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Insecticidal effect of silica and silver nanoparticles on the cowpea seed beetle, *Callosobruchus maculatus* F. (Col.: Bruchidae)

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Abstract

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 (SNP) and silver nanoparticles (AgNP) on larval stage and adults of *Callosobruchus maculatus* on cowpea seed. Nanoparticles of silica and silver were synthesized through a solvothermal method and different concentrations (1, 1.5, 2 and 2.5 g kg^{-1}) of them were tested on *C. maculatus*. In the experiments, the LC_{50} value for SiO_2 and Ag nanoparticles were calculated 0.68 and 2.06 g kg^{-1} cowpeas on adults and 1.03 and 1.00 g kg^{-1} on larvae, respectively. Result showed that, the both nanoparticles (silica and silver) were highly effective on adults and larvae with 100% and 83% mortality, respectively. The result also showed that SiO_2 nanoparticles can be used as a valuable tool in pest management programs of *C. maculatus*.

Key words: *Callosobruchus maculatus*, nanosilver, nanosilica, storage pest

Introduction

The cowpea seed beetle, *Callosobruchus maculatus* F. (Col: Bruchidae), is the most important storage pest of cowpea throughout the tropics. The control of stored grain pests stands mostly on broad action insecticides and fumigants. Unfortunately, this leads to contamination of food with toxic pesticide residues (Debnath *et al.,* 2011). Moreover, the main problem in controlling pests in stored grain is the resistance to pesticides. 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.

One alternative is the use of Diatomaceous Earths (DEs), composed mainly of amorphous silica and derived from fossilized phyto-plankton (Subramanyam & Roesli, 2000; Mewis & Ul-richs, 2001). DE becomes more effective against insects if it possesses high silica content with uniform size distribution (Korunik, 1997) and a number of physical properties of materials change as their size approach nanoscale (Debnath *et al.,* 2011).

Nanoparticles represent a new generation of environmental remediation technologies that could provide cost-effective solution to some of the most challenging environmental clean-up problems (Chinnamuthu & Murugesa Boopathi, 2009). Nanoparticles help to produce new pesticides, insecticides and insect repellants (Owolade *et al.,* 2008). Also, researchers believe that nanotechnology will revolutionize agriculture including pest management in the near future (Bhattacharyya *et al.,* 2010). Although there have been numerous studies on the toxicity effects of nanoparticles on bacteria, fungi, and animal pathogens (Bragg & Rannie, 1974; Feng *et al*., 2000; Samuel & Guggenbichler, 2004; Elchiguerra *et al*., 2005; Reddy *et al*., 2007). Little research has

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been carried out to investigate the toxicity effect of nanoparticles on insects specially storage pest. Wan *et al.* (2005) studied the effect of action of mixture of two nanoparticles with two insecticides to the pest mite (*Epitrimerus pyri* (Nalepa)). According to the authors cypermethrin and alpha Terhienyl mixed with nano-particled zinc oxide and copper oxide was effective on the tested mite. Yang *et al.* (2009) expressed that nanoparticles loaded with garlic essential oil is efficacious against *Tribolium castaneum* (Herbst). Stadler *et al.* (2010) showed that nano alumina could be successfully used to control stored grain pests.

These cues led us to investigate the entomotoxicity of silica and silver nanoparticles against *C. maculatus*.

Material and Methods

Synthesis of silica and silver nanoparticles

To synthesis the silica and silver nanoparticles, Mathew & Narayanankutty (2010) and Aslani *et al.,* (2003) methods with some modification were used, respectively. Nanosilica was synthesized by acid hydrolysis of sodium silicate using dilute hydrochloric acid 15%. Sodium silicate solution was prepared by 1% polyvinyl alcohol solution. Then 0.5 NHCl, was added to it slowly with stirring at the temperature of 60^{o}C. The pH of the mixture was maintained between 1and 2. The solution was stirred at 60^{o}C for 30 minute 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^{o}C and then muffled at 600^{o}C.

