

EGYPTIAN ACADEMIC JOURNAL OF

# F

# BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL



ISSN 2090-0791

WWW.EAJBS.EG.NET

Vol. 13 No. 1 (2021)

www.eajbs.eg.net

# Egypt. Acad. J. Biolog. Sci., 13(1):217- 226 (2021)



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



# Toxicity of Nanosilica Particles on *Eobania vermiculata* and Their Effects on Biochemical Changes in Rats.

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#### ARTICLE INFO

Article History Received: 15/1/2021 Accepted: 21/3/2021

# Keywords:

Organic, inorganic nanosilica, *Eobania* vermiculata, Rats, hematological parameters.

#### ABSTRACT

In the present study, organic and inorganic nanosilica were evaluated against the terrestrial snail Eobania vermiculata including mortality percentage, some life-cycle aspects studied included, also their effects on change in some biochemical parameters in albino Wistar rats under laboratory conditions.LC<sub>50</sub> values for organic and inorganic nanosilica treatments on the snail were 303.92µg/ml and 6.282×10<sup>4</sup> µg/g after five and three days of treatment respectively. Data showed significant results against E. vermiculata life- cycle aspects and new generation where treatments caused a reduction of copulation, oviposition and larva stage percentages reached {(50 and 25), (70.92 and 81.21) and (92.37 and 95.69) %} for organic and inorganic nanosilica respectively. On rats, there were highly significant changes in hematological parameters in rats, as there were a very high significant decreased in WBCs, 4.70 and 4.87 (10<sup>3</sup>/µl) when treated with organic and inorganic nanosilica respectively, compared with  $10.80 (10^3/\mu l)$  in the control, a very high significant decreased in MCV by 73.63 and 74.0 (fl) respectively, compared with 91.30 (fl) in the control, a significant increase in RBCs treated with organic nano silica by 7.30  $(10^6/\mu l)$  and a very high significant increase reached 7.90  $(10^6/\mu l)$  in treated with inorganic nanosilica compared with 6.77 (10<sup>6</sup>/µl) in the control. Also, a very high significance was detected in PLT treated with organic nanosilica reached 832.0 (10<sup>3</sup>/µl) and a highly significant increase in treated with inorganic nanosilica by 811.07 (10<sup>3</sup>/µl) compared with 478.0  $(10^3/\mu l)$  in the control.

# INTRODUCTION

Nanotechnology is a promising field of research that opens up in the present decade it is expected to give major impulses to technical innovations in a variety of industrial and agriculture sectors in the future. The potential uses and benefits of nanotechnology are enormous. These include agricultural productivity enhancement for efficient dosage of water and fertilizer. The atom-by-atom arrangement allows the manipulation of nanoparticles thus influencing their size, shape and orientation for reaction with the targeted tissues (Bhattacharyya *et al.*, 2010). Nanoparticles exhibit different physical strength, chemical reactivity, electrical conductance and magnetic properties (Nykypanchuk *et al.*, 2008). Thus, nanotechnology will revolutionize agriculture including pest management in the near future. Over the next two decades, the green revolution would be accelerated by

Citation: Egypt. Acad. J. Biolog. Sci. (F.Toxicology & Pest control) Vol.13(1)pp217-226(2021)

DOI: 10.21608/EAJBSF.2021.162947

means of nanotechnology. A few years ago; the uses of synthetic pesticides in modern agriculture production promoted the widespread of environmental contamination, toxicity to human food as well as the resistance of targeted pest was developed and hazards to human health occurred (Pretty *et al.*, 2009). Hence, controlling pests required a modern technology of fewer hazards to human health with no residues in their food and less resistance to the pest.

Land snails are one of the most important invertebrate pests that spread in the world. In recent years, these pests spread widely in many Arab countries, especially Egypt. The importance of these gastropods pests is that they can feed on all parts of many plant species without discrimination (Al-Akraa and Mohammed, 2015 and Gazzy *et al.*, 2019). Besides, these animals transmit a number of bacterial, fungal and viral diseases to infected plants, through a mucous substance secreted during feeding and movement, thus increasing the damage to plants. Some of these pests have a very important role in transmitting some worms to humans and other animals (Lu *et al.*, 2011). Many control methods have been used in recent years to reduce the dangers of these pests (Sharaf *et al* 2015). Using nanomaterials became one of the modern and new technologies to combat the different pests quickly and effectively (Safaa *et al.*, 2015).

