuclear pyknosis and necrosis of tubules. In addition, Mustafa (2018) found vacuolated cytoplasm and degenerated nuclei of salivary gland treated with LC_{90} of thymol against *L. maximus.* Abdel-Haleem and El-Kassas (2013) found cytoplasmic vacuolation, swelling of secretory cells and accumulation of residual bodies when freshwater snail *B-alexandrina* and *B-truncates* treated with plant extracts from *E. splendens, Z. spina – christia* and *A. maritimaon*. Moreover, Ustina, *et al.*, (2018) found histological changes and ultrastructural abnormalities such as cytoplasmic vacuolation, scattered toxic agents, degeneration of some nuclei and cells, rupture of microvilli, increasing of calcium shperules inside secretory cells and wide fused vacuoles in the digestive gland of the Egyptian slug, *Limax maximus* treated with botanic molluscicide thymol. Hamed, *et al.*, (2007) reported that methomyl and Methiocarb caused histological changes as suffering of digestive gland tissues suffered from hemocytes infiltration, and bizarre nuclei ranging from karyolysis to severe karyorrhexis and complete

pyknosis. Also, Hamlet, *et al.,* (2012) studied the effect of thiometoxam on the histology of *Helix asppersa* as well as degeneration of the digestive tubules and breakdown of the basement membrane.Backry (2009) also, reported damages in the digestive glands of *Biomphalaria alexandrina* after exposure to the methanolic extract of *Guayacum officinalis*, *Atriplex styllosa* and *Euphorbia splendens*. The authors observed that epithelial cells of treated snails lost their regular shape and appeared empty, while digestive tubules and connective tissues were also damaged.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology,* 18: 265-267.

Abdel-Haleem, A.A. and El-Kassas, N. B. (2013). Ultrastructure and histopathological effects of some plant extracts on digestive gland of *Biomphalaria alexandrina* and Bulinus truncates. *The Journal of Basic & Applied Zoology, 66*(2):27-33.

Abdel-Rahman Hussein Amin; Alaa El-Din Bayoumi and Nadia Zikry Dimetry. (2019). Efficiency of Nano-formulations of Neem and peppermint oils on the biohomics and enzymatic activities of *Agrotis ipsilon* larvae (Lepidoptera: Noctuidae). *International Journal of Natural Resource Ecology and Management,* 4(5): 102-111.

Abdin, M. I. (1979). Standard technique for ass rearing of the black cutworm *Agrotis ipsilon*. M.Sc. Thesis, Fac. Agric., Al Azhar Univ., Egypt.

Abo El-Ghar, G.E.; Khalil, M.S. and Eid, T.M. (1994). Effect of plant extracts on development and fecundity of *Agrotis ipsilon* (*Lepidoptera*: *Noctuidae*). *Bulletin of Entomological Society of Egypt, Economic Series,* 21:171-190.

Ahmed, S.S.; Abd El-Rahman, S.F. and Abdel-Kader, M.H. (2018). Field evaluation of some photosensitizers and nanocomposites against cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae. *Middle East Journal of Applied Sciences,* 8(4): 1471-1479.

Ali, M.A. (2017). Comparison among the toxicity of Thymol and certain pesticides on adults' survival and egg hatchability of the glassy clover snail *Monacha cartusiana*. *Journal of Plant protection and Pathology, Mansoura University,* 8(4):189-194.

Awad, M.; Ibrahim, E. D.S.; Osman, E.I.; Elmenofy, W.H.; Mahmoud, A.W.M.; Atia, M.A.M. and Moustafa, M.A.M. (2022). Nano-insecticies against the black cut worm *Agrotis ipsilon* (*Lepidoptera*: Noctuidae): Toxicity, development, enzyme activity and DNA mutagenicity. *PLOS ONE,* 17(2): 1-21.

Badawy, M. E.I.; E. I. Rabea; T. M. Rogge; C.V. Stevens; W. Steurbaut; M. Hofte and G.Smagghe (2005). Fungicidal and insecticidal activity of O-acyl chitosan derivatives. *Polymer Bulletin*, 54:279-289.

Bakery, A.F. (2009). Impact of some plants extracts on histological structure and proteins patterns of *Biomphalaria alexandrina* snails Global. *Journal of Molecular Sciences,* 4(1):34-41.

Bergmeyer, H. (1963). Methods of enzymatic analysis. Academic Press. In New York, PP. 886.

