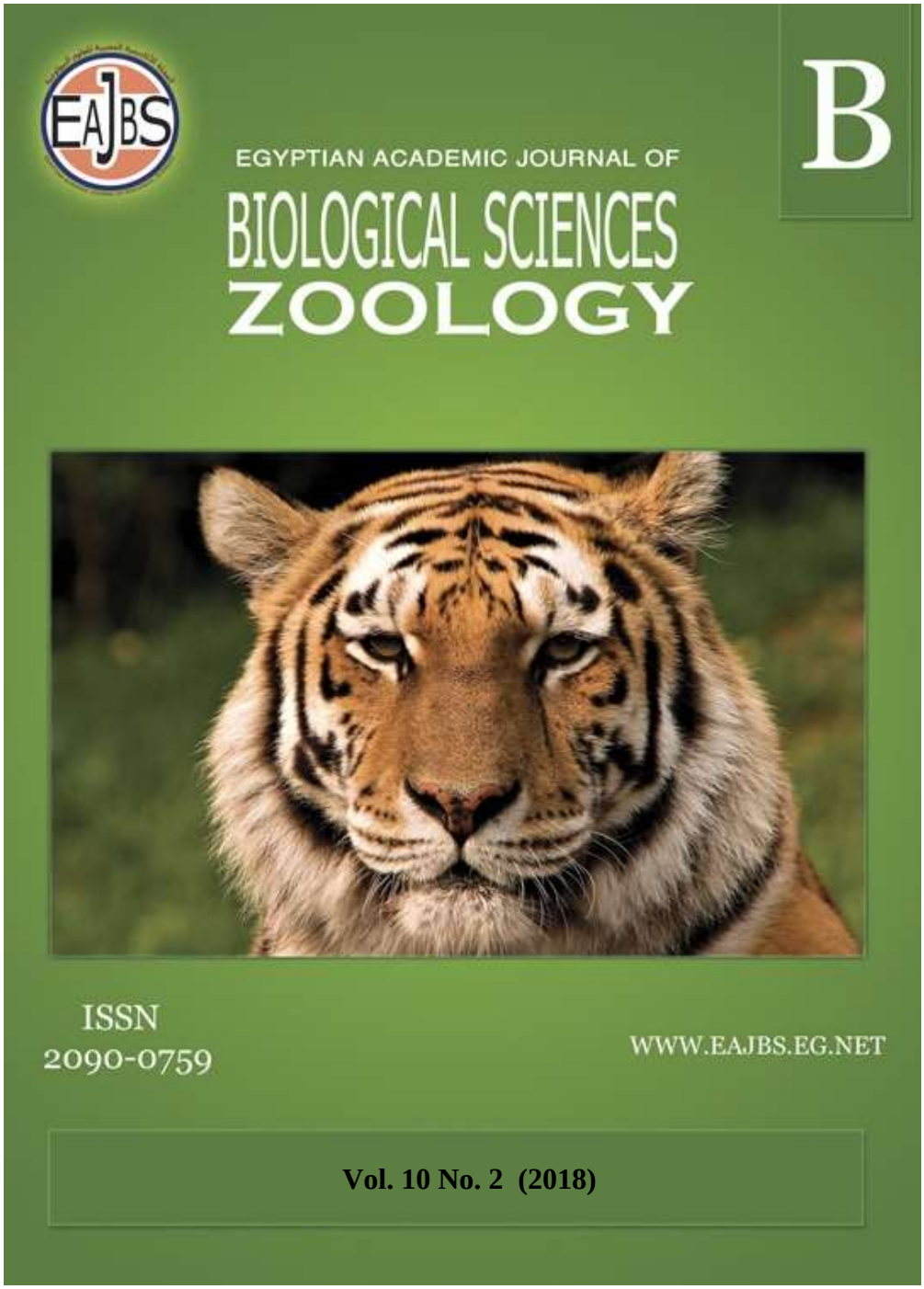

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Chitosan and Nano-Chitosan Efficacy Against the Land Snails *Eobania vermiculata* and *Monacha obstructa* (Muller) Under Laboratory Conditions

Eman kamel Khidr
Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt.
E.Mail:: dremankamel@yahoo.com