The present study is a trial to characterize the selected organic and inorganic nanosilica particles using a transmission electron microscope (TEM) followed by an evaluation of the molluscicidal efficacy of these particles against *Eobania vermiculata* snail under laboratory conditions. Also, the effect of these materials on rats was studied.

# MATERIALS AND METHODS

# **Tested Materials:**

- organic and inorganic nanosilica (Sio2)
- Size (TEM):100nm
- Purity: 99%
- Form: liquid for organic and powder for inorganic nanosilica.
- Source: purchased from Nano Way Company, Egypt.
- Size and shape characterization of nanoparticles.

The nanoparticles sizes were analyzed by the Transmission Electron Microscope of the Nanotechnology and Advanced Material Central Lab, (NAMCL), National Research Center (NRC). Gun type: LaB6 Gun.

Model: Tecnai G20, Super twin, double tilt. Applied voltage: 200 Kv.

# Preparation of *Eobania vermiculata* Land Snail in The Laboratory:

Adult of the snail *Eobania vermiculata* individuals were brought from multiple plants to the Agricultural Animal Laboratory of the Faculty of Agriculture, Benha University. The animals were thoroughly washed with water to remove traces of mud and dirt, and left until the animals moved to distinguish the injured from the healthy individuals. The best healthy individuals were selected from almost the same size and placed in plastic cages feeding on fresh lettuce plants while covering the cages with a light perforated cloth to help the animals breathe and prevent the individuals from escaping (Mohammed 2018). The animals were starved for a week before the start of the experiments.

# Toxicity Test Against E. vermiculata Land Snail:

Serial concentrations of tested materials were prepared at 1000, 800, 600, 400 and 200  $\mu$ g/ml for organic nanosilica and  $32\times10^4$ ,  $16\times10^4$ ,  $8\times10^4$ ,  $4\times10^4$ ,  $2\times10^4$   $\mu$ g/g for inorganic nanosilica, three replicates were used for each concentration, each of five adults. Fresh lettuce leaves were mixed with the different concentrations for two tested nanomaterials and placed inside the replicates with *E. vermiculata* individuals and covered

to prevent escape. Mortalities were recorded after 1, 2, 3, 5, 7 and 10 days of treatment .the recorded mortalities were corrected according to Abbott's formula (1925).

# Aspects of Eobania vermiculata Life-Cycle:

Lettuce leaves were mixed with LC<sub>50</sub> of each of the tested nanomaterials and submitted for *E. vermiculata* adults to clarification the effect of these materials on some lifecycle aspects. Four replicates for each material and control. Two snail adults for each replicate and follow-up took place daily. After copulation, each snail was translocated to a separate cup containing wet clay and watched until eggs laying (Mohammed 2004). Copulation percentage, copulation period, pre-laying eggs period, egg-laying percentage, the average total number of laid eggs, percentage of eggs in clutches form, the average number of eggs in each clutch, an average of clutch depth, percentage of eggs in solitary form, an average of eggs weight, average of egg diameter, incubation period, hatching percentage and the average number of alive larvae were estimated. Finally, the reduction percentages were calculated for copulation, oviposition and larva stage. Hatchability percentage was calculated according to Mohammed (2004) but the inhibition of copulation, oviposition and larva stage were calculated according to Zhang *et al.*, (2013)

# **Biosafety Experiment:**

Twenty-seven males of albino Wistar rats were obtained from the Faculty of Veterinary Medicine, Benha University. Rats were clinically healthy. Those were acclimatized one week before the experiment, 80 - 100 gm weight/rat. Rats were randomly assigned to three groups; the first group fed on grain mixed with organic nanosilica, the second group fed on grain mixed with inorganic nanosilica and a third group was fed on untreated grain as control. Three replicates were tested out for each group. The samples of blood were taken after ten days of treatment.

# Analysis of Hematology and Biochemical Parameters:

Blood samples were taken after ten days of treatments directed from heart rats. The samples were centrifuged at 3000 rpm for 15 minutes. The serum was collected and kept in the freezer at -20 C<sup>0</sup>. Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Creatinine (CREA), White Blood cells (WBC), Red Blood cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Platelets (PLT), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) according to Raoofi *et al.*, (2016).

