Insecticidal efficacy of silica nanoparticles against *Rhyzopertha dominica* F. and *Tribolium confusum* Jacquelin du Val

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Abstract: Bioassays were conducted to assess the effects of two silicon dioxide nanoparticles of Aerosil® and Nanosav against adults of *Rhyzopertha dominica* F. and *Tribolium confusum* Jacquelin du Val. Silica nanoparticles were applied at the rates of 50, 100, 200 and 300 mg · kg^{-1} on wheat and peeled barley. The mortality was counted after 1, 2, 3, and 7 days of exposure. Another experiment was carried out to evaluate the effect of food source on the survival of beetles after exposure to silica nanoparticles. Adults were exposed to silica nanoparticles at the rate of 0.2 mg · cm^{-2} for 1 and 2 days on filter paper inside plastic Petri dishes, respectively. After exposure, the initial mortality was counted and live individuals of both species were held for a week in empty glass vials or vials containing wheat and wheat flour, respectively. Silica nanoparticles have high toxicity on *R. dominica* and *T. confusum* adults. *Rhyzopertha dominica* was more susceptible than *T. confusum*. However, the mortality of both species increased with increasing concentrations and time exposed to each concentration. At low concentrations, Aerosil® was more effective than Nanosav. Silica nanoparticles were more effective in wheat grains than barley. Results indicated that the initial mortality was so high that the impact of food source on delay mortality was unclear in most cases. Silica nanoparticles were efficient against tested species and can be used effectively in a stored grain integrated pest management program.

Key words: Aerosil®, barley, Nanosav, pest control, protection, wheat

Introduction

Wheat (*Triticum aestivum* L.) is an important crop used for human food and livestock feed. Therefore, much effort should be made towards sustainable production of wheat and increase its quality by reducing the use of chemical pesticides (Shewry 2009). After wheat, rice and maize are of prime importance. Barley (*Hordeum vulgare* L.) ranks fourth in world cereal crop production after wheat, maize and rice. Barley is used for animal feed, malts and human food (Akar *et al.* 2004).

Insect pests of cereals cause damages and weight loss of grains. *Rhyzopertha dominica* Fab., a lesser grain borer, is one of several serious pests of stored grains and other foodstuffs worldwide. It is known as a primary pest of stored grain because it eats the grains especially the germinal region, causing economic loss (Hill 2002; Kłys 2006; Shafighi *et al.* 2014).

Tribolium confusum Jacquelin du Val, confused flour beetle, is also one of the most important pests in flour mills and causes damage to commercial grain products, oilseeds, nuts, dried fruits, spices, pulses, beans, cacao, cottonseed, and forest products. They are known as secondary pests that feed on broken kernels, seed embryos, and grain dust (Mahroof and Hagstrum 2012).

Diatomaceous earth (DE) has long been used for protection of stored products; from pest infestations. Diatomaceous earths have low mammalian toxicity, high stability and provide long lasting protection (Fields 1998). They do not affect grain end-use quality and do not break down rapidly. According to the physical mode of action of DEs, it is possible that physiological resistance of insects to DEs has not occurred (Fields and Korunic 2000). Desmarchelier and Dines (1987) reported that 98% of DE Dryacide can be removed from wheat grains by commercial cleaning processes and milling. Trace amounts of DE particles do not pose any risks to consumer health and safety (Subramanyam and Roesli 2000).

The efficacy of DEs depends on different properties. DEs with smaller particle sizes are more toxic than the larger ones. Besides particle size, other properties influenced DEs insecticidal efficiency, such as active surface and oil adsorption capacity, SiO_2 content, moisture content, etc. (Korunic 1997; Mohitazar *et al.* 2009; Vayias *et al.* 2009; Ziaee and Moharramipour 2012). The main chemical composition of silica nanoparticles and DEs is silica which may cause their common properties. However, DEs are of micron particle size which seems to reduce their insecticidal effect more than the silica nanoparticles (Debnath *et al.* 2011).

During recent years, the tendency to apply nanoparticles for pest control has increased. Surface-functionalized silica nanoparticles were found to be highly toxic against *Sitophilus oryzae* (L.) adults (Debnath *et al.* 2011). Silica nanoparticles indicated high toxicity on mosquitos, in-

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cluding *Anopheles stephensi* Liston, *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* Say (Barik *et al*. 2012).