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ABSTRACT

The effect of chitosan and nano-chitosan on the land snails *Eobania vermiculata* and *Monacha obstructa* was investigated. Under laboratory conditions, the LC_{50} values recorded, 8.6%. and 6.9 % for *E. vermiculata* and *M. 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. The effects of both compounds on certain biochemical parameters were recognized. When chitosan and nano-chitosan were applied at the LC_{50} level, the total protein activities showed 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 32.5 to 52.5 g/dL after the treatment. While nano-chitosan increased it up to 78.33 g/dL for *E. vermiculata*. Also, natural chitosan increased the total lipid from 25.0 to 37.5 g/dL for *M. obstructa* compared to an increase of 52.5g/dL attained by the effect of nano-chitosan particles.

INTRODUCTION

Land snails are considered as economic important pests attack different types of plants. In Egypt, terrestrial snails attack vegetables, field crops, orchard trees as well as ornamental and medical plants. *Eobania vermiculata* and *Monacha obstructa* are the important snail species in the Egyptian governorates attacking various plantations (Miller *et al.*, 1988; Eshra 2013). These animals attack the different kinds of plants e.g., Cereal, vegetables, fruit orchard and ornamental plants at the different growth stages reducing their yields (El-Okda, 1980). In Sharkia Governorate, land snails considered one of the dangerous crop pests casing severe damage especially in vegetables and field crops.(Ghamry *et al.*, 1993, Arafa 1997, Ismail, 1997, El-Massry, 1997).

Several attempts have been paid to control its dispersal by using synthetic pesticides and different plant products against the snails (Ismail *et al*. 2005 and Genena *et al*.(2008). Natural products from plant origin have received much attention as potentially useful bio-active compounds in an effort to develop alternatives to the conventional pesticides. A large number of plant products which possess molluscicidal activity on both terrestrial and fresh water snails have been studied

(Singh and Singh 2004; Gabr *et al*. 2006 and Shoaib *et al.* 2009).The use of conventional insecticides is the effective method of controlling for the insect. Frequent use of insecticides causes a major problem of pest resistance which facing the using of traditional insecticides 4 (Salama, *et al*. 1970). The development of pest resistance to one molecular target means that many insecticides are rendered ineffective for pest control, the increase of costs, and many problems of environmental / personal exposure. The control of insects and other vectors with bulk form of insecticides has led to the contamination of ground waters, plants, soil, animals and damaging beneficial non-target organisms. (Kuzma, *et al.* 2006).

Nano-chitosan form presents an attractive solution to this problem, because their effective concentration is expected to be much lower compared to that of volume materials and they can be formulated in water without organic solvents. (Bhattacharyya, *et al.* 2010 and Ju-Nam, and Lead, 2008).

Nanopesticides is the most important property which results in high surface reactivity for many deferent nanoparticle types. Metal nanoparticles such as copper, are unique because they offer the possibility of altering their surfaces in order to introduce specific functionalities for environmental applications. (Schmid, and Corain, 2003 and Klaine, *et. al.* 2008).

The ultimate target of nanocopper composition for chitosan necessary world applications is to obtain nanoparticles with the following characteristics: (a) constant and lean size distribution, (b) well-known shape, (c) known chemical structure with no impurities, and (d) no congregation or clot. (Kalsi, 2002 and Sooresh *et. al.* 2011).The using capping agent of acts as a colloidal stabilizer and enhances water suspend ability. These are very eligible characteristics can be carried out for copper. (Poole and Owens 2003).

This research presented a try to synthesize copper nanoparticles stabilized by starch as the matrix for the encapsulation of synthetic chitosan , inexpensive and reproducible method.

The aim of the present study is to spotting a light on the efficiency of chitosan and nano-chitosan against of *Eobania vermiculata* and *Monacha obstructa* under laboratory conditions.

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MATERIALS AND METHODS

Tested Compound:

Chitosan was obtained from Egyptian-Canadian Company for Humate Technology and Agricultural Consultancy.

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 human, biodegradable and it may possess insecticidal properties (Rabea *et a*., 2003 and Badawy *et al*., 2005)

Chemicals:

Copper sulphate ($CuSO_4.5H_2O$), 98%, produced by Adwic Chemika co., glucose ($C_6 H_{12} O_6$, with average molecular weight =180.2) produced by El-Nasr Pharmaceutical Chemicals Co., starch soluble 99% powdered solid ($(C_6 H_{10} O_5)_n$, with average molecular weight =81.37) produced by Chemajet Pharmaceutical co.