# **Statistical Analysis:**

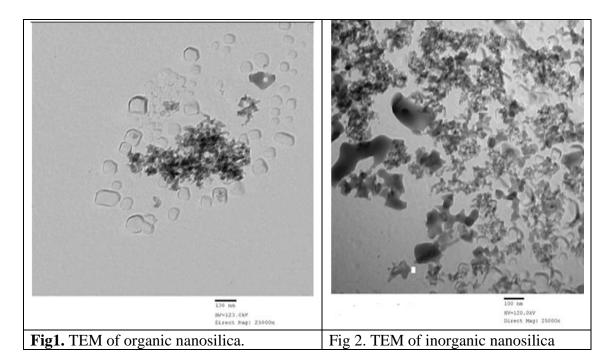
The obtained mortality data were subjected to Probit analysis (Finney, 1971), using a computer Program of Noack and Reichmuth (1978).

The statistical analysis was carried out using one-way ANOVA using SPSS, ver. 25 (IBM Corp. Released 2013). Data were treated as a complete randomization design according to Steel *et al.*, (1997). The significance level was set at < 0.05.

# **RESULTS AND DISCUSSION**

# Characterization of Organic and Inorganic Nanosilica:

The characterization and morphology of organic and inorganic nanosilica particles visualized using transmission electron microscopy (TEM). The particles appeared spherical and round in shape. The average size of the nanoparticles of organic and inorganic nanosilica were 150 and 100 nm respectively, (Figs., 1 and 2).



# Toxicities of Organic and Inorganic Nanosilica on E. vermiculata:

The LC50 values for organic and inorganic nanosilica after five and three days, respectively on *E. vermiculata* were 303.92 $\mu$ g/ml and 6.282×10<sup>4</sup>  $\mu$ g/g, respectively (Table,1). The correspondent values of LC90 were much lower in inorganic nanosilica treatment (34.909×10<sup>4</sup>  $\mu$ g/g) than that of the organic nanosilica (919.78 $\mu$ g/ml), respectively.

These results agree with those of Fahmy *et al.*, (2014) found that the LC50 and LC90 of zinc oxide nanoparticles against the snail *Biomphalaria alexandrina* were 145 and 2700 µg/ml. It was reported that zinc oxide NPs showed molluscicidal activity against this snail induce malondialdehyde and nitric oxide with decreasing of glutathione and glutathione Stransferase levels in hemolymph and soft tissues so the death of this snail.

**Table1**. LC<sub>50</sub> and LC<sub>90</sub> values of organic and inorganic nanosilica on *E. vermiculata*.

Treatments	$\mathrm{LC}_{50}$	$LC_{90}$	SLOP±SE		
	(lower -upper)	(lower -upper)			
Organic nonosilica	303.92	919.78	2.665±0.97		
	(226.2-369.8)	(728.5-1362.9)			
Inorganic nanosilica	6.282×10 <sup>4</sup>	34.909×10 <sup>4</sup>	1.721±0.99		
	(2.901×10 <sup>4</sup> -11.45×10 <sup>4</sup> )	(16.9×10 <sup>4</sup> -39.77×10 <sup>4</sup> )			

SE=standerd error of the mortality regression line.

Nanosilica is one of the most spreading materials on earth; it possesses a highly adhesive property to the cell membrane, so, it affects the membrane structures. Furthermore, this nanosilica can be absorbed by phospholipid present in cuticle of the larval instar by physisorption and lysis so cause death (Barik *et al.*, 2008 and Tiwari and Behari, 2009).

Hydrophilic NSP was selected and identified by TEM to appear spherical in shape with a size range of 80 nm in diameter. This size proved that they are able to adhere and absorb by the surface of target agents inducing mortality (Barik *et al.*, 2008 and Salunkhe *et al.*, 2011).

The ability of the tested NSP to cause mortalities in snails may be related to this suggested mode of actions (Barik *et al.*, 2008 and Barik *et al.*, 2012) as NSP can be absorbed by phospholipid found in the cuticle of larval instar by physisorption and lysis so cause death

to larval insects (Barik *et al.*, 2008 and Barik *et al.*, 2012). For this reason; their effect was high as the exposed tegument is thin. The LC50 and LC90 of hydrophilic nanosilica to *Biomphalaria alexandrina* were 590 ppm/6h and 980 ppm/48h, respectively (Attia *et al.*, 2017).