For synthesis nanosilver, sodium hydroxide solution (4 M, 10 ml) was added to a solution of $AgNO_3$ (2 mmol) in EtOH/H_2O solvent (25 ml). To investigate the role of surfactants on the size and morphology of nanoparticles, 0.5g of polyethylene glycol (PEG) was used in the reactions with optimized conditions. The obtained mixtures were sonicated for 150 min with ultrasound powers followed by centrifugation and separation of the solid and liquid phases. The morphology and size of samples were characterized by Scanning Electron Microscope[*].

Bioassay

Adults of *C. maculatus* were reared at 30±1^{o}C and 65±5% RH in the dark on cowpea seed. Insects were obtained from cultures maintained in the Kerman Agricultural Organization laboratory for at least three years, with no history of exposure to insecticides. Adults with less than 2 weeks old and first instar larvae (Negahban & Moharramipour, 2007) were used for the experiments.

Effects of the nanosilica and nanosilver on adults and larvaes of *C. maculatus* were determined by contact toxicity assay at three doses of 1, 1.5 and 2 g nanoparticle kg^{-1} cowpea seed. The experiments were carried out in Completely Randomized Design with five replications each consisted of 20 adults or larvae of *C. maculatus* in small plastic screw capped jars containing 20 g of cowpea seed. Seeds in each jar were treated individually with nanoparticles. Then, the jars were shaken manually for approximately 1 min to achieve equal distribution of nanoparticles on cowpea seed (Subramanyam & Roesli, 2000). In one additional set no nanoparticle was mixed with cowpea and this set served as control. After 24 h, 20 unmated adults of *C. maculatus* were introduced into each jar. All bioassays were performed at 30±1^{o}C and 65±5% RH. Insect mortality was checked after 1, 2, 4, 7 and 14 days for adults and 48 hours for larvae stages.

Statistical Analysis

The mortality data was analyzed with SPSS 16 software followed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests. The results were expressed as means (±SE) of untransformed data and considered significantly different at $P<0.05$. Probit analysis was conducted to estimate LC_{50} values with their fiducially limits by Probit analysis software.

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* Model Philips XL 30

Results

Structural study of nanoparticles

The shape and size of the nanoparticles prepared in this study were checked by Scanning Electron Microscope (SEM). Figure 1, indicates that the original morphology of the silica and silver nanoparticles are approximately spherical with the diameter varying between 20 to 60 nm.