Synthesis of starch–copper nanoparticle-encapsulated:

Starch–copper nanoparticle-encapsulated the formulated according to ref.[18](Nnemeka, E. I. & *et. al.* 2016), with the modification that encapsulation was

completed in situ during synthesis of the copper nanoparticles by direct physical gelation. (Nnemeka, E. I. and *et. al.* 2016 and Tali, D. 2009) The synthesis was carried out via chemical reduction of Copper sulphate by glucose as follows: to a mixture of 5% $CuSO_4.5H_2O$ and 0.2 M glucose solution (1 : 3 volume ratio) in a loosely covered flask containing 1% starch dispersion (1 g in 100 ml distilled water), were added 10 ml of formulation of 16 %, respectively. This was stirred and heated at in a fume cupboard for3 hours. The resulting nanocomposite was finely ground, kept in a sample bottle, and stored in a vacuum desiccator in the dark for further use and characterization.

Characterization of chitosan:

UV-visible spectral analysis and TEM.

All prepared samples were characterized by Transmission Electron Microscopy (TEM) as a base tool for scaling the particles size, structure and style, and the plasmonic effect was detected by UV-VIS spectroscopy. The nature of linkage between chitosan and CUNPS were investigated using IR-Spectroscopy (Figs.1- 4).

Tested animals

Adult individuals of the two terrestrial snail species, *Eobania vermiculata* and *Monacha obstructa* were collected from the infested ornamental plants at Qalubia Governorate. Animals were transferred to the laboratory, kept in glass boxes and fed on fresh lettuce leaves for two weeks before treatment for acclimatization (El-Okda, 1981). For each treatment, thirty healthy animals were allocated and divided into three replicates (each of 10 individuals) For each treatment(Ghamry *et al*. 1993) and another for control**.**

Laboratory experimental

1. Contact application

Thin film technique was used as the method of application in this investigation **(Ascher and Mirian, 1981)**. Whereas the tested concentrations were applied using water to the surface of Petri- dish (9 cm diameter). Two ml of each concentrations of the tested compound were spread on the inner surface of Petri- dish by moving the dish gently in circles. The water was evaporated under room conditions in a few minutes leaving a thin layer film of the tested compound. Animal of each species was exposed for 7 days in Petri- dish. A parallel control test was conducted using plain water. The killed animals were daily counted and removed. Mortality percentages were calculated and LC_{50} value was determined.

2. Biochemical studies:

Animals were treated with LC_{50} of each compound to determine their effect on some biochemical parameters i.e. total protein and total lipid contents. These parameters were measured at 3 days post-treatment. A parallel control test was conducted.

- Sample Preparation: Ten animals of each species at 1g weight were used according to the method of Bergmeyer (1963).

a. Determination of total protein: Colorimetric method of soluble protein was done according to the method of Henry (1964) using Buiret reagent.

b. Determination of total lipid: Lipids are hydrolysed by sulphoric acid, then treated with phosphovanilin mixture to produce sulphophosphovanilin complex of rose coloration which is measured photometrically according to Zollner and Kirsch (1962).