# Effect of Organic and Inorganic Nanosilica on E. vermiculata Life-Cycle Aspects:

Data in table (2) indicated that the two tested materials had a high effect on many aspects of life for E. vermiculata. At the beginning, no significant difference was found between these materials and control in the occurrence of copulation, despite the low mating rate of 50, 75 and 100 % for organic nanosilica, inorganic nanosilica and control, respectively. Reduction Percentage of copulation reached to 50 and 25 % for organic nanosilica, inorganic nanosilica, respectively. On the other hand, there was a highly significant difference in the copulation period spent by snails in case of inorganic nanosilica were increased to 16.13 hr. versus 12.66 and 10.95 hr. for inorganic nanosilica and control, respectively. Besides the period E. vermiculata spent before laying of eggs were high and very high significant between the two tested materials and control, respectively showing 14.33, 15.25 and 11.38 days for organic nanosilica, inorganic nanosilica and control, respectively. The rates of laying eggs were no significant between organic nanosilica, inorganic nanosilica and control being 75, 66.67 and 100 %, respectively but there was a clear decrease in the average total number of eggs laid by treated individuals of E. vermiculata compared to untreated ones, especially in case of inorganic nanosilica article where a highly significant difference appeared with mean of 28 eggs only, while untreated individuals were able to lay 111.75 eggs on average. Thus, it appeared that there was clear inhibition in oviposition of treated E. vermiculata individuals that reached 70.92 and 81.21% after treatment by organic and inorganic nanosilica respectively. A significant difference was confirmed for the percentage of eggs laid in the clutch form in the case of inorganic nanosilica with 25 % only, while no significant difference occurred between organic nanosilica and control. In terms of the number of eggs per clutch. It was also evident from results that high significant differences occurred between the two tested nano materials and control, and it was very highly significant for inorganic nanosilica with means of 45.50, 37 and 69.67 eggs/clutch for organic nanosilica, inorganic nanosilica and control respectively. Tested materials also showed a clear effect on the depth of laid eggs in the soil. In this respect, data showed very high significant differences between treated and untreated individuals, where the depth of hole decreased reached 3.80 cm when individuals were treated by organic nanosilica compared to control 5.57 cm. While individuals treated by inorganic nonosilica couldn't burrow in the soil. Regarding the percentage of eggs laid in solitary form, it increased to 75% when E. vermiculata individuals were treated with inorganic nanosilica. About the weight and diameter of eggs, a highly significant difference was detected, when individuals were treated with inorganic nanosilica, the mean weight of 10 eggs decreased to 0.31g and the average egg diameter decreased to 3.08 mm. Besides, the incubation period of eggs was longer in case of individuals treated with organic and inorganic nanosilica than individuals in control, being 16.67, 18.25 and 13.88 days respectively, thus indicating high and very high significant differences between transactions and control. Concerning eggs hatching percentage and the average number of larvae that survived after hatching, the results showed that there was a very high significant difference between untreated and treated E. vermiculata individuals, where hatchability rate and mean of the total number of alive larvae decreased from 92.19% and 45.88 larvae in control to 53.27 and 46.30%, and 9.33 and 4 larvae for organic and inorganic nanosilica, respectively resulting in a decrease in population of a new generation of E. vermiculata due to inhibition in larva percentage that increased to 92.37 and 95.64 % when snails were treated with the same two tested materials, respectively.

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	Some of life cycle aspects of <i>E. vermiculata</i> land snail																
Treatments	CO (%)	P of CO (hr.)	PBLE (day)	LE (%)	Mean of TNE	E (CH) (%)	Mean of E/CH	Mean of DCH (cm)	E (S) (%)	Mean of 10 EW (g)	Mean of ED (mm)	Mean of IP (day)	H E (%)	Mean of TNLL	I. C (%)	I. O (%)	I. L (%)
Organic nanosilica	50.00± 28.87	12.66± 2.55	14.33± 0.67**	75.00± 25.00	65.00± 24.00	66.70± 0.00	45.50± 4.50**	3.80± 0.70***	33.35± 0.30	0.46± 0.04	3.47± 0.49*	16.67± 0.88**	53.27± 4.00***	9.33± 1.20***	50	70.92	92.37
Inorganic nanosilica	75.00± 25.00	16.13± 1.02**	15.25± 0.48***	66.67± 16.67	28.00± 10.60**	25.00± 0.00*	37.00± 0.00***	0.00± 0.00***	75.00± 0.00*	0.31± 0.03**	3.08± 0.05**	18.25± 0.63***	46.30± 3.78***	4.00± 2.38***	25	81.21	95.64
Control	100.00± 0.00	10.95± 1.40	11.38± 0.38	100.00± 0.00	111.75± 19.72	75.00± 14.43	69.67± 4.42	5.57± 0.24	25.00± 14.43	0.55± 0.05	4.08± 0.16	13.88± 0.64	92.19± 2.18	45.88± 8.21	-	-	-