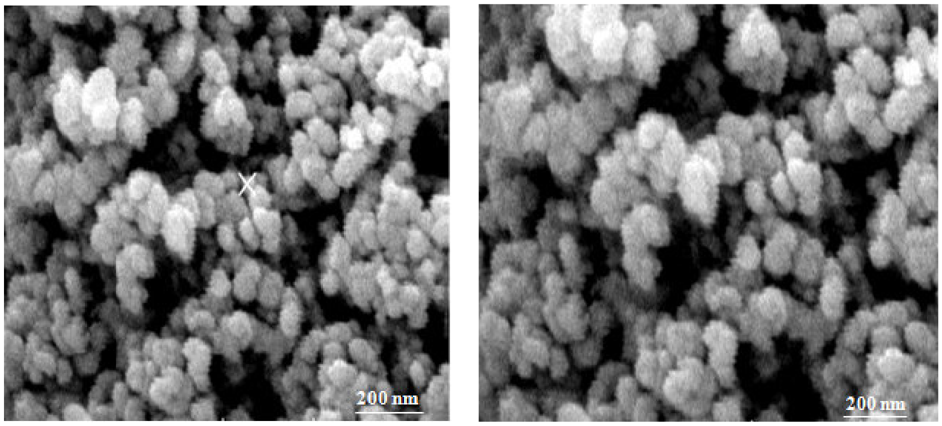

Fig. 1- The SEM images of synthesized Sio_2 (left) and Ag (right) nanoparticles

Contact toxicity bioassay

Mortality of adults treated with the nanoparticles started after 18 hours under the laboratory conditions. The presence of nanosilica and nanosilver resulted 100 and 75% mortality at the highest concentration of 2.5 g kg^{-1} on day 14, respectively (Table 1). The results showed that there was significant interaction effects between dates and treatments. Based on this finding the effect of treatments on different days were reported, separately. The analysis of variance showed that the effect of nanosilica on adults on first day ($F_{9,20}$ = 53.92, P = 0.0000), second day ($F_{9,20}$ = 123.68, P = 0.0000), fourth day ($F_{9,20}$ = 126.97, P = 0.0000), seventh day ($F_{9,20}$ = 119.51, P = 0.0000), fourteenth day ($F_{9,20}$ = 115.56, P = 0.0000), SiO_2 on larvae ($F_{4,20}$= 309.75, P = 0.0000) and Ag on larvae ($F_{4,20}$= 106.52, P = 0.0000) had a significant difference at 1% level. In all treatments using NP, percentage of mortality of adults and larvae (Tables 1 and 2) increased with increase in NP concentrations.

Table 1 shows that on the first day, nanosilica was not at all proper effect on adults. Since the mortality less than 50% is not efficient, the efficiency of nanosilica was observed on day 4 with concentration of 1.5 g kg^{-1}. At the dose of 2.5 g kg^{-1} more than 85% and 96% of beetles died in case of nanosilica after 4^{th} and 7^{th} days of exposure, respectively. Also these results showed that SNP at 2 and 2.5 g kg^{-1} could kill all insects after 14 days. The highest percent mortality of silver nanoparticles (51.66) was obtained on day 14 with concentration of 2.5 g kg^{-1}. However, at the similar concentration, nanosilica killed 100% of *C. maculatus* adults.

Application of NPs on larval stage showed higher killing effect of AgNP in all rates of applications (Table 2). Both nanoparticles had the highest mortalities at concentration of 2.5 g kg^{-1}.

Table 1- Percent mortality (mean ± SE) of *C. maculatus* adults treated with silica and silver nanoparticles

Concentration (g kg^{-1})	Nanoparticles	Mortality (%)				
		Day				
		1	2	4	7	14
1	SiO_2	11.65±3.33^{d}	28.3±4.40^{c}	41.6±1.66^{c}	61.6±6.00^{c}	78.3±3.33^{c}
	Ag	1.65±1.66^{e}	3.3±1.66^{eg}	8.3±1.66^{e}	23.3±1.66^{g}	33.3±3.33^{c}
1.5	SiO_2	23.3±1.66^{c}	33.3±4.40^{c}	58.3±4.40^{b}	75.0±5.00^{b}	91.6±4.40^{c}
	Ag	3.3±1.66^{e}	6.65±1.66^{deg}	13.3±3.33^{e}	28.3±1.66^{g}	43.3±1.66^{c}
2	SiO_2	33.3±3.33^{b}	63.3±1.66^{b}	80±2.88^{a}	93.00±3.33^{a}	100.0±0.00^{c}
	Ag	3.3±1.66^{e}	8.3±1.66^{de}	28.3±3.33^{d}	41.6±1.66^{e}	55.0±5.00^{c}
2.5	SiO_2	43.33±3.33^{a}	71.6±1.66^{a}	85.0±0.00^{a}	96.0±1.66^{a}	100.0±0.00^{c}
	Ag	5.0±0.00^{a}	11.6±1.66^{d}	40.6±2.88^{c}	51.6±1.66^{d}	75.0±2.88^{c}
0	SiO_2 - Ag	0.0±0.00^{e}	0.0±0.00^{g}	6.6±1.66^{e}	10.0±10.00^{h}	15.0±2.88^{c}

* Means followed by same letter in column are not significantly different at 5% level

Table 2- Percentage mortality (mean ± SE) of *C. maculatus* larvae treated with silica and silver nanoparticles

Concentration (g kg^{-1} of cowpea)	Mortality (%)	
	SNP	AgNP
1	37.00±2.00^{c}	33.00±1.22^{c}
1.5	52.00±2.54^{b}	58.00±1.22^{b}
2	61.00±1.87^{a}	71.00±2.44^{a}
2.5	65.00±2.23^{a}	73.00±2.00^{a}
0	2.00±1.22^{d}	2.00±1.22^{d}

* Means followed by same letter in column are not significantly different at 5% level

The LC_{50} value for SiO_2 and Ag nanoparticles on larvae and adults of *C. maculatus* is shown in Table 3 and 4. According to these values, adults of *C. maculatus* were more sensitive to SiO_2 than Ag nanoparticles. However, there was not any significant difference between LC_{50} of Ag and SiO_2 nanoparticles. These results indicated that LC_{50} decreased with increasing in NP concentrations (Table 1 and 3). However the mortality increased with increasing NP concentrations. Due to the highest mortality in superlative concentration LC_{50} was compared after 14 days and 48 hours for adults and larvae, respectively.

