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Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Synthesis and entomotoxicity assay of zinc and silica nanoparticles against *Sitophilus granarius* (Coleoptera: Curculionidae)

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Vol. 59, No. 1: 26–31, 2019

DOI: 10.24425/jppr.2019.126033

Received: Septermber 1, 2018
Accepted: January 28, 2019

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Abstract

The granary weevil, *Sitophilus granarius* (L.), is one of the most important internal feeders of stored grain. Nanotechnology has become one of the most promising new approaches for pest control in recent years. In our screening program, laboratory trials were conducted to determine the effectiveness of silica nanoparticles (SNPs) and zinc nanoparticles (ZNPs) against the larval stage and adults of *S. granarius* on stored wheat. Nanoparticles of silica and zinc were synthesized through a solvothermal method. They were then used to prepare insecticidal solutions of different concentrations and tested on *S. granarius*. Silica nanoparticles (SNPs) were found to be highly effective against *S. granarius* causing 100% mortality after 2 weeks. ZNPs were moderately effective against this pest.

Keywords: insecticidal, nanoparticles, silica, *Sitophilus granarius*, zinc

Introduction

The granary weevil, *Sitophilus granarius* (L.), also known as the grain weevil, occurs all over the world. It is a small (2.5–4 mm), reddish brown to dark brown beetle infesting stored grain, mainly wheat grain (Nowaczyk *et al.* 2008). The granary weevil is most often found wherever grain and wheat products are stored, as they are the main sources of food for both larvae and adults. If there is a large population, they can cause a great deal of damage. Fumigants and residual insecticides are commonly used for protection of stored grains against pest infestation. Unfortunately, this leads to contamination of food with toxic pesticide residues (Shojaaddini *et al.* 2008; Debnath *et al.* 2011). Moreover, the main problem in controlling pests in stored grain is their resistance to pesticides (Lorini *et al.* 2006). Regarding the resistance of grain pests and pesticide residues, it seems that chemical control is not an appropriate approach for controlling the population of these pests. Therefore, in recent decades researchers have investigated alternative strategies to eliminate this problem, such as the use of plant extracts, diatomaceous earths (DEs) and nanoparticles (NPs) (Chanbang *et al.* 2007; Liu *et al.* 2010; Debnath *et al.* 2011; Shafighi *et al.* 2014; Ziaee and Ganji 2016).

Diatomaceous earth is an inert dust that has been extensively tested for control of stored-grain insect pests (Subramanyam and Roesli 2000; Shafighi *et al.* 2014; Sabbour and Abd-El-Aziz 2015). Diatomaceous earths is processed of amorphous silica from fossilized phytoplankton and the insecticidal effect of DE referred to silica content (Fields and Korunic 2000;

Mewis and Ulrichs 2001). In several recent tests DE formulations have been successfully evaluated against several stored-product pest species (Arthur and Throne 2003; Chanbang *et al.* 2007; Iatrou *et al.* 2010; Sabbour and Abd-El-Aziz 2015). Diatomaceous earths becomes more effective against insects if it possesses a high amorphous silica content with uniform size distribution (Korunik 1997; Debnath *et al.* 2011). Decreasing the size of materials to nanometer scale causes prominent mutation of their properties. Debnath *et al.* (2011) described a number of physical properties of materials which change as their size approaches nanoscale. Nanoparticles represent a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental cleanup problems (Chinnamuthu and Murugesa Boopathi 2009). Nanoparticles show promise in different fields of agricultural biotechnology (Majumder *et al.* 2007; Rahman *et al.* 2009). Nanoparticles help 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 *Tribolium castaneum* Herbst. Stadler *et al.* (2010) showed that nano alumina can be successfully used to control stored grain pests.

In this work, we studied the mortality effect of silica (SNPs) and zinc (ZNPs) nanoparticles on *S. granarius*. We synthesized nanoparticles of SNPs and ZNPs by using a solvothermal method. Then we investigated the insecticidal effect of synthesized SNP and ZNP on *S. granarius*.

Materials and Methods

Synthesis of zinc nanoparticles

A sodium hydroxide solution was stirred into a solution of $Zn(AC)_2 \cdot 2H_2O$ in $EtOH/H_2O$ solvent at room temperature. After 30 min of stirring, the mixture was transferred into Teflon lined stainless steel autoclaves, sealed, and maintained at 150°C for 12 h. Subsequently, the reactor was immediately cooled down to −15°C. The resulting gray solid products were centrifuged, washed with distilled water and ethanol to remove the ions possibly remaining in the final products, and air dried at 60°C.

