Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Insecticidal efficiency and safety of zinc oxide and hydrophilic silica nanoparticles against some stored seed insects

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Vol. 60, No. 1: 77–85, 2020

DOI: 10.24425/jppr.2020.132211

Received: April 21, 2019
Accepted: October 7, 2019

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Abstract

The present study was conducted to evaluate the insecticidal efficiency and safety of zinc oxide nanoparticles (ZnO NPs) and hydrophilic silica nanoparticles (SiO_2 NPs) against: adults of rice weevil (*Sitophilus oryzae* L.); red flour beetle (*Tribolium castaneum* Herbst.) and cowpea beetle (*Callosobruchus maculatus* F.) results showed that, both ZnO NPs and hydrophilic SiO_2 NPs exhibited a significant toxic effect (*df*, *F* and $p < 0.5$) against *S. oryzae* and *C. maculatus* at the highest concentration while *T. castaneum* showed high resistance against the two tested materials. At the end of the experiment, recorded mortality was: 81.6, 98.3 and 58.3% at the highest concentration used for each insect (0.3, 2 and 8 $gm \cdot kg^{-1}$ of SNPs with *C. maculatus*, *S. oryzae* and *T. castaneum,* respectively), while mortality was 88.3, 100 and 38.3% at the highest concentration used for each insect (0.6, 2.5 and 8 $gm \cdot kg^{-1}$ of ZnO NPs with *C. maculatus*, *S. oryzae* and *T. castaneum,* respectively). Both tested materials caused high reductions in F1-progeny (%) with *C. maculatus* and *S. oryzae*. Histopathological examination of male mice livers showed hepatic architecture with congested blood sinusoids, binucleated hepatocytes nuclei, dilated central vein and marginated chromatin in some nuclei. Histopathological assessment of the lungs showed normal histoarchitecture. There were no differences in alveolar septa, bronchiolar and epithelium of the treated and untreated animals. Silica and zinc oxide nanoparticles have a good potential to be used as stored seed protectant alternatives if applied with proper safety precautions.

Keywords: *Callosobruchus maculatus*, hydrophilic silica nanoparticles, *Sitophilus oryzae*, *Tribolium castaneum*, wheat, zinc oxide nanoparticles

Introduction

According to FAO approximately 10 to 25% of harvested food worldwide is destroyed annually by insects and rodent pests (Anonymous 1980). Grain damage arising from direct feeding of insects on endosperm and grain embryos increases the exposure of grain to rot because scratches lead to unpleasant odors which cannot be accepted by humans and animals (Ismail 2014).

The rice weevil (*Sitophilus oryzae* L.) (Coleoptera: Curculionidae) is considered to be a primary insect pest of stored grains and causes high losses in warm climate areas (Batta 2004).

The cowpea weevil (*Callosobruchus maculatus* F.) (Coleoptera: Bruchidae), is a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of cowpea in the tropics (Caswel 1985).

The red flour beetle (*Triboleum castaneum* Herbst.) (Coleoptera: Tenebrionidae), is a polyphagous, cosmopolitan pest of stored grains. In severe infestation the flour turns grayish and moldy and has a pungent, disagreeable odor making it un-fit for human consumption (Suresh *et al*. 2001).

In recent years, consumer awareness of health hazards from fumigants and residual toxicity of insecticides which are commonly used to control stored grain pests and the growing problem of insect resistance to these conventional insecticides have led researchers to look for alternate strategies for stored grain protection (Debnath *et al*. 2011).

More recently, materials including diatomaceous earth, silica aerogels and silica nano particles have been increasingly finding use in commercial storage in the developed world, replacing conventional chemicals (Golob 1997).

Nanotechnology is a new and promising field of research in a wide range of various fields like insecticides, agriculture and pharmaceuticals (Ragaei and Sabry 2014). Nanotechnology could provide green and efficient alternatives for the management of insect pests in agriculture without harming nature (Rai and Ingle 2012).

Nanoparticles show promise in different fields of agricultural biotechnology (Majumder *et al*. 2007; Rahman *et al*. 2009). Nanoparticles have helped to produce new pesticides, insecticides and insect repellants (Owolade *et al*. 2008). Yang *et al*. (2009) found that nanoparticles loaded with garlic essential oil is efficacious against *T. castaneum*. Stadler *et al*. (2010) showed that nano alumina can be successfully used to control stored grain pests.

According to relevant specialized encyclopedias, the definition of nanoparticles (NPs) is: "Nanoparticles are solid colloidal particles ranging in size from 1 to 100 nm" (Ball 2002; Roco 2003).

Some researchers believe that nanoparticles could inhibit plant growth. Yang and Watts (2005) reported that nano alumina in ground water inhibited the growth of carrot, cabbage, cucumber and soybean but SiO_2 NPs have no such adverse effect on plant health (Debnath *et al*. 2010). Instead of having negative effects, silica enhances structural rigidity and plant strength (Epstein 1994). This may be one of the possible reasons for an age-old tradition of using silica dust as a protecting agent for stored seeds by different ethnic races all over the world (Debnath *et al*. 2010).