The LC_{50} values for SiO_2 and Ag nanoparticles on adults and larvae after 14 days and 48 hours were estimated 0.68, 1.03, 2.06 and 1.00 g kg^{-1} cowpea, respectively (Table 4). The LC_{50} value comparison using the LC_{50} ratio (0.328) and their lower and upper 95% confidence limits (0.211-0.511) showed that there was a significant difference between LC_{50} value for SiO_2 (0.68 g kg^{-1}) and this value for Ag nanoparticles (2.06 g kg^{-1}) on adults.

Comparision between SiO_2 and Ag nanoparticles on larvae, the LC_{50} ratio (1.023) with their lower and upper 95% confidence limits (0.0803-1.303) showed that there was no significant difference between the LC_{50} value for SiO_2 (1.03 g kg^{-1}) and Ag nanoparticles (1.00 g kg^{-1}). For the all nanoparticles the statistic t ratio was >1.96, the g factor was <0.5 and the heterogeneity factor was <1.

Because of no significant difference between the LC_{50} value for SiO_2 and Ag nanoparticles on larvae, silica nanoparticles could be more appropriate to control *C. maculatus*.

Table 3- LC_{50} (g kg^{-1} Seed weight) value of silica and silver nanoparticles against adults of *C. maculatus*

Nanoparticles	Day				
	1	2	4	7	14
SNP	2.91	1.67	1.27	0.92	0.68
AgNP	84.12	15.00	2.95	2.80	2.06

Table 4- LC_{50} (g kg^{-1} Seed weight) value of silica and silver nanoparticles against larvae after 48 hours and adults of *C. maculatus* after 14 days

Nanoparticles	Stage	LC_{50}	Slop (± SE)	Limits 95%	Chi square (χ^2)
SNP	Adults	0.68	3.91 ± 0.97	0.30 - 0.89	0.49
	Larvae	1.03	2.82 ± 0.54	0.94 – 1.11	1.73
AgNP	Adults	2.06	2.70 ± 0.74	1.80 – 2.50	0.24
	Larvae	1.00	4.90 ± 0.67	0.76 – 1.17	0.91

Dose–response gradient was used to evaluate the relationship between pesticide and mortality. The presence of a dose-response gradient is one of the key criteria for determining the effectiveness of toxicity is causal (Robertson & Rappaport, 1979). According to Fig. 1 gradient of mortality in higher doses was more than low doses in all treatments. The dosage-response gradient for SiO_2 and Ag on adults and larvae were estimated 3.91, 2.70, 2.93 and 4.90 based on calculated LC_{50}, respectively. These gradients also demonstrates that SiO_2 (3.91) is more effective on adults than Ag (4.90) is more effective on larvae than SiO_2 (Fig. 1).