Buffle, J. (2006). The key role of environmental colloids/nanoparticles for the sustainability of life. *Environmental Chemistry,* 3(3): 155-158.

Du, J.; Yu, W.; Wang, M.; Zhang, C. and Katase, W. (2013). Selective toxicity of three amide pesticides to black cutworm *Agrotis ipsilon* and earthworm *Eisenia foctida*. *Acta Phytopphlacica Sinica;* 40:266-272.

El-Kady, M.B.; Tayab, E.H.; Mesbah, H.A. and Saad, A.A. (1990). The relative efficiency of the pregnancy inhibitory hormone medroxy progesterone acetate (MPA) and insect growth inhibitor (XRD) on the greasy cut worm, *Agrotis ipsilon* (Huf.) (Noctuidae, Lepidoptera). *Bulletin of Entomological Society of Egypt, Economic Series,* 118:93-104.

Ellman, G.L.; V.J. Courtuerg- Adersand and R.M. Feat Feathersone (1961). A new rapid colorimetric determination of acetylcholine esterase activity. *Biochemical Pharmacology,* 7: 88-93.

El-Okda, M.M. (1981). Response of two land Mollusca to certain insecticides. *Bulletin of Entomological Society of Egypt, Economic Series*, 12: 53- 57.

Eman Kamel Khidr (2018). Chitosan and nano-chitosan efficacy against the land snails *Eobania vermiculata and Monacha obstructa* (Muller) under laboratory conditions. *Egyptian Academic Journal of Biological Sciences,B.Zoology,* 10(2):15-25.

Eshra, E.H. (2013): Survey and distribution of terrestrial snails in fruit orchards and ornamental plants at Alexandria and El-Behira Governorates. *Egyptian, Alexandria Science Exchange, Journal;* 34(2): 242-248.

Farkas, J.P.; Farkas, P. and Hyde, D. (2004). Liver and Gastroenterology Tests. In: Lee M. (ed.) Basic Skills in interpreting laboratory Data (3rd edn.) American Society of Health system pharmacists inc., USA.

Finney, D.J. (1971). Probit analysis 3rd ed. London: Cambridge University Press.

Galal, O. A. and M.F.M. El-Samahy (2012). Genetical effects of using silica nanoparticles as biopesticide on *Drosophita melanogaster*. *Egyptian Journal of Genetics and Cytology,* 41(2012), pp. 87-106.

Gamed, S. S.; Abdelmeguid, E.; Essawy, E.A.; Radwan, A.H. and Hegazy, E.A. (2007). Histological and ultrastructural changes induced by two carbamate molluscicides on the digestive gland of *Eobania vermiculata*. *Journal of Biological Science*, 7:1017-1037.

Ghamry, E.M.; El-Deeb, H.I. and Kokab, Y.A. (1993). Ecological and Morphological studies on certain land snails at Sharkia Governorate, Egypt. *Journal of Applied sciences,* 8(1):213-225.

Goodwin, B.; T. Babb; S. Kaffka and L. Godfrey (2007). Biorational management of beet armyworms in sugar beets in the central valley, Larry Godfrey and Tom Babb. Uc DAVIS, University of California. http//sugar beet. Ucdavis.edu.

Gornall, J. G.; G.J. Barduill and M.M. David (1968). Determination of serum protein by means of biuret reaction. *Journal of Biochemistry,* Pp. 177.

Hamed, S.S.; Abdel-Meguied, N.E.; Essawy, A.E.; Radwan, M.A. and Hegazy, A.E. (2007). Histological and ultrastructural changes induced by two carbamate molluscicides on the digestive gland of *Eobania vermiculata*. *Journal of Biological Sciences,* 7(6):1017-1037.

Hamlet, A.S.; Bensultan, S.; Djekoun, M.; Yassi, F. and Berrebbah, H. (2012). Histological changes and biochemical parameters in the hepatopancreas of terrestrial gastropodah *Helix aspersa* as biomarkers of neonieotinoid insecticide exposure. *African Journal of Biochemistry,* pp: 16277-16283.

Hanan Alfy; Rehab, Y. Ghareeb and Doaa, A. Farag (2020). Impact of chitosan nanoparticles as insecticide and nematicide against *Spodoptera littoralis*, *Locusta mirgatoria* and *Meloidogyne incognita*. *Plant cell Biotechnology and Molecular Biology,* 21(69-70): 126-140.

Humason, G.L. and W.H. Freeman (1974). Animal tissue technique. 4th edition, San Francisco, USA.