Nanoparticles may interfere with plant metabolism in several ways, such as, by providing micronutrients (Liu and Lal 2015), regulating genes (Nair and Chung 2014), or interfering with different oxidative processes in plants which results in oxidative burst (Hossain *et al*. 2015).

The objective of our study was to evaluate the insecticidal efficiency and safety of zinc oxide nanoparticles (ZnO NPs) and hydrophilic silica nanoparticles (SiO_2 NPs) against three stored grain insects: *S. oryzae*, *T. castaneum* and *C. maculatus*.

Materials and Methods

Experiments were conducted in the laboratory of the Plant Protection Research Institute – Agriculture Research Center, Egypt at 30 ± 2°C and 65 ± 5% relative humidity (RH).

Tested materials

Varieties *Vigna sinensis* L. (cowpea dokki 331) and *Triticum sativum* (wheat Sakha 93) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. Zinc oxide and hydrophilic SiO_2 NPs were obtained from the Nanotech Egypt Company, Cairo, Egypt. The aqueous suspensions of the studied nano particles were characterized with a Transmission Electron Microscope (TEM) (Bonevich and Haller 2010).

Insect rearing

Laboratory strains of the target insect species *S. oryzae*, *T. castaneum* and *C. maculatus* were reared for several generations in glass jars (each approximately 500 ml in size) containing about 250 gm of insect feed. Each jar was covered with muslin cloth and fixed with rubber bands.

To obtain a primary population of insect adults homogenous in age, about 500 adults were introduced into jars containing seeds for egg laying and then kept in an incubator at 30 ± 2°C and 65 ± 5% RH.

Sitophilus oryzae was reared on wheat seeds, *T. castaneum* was reared on wheat flour or crushed wheat and *C. maculatus* was reared on cowpea seeds. They were kept in an incubator at 30 ± 2°C and 65 ± 5% RH until the 5th generation at the stored product laboratory.

Wheat seeds, wheat flour and cowpea seeds were sterilized by cooling at 10°C for 2 weeks to ensure that feeding materials were free from any previous infestation.

Insecticidal efficiency tests

Experiments were conducted to test the insecticidal activity of ZnO NPs and SiO_2 NPs on *S. oryzae, T. castaneum* (2 weeks old) and *C. maculatus* (24 h old).

Experiments were carried out with three replicates. Each consisted of 20 male and female insect adults in small plastic screw capped jars containing 10 g of either wheat seeds for *S. oryzae*, wheat flour for *T. castaneum* or cowpea seeds for *C. maculatus*. Seeds in each jar were treated individually with different concentrations (0.05–8 gm · kg^{-1}) of ZnO NPs or SiO_2 NPs. Then, the jars were shaken manually for approximately 1 min to achieve equal distribution on the sample, then incubated at 30 ± 2°C and 65 ± 5% RH. In addition, three untreated replicates were used as control (Subramanyam and Roesli 2000).

Insect mortality (%) was checked after 1, 2, 3, 4, 5, 7 and 10 days for *S. oryzae*; 1, 2, 4, 6, 8, 10, 14 and 21 days for *T. castaneum* and 2, 3, 4 and 5 days for *C. maculatus* according to the usual practice.

At the end of the experiments live insects were removed and the glass jars were incubated for 60 days for *S. oryzae* and *T. castaneum* and 30 days for *C. maculatus* at 30 ± 2°C and 65 ± 5% RH to obtain the first progeny. Then the reduction percent of F1-progeny was calculated according to (El-Lakwah *et al.* 1996). The percent of insect mortality was calculated using the corrected Abbot's formula (Abbot 1925).

Toxicity experiment of hydrophilic SiO_2 NPs on mice

The toxicity of hydrophilic SiO_2 NPs is of concern for public and consumer health. Accordingly, this study tested the diet toxicity at LC_{90} concentration (0.3 gm · kg^{-1}) of hydrophilic SiO_2 NPs (the most effective against tested insects) on mice lung and liver function over a period of 28 days (feeding on wheat grains).

For the *in vivo* studies, 12 to 16-week-old male mice, weighing 20 ± 5 gm, were purchased from Alroaa Laboratory (Cairo, Egypt) and were maintained in a controlled environment (23 ± 1.5°C; 12 h light/dark cycle) with access to wheat and water. The mice were left to adapt to the new environment for 1 week before commencing with the experiment. They were then divided into two groups (five mice in each group): the first group, used as control, was fed untreated wheat for 28 days and the second group was fed wheat mixed with hydrophilic SiO_2 NPs for 14 days. They were then fed untreated wheat for another 14 days for a total of 28 days. At the end of the experiment, liver functions [Alkaline Phosphatase (ALP), Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST)] were investigated, along with a histopathological examination of livers and lungs. All animals were observed once daily after treatment for health, death, and any clinical signs of toxicity.

Feed and water consumption were noted daily after the start of treatment. Consumption was calculated from the differences between the provided amounts and the remaining amounts measured the next day.