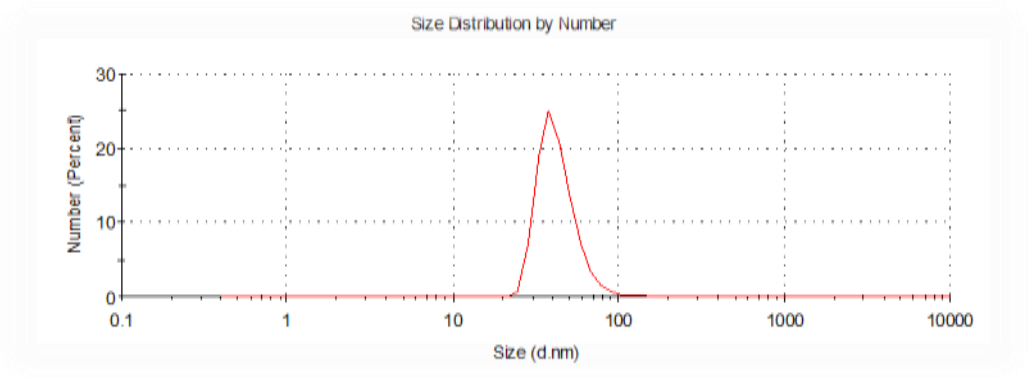
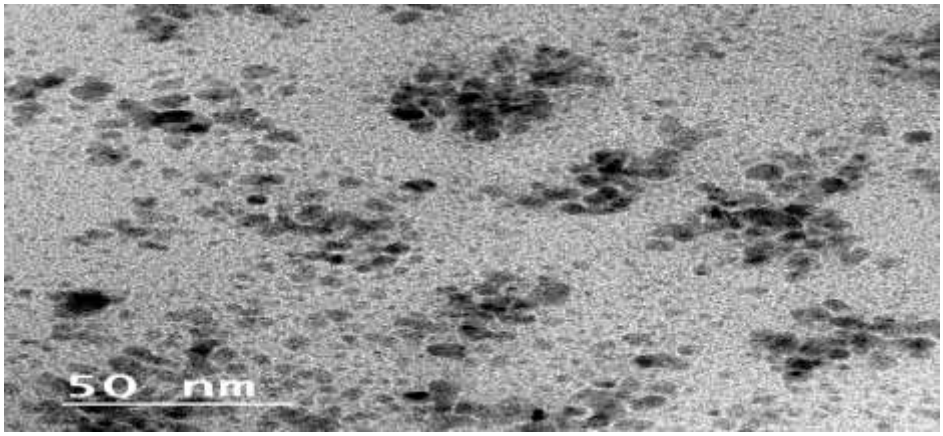


Fig. 1. Volume of nanoparticles of copper

A



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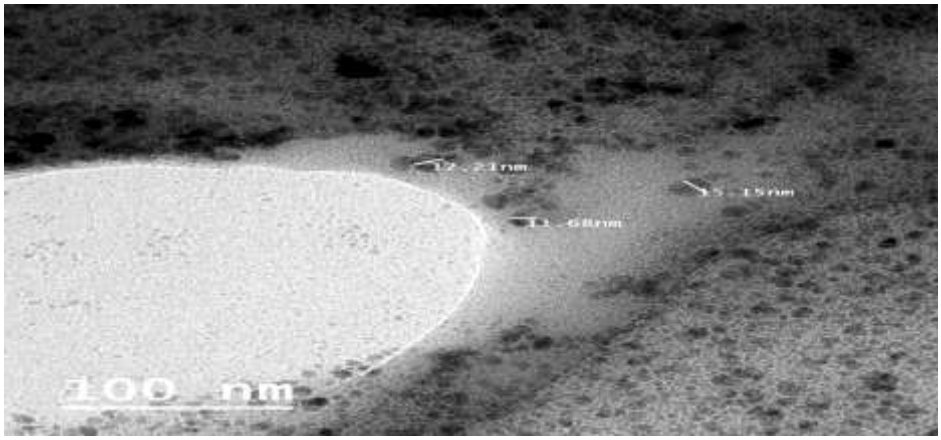


Fig. 2. Scanning electron microscopy of nano copper particles (A: the nano copper at 50 nanometer, B: at 100 nanometer).