Hussein, M.A. and Sabry, A.H. (2019). Assessment of some new pesticides as molluscicides against the adult and eggs of chocolate banded snail, *Eobania vermiculata*. *Bulletin of the National Research Centre,* 43(75):1-5.

Jyothsna Yasur and Pathipati Usha Rani (2015). Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physiology. *Chemosphere*, 124(1): 92-102.

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? Egyptian *Journal of Agricultural Research*,92:53-73

Khaled Sayed Ahmed; Wafai, Z.A. Mikhail; Hassan M. Sobhy; Eman Mohamed Mostafa Radwan; Taher Salah El-Din and Ahmed M. Youssef (2019). Effect of lambada-cyhalothrin as Nanopesticide on cotton leaf worm, *Spodoptera littoralis* (Boisd.). *Egyptian Journal of Chemistry*,62(7):1263-1275.

Khaled Yassin; K. Y. Abdel-Halim; Safaa Ramadan Osman and Heba Mohamed El-Danasoury and Gihan Fathy Aly (2021). Comparative toxicity of abamectin and nano-derived form on land snail, *Helix aspersa* in attributing to cytotoxicity and biochemical alterations. *World Journal of Advanced Research and Reviews,* 10(01): 296-311.

Khater, A.A.; El-Sheakh, A.A.; El-Sheamy, M.K. and M.Z. Hussein (1990). Biochemical effects of lannate and layin on *Tilapia nilotica* fingerlings. *Egyptian Journal of Applied Science,* 5(8): 227-235.

Kheiri, A.; S.M. Jorf; A. Malihipour, H. Saremi and M. Nikkhah (2017): Synthesis and characterization of chitosan nanoparticles and their effect on fusarium head blight and oxidative activity in wheat. *International Journal of Biological Macromolecules,* 102:526-538.

Kurita, K.; Kojima, T.; Nishiyama and Shimojoh, M. (2000). Synthesis and some properties of no nature amino polysaccharides: Branched chitin and chitosan. *Macromolecular research,* 33:4711-4716.

Lopes, M.; Rodrigues, A. and Marigomes, I. (2001). Morphology and histology of the digestive gland of oxychilus (Dorouetia) at lanticus (morelet, drouet) (Gastropoda: pulmonated) Arquipelago, *Life and Marine Sciences*, 2(B): 71-76.

Mahrous, M.E.; Ibrahim, M.H. and Abdel-Aal, E.M. (2002). Ecological aspects of the glassy clover snail, *Monacha cartusiana* under field conditions in Sharkia Governorate. Egypt. 2nd Intern. Conf. PPRI. Cairo, Egypt, 21-24 December, 1: 107-114.

Memarizadeh, N.; Ghadamyari, M.; Adeli, M. and Talebi, K. (2014). Biochemical biomarkers of *Glyphades pyloalis* walker (Lepidoptera: pyralidae) in exposure to Tio_2 nanoparticles. *Invertebrate survival Journal*, 11: 47-53.

Merghany, M.M.; Abdel-Gawad, K.F.; Tawfic, G.A. and Ahmed, S.S. (2019). Yield, quality and leaves anatomy structure of spring onion sprayed by nanocomposite to control *Thrips tabaci*. *Plant Archives*, 19(1):1839-1849.

Miller, E.; Swails, S.; Swaila D. and Staten R.T. (1988). White garden snail *Theba pisana* (Muller). Efficacy of selected bait and spayable moluscicides. *Journal of Agricultural Entomology,* 5(3):189-197.

Mobarak, S.A. (2014). Biochemical impacts of various compounds on two land snail species *Ebania vermiculata* and *Monacha obstructa* Bulletin Faculty of Agriculture, Cairo. University. Neighboring regions. *Journal of Agricultural Research and Reviews*, 58(1): 79-86.

Mohamed Gh. R. (2018). Some laboratory studies on the efficacy of some plant extracts on biochemical and biological aspects of *Monacha cartusiana* land snail

(Stylommatophora: Helicidae). *Middle East Journal of Agriculture Research,* 7(4): 1867-1873.

Monika Mishra, Kamal Kumar Gupta and Sarita kumar (2015). Impact of the stem extract of *Thevetia neriifolia* on the feeding potential and histological architecture of the mid gut epithelial tissue of early fourth instars of *Helicoverpa armigera* Hubner. *International Journal of Insect Science,* 7-53.