Biochemical examination

The determined enzymes (ALP, ALT and AST) were measured with commercially available kits according to the manufacturer's protocols (Biotech Laboratory, Cairo, Egypt).

Histological examination

Lung and liver (internal organs) of three animals from each group were removed and fixed with 4% paraformaldehyde. After sectioning, thin tissue sections were stained with hematoxylin and eosin for histological observations (Biotech Laboratory, Cairo, Egypt).

Statistical analysis

Insect mortality data were subjected to one way completely randomized analysis of variance and differences using ANOVA test (a computer program costate). Mean values were adjusted by Duncan's Multiple Range test (Duncan 1951) at 0.05% level of significance with statistical software version 6.3.0.3.

The mortality (%) of *S. oryzae* on the 7th day was probit analyzed using a computer program named Ldp-line according to (Finney 1947). From this the toxicity values (LC_{50} and LC_{90}) were calculated.

Results and Discussion

Structure study

The size and shape of hydrophilic SiO_2 NPs, observed with a TEM (Fig. 1), indicated that the particles were approximately spherical, and less than 100 nano rods/sheets in size while ZnO NPs were spherical in shape with sizes ranging between 12–21 nano TEM (Fig. 2).

Insecticidal toxicity bioassay

According to results (Tables 1–3) accumulative mortality (%) increased with increased concentrations and exposure periods. Results showed that both ZnO NPs and hydrophilic SiO_2 NPs exhibited a significant toxic effect (*df*, *F* and $p < 0.5$) against *S. oryzae* and *C. maculatus* at the most effective concentration while with *T. castaneum* both tested materials caused low mortality (%).

An analysis of results of variance showed that the effect of ZnO NPs on *S. oryzae* on day 2 (F3,8 = 1.27, $p = 0.35$), day 3 (F3,8 = 7.00, $p = 0.01$), day 4 (F3,8 =14.67, $p = 0.00$), day 5 (F3,8 = 35.08, $p = 0.00$), day 7 (F3,8 = 87.3, $p = 0.00$) and day 10 (F3,8 = 106.30, $p = 0.00$) (Table 1) had a significant difference at 5% except day 2 had no significance and *C. maculatus*; on day 2 (F3,8 = 1.83, $p = 0.22$), day 3 (F3,8 = 7.48, $p = 0.01$), day 4 (F3,8 = 7.92, $p = 0.01$), day 5 (F3,8 = 23.75, $p = 0.00$) (Table 2) had a significant difference at 5% except day 2 had no significance and on *T. casteneum*; on day 8 (F2,6 = 6.5, $p = 0.32$), day 10 (F2,6 = 16.33, $p = 0.00$), day 14 (F2,6 = 22.4, $p = 0.00$),

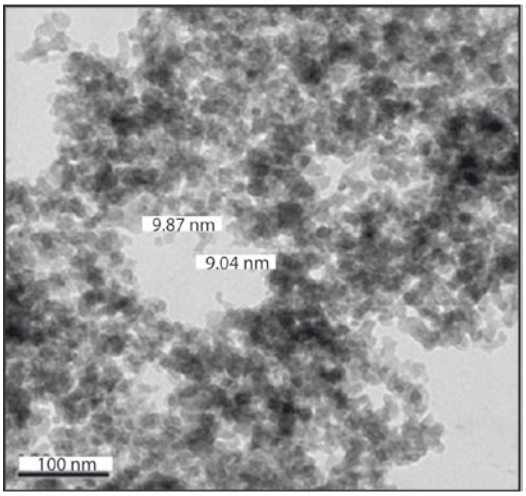

Fig. 1. The Transmission Electron Microscope (TEM) images of hydrophilic silica nanoparticles (x = 40 k); X – magnification power

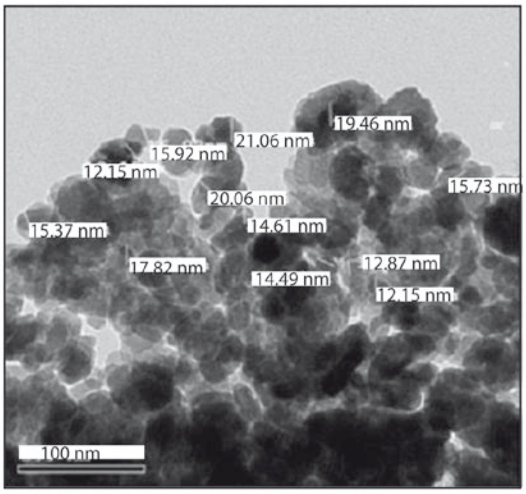

Fig. 2. The Transmission Electron Microscope (TEM) images of zinc oxide nanoparticles (x = 40 k); X – magnification power

Table 1. Mortality percent (mean ± SE) of *Sitophilus oryzae* adults treated with different concentrations of zinc oxide nanoparticles (ZnO NPs) and hydrophilic silica nanoparticles (SiO_2 NPs) for 10 days