The aims of the current study were 1) to evaluate the insecticidal efficacy of two nanosilica against *R. dominica* and *T. confusum* adults on wheat and barley, 2) to assess initial and delay mortality of adults after a post-treatment period with or without food.

Materials and Methods

Insects

The *R. dominica* and *T. confusum* adults used in the experiment were reared on wheat (var. Chamran) and wheat flour plus 5% brewer's yeast (by weight), respectively. The insects were kept at 27±1°C and 65±5% relative humidity (RH) in continuous darkness. Adults used in the experiments were 7–14 days old of mixed sex.

Commodity

Wheat variety Chamran and peeled barley variety Jonoob were purchased from Safiabad Agricultural Research Center of Dezful and used in the experiments. Clean uninfested grains were stored at –24°C for at least 2 days. Before the experiments, kernels were kept for a week in incubators set at 27±1°C and 55±5% RH to achieve the moisture content (m.c.) related to environmental RH. The moisture content of the grains was measured by milling, and then drying 10 g of wheat or barley in a ventilated oven set at 110°C. The m.c. of wheat and barley was 11.5 and 10.8, respectively. For *T. confusum,* whole plus cracked grains at a ratio of 9 : 1 were used for the experiments.

Nanosilica

Two different silicon dioxide nanoparticles were applied in the experiments. Silicon dioxide nanoparticles of Aerosil® were purchased from Evonik Degussa GmbH Company with mean particle sizes of 12 nm. The X-ray diffraction (XRD) analysis indicated SiO_2 > 99%, Ti < 120 ppm, Ca < 70 ppm, Na < 50 ppm and Fe < 20 ppm. Scanning (SEM) and transmission electron microscopy (TEM) images of Aerosil® are presented in Figure 1. The XRD analysis, SEM and TEM images of Aerosil® were obtained from the company.

Iranian silicon dioxide nanoparticles (code: 20201) were purchased from Nanosav Company with mean particle sizes of 20–30 nm. Nanosav particles composed of SiO_2 > 98%, loss on ignition < 2%, 0.328% Na content as Na_2O, 0.393% Ca content as CaO, 0.294% Fe content as Fe_2O_3, and 0.185% sulfate content as SO_3. Transmission electron microscopy images of Nanosav Silicon dioxide nanoparticles indicated particles less than 30 nm (Fig. 2). The XRD analysis and TEM images of Nanosav were obtained from the company.

Insecticidal efficacy on wheat and barley

The effectiveness of Aerosil® and Nanosav nanoparticles was evaluated against *R. dominica* and *T. confusum* adults. Twenty grams of wheat or peeled barley grains were poured into glass vials. The grains were treated with 50, 100, 200 and 300 mg · kg^{-1} of silica nanoparticles with four replications, and the control group (without nanoparticles) was used for monitoring. The vials were shaken for 5 min to achieve equal distribution in the entire grain mass. Then, 20 adults of each species were added into each vial. The vials were covered with muslin cloth for sufficient ventilation. The vials were placed in an incubator set at 27±1°C, 55±5% RH and continuous darkness. The mortality was counted after 1, 2, 3 and 7 days of exposure. Insects were considered dead when no leg or antenna movements were observed after prodding with a fine brush.

Insecticidal efficacy after post-treatment period with food

The method of the experiment was the same as Arthur 2000; Ziaee and Khashaveh 2007 with some modifications. Plastic Petri dishes with an internal radius of 8.8 cm and an area of 62 cm^2 served as the exposure arena. Silica nanoparticles were applied at the rate of 0.2 mg · cm^{-2}, therefore, the rate for the area of the Petri dish was 12.4 mg. Filter papers were placed inside Petri dishes and treated with silica nanoparticles.