**Table 2:** Effect of organic and inorganic nanosilica on some aspects of *Eobania vermiculata* life- cycle under laboratory conditions

N on significant (P>0.05) \*: Significant (P<0.05)

\*\*: High significant (P<0.01)

\*\*\*: Very high significant (P<0.001)

CO: Copulation (%) LE: The Laying of Eggs E/CH: Eggs in each Clutch EW: Egg Weight (g)

HE: Hatching eggs (%) I. C: Inhibition of Copulation (%) P of CO: Period of Copulation (hours) **TNE: The Total Number of Eggs** DCH: Depth of Clutch (cm) ES: Egg Diameter

**TNLL: Total Number of Living Larva stage** I. O: Inhibition of Oviposition (%)

PBLE: The Period before Eggs laying (days).

E (CH): Eggs laid Clutch (%) E (S): Eggs laid Solitary (%) **IP: Incubation Period** 

I. L: Inhibition of Larva stage (%)

Generally, the formerly explained results confirmed that organic and inorganic nanosilica had a clear and significant effect on many aspects of E. vermiculata life-cycle, especially in case of inorganic nanosilica which had the greatest effect. Finally, using these materials in E. vermiculata control led to a reduction in the copulation and fertility of snail, where a small number of eggs were laid as well as the hatchability rate of the resulting eggs was inhibited. This, in turn, ultimately reduced the number of larvae in the next generation. These results agree with Sana et al., (2016) where used iron oxide powder (Fe2O3) nanoparticles against *Helix aspersa* land snail greatly significantly, affected the life-cycle of the terrestrial snail, as the treatments resulted in the appearance of deformation of the laid eggs and there was also a significant decrease in the percentage of hatching eggs. Silver nanoparticles (AgNPs) were used to control Eobania vermiculata land snail by Safaa et al., (2015). Their results showed that these materials affected the snail activity in the soil. Also, silver nanoparticles (AgNPs) showed great effectiveness on the life cycle of the gastropod freshwater snail *Physa acuta* by Sandra *et al.*, (2017).

# Hematological Parameters in Control and Tested Materials on Rates:

The results of the present study showed that the change in hematologic parameters in serum concentrations of WBCs, HGb, RBCs, MCV, HCT, MCHC and PLT in rat's animals treated with organic nanosilica and inorganic nanosilica, Table 3.

Data showed that there was no significant effect in HGb % of rats when treated with organic and inorganic nanosilica the values retched to 15.43 and 15.70 % respectively, compared with 15.30 % in the control. While there was a very high significant decrease in WBCs in rats' animals, the values reached 4.70 and 4.87 ( $10^3/\mu l$ ) when treated with organic and inorganic nanosilica respectively, compared with  $10.80 (10^3/\mu l)$  in the control. Also, rats treated with organic and inorganic nanosilica showed a very high significantly decreased in MCV by 73.63 and 74.0 (fl) respectively, compared to 91.30 (fl) in the control treatments. In the same trend, a very high significant decrease in HCT % treated with organic and inorganic nanosilica reached 48.63 and 46.30 respectively, compared to 50.30 % in the control. On contrary, there was a significant increase in RBCs treated with organic nanosilica by  $7.30 (10^6/\mu l)$  and a very high significant increase reached  $7.90 (10^6/\mu l)$  in treated rats with inorganic nanosilica compared with 6.77 (10<sup>6</sup>/µl) in the control. Also, a very high significance showed in PLT of treated rats with organic nanosilica reached 832.0 (10<sup>3</sup>/µl) and a highly significant increase in rats treated with in organic nanosilica by  $811.07 (10^3/\mu l)$ compared with 478.0 ( $10^3/\mu l$ ) in the control.