Synthesis of silica nanoparticles

Nanosilica was synthesized by acid hydrolysis of sodium silicate using dilute hydrochloric acid as suggested by Kotoky and Dolui (2004). A 15% sodium silicate solution was prepared with 1% polyvinyl alcohol solution. Then, 0.5 N HCl was stirred into it slowly at 60°C. The pH of the mixture was maintained between 1 and 2. The solution was stirred at 60°C for 30 min to carry out acid hydrolysis of sodium silicate. The sol-gel mixture was then washed well to remove all the sodium chloride formed. It was dried at 50°C and then muffled at 600°C.

Insects

Larvae and adults of *S. granarius* were tested. Insects were reared at 30 ± 1°C and 65 ± 5% relative humidity (RH) in the dark on wheat. Insects were obtained from cultures maintained in the laboratory for at least 3 years, with no history of exposure to insecticides. Adults less than 2 weeks old and one-day old first instar larvae were used for the experiments.

Bioassay

Effects of the SNPs and ZNPs on one-day old first instar larvae and adults of *S. granarius* were determined by contact toxicity assay at three dose rates: 1, 2 and 3 g nanoparticles $\cdot$ kg^{-1} wheat. For this purpose, the bioassay was performed in small plastic screw capped jars. Twenty grams of wheat was placed in each jar. Wheat in each jar was treated individually with nanoparticles. Then, the jars were shaken manually for approximately 1 min to achieve equal distribution of nanoparticles on the wheat. The jars were kept for 24 h before 20 unsexed adults and first instar larvae of *S. granarius* were introduced into each jar. All bioassays were performed at 30 ± 1°C and 65 ± 5% RH. Insect mortality was checked after 1, 2, 4, 7, and 14 days for adults and after 48 h for the larvae stage.

Statistical analysis

The morphology and size of samples were characterized by Scanning Electron Microscope (SEM) (Philips XL 30) with gold coating. The mortality data were analyzed with SPSS 16 software followed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests to compare effects between treatments. The results were expressed as means (± SE) of untransformed data and considered significantly different at $p < 0.05$.

Results

Structural study of nanoparticles

The morphology, structure and size of the samples were investigated by Scanning Electron Microscopy (SEM). Figures 1 and 2 indicate that the original morphology of the SNPs and ZNPs was approximately spherical with diameters of 25 and 34 nm, respectively.

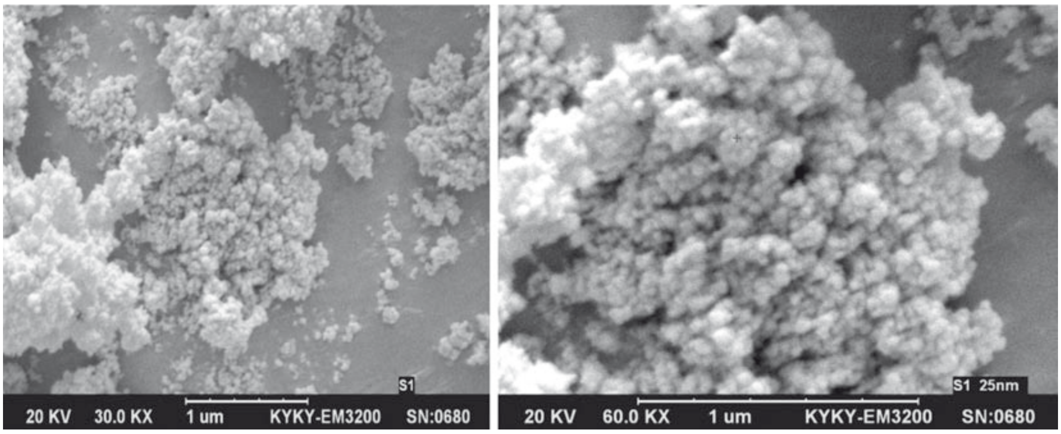

Fig. 1. Scanning Electron Microscopy (SEM) images of synthesized silica nanoparticles in different zoom