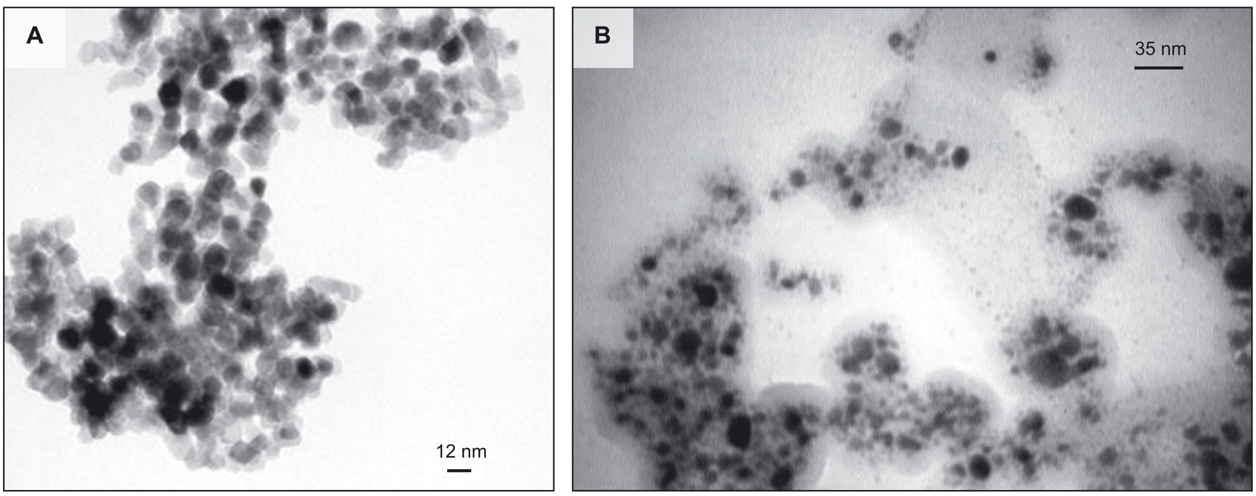

Fig. 1. Scanning (A) and transmission (B) microscopic images of Aerosil® silicon dioxide nanoparticles

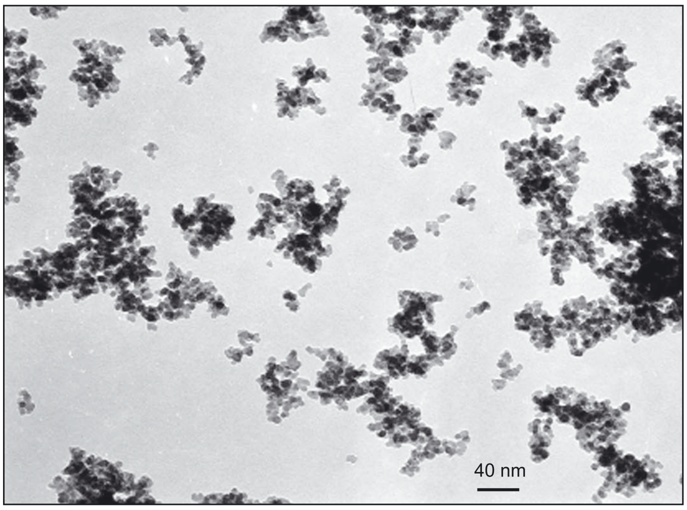

Fig. 2. Transmission microscopic images of Nanosav silicon dioxide nanoparticles

The dishes were shaken for a minute to distribute the particles which due to static electricity tend to stick to the filter paper. The Petri dishes were left undisturbed for at least 1 min to settle the particles on the filter paper. For each silica formulation, 20 unsexed adults of each species were placed on separate Petri dishes and the dishes were covered with lids. There were six treated replications. Untreated Petri dishes served as the control. The Petri dishes were placed in an incubator set at 27±1°C, 55±5% RH and continuous darkness. The initial mortality was counted after one day of exposure to silica nanoparticles for *R. dominica,* and after 1 and 2 days of exposure in the case of *T. confusum*. Subsequently, live *R. dominica* and *T. confusum* were held for a week in glass vials containing 20 mg whole wheat and wheat flour, respectively under the same conditions. The moisture content of wheat and wheat flour was measured with the method described above and were 11.5 and 13.1% m.c., respectively. After one week, the beetles were classified as live or dead and then discarded.

Insecticidal efficacy after post-treatment period without food

The conditions of the experiment were the same as above; except that *R. dominica* and *T. confusum* adults were transferred to empty glass vials after 1 and 2 days of exposure, respectively and kept for one week without food. After this 1-week holding period, the insects were removed from the vials and the number of live and dead individuals was assessed as above.