Tested materials	Concentration [gm · kg^{-1}]	Adult mortality after indicated days [%]						Reduction in F1 progeny [%]
		2nd	3th	4th	5th	7th	10th	
ZnO NPs	2.5	5 a ± 2.8	28.3 a ± 4.4	43.3 a ± 9.3	61.7a ± 1.6	88.3 a ± 1.6	100 a ± 0	94.0
	2	1.6 a ± 1.6	23.3 ab ± 1.6	38.3 a ± 4.4	53.3a ± 4.4	70 b ± 2.9	82 b ± 1.6	93.2
	1.5	1.6 a ± 1.6	13.3 bc ± 4.4	25 b ± 1.6	38.3b ± 1.6	50 c ± 2.9	58.3 c ± 4.4	90.0
	1	00 a ± 00	10 c ± 0	13.3 c ± 3.3	16.7c ± 4.4	28.3 d ± 3.3	35 d ± 2.9	86.6
	LSD 5%	6	10.5	11.5	10.8	9	9	
Hydrophilic SiO_2 NPs	2	15 a ± 2.9	26.6 a ± 3.3	41.6 a ± 1.6	61.6 a ± 1.6	83.3 a ± 3.3	98.3 a ± 1.6	95.0
	1.5	5 b ± 2.9	21.6 a ± 4.4	36.6 a ± 1.6	55 a ± 2.9	71.6 b ± 1.6	79 b ± 2.9	93.0
	1	3.3 b ± 1.6	11.6 b ± 1.6	25 b ± 2.8	41.6 b ± 6.0	46.6 c ± 4.4	49 c ± 4.4	90.6
	0.5	3.3 b ± 1.6	8.3 b ± 1.6	11.6 c ± 1.6	13.3 c ± 1.6	18.3 d ± 1.6	24.5 d ± 1.6	88.8
	LSD 5%	7.6	9.8	6.6	11.5	9.8	9.4	

*means within a column followed by the same letter are not significantly different ($p < 0.05$)

Table 2. Mortality percent (mean ± SE) of *Callosobruchus maculatus* adults treated with different concentrations of zinc oxide nanoparticles (ZnO NPs) and hydrophilic silica nanoparticles (SiO_2 NPs) for 5 days

Tested materials	Concentration [gm · kg^{-1}]	Adult mortality after indicated days [%]					Reduction in F1 progeny [%]
		1 st	2nd	3th	4th	5th	
ZnO NPs	0.6	00 ± 00	3.3 a ± 1.6	30 a ± 2.9	66.6 a ± 6.0	88.3 a ± 4.4	86.4
	0.5	00 ± 00	1.6 a ± 1.6	13.3 b ± 6.0	51.6 ab ± 4.4	83.3 a ± 4.4	83.0
	0.4	00 ± 00	00 a ± 00	11.6 b ± 1.6	48.3 bc ± 4.4	56.6 b ± 4.4	80.9
	0.3	00 ± 00	00 a ± 00	8.3 b ± 1.6	33.3 c ± 4.4	43.3 b ± 4.4	79.2
	LSD 5%		3.8	10	15.8	14.4	
Hydrophilic SiO_2 NPs	0.3	6.6 a ± 1.6	26.6 a ± 1.6	40 a ± 2.9	62.5 a ± 2.9	98 a ± 3.3	92.0
	0.2	5 ab ± 2.9	20 ab ± 7.6	21.6 b ± 11.7	46.5 b ± 5.8	76.5 b ± 6.0	85.0
	0.1	1.6 ab ± 1.6	6.6 bc ± 1.6	13.3 c ± 1.6	28.6 c ± 1.6	43.1 c ± 2.9	83.0
	0.05	00 b ± 00	5 c ± 2.9	8.3 c ± 4.4	14.3 d ± 2.9	15.7 d ± 1.6	82.2
	LSD 5%	6.07	13.8	8	11.8	11.5	

*means within a column followed by the same letter are not significantly different ($p < 0.05$)

Table 3. Mortality percent (mean ± SE) of *Tribolium castaneum* adults treated with different concentrations of zinc oxide nanoparticles (ZnO NPs) and hydrophilic silica nanoparticles (SiO_2 NPs) for 21 days

Tested materials	Concentration [$gm \cdot kg^{-1}$]	Adult mortality after indicated days [%]				Reduction in F1 progeny [%]
		8th	10th	14th	21st	
ZnO NPs	8	6.6 a ± 1.6	16.6 a ± 1.6	26.6 a ± 1.6	36 a ± 1.6	6.8
	6	1.6 b ± 0.96	8.3 b ± 1.6	20 a ± 2.9	31 a ± 4.4	5.4
	4	0 b ± 0	3.3 b ± 1.6	6.6 b ± 1.6	15.5 b ± 1.6	1.1
	LSD 5%	4.7	5.7	7.4	9.9	
Hydrophilic SiO_2 NPs	8	6.6 a ± 1.6	15 a ± 2.9	36.6 a ± 1.6	57 a ± 3.3	9.1
	6	1.6 a ± 1.6	8.3 a ± 1.6	21.6 b ± 1.6	41.3 b ± 4.4	6.6
	4	1.6 a ± 1.6	8.3 a ± 1.6	15 b ± 2.9	20.6 c ± 4.4	2.5
	LSD 5%	5.7	7.4	7.4	14	

*means within a column followed by the same letter are not significantly different ($p < 0.05$)

day 21 ($F2,6 = 13$, $p = 0.00$) (Table 3) had a significant difference at 5%.