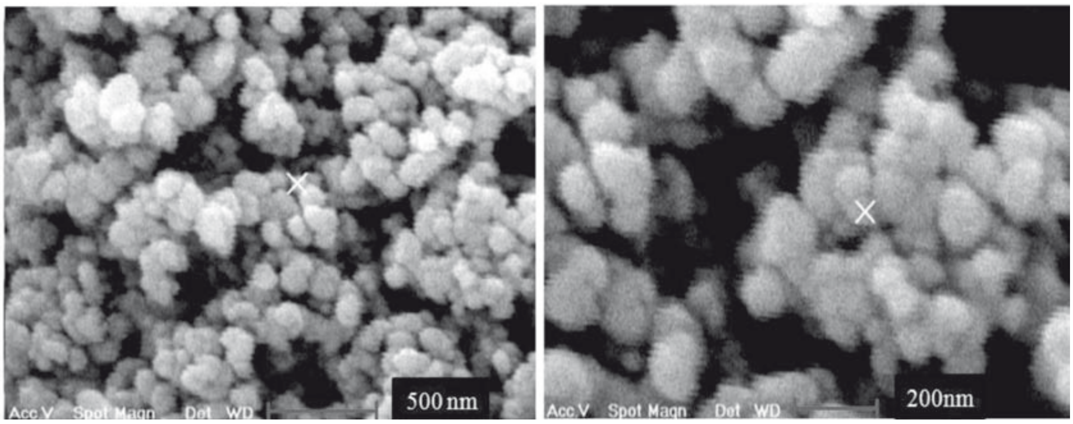

Fig. 2. Scanning Electron Microscopy (SEM) images of synthesized zinc nanoparticles in different zoom

Contact toxicity bioassay

The analysis results of variance showed that the effect of SNPs on adults on day 1 ($F_{3,8}$ = 44.79, p = 0.00), day 2 ($F_{3,8}$ = 53.04, p = 0.00), day 4 ($F_{3,8}$ = 130.30, p = 0.00), day 7 ($F_{3,8}$ = 104.71, p = 0.00) and day 14 ($F_{3,8}$ = 335.42, p = 0.00) , and on larvae ($F_{3,16}$ = 173.93, p = 0.00) had a significant difference at 1% level (Table 1). The effect of ZNPs on adults on day 2 $F_{3,8}$ = 14.25, p = 0.01), day 4 ($F_{3,8}$ = 14.13, p = 0.01), day 7 ($F_{3,8}$ = 58.00, p = 0.00) and day 14 ($F_{3,8}$ = 64.33, p = 0.00), and on larvae ($F_{3,16}$ = 352.25, p = 0.00) had a significant difference at 1% level but did not have a significant difference on day 1 ($F_{3,8}$ = 0.66, p = 0.59) (Table 1). In all the tested NPs, mortality of larvae and adults increased with increased NPs concentrations. In other words, the mortality of *S. granarius* increased as a function of the NPs concentrations.

Table 2 shows that on day 1, SNPs were not at all effective on the adult insects. More than 50% mortality was obtained with SNPs when the dosage rate was 2 g · kg^{-1} after 2 and 4 days of exposure, respectively.

Table 1. Means (± SE) of the effect of NPs on *Sitophilus granarius* adults and larvae

NPs		Days				
		1	2	4	7	14
Adults	SNPs	44.79 ± 0.00	53.04 ± 0.33	130.30 ± 0.33	104.71 ± 0.88	335.42 ± 0.33
	ZNPs	0.66 ± 0.59	14.25 ± 0.88	14.13 ± 0.57	58.00 ± 0.92	64.33 ± 0.00
Larva	SNPs	173.93 ± 0.00	–	–	–	–
	ZNPs	352.25 ± 0.00	–	–	–	–

NPs – nanoparticles; SNPs – silica nanoparticles; ZNPs – zinc nanoparticles

Table 2. Mortality (mean ± SE) of *Sitophilus granarius* adults treated with silica nanoparticles

Concentration [g · kg^{-1}]	Days				
	1	2	4	7	14
1	44.79 ± 0.00 b	5.66 ± 0.33 b	8.66 ± 0.33 c	12.33 ± 0.88 c	16.33 ± 0.33 b
2	4.66 ± 0.33 b	7.33 ± 0.88 b	13.00 ± 0.57 b	15.33 ± 0.92 b	20.00 ± 0.00 a
3	7.66 ± 0.88 a	11.33 ± 0.88 a	16.66 ± 0.88 a	20.00 ± 0.00 a	20.00 ± 0.00 a
0	0.00 ± 0.00 c	0.00 ± 0.00 c	1.33 ± 0.33 d	2.00 ± 0.00 d	3.00 ± 0.57 c

The same letters in a column indicate the lack of significant difference at 5%

As shown in Table 2, application of SNPs at 3 g · kg^{-1} could kill more than 80 and 100% of the insects after 4 and 7 days of exposure, respectively. Nearly 77% of the insects were killed when the wheat was treated with SNPs at 2 g · kg^{-1} after 7 days. Also, these results showed that SNPs at 2 g · kg^{-1} could kill all insects after 14 days.