Data analysis

Control mortality was zero and no corrections were necessary. Mortality percentages were transformed into square root of arcsine to normalize the data, but non-transformed data are presented in the tables. The data were analyzed by using analysis of variance (ANOVA) and means were separated by using the Tukey-Kramer (HSD) test at $p < 0.05$ using SPSS software version 16. To estimate lethal concentrations (LC_{50}), data were subjected to Probit analysis (Finney 1971); using SPSS software version 16.0 (SPSS 2007).

Results and Discussion

Mortality of the exposed *R. dominica* after 1 day of exposure was higher on wheat than on barley. However, a noticeable increase in mortality was recorded 24 h later. On wheat treated with Aerosil®, there were no significant differences between different concentrations; and a high level of mortality was observed even at the rate of 50 mg · kg^{-1}. For Nanosav 50 mg · kg^{-1} was not sufficient to cause satisfactory results. Despite the high levels of mortality on wheat (95%), 50 mg · kg^{-1} had significant differences with three concentrations tested after 7 days of exposure (Table 1). The mortality of *T. confusum* adults increased with increasing concentrations and time exposed to each concentration. On barley, 24 h after the introduction of the beetles, the mortality was very low. However, the mortality was 100% after 7 days of exposure to 200 mg · kg^{-1} of both tested nanosilica (Table 2). Based on the results of the present study, silica nanoparticles have high toxicity on *R. dominica* and *T. confusum* adults. At low concentrations, Aerosil® was more effective than Nanosav.

Sabbour (2013) found that adults of *S. oryzae* were susceptible to silica gel Cab-O-Sil-750 and silica gel Cab--O-Sil-500 nanoparticles and caused significant reduction of the number of eggs laid per female. They reported that silica nanoparticles protected rice seeds from beetle infestation for 120 days during storage. In the same trend, silica nanoparticles sized 20–30 nm, were effective on *S. oryzae* adults (Debnath *et al.* 2011). They noted that silica nanoparticles can be applied to protect plant crops in fields; because they do not have adverse effects on plant growth, but also enhance structural rigidity and strength of plants. Silica nanoparticles have a physical mode of action and act like DEs. The particles absorb the insect wax layer, causing death through desiccation and to a lesser degree by abrasion (Ebeling 1971). Athanassiou and Ka-

Table 1. Mean mortality (%±SE) of *Rhyzopertha dominica* adults, exposed on wheat and barley treated with Aerosil® and Nanosav nanosilica at four concentrations

Exposure time [d]	Nanosilica/Concentration [mg · kg⁻¹]								$F_{7,24}$, p-value
	Aerosil®				Nanosav				
	50	100	200	300	50	100	200	300	
Wheat									
1	71±5.1 bcAB	82±4.3 bA	89±2.3 bcA	90±4.0 abA	25±3.5 dC	54±5.1 bcB	70±3.5 cAB	82±5.9 bA	24.7, 0.00
2	87±6.6 abA	94±4.7 abA	97±2.5 abA	99±1.2 aA	50±2.0 cB	87±3.2 aA	91±4.2 abA	97±2.5 aA	18.0, 0.00
3	94±4.7 abA	99±1.2 abA	100±0 aA	100±0 aA	69±2.3 bB	95±2.9 aA	94±2.4 abA	100±0 aA	20.0, 0.00
7	99±1.2 aAB	100±0 aA	100±0 aA	100±0 aA	95±2.0 aB	100±0 aA	100±0 aA	100±0 aA	4.3, 0.00
Barley									
1	16±5.5 eCD	54±5.5 cB	82±4.7 cA	86±5.1 bA	0±0 eD	31±6.2 cBC	82±5.2 bcA	89±3.1 abA	53.2, 0.00
2	36±6.5 deC	85±4.5 abA	97±1.4 abA	99±1.2 aA	31±4.2 dC	64±8.5 bB	97±1.4 aA	100±0 aA	41.5, 0.00
3	56±6.5 cdB	97±1.4 abA	100±0 aA	100±0 aA	50±4.5 cB	90±5.7 aA	99±1.2 aA	100±0 aA	34.8, 0.00
7	86±4.7 abB	100±0 aA	100±0 aA	100±0 aA	77±3.2 bB	100±0 aA	100±0 aA	100±0 aA	18.4, 0.00
$F_{7,24}$, p-value	30.0, 0.00	20.5, 0.00	9.3. 0.00	5.0, 0.00	99.3, 0.00	26.8, 0.00	13.2, 0.00	7.0, 0.00	