Moustafa, M. A.; Mossalem, H.S. and Hassan, E. M. (2018). The potential effects of silver and gold nanoparticles as molluscicides and cercaricides on *Schistasoma mansoni*. Parasitology *Research,* 117, 3867-3880.

Mustafa, O.M. (2018). Effect of a plant product (Thymol) on the salivary gland of the giant slug *Limax maximus* in Egypt (Histological and ultra-structural study). *Egyptian Journal of Aquatic Biology and Fishers,* 22(3):55-59.

Mustafa, O.M. and Awad, I.R. (2018). Studies on the histology, histochemistry and ultrastructure of the digestive gland of the slug *Lehmannia marginata* (Gastropoda: Pulmonata). *Egyptian. Journal of Chemistry,* 2001, 69:43-53.

Osman, H.H; H. F. Abdel-Hafez and A.A. Khidr (2015). Comparison between the efficacy of two nano- particles and effective microorganisms on some biological and biochemical aspects of *Spodoptera littoralis*. *International Journal of Science and Research (IJSR).,*3:1319-1473.

Owolade, O. F.; Ogunleti, D.O. and Adenekan, M.O. (2008). Titanium dioxide affects disease development and yield of edible cowpea. Electronic Journal of Environmental, *Agricultural and Food chemistry,* 7(50): 2942-2947.

Radwa, G. Attia and Shireen, A.M. Mammoun (2020). Effect of cinnamon oil encapsulated with silica nanoparticles on some biological and biochemical aspects of the rice moth, *Corcyra cephalonica* (stain) (Lepidoptera: Pyralidae) .*Annals of Agricultural Sciences;* 65(1):1-5.

Robea, E.I.; Badawy, M. E.I.; Stevens, C.V.; Smagghe, G. and Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules,* 4:1457-1465.

Romeis, B. (1989). Mikroskopische Technik. Auflage, urban and schwarzenberg, *Munchenwien-Baltimore.* 17(235-236).

Rouhani, M.; Samih, M.A. and Kalantari, S. (2012). Insecticidal effect of silica and silver nanoparticles on cowpea seed beetle, *Callosobruchus maculates* F., (Coleoptera: Bruchidae). Journal of Entomology, 4(4):297-305.

Saadiy, M. Said; S.M. El-Sayed; Hoda E.A.; Farid and Medhat M. Abozid (2011). Insecticidal effect of chitosan prepared by different chemical processing sequences against *Galleria melleonella* A.Z. *Journal of Pharmaceutical Sciences,* 43:123-132.

Sabbour, M.M. and N.Y. Solieman (2016). The efficacy effect of using chitosan and nano-chitosan against *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Chemical and Pharmaceutical Research,* 8(3): 548-554.

Safaa, M. Ali.; Naeima, M. Yousef and Nivien, A. Nafady (2015). Application of biosynthesized silver nano particles for the control of land snail *Eobania vermiculata* and some plant pathogenic fungi. Journal of Nano materials, Volume 2015, Article ID 218904, 10 pages.

Sahib, A. F.; Waly, A.I.; Sabbour, M.M. and Lubnas. Nawar (2015). Synthesis, antifungal and insecticidal potential of chitosan nanoparticles against some seed borne fungi and insects of soybean. *International Journal of Chem. Tech. Research,* 8(2):589-598.

Saxena, P.K.; Snigh, V.P.; Kondal, J.K. and Soni, G.L. (1989). Effect of some pesticides on in vitro lipid and protein synthesis by the liver of the freshwater teleast, channa punctatus (ML.). *Environmental Pollution,* 58:273-276.

Sayed, R.M.; Khidr, A.A. and Moustafa, H.Z. (2014). Nano chitosan efficacy against the cotton leaf worm *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 24(2), 461-464.

Schmid, G. and Corain, B. (2003). Nanoparticulated gold: synthesis, structures, electronics and reactivities. *European Journal of Inorganic. Chemistry*, 17,3081-3098.

Sharaf, H. M.; Salama, M.A. and Abd El- Atti, M.S. (2016). Biochemical and histological alternations in the digestive gland of land snail *Helicella vestalis* exposed to Methiocarb and chlorpyrifos in the laboratory. *International Journal of Science and Research (IJSR).*, 2319-7064.

Sharaf, H.M.; Salama, M.A. and Abd El-Atti, M.S. (2015). Biochemical and histological alterations in the digestive gland of the land snail *Helicella vestalis* (Locard, 1882) exposed to Methiocarb and chlorpyrifos in the laboratory. *Journal of Cytology and Histology*, 6(3):334-343.