Parameters	Control	Organic Nanosilica	Inorganic Nanosilica			
HGb (%)	15.30±0.17	15.43±0.03	15.70±0.12			
WBCs (10 <sup>3</sup> /μl)	10.80±0.06	4.70±0.12***	4.87±0.03***			
MCV (fl)	91.30±0.12	73.63±0.20***	74.00±0.00***			
HCT (%)	50.30±0.12	48.63±0.03***	46.30±0.06***			
RBCs (106/μl)	6.77±0.07	7.30±0.17*	7.90±0.06***			
PLT (10 <sup>3</sup> /μl)	478.00±1.73	832.00±1.15***	811.07±0.03**			
MCH (Pg)	33.60±0.06	32.14±0.01***	36.31±0.01***			
MCHC (%)	46.40±0.23	44.23±0.15***	47.03±0.02*			

**Table 3**: Hematological parameters in rats treated by tested materials.

Nonsignificant (P>0.05) \*: Significant (P<0.05) \*\*: High significant (P<0.01) \*\*\*: Very high significant (P<0.001)

WBC: white blood cell count, HGb: hemoglobin, RBC: red blood cell count, HCT: Platelets, PLT: Mean Corpusclarn Hemoglobin, MCH: Mean Corpusclarn Volume, MCHC: Mean Corpusclarn Hemoglobin Concentration, MCV: mean cell volume.

On the other hand, rats treated with organic and inorganic nanosilica showed variable effects in the MCH (Pg) and MCHC %.

MCH recorded a very high decrease by 32.14 (Pg) treated with organic nanosilica, but inorganic nanosilica treatment showed a significant increase by 36.31 (Pg) compared with 33.60 (Pg) in the control. The same effect occurred in MCHC % showed a very high significant decrease reached to 44.23 % in case of organic nanosilica application and showed a significant increase reached to 47.03 % for treatment with inorganic nanosilica compared with 46.40 % in the control (Table,3).

# **Biochemical Parameters in Control and Tested Materials on Rats:**

The effects of tested compounds on serum biochemical analysis indicated a very high significant decrease in creatinine (mg/dl) reached 0.4 and 0.42 (mg/dl) when treated with organic and inorganic nanosilica respectively, compared with 0.56 (mg/dl) in serum of the control rats. Also, ALP (U/L) showed a very high significant decrease after treatment with organic and inorganic nanosilica showing 3.52 and 3.7 (U/L) respectively, compared with 4.96 (U/L) in the control.

On the other hand, the effect on ALT (U/L) recorded a very high significant increase when treatment took place with organic or inorganic sanosilica the values reached 120.01 and 121.07 (U/L) respectively, compared with 25.07 (U/L) in case of the control, (Table,4).

The obtained results agree with Song *et al.*, (2009) who stated that exposure to nanosilica, resulted in a downward trend in WBC, neutrophils, and monocyte counts in the blood of rats, as well as a significant fall in neutrophils. In addition, compared to control, ALT and LDH presented an upward trend in exposure groups, thus indicating the existence of liver damage after exposures. The present results indicate that nanosilica materials have similar toxicity, which is consistent with the increase of ALT.

The liver is an important organ for detoxification in the body and plays a vital role in the metabolism of nanomaterials. It has been shown that tail vein injection by nanosilica resulted in a significant accumulation of nanoparticles in the liver and resulted in liver injury (Lu *et al.*, 2011 and Xie *et al.*, 2010). Also, Oxidative stress in mice livers was notably prevalent after exposure to nanosilica, resulting in hepatic injury (Steel *et al.*, 1997). The results of serum ALT and LDH levels in the exposed animals in the present study supported liver-damaging by silica-containing nanoparticles (So *et al.*, 2008 and Ye *et al.*, 2010).

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#### ARABIC SUMMARY

سمية جسيمات النانو سليكا على Eobania vermiculata وتأثيراتها على التغيرات البيوكيميائية في الفئران.

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في هذه الدراسة تم تقييم النانوسيليكا العضوية و غير العضوية ضد قوقع Eobania vermiculata نسبة الوفيات، وشملت الدراسة بعض جوانب دورة الحياة وأيضا تأثير ها على بعض المتغيرات البيوكيميائية في فئران نسبة الوفيات، وشملت الدراسة بعض جوانب دورة الحياة وأيضا تأثير ها على بعض المتغيرات البيوكيميائية في فئران Albino Wistar ميكروجرام / مل و  $10^4 \times 6.282 \times 10^4 \times 6.282$  ميكروجرام / مل و  $10^4 \times 6.282 \times 10^4 \times 6.282$  هي 203.9 والجيل النواية أيام من المعاملة على التوالي أظهرت البيانات نتائج معنوية ضد جوانب دورة حياة لقوقع E. vermiculata والجيل الجديد حيث تسببت المعاملات في خفض معدل التزاوج، وضع البيض والنسب المئوية لمرحلة البرقة  $10^4 \times 10^4 \times$