At 1 g · kg^{-1} ZNPs did not have a mortality effect on the adult insects after 2 days and did not show 50% mortality at 1 and 2 g · kg^{-1} even after 14 days. Almost 60% of the insects died when ZNPs were applied at a dose of 3 g · kg^{-1} after 14 days of exposure (Table 3).

Application of NPs on *S. granarius* showed that ZNPs on larvae instar were more effective than on adults. More than 60 and 70% larvae mortality was found at 3 g · kg^{-1} after 48 h for ZNPs and SNPs, respectively (Table 4). There was a significant difference between dose and mortality, and the greatest larvae mortality was found at the highest dose.

Discussion

In this study, the potential use of ZNPs and SNPs for control of *S. granarius* was investigated. Usually larger doses of insecticide inflict the highest mortality on pests. In this research, insect mortality increased significantly with increased NPs concentrations. In our results SNPs had excellent performance in the control of *S. granarius*.

Application of silica (as diatomaceous earth) has been increasingly used as a stored grain protectant (Golob 1997). The efficacy of commercial formulations of diatomaceous earth, particularly those based on silica, has been documented for a number of insect species on various stored grains. Fundamental studies were conducted on the contact toxicity of NPs which showed that the mortality effect of nanoparticles is more than bulky scales (Stadler *et al.* 2010; Debnath *et al.* 2011). But the use of nanomaterials in agriculture is still at a rudimentary stage (Debnath *et al.* 2011). Little research has been carried out to investigate the toxicity effect of nanoparticles on stored-grain insect pests. In our studies insect mortality increased significantly with increased NPs concentrations of both SNPs and ZNPs as pesticides on the larval stage and adults, indicating excellent performance of these NPs for control of *S. granarius*. These findings are consistent with other reports (Yang *et al.* 2009; Stadler *et al.* 2010; Debnath *et al.* 2010, 2011; Ziaee and Ganji 2016).

Table 3. Mortality (mean ± SE) of *Sitophilus granarius* adults treated with zinc nanoparticles

Concentration [g · kg^{-1}]	Days				
	1	2	4	7	14
1	0.00 ± 0.00 b	0.00 ± 0.00 b	1.66 ± 0.33 c	5.00 ± 0.57 c	7.66 ± 0.33 b
2	0.33 ± 0.33 a	2.00 ± 0.57 b	3.33 ± 0.66 b	6.33 ± 0.33 b	9.33 ± 0.33 a
3	0.33 ± 0.33 a	2.33 ± 0.33 a	5.66 ± 0.66 a	8.00 ± 0.00 a	12.00 ± 0.57 a
0	0.00 ± 0.00 c	0.00 ± 0.00 c	1.33 ± 0.33 d	2.00 ± 0.00 d	3.00 ± 0.57 c

The same letters in a column indicate the lack of significant difference at 5%

Table 4. Mortality (mean ± SE) of *Sitophilus granarius* larva treated with silica and zinc nanoparticles

Concentration [g · kg^{-1}]	Mortality	
	SNPs	ZNPs
1	7.40 ± 0.40 c	6.60 ± 0.24 c
2	10.40 ± 0.50 b	11.60 ± 0.24 b
3	12.20 ± 0.37 a	14.20 ± 0.48 a
0	0.40 ± 0.24 d	0.40 ± 0.24 d

SNPs – silica nanoparticles; ZNPs – zinc nanoparticles
The same letters in a column indicate the lack of significant difference at 5%

In addition, our study demonstrated that with time the application of SNPs could significantly increase the mortality effect of NPs, indicating that SNP has 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 and 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 (Ziaee and Ganji 2016). Stadler *et al.* (2010) demonstrated an insecticidal effect of alumina nanoparticles on *Sitophilus oryzae* and *Rhyzopertha dominica*. 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 SNPs 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).

Our study showed that the entomotoxicity effect of ZNPs is not salient, and it is less than SNPs effect on *S. granaries*. A literature review showed that no records were available that related to the application of ZNPs against stored-grain pests. But Samih *et al.* (2011) evaluated the effect of NPs (as ZNPs) and nano-amitraz on *Agonoscena pistaciae* and reported that the mortality effect of nano-amitraz is more than ZNPs.

Conclusions

Motivated by the fact that little is known regarding the effects of NPs on stored-grain pests, we evaluated the insecticidal activity of silica nanoparticles and zinc nanoparticles against *S. granaries*. In our study, SNPs and ZNPs were synthesized by a solvothermal method. Our results suggest the possibility of using SNPs nanoparticles to eradicate stored-grain pests. While the environmental effects of using SNPs and ZNPs as insecticides needs further study, one clear advantage of using them as insecticides is the low risk of developing resistance by the stored-grain insects with long term use.

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