Sharma, A.V.; Kumar; B. Shahzad; M. Tanveer; G.P. S. Sidhu; N.Handa; S.K. Kohli; P. Yadv.; A.S. Bali and R.D. Parihar. (2019). Worldwide pesticide usage and its impacts on ecosystem. *Applied Sciences*, 1; 1446.

Sokal R.R. and Rohilf. P.J. (1981). "Biometry: The principles and practice of statistics in biological research". Freeman and Co New York.

Sooresh, A.; Hyeogsun, K.; Robert, T.; Patricia, P.; Michelle, P. and Christie, M.S. (2011). Surface functionalization of silver nanoparticles: Novel Applications for Insect Vector Control. *Access Applied Materials and Interfaces*, 3, 3779-3787.

Thulasiraman Manimegalai; Krishnan Raguvaran; Manickam Kalpana and Rajan Maheswaran (2020). Green synthesis of silver nanoparticle using *Leonotis nepetifolia* and their toxicity against vector mosquitoes of *Aedes aegyptica* and *culex quinquefaseiatus* and agricultural pests of *Spodoptera litura* and *Helicoverpa armigera*. *Environmental Science and Pollution Research*, 27:43103-43116.

Tuncsoy, B.S. (2018). Toxicity of nanoparticles on insects: A Review. *Adana Bilim Veteknolo universitesi fen Bilimleri Dergisi*, 1(2): 49-61.

Ustina, N.T.; Habib; Ahmed, A.; Abdel-Haleem; Omaima, M. Mustafa and Eman, H. Ismail (2018). Histological and ultrastructural alternations in the digestive gland of the Egyptian slug, *Limax maximus* (Linnacus, 1758) treated with botanic molluscicidal thymol, with references to biological studies. *Egyptian Journal of Aquatic Biology and Fisheries*, 22(5):137-148.

Yu, W.; Du, J.; Hu, Y.; Shen, R. and Mu, W. (2012). Toxicity of six insecticides to black cutworm *Agrotis Ypsilon* (Rottemberg) and safety evaluation to oil organisms. *Acta phytophylacica sinica*, 39:277-282.

Zollner, N. and K. Kirsch (1962). Determination of lipids (micro method) by means of the sulfophovanilin reaction common to many natural lipids (all know plasma lipids). *Zeitschrift fur die gesamate. Experimentelle Medizin*, 135:545-561.

ARABIC SUMMARY

كفاءة الكيتوزان، الفضة وجزيئاتها النانومترية على الأنشطة الهستولوجية والأنزيمية للدودة القارضة وقوقع البرسيم

أماني رشوان مرسى – غادة محمد رفعت - أميرة محمد الشيوى
قسم وقاية النبات، كلية الزراعة، جامعة بنها

أجريت هذه الدراسة بغرض تقييم فاعلية الكيتوزان، الفضة وجزيئاتها النانومترية على الأنشطة الهستولوجية والأنزيمية ضد الدودة القارضة وقوقع البرسيم. تم تقدير ثلاث أنواع من الأنزيمات وهي انزيمات AST, ALTوAChE بجانب تقدير محتوى البروتين الكلى والليبيدات الكلية.

أوضحت نتائج الاختبارات المعملية أن مركب الكيتوزان والفضة أدى الى زيادة نشاط انزيمات AST, ALT and AChE بينما على العكس أدت مركبات الكيتوزان والفضة النانومترية الى حدوث انخفاض في نشاط نفس الأنزيمات. على الجانب الأخر أدت جميع المركبات المختبرة سواء في صورتها العادية أو النانومترية الى حدوث انخفاض معنوي في محتوى البروتين والليبيدات الكلية. وبصفة عامة أثرت المركبات النانومترية على نشاط الأنزيمات ومحتوى البروتين والليبيدات الكلية مقارنة بالكنترول ضد كلا من الحشرة والقوقع المختبرة. وفى نفس الوقت لوحظت بعض التغيرات الهستولوجية على المعي الوسطى للدودة القارضة والغدة الهضمية لقوقع البرسيم بعد المعاملة بالتركيز النصف مميت لكلا من مركبات الكيتوزان والفضة النانومترية. لذلك تقترح هذه الدراسة إمكانية استخدام الكيتوزان والفضة كبدائل لمبيدات الأفات التقليدية بالتوافق مع ممارسات المكافحة المتكاملة للأفات.