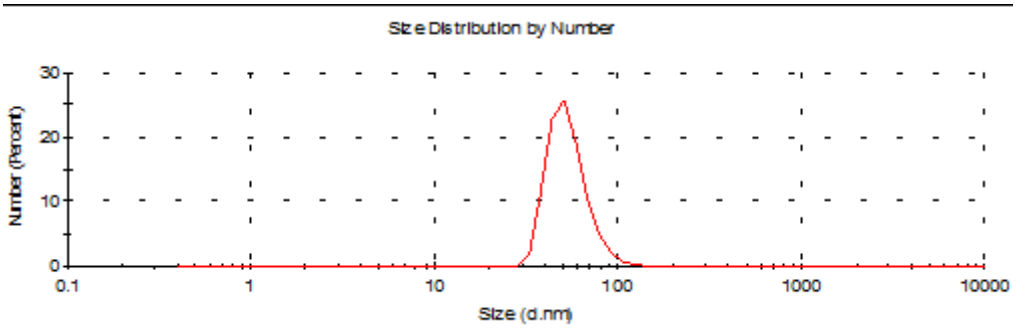
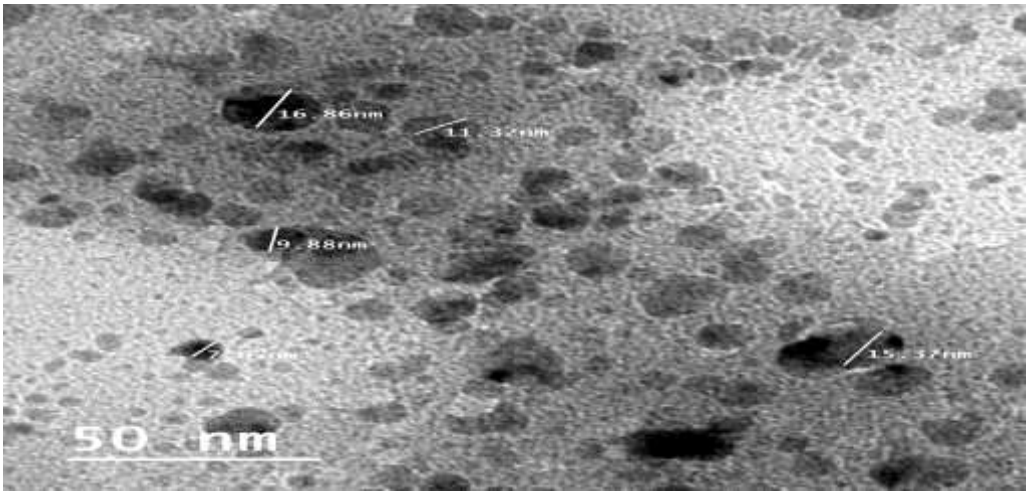
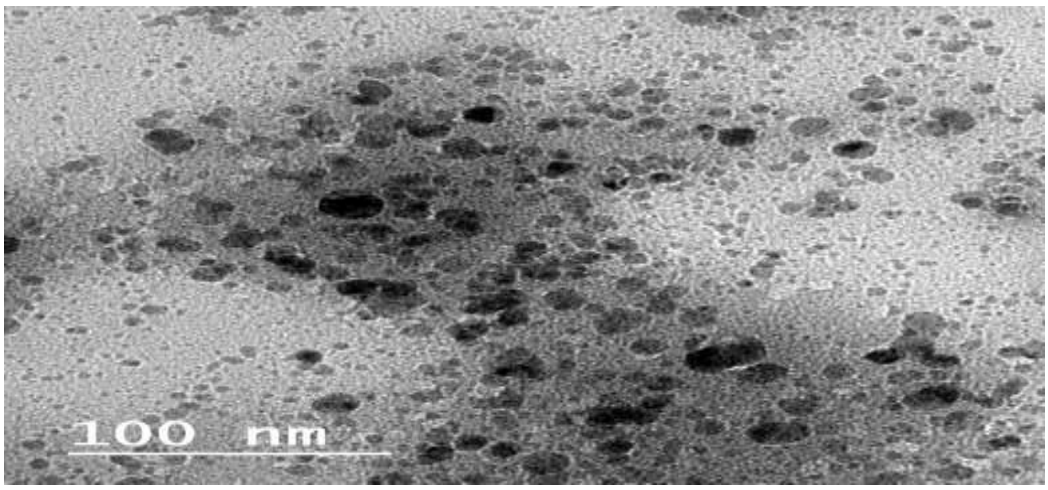


Fig. 3. Volume of chitosan nanoparticles (The size of nanoparticles of chitosan loaded on nano copper particles)



A



B

Fig. 4. The nano chitosan particles using scanning electron microscopy. (A and B: the nano chitozan at 50 and 100 nanometer.

RESULTS AND DISCUSSION

Data in Tables 1&2 show the efficacy of chitosan against the land snails, *E. vermiculata* and *M. obstructa* using contact technique. Results showed a gradual increase in the mortality percentage with the increase of compound concentrations. Chitosan concentrations of 4, 6, 8, 10, 12, 14 and 16 % caused 20, 46.7, 50, 66.7, 80, 86.7 and 100 mortality for *M. obstructa,* respectively. The same concentrations gave 10 , 20 , 50 , 60 ,70 ,70 and 100% mortality for *E. vermiculata*, respectively.