An analysis of results of variance showed that the effect of hydrophilic SiO_2 NPs on *S. oryzae* on day 2 ($F3,8 = 5.66$, $p = 0.02$), day 3 ($F3,8 = 8.07$, $p = 0.00$), day 4 ($F3,8 = 42.83$, $p = 0.00$), day 5 ($F3,8 = 36.65$, $p = 0.00$), day 7 ($F3,8 = 92.10$, $p = 0.00$) and day 10 ($F3,8 = 114.30$, $p = 0.00$) (Table 1) had a significant difference at 5%; on *C. maculatus* on day 1 ($F3,8 = 2.66$, $p = 0.12$), day 2 ($F3,8 = 6.08$, $p = 0.02$), day 3 ($F3,8 = 30.96$, $p = 0.00$), day 4 ($F3,8 = 29.10$, $p = 0.00$), day 5 ($F3,8 = 114.30$, $p = 0.00$) (Table 2) had a significant difference at 5% except day 1 had no significance; on *T. castaneum* on day 8 ($F2,6 = 3$, $p = 0.12$), day 10 ($F2,6 = 3.2$, $p = 0.11$), day 14 ($F2,6 = 26.6$, $p = 0.00$), day 21 ($F2,6 = 18.5$, $p = 0.00$) (Table 3) had a significant difference at 5% except days 8 and 10 had no significance.

At the end of the experiment, mortality (%) recorded was 98.3, 98 and 57 at the highest concentration 2, 0.3 and 8 $gm \cdot kg^{-1}$ of hydrophilic SiO_2 NPs with *S. oryzae, C. maculatus* and *T. castaneum,* respectively, while mortality (%) recorded was 100, 88.3 and 36 at the highest concentration 2.5, 0.6 and 8 $gm \cdot kg^{-1}$ of ZnO NPs with *S. oryzae, C. maculatus* and *T. castaneum,* respectively.

Both tested materials caused a high reduction (%) in F1 progeny with *S. oryzae* (94% at 2.5 $gm \cdot kg^{-1}$ ZnO NPs and 95% at 2 $gm \cdot kg^{-1}$ hydrophilic SiO_2 NPs) and with *C. maculatus* (86.4% at 0.6 $gm \cdot kg^{-1}$ ZnO NPs and 92% at 0.3 $gm \cdot kg^{-1}$ hydrophilic SiO_2 NPs).

The reduction (%) in F1 progeny caused by ZnO NPs ranged from 79.2 to 86.4% and 86.6 to 94% with *C. maculatus* and *S. oryzae,* respectively, while with hydrophilic SiO_2 NPs reduction (%) in F1 progeny ranged from 82.2 to 92 and 88.8 to 95% with *C. maculatus* and *S. oryzae,* respectively. Both tested materials caused a slight reduction (%) in F1 progeny (6.8% at 8 $gm \cdot kg^{-1}$ ZnO NPs and 9.1% at 8 $gm \cdot kg^{-1}$ hydrophilic SiO_2 NPs) with *T. castaneum.*

Our study demonstrated that with time the application of ZnO NPs and hydrophilic SiO_2 NPs could significantly increase the mortality effect of NPs, indicating that hydrophilic SiO_2 NPs have a high potential as a pesticide. Debnath *et al.* (2011) showed that the mortality effect of hydrophilic and hydrophobic SiO_2 increased from day 1 to day 14 and greater mortality was observed with the highest dose at the end of 2 weeks. Silica nanoparticles were reported against stored species (*Rhyzopertha dominica* and *Tribolium confusum*) and showed that they can be used effectively in a stored grain integrated pest management program.

Also the findings of our study showed that the entomotoxicity effect of ZnO NPs was not salient, and it was less than the effect of hydrophilic SiO_2 NPs on tested insects. This finding is in harmony with that of (Rouhani *et al.* 2019) who reported that hydrophilic SiO_2 NPs were found to be highly effective against *Sitophilus granarius*, causing 100% mortality after 2 weeks. ZnO NPs were moderately effective against this pest. Another study by Debnath *et al.* (2011) reported that silica nanoparticles were found to be highly effective against *S. oryzae* causing more than 90% mortality, indicating the effectiveness of hydrophilic SiO_2 NPs to control insect pests.