Means followed by the same lowercase letter in each column and uppercase letter within each row are not significantly different using Turkey's test at $p < 0.05$

Table 2. Mean mortality (%±SE) of *Tribolium confusum* adults, exposed on wheat and barley treated with Aerosil® and Nanosav nanosilica at four concentrations

Exposure time [d]	Nanosilica/Concentration [mg · kg⁻¹]								$F_{7,24}$, p-value
	Aerosil®				Nanosav				
	50	100	200	300	50	100	200	300	
Wheat									
1	21±6.5 deCD	67±5.9 bB	85±4.5 bcAB	96±3.7 abA	2±1.4 fD	7.5±1.4 eD	24±5.9 cCD	34±5.5 bC	56.2, 0.00
2	55±2.0 bB	99±1.2 aA	100±0 aA	100±0 aA	25±2.0 cdC	47.5±6.6 cB	57±5.9 bB	97±1.4 aA	78.2, 0.00
3	85±5.7 aA	100±0 aA	100±0 aA	100±0 aA	57±6.6 bB	85±2.0 abA	91±4.2 aA	100±0 aA	17.3, 0.00
7	94±3.7 aA	100±0 aA	100±0 aA	100±0 aA	97±2.5 aA	100±0 aA	100±0 aA	100±0 aA	2.0, 0.09
Barley									
1	1.2±1.2 eC	15±5.4 cB	15±2.0 dB	44±3.7 cA	1.2±1.2 fC	8.7±2.3 eBC	17±3.2 cB	21±1.2 cB	21.5, 0.00
2	16±2.3 deD	52±3.2 bC	75±4.5 cB	89±3.1 bAB	10±2.0 efD	26±2.3 dD	87±5.9 aAB	96±3.7 aA	92.8, 0.00
3	30±5.4 cdBC	90±4.5 aA	95±2.0 abA	97±2.5 abA	21±1.2 deC	40±2.0 cdB	95±2.8 aA	100±0 aA	125, 0.00
7	47±4.3 bcC	99±1.2 aA	100±0 aA	100±0 aA	37±3.2 cC	84±4.7 bB	100±0 aA	100±0 aA	101.8, 0.00
$F_{7,24}$, p-value	57.9, 0.00	77.4, 0.00	136.8, 0.00	67.9, 0.00	114.8, 0.00	117.4, 0.00	65.7, 0.00	182.8, 0.00	

Means followed by the same lowercase letter in each column and uppercase letter within each row are not significantly different using Turkey's test at $p < 0.05$

vallieratos (2005) reported that 1,500 mg · kg^{-1} of PyriSec® (an enhanced DE) cause 95 and 80.5% mortality of *R. dominica* on wheat and peeled barley after 7 days of exposure, respectively. However, according to our results, 100% mortality of *R. dominica* was observed on wheat and barley treated with 100 mg · kg^{-1} tested silica nanoparticles, after 7 days of exposure. The high insecticidal potential of silica nanoparticles could be attributed to the SiO_2 content and nanometer size range of the particles which increases the ratio of the surface area to volume. High surface-to-volume ratio increased insect contact with particles leading to more cuticle desiccation and death.

In all tests, *R. dominica* adults were more susceptible than *T. confusum.* However, the mortality of both species increased with increasing concentrations and time exposed to each concentration. *Rhyzopertha dominica* was reported to be more susceptible than *T. confusum* when exposed to maize treated with three different DE formulations (Insecto®, PyriSec® and Protect-It®) (Athanassiou *et al.* 2007). Similar results have been reported by Athanassiou and Korunic (2007). They stated that *Cryptolestes ferrugineus* (Stephens) is considered to be the most susceptible species to DEA (an abamectin-enhanced DE) and DEBBM (a bitterbarkomycin-enhanced DE) formulations of DEs was followed by *S. oryzae*, *R. dominica* and *Tribolium castaneum* Herbst.

Lethal concentrations of Aerosil® and Nanosav nanosilica which caused 50% mortality on *R. dominica* and *T. confusum* are presented in Table 3. Based on LC_{50} values, the effectiveness of silica nanoparticles was more in wheat grains than peeled barley. This agrees with previous studies which evaluated the influence of grain type on the toxicity of diatomaceous earth formulations against insect pests of different stored products (Athanassiou and Kavallieratos 2005; Athanassiou *et al.* 2008; Ziaee 2015). According to Athanassiou and Kavallieratos (2005) the efficacy of PyriSec® DE formulation was low on peeled barley compared with other tested grains. They assumed that the removal of the seed coat reduced the degree of DE adherence to the grain kernels. Hence, the possibility of the insects contacting DE particles is reduced leading to decreased DE effectiveness.