Concerning the toxic effect of nano-chitosan, the data showed that the compound caused 26.7 ,66.7 ,86.7 , 86.7 , 100 and 100% mortality on *M. obstructa* , while the mortality reached 0.0%, 0.0%, 33.3%, 66.7%, 66.7% and 100% on *E. vermiculata* using concentration levels of 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2% respectively.

The LC_{50} values for chitosan were calculated as 6.9% for *M. obstructa*, and 8.6% for *E. vermiculata*, while reached 0.1571% and 1.3843% for *M. obstructa* and *E. vermiculata* respectively, using chitosan nanoparticles.

The attained results agreed with that of Sabbour (2016), using chitosan and nano chitosan on *Schistocerca gregaria* adult and nymphal stages under laboratory conditions. He recognized their negative effect on survival and fertility.

Similarly, nano chitosan, under laboratory conditions, gave LC50 values of 19, 27, 29,43, 44,66 and 69, respectively for *Tuta . absoluta* comparing to high values of 49, 67, 87, 101, 122,133 and 135 ppm for chitosan (Sabbour and Solieman2016).

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

Also, Zhang *et al.* (2003) stated that chitosan was active against lepidopterous and homopterous insects; causing up to 80% mortality.

-Total protein:

The response of total protein after the treatment with LC_{50} values of chitosan and nano chitosan is summarized in Table (3). Total protein activities showed a significant increase compared to untreated *E. vermiculata*. The same compounds showed a significant. decrease in total protein levels of *M. obstructa* . Khater *et al.* (1990) and Mobarak,. (2014) reported that the increase in total protein of *M. obstructa* and *E. vermiculata* could be attributed to the increased biosynthesis process occurred by high enzyme stress. Comparatively, Kandil *et al.,* (2014) reported that acetylsalicylic acid exhibited a highest effect on the total protein.

-Total Lipids:

The effect of the tested compounds on total lipid was differ when used as a contact molluscicidal against both snail species (Table, 4). Natural chitosan increased the level of total lipid from 32.5 to 52.5 g/dL after the 3 days of treatment, while nano chitosan increased it up to 78.33 g/dL for *E. vermiculata*. Also, natural chitosan increased the total lipid from 25.0 to 37.5 g/dL for *M. obstructa* compared to an increase of 52.5g/dL attained by the effect of nano-chitosan particles. The depression in total lipids may be due to decline in lipid synthesis capacity and/or attribute to an increase in the hydrolysis of hepatic lipid to compact the stress conditions as reported by Saxena *et al.* (1989). Kandil *et al.,* (2014) reported that acetylsalicylic acid exhibited the highest effect on the total lipid which important for the synthesis of shell and mucus. Also, Chitosan is generally similar to magnets where they carry Positive charges along its fibers, attracting any adjacent negative charges such as fat by Mohamed, (2013).

As a conclusion, both compounds appeared a promising role in controlling the investigated land snails, with a unique role for chitosan nano particles.

Table 1. The difference between chitosan and nano-chitosan compounds in the effect on the land snail, *Monacha obstructa,* using contact techniques after three days of treatment under laboratory conditions.

Technique	**Type of compound**	***M. obstructa***			
		Concentration (%)	**Mortality% after 3days**	**LC_{50} (%)**	**Slope**
Contact	Control	0.0	0.0	6.9475	3.884± 0.5368
	Natural chitosan	4	20		
		6	46.7		
		8	50		
		10	66.7		
		12	80		
		14	86.7		
		16	100		
	Chitosan nano particles	0.1	26.7	0.1571	2.2340±0.4891
		0.2	66.7		
		0.4	86.7		
		0.8	86.7		
		1.6	100		
		3.2	100		

Table 2. The difference between chitosan and nano-chitosan compounds in the effect on the land snails, *Eobania vermiculata*, using contact techniques after three days of treatment under laboratory conditions.