Several studies have reported the potential of nanomaterials as insecticides against stored grain insects. A study by Sabbour (2013) revealed that ZnO NPs were more effective in decreasing the infestation of *S. oryzae* under laboratory and store conditions. Another finding by Doaa and Nilly (2015) revealed that fumed silica, Aerosil 200 NPs had a significantly strong toxic effect ($p < 0.05$) with the highest concentration: 1 $gm \cdot kg^{-1}$ for *C. maculatus*, 1.5 $gm \cdot kg^{-1}$ for *R. dominica* and 2.5 $gm \cdot kg^{-1}$ for *S. oryzae.* It also reduced the

Table 4. Changes in mean bodyweight of control and treated mice exposed to hydrophilic SiO_2 NPs 2 and 4 weeks after the beginning of treatment

Treatment	Mice weight [gm]	Mean increase in mice body weight [%]	
	0 time	after 2 weeks	after 4 weeks
Control	19.8	45.3	64.0
Treated	19.0	33.8	60.5

Table 5. Blood biochemical indices of the experimental mice feeding on wheat grains untreated and treated with hydrophilic SiO_2 NPs

Treatment	Sample	Alanine amino-trans-ferase [$IU \cdot l^{-1}$]	Aspartate amino-transferase [$IU \cdot l^{-1}$]	Alkaline phosphatase [$IU \cdot l^{-1}$]
After 2 weeks	Control mean ± SE	41.9 ± 0.8	84.7 ± 1	3 ± 0.1
	Treated mean ± SE	40.9 ± 0.9	74.5 ± 0.7	3.2 ± 0.1
After 4 weeks	Control mean ± SE	47.5 ± 5.9	116 ± 7.2	3.8 ± 0.03
	Treated mean ± SE	63.1 ± 2.2	87.9 ± 11.9	3.7 ± 0.2

progeny by 100% at the highest concentration. Also El-Bendary and El-Helaly (2016) reported that hydrophilic nano silicate at 20 mg · kg^{-1} was an efficient candidate to control rice weevil, *S. oryzae*.

In this connection, inert dusts have been shown to control a variety of common storage insect pests. They are most effective under conditions of low humidity because they induce mortality by causing desiccation; water is lost due to destruction of the waxy layer of the cuticle by adsorption (Ebeling 1971). This led agrochemical researchers to reappraise the use of inert dusts as alternative insecticides for crop protection.

Toxicity of hydrophilic SiO_2 NPs on male mice

Changes in mice weight

Results recorded in Table 4 showed the mean increase (%) in mice body weight after feeding on wheat treated with LC_{90} concentration of hydrophilic SiO_2 NPs (the most effective against *S. oryzae* from previous experiments) for 2 weeks and resumed feeding on untreated wheat for another 2 weeks.

There were no obvious differences in the mean weight (%) of treated male mice compared to the control mice 2 and 4 weeks after the beginning of the initial experiment.

Biochemical examination

Observations from pathological examination showed that the liver was the target organ for nanoparticles of silica via diet exposure. Hence, blood biochemical parameters (ALT, AST and ALP) that reflect hepatic functions were further investigated (Table 5). No distinct differences between the control and hydrophilic SiO_2 NPs treated mice groups were observed, except a very slight decrease of AST in the mice of treated groups.

Histopathological examination

Histopathological examination of the male mice livers (Fig. 3) showed hepatic architecture with congested blood sinusoids, binucleated hepatocytes and many pyknotic nuclei (Fig. 3D), hepatic architecture with a dilated central vein, congested blood sinusoids, binucleated hepatocytes and margainated chromatin in some nuclei (Fig. 3E), congested blood sinusoids and many pyknotic nuclei (hematoxylin and eosin stain, 400×).

Histopathological assessment of the lungs (Fig. 4) showed normal histoarchitecture. There were no difference in alveolar septa (Fig. 4D), bronchiolar (Fig. 4E) and epithelium (Fig. 4F) of the treated animals compared to the control set.

In the current study, although no morphological differences appeared between treated and untreated mice (Table 4) the results indicated that the control group showed normal shapes of mice organs (liver and lung) as shown in the illustrated replicates A, B and C. Whereas, in the treated group (Figs. 3 and 4, respectively). Figure 3 (with the three replicates: D, E and F) showed the liver infiltration of inflammatory cells, depletion of glycoprotein, hepatic architecture with binucleated hepatocytes, hemorrhage (an escape of blood from a ruptured blood vessel) and infiltration of lymphocytes (margainated) chromatin in some nuclei. As regarding the lungs of treated mice no changes were detected (Fig. 4 with the three replicates: D, E and F). The toxic effect of hydrophilic SiO_2 NPs (0.4 gm · kg^{-1}) indicated that some toxic metabolites may be transported from the intestine to the liver causing these changes. In agreement (Benjamin *et al*. 2006) reported that the presence of definite necrosis indicated the capability of the toxic metabolites to cause cell death.

In support results which found differences in the histology of liver with no changes in the morphology of mice, Kim *et al.* (2014) reported that, histopathological findings with nano treatment, no hydrophilic SiO_2 NPs treatment-related changes were observed in the appearance or morphology of the treatment groups. Similar results were obtained by (Aderem 2003) who stated that histological analysis showed that apparent pathological changes, including lymphocytic infiltration at the portal area and hepatocytes necrosis at the portal triads, were observed in the livers of tested mice treated with nanoparticles.