The toxicity of Aerosil® nanosilica was more than Nanosav one day after exposure (Table 3). It should be noted that the presence of a food source after the post-treatment period to Nanosav silica nanoparticles reduced insect mortality. The mortality of *T. confusum* adults when exposed to Aerosil® and Nanosav nanosilica for 2 days was about 70 and 20%, respectively. However, mortality increased after the 1-week holding period even with a food source. For Nanosav, 65% mortality was recorded when exposed to food for one week; however, complete mortality was recorded when no food was provided for the beetles (Table 4). In the case of Nanosav, the insects may compensate their water losses in one week period on food and replenish the lost protective waxy layer (Ziaee and Khashaveh 2007). However, for Aerosil®, a high level of mortality was observed in the first and second days of *R. dominica* and *T. confusum* treatment, respectively. As a result, the effect of a food source on insect survival was not significant after one week.

Table 3. LC_{50} values of Aerosil® and Nanosav nanosilica applied against *Rhyzopertha dominica* and *Tribolium confusum* after 1 day of exposure

Species	Nanosilica	Commodity	LC_{50} [ppm]	Cl [ppm]		Slope	χ^2	p-value
				lower	upper			
R. dominica	Aerosil®	wheat	12.1	0.52	29.3	0.96	0.29	0.86
		barley	102.0	88.04	116.3	2.75	3.27	0.19
	Nanosav	wheat	101.5	82.9	120.5	1.98	0.92	0.62
		barley	136.6	70.1	222.7	4.44	7.23	0.37
T. confusum	Aerosil®	wheat	82.7	71.5	93.8	3.13	3.27	0.19
		barley	413.0	–	–	2.13	8.76	0.02
	Nanosav	wheat	469.0	346.9	830.2	2.05	0.21	0.90
		barley	854.6	491.7	3656.6	1.59	1.32	0.51

Cl – Confidence limit (95%); "–" – cannot be calculated

Table 4. Initial and delay mean mortality (%±SE) of *Rhyzopertha dominica* and *Tribolium confusum* adults after post-treatment period with or without food

On food for 1 week	Nanosilica	*R. dominica*		*T. confusum*		
		1 day	1 week	1 day	2 days	1 week
Yes	Aerosil®	90.0±3.6 a	97.5±1.1 a	9.1±4.5 a	70.8±2.7 a	90.0±2.8 a
	Nanosav	77.5±2.8 b	92.5±1.7 b	3.3±1.6 a	20.0±4.6 b	65.0±4.0 b
No	Aerosil®	91.6±3.3 a	100±0 a	5.8±3.0 a	73.3±3.8 a	97.5±2.5 a
	Nanosav	83.3±2.1 ab	100±0 a	0.83±0.8 a	20.0±3.6 b	100±0 a
$F_{3,21}$, p-value		4.57, 0.01	12, 0.00	1.52, 0.23	63.7, 0.00	32.7, 0.00

Means followed by the same letter in each column are not significantly different using Turkey's test at $p < 0.05$

Athanassiou *et al.* (2008) declared that the type of food source influenced both the developmental rate and progeny production and also the insecticidal efficacy of DEs against stored-product pests. Therefore, silos and warehouses should be clean and without cracks or crevices to prevent insects having access to food.

Barik *et al.* (2012) using hydrophobic nanosilica against different mosquito species, found larvicidal and pupicidal activity of silica nanoparticles. They demonstrated that nanosilica could be applied in mosquito vector control. Debnath *et al.* (2012) studied the in vitro cellular toxicity of silica nanoparticles in human fibroblast cell lines and acute oral toxicity in mice. They declared that the nanosized form is relatively non-toxic. However, further studies are required to confirm the non-toxicity of nanosilica.

Conclusions

It can be concluded that silica nanoparticles could be applied for protection of stored grains at low concentrations. However, additional experiments are required to clarify silica nanoparticles properties, their potential toxicity on different insect species, in various commodities, and different environmental conditions.

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