Technique	**Tested compound**	***E. vermiculata***			
		Concentration (%)	**Mortality% after 3days**	**LC_{50} (%)**	**Slope**
Contact	Control	0.0	0.0		
	Natural chitosan	4	10	8.6099	4.3364± 0.7294
		6	20		
		8	50		
		10	60		
		12	70		
		14	70		
		16	100		
	Chitosan nano particles	0.1	0.0	1.3843	2.085± 0.3485
		0.2	0.0		
		0.4	33.3		
		0.8	66.7		
		1.6	66.7		
		3.2	100		

Table 3. Effect of chitosan and nano-chitosan at LC_{50} on total protein concentration levels of *Eobania vermiculata* and *Monacha obstructa* land snails (mean±SD) after three days of treatment.

Snails species	Tested compounds			LSD
	Control (mean±SD)	Natural chitosan	Chitosan nano particles	
E. vermiculata	$0.11^{a} \pm 0.01$	$0.66^{C} \pm 0.05$	$0.1767^{b} \pm 0.01$	0.0221
M. obstructa	$0.3^{a} \pm 0.03$	$0.19^{b} \pm 0.01$	$0.127^{C} \pm 0.03$	0.0266

*Means followed by the same letter in the same row are statistically non-significant. No. of tested snails = 10 per one replicate; SD = standard deviation; a = non-significant (P>0.05); b = significant (P<0.05); c = highly significant (P<0.01).

Table 4. Effect of chitosan and nano-chitosan at LC50 on total lipid of *Eobania vermiculata* and *Monacha obstructa* land snails (mean±SD**)** after three days of treatment.

Snails species	Tested compounds (mean±SD)			LSD
	Control (mean±SD)	Natural chitosan	Chitosan nano particles	
E. vermiculata	$32.5^{a} \pm 2.02$	$52.5^{b} \pm 2.53$	$78.33^{c} \pm 5.01$	12.679
M. obstructa	$25.0^{a} \pm 3.00$	$37.5^{b} \pm 0.82$	$52.5^{c} \pm 0.44$	10.7899

*Means followed by the same letter in the same row are statistically non-significant. No. of tested snails = 10 per one replicate; SD = standard deviation; a = non-significant (P>0.05); b = significant (P<0.05); c = highly significant (P<0.01).

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ARABIC SUMMERY

فاعلية الكيتوزان والنانوكيتوزان تجاه نوعى القواقع الارضيه ايوبانيا فيرميكيولاتا وموناكا ابوستركتا تحت الظروف المعمليه

إيمان كامل خضر
معهد بحوث وقاية النباتات، الدقى، الجيزة، مصر

اوضحت نتائج الاختبارات المعمليه لتاثير الكيتوزان والنانو كيتوزان على نوعي القواقع الارضيه، قوقع الحدائق البنى (*Eobania vermiculata*) وقوقع البرسيم (*Monacha obstructa*) تحت الظروف المعمليه ان قيمة ال LC_{50} للكيتوزان ٨،٦% بالنسبه لقوقع الحدائق البنى و ٦،٩% بالنسبه لقوقع البرسيم بينما للنانوكيتوزان كانت قيمة ال LC_{50} ١،٤% بالنسبه لقوقع الحدائق البنى و ٠،١٦% بالنسبه لقوقع البرسيم. و بالنسبه للتاثيرات البيوكيميائيه باستخدام ال LC_{50} لكلا المركبين فقد كانت هناك زياده كبيره فى مستوى البروتين الكلى بالنسبه لقوقع الحدائق البنى وانخفاض البروتين الكلى بالنسبه لقوقع البرسيم مقارنة بالكنترول .وادى استخدام الكيتوزان الطبيعى الى زياده فى مستوى الدهون الكليه من ٣٢،٥ الى ٥٢،٥ g/dl بعد المعامله بينما ادت المعامله بالنانوكيتوزان الى زياة فى مستوى الدهون الكليه بقيمه وصلت الى ٧٨,٣٣ g/dl بالنسبه لقوقع الحدائق البنى. بينما كانت الزياده من ٢٥ الى ٣٧،٥ g/dl بالنسبه لقوقع البرسيم باستخدام الكيتوزان الطبيعى. اما بالنسبه للنانوكيتوزان فقد وصلت الزياده في مستوى الدهون الكليه الى ٥٢،٥ g/dl .