With respect to the analyzed enzymes (ALT, AST and ALP) that reflect hepatic functions (Table 5), no differences between the control and hydrophilic SiO_2 NPs treated mice group were observed, except a very slight decrease of AST in the mice of treated groups. In this connection, the level of LDH in serum is often tested along with ALP and ALT to evaluate whether the liver is damaged/diseased or healthy. When the liver is dysfunctioning, the levels of the above serum enzymes will rise (Kellerman 1987).

On the other hand Xie *et al.* (2010) reported that the results conclusively demonstrate toxicity of exposure to hydrophilic SiO_2 NPs in mice leads to an accumulation of nanoparticles in the liver.

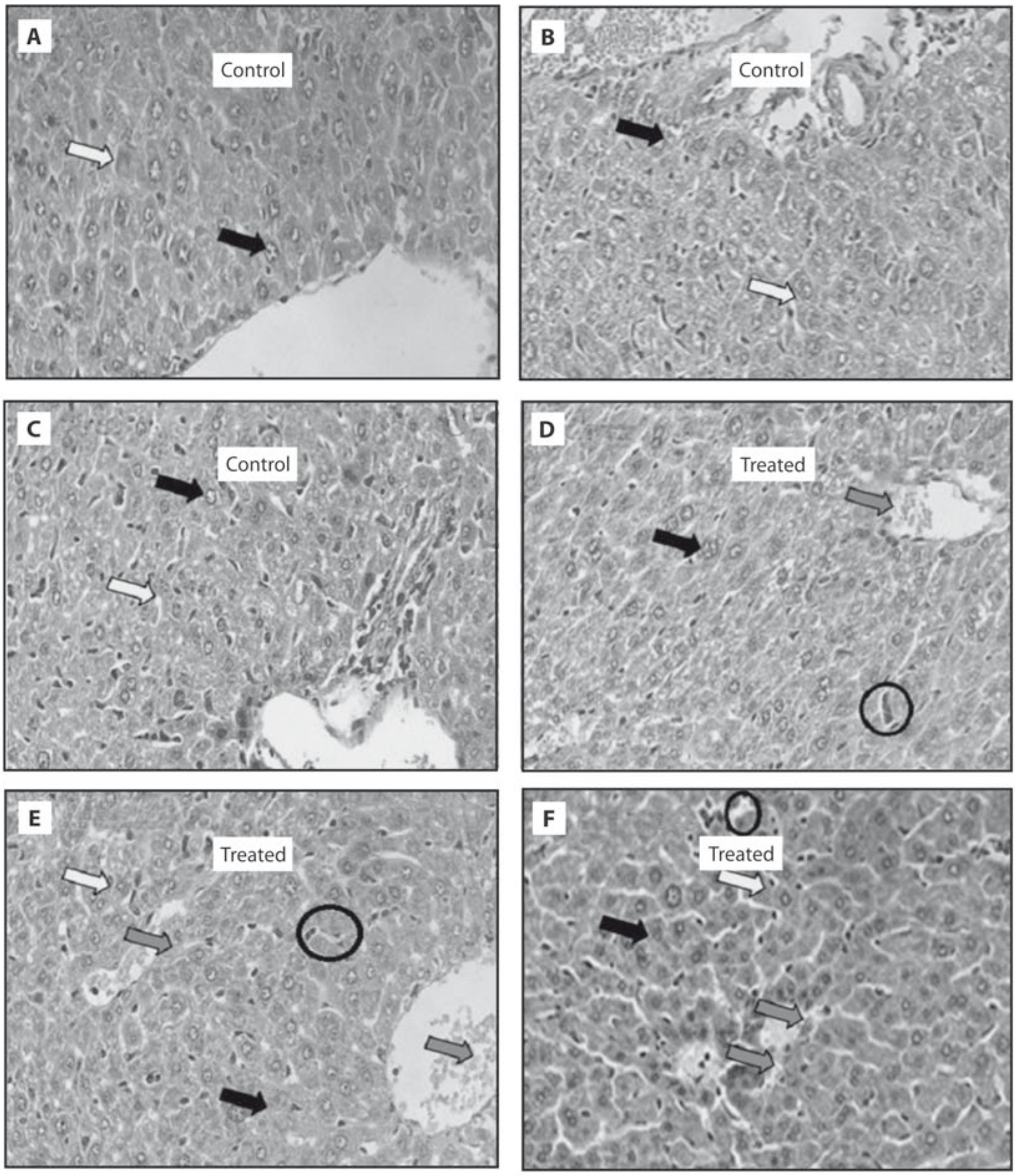

Fig. 3. Histological images of livers from three animals feeding on wheat grains untreated and treated with hydrophilic SiO_2 NPs after 28 days A, B and C – cross sections of three control mice livers showing normal shape composed of central veins (white arrows) and hepatocytes (black arrows). D, E and F – cross sections at a dose of 0.4 gm · kg^{-1}. Hydrophilic SiO_2 NPs in mice livers at 14 days showing depletion of glycoproteinan (white arrows) infiltration of inflammatory cells and hepatic architecture with binucleated hepatocytes (black arrows) hemorrhage (an escape of blood from a ruptured blood vessel) and infiltration of lymphocytes (grey arrows) marginated chromatin in some nuclei (black circle) (hematoxylin and eosin stain, 400×)

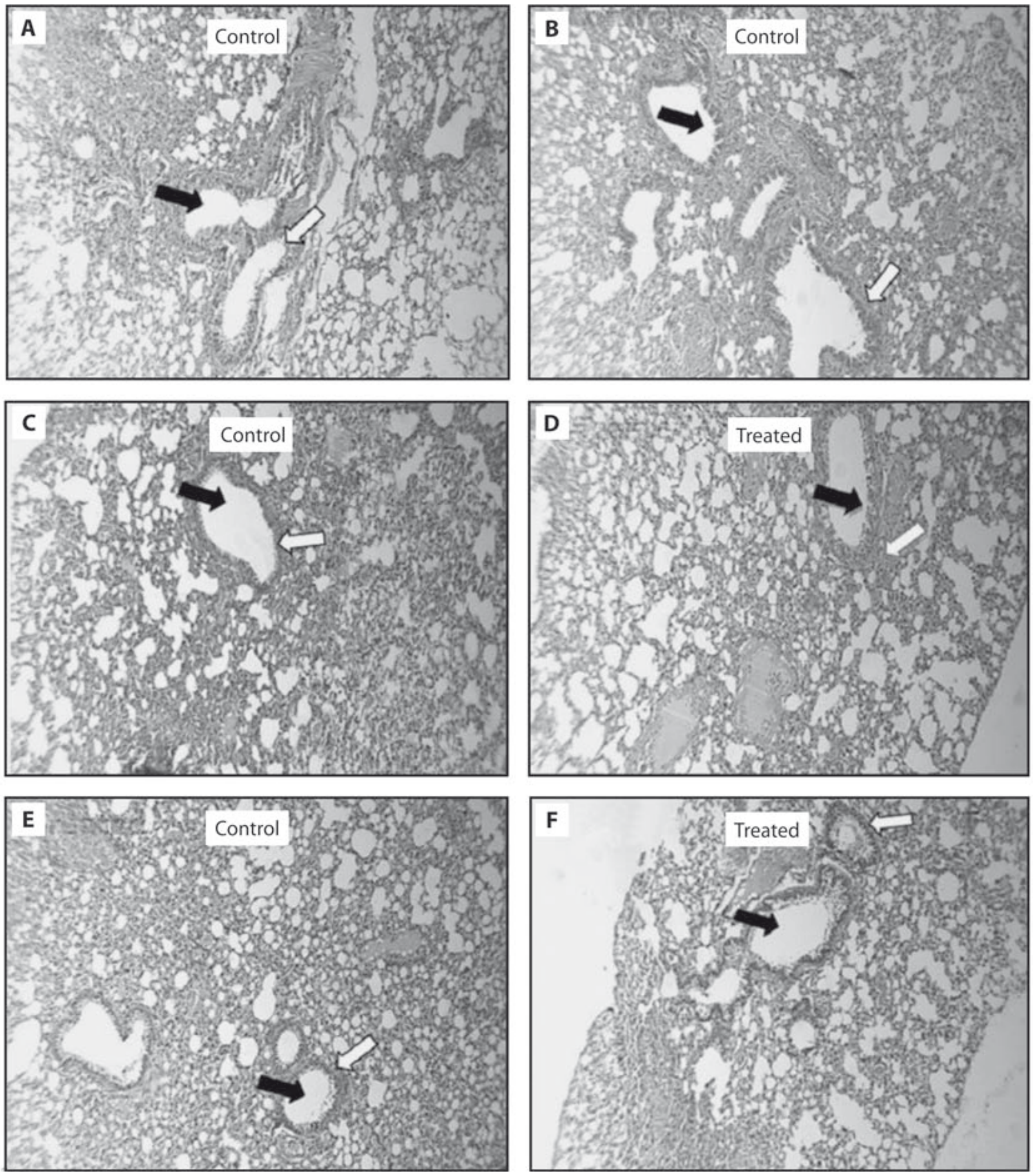

Fig. 4. Histological images of lungs from three animals feeding on wheat grains untreated and treated with hydrophilic SiO_2 NPs after 28 days. A, B and C – lung sections from control mice with normal lung histology of alveolar septa (black arrows) and bronchiolar (B) epithelium (white arrows). D, E and F – cross sections at a dose of 0.4 gm · kg^{-1}. Hydrophilic SiO_2 NPs in mice livers at 14 days showing no differences between treated and untreated animals. Mice had normal lung histology of alveolar septa (black arrows) and bronchiolar (B) epithelium (white arrows) (hematoxylin and eosin stain, 400x)

Conclusions

Silica and zinc oxide (NPs) have a good potential to be used as stored seed protectant alternatives if applied with proper safety precautions. Results also suggest the need for a complete risk assessment of any new engineered nanoparticles before being introduced to the consumer.

Acknowledgements

Authors are grateful to Alroaa Laboratory for rearing mice and sections photography.

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