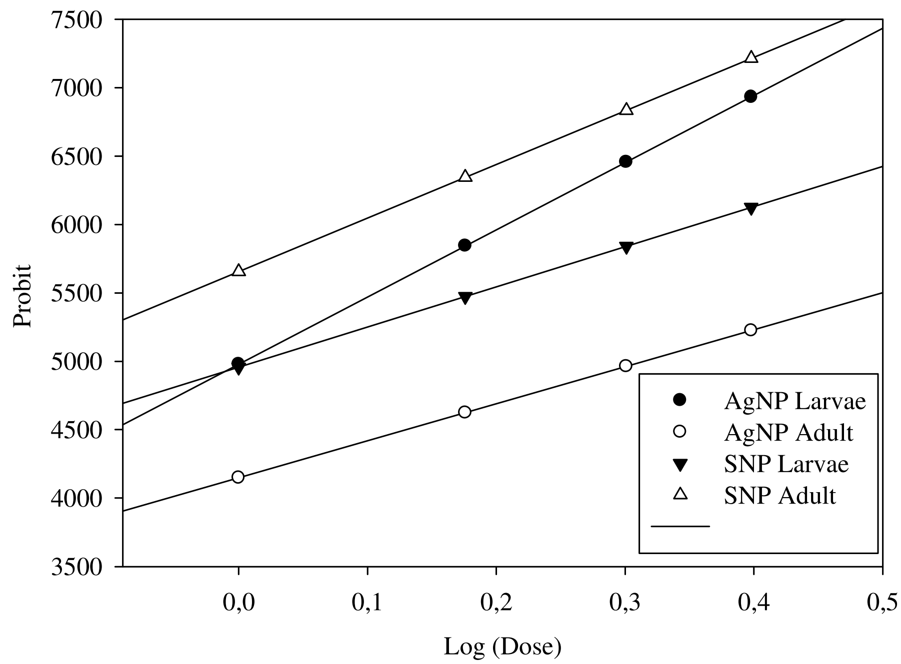

Fig. 1- Dose-response gradient of silica and silver nanoparticles against *C. maculatus*

Discussion

Management of stored-grain pests stands traditionally on use of synthetic insecticides and long term application of these chemicals develops resistance to pesticides. In recent years, nanoparticles have received much attention for controlling of pathogens in agriculture (Eleka *et al.,* 2010; Guan *et al.,* 2008; Sang Woo *et al.,* 2009). The use of nanomaterials in agriculture is still at a rudimentary stage. Stadler *et al.* (2010) applied successfully nano alumina against two stored grain pests. However, Yang & Watts (2005) showed that nano alumina in ground water inhibits the growth of carrot, cabbage, cucumber, corn, and soybean. This study showed that NPs could be applied to facilitate pest control management (IPM) of stored grain pests as *C. maculatus.* Comparison of our results with earlier investigations (Debnath *et al.,* 2011) demonstrates that application of SNP could significantly increase mortality effect of NPs with increasing the time after application. They reveal that SNP has a high potential as pesticide. This may be one of the possible reasons for which there is an age-old tradition of using silica dust as protective agent for stored seeds by different ethnic races all over the world (Ebeling, 1971). On the basis of our results, mortality effect of silica was far more effective on adults than larvae, which this mortality could be attributed to the impairment of the digestive tract (Smith, 1969) or to surface enlargement of the integument as a consequence of dehydration or blockage of spiracles and tracheas. Also it refers to their enormously increased exposed surfaces which could interact with the insect cuticle. Damage occurs to the insects' protective wax coat on the cuticle, both by sorption and abrasion.

It is well obvious from our results that nano silver is not effective on adults; this may be due to cuticle but it shows effective entomotoxic potential against larval stage. This reaction could refer to various toxicity mechanisms of Ag, which have been reported. Examples of such mechanisms include generation of reactive oxygen Types, oxidative stress, membrane disruption, protein unfolding, and/or inflammation (Bragg & Rannie, 1974; Feng *et al*., 2000; Samuel & Guggenbichler, 2004; Elchiguerra *et al*., 2005; Reddy *et al*., 2007; Meng *et al*., 2009; Donaldson *et al*., 2009).

Insect mortality effect of SiO_2 nanoparticles was slightly more than that of Ag nanoparticles on adults. These results also showed that Ag nanoparticles was more than that of SiO_2 nanoparticles on larvae but there was no significant difference between LC_{50} of Ag and SiO_2 against larvae. According to these findings, it is well obvious from our results that Sio_2 could be selected as a good agent to control this pest. This should be done whether the nanoparticles can act as a good method of control and bring stored grain pests populations under control following EIL or not. Many researchers have studied the effect of nanoparticles, especially that of against pathogens (Debnath *et al.,* 2011; Feng *et al.,* 2000; Reddy *et al.,* 2007). Our finding is consistent with other researchers' reports (Debnath *et al.,* 2011; Stadler *et al.* 2010; Rouhani *et al.* 2011). According to Debnath *et al.* (2011), the mortality of *Sitophilus oryzae* (Linnaeus) was significantly affected by hydrophilic and hydrophobic of Sio_2. Finding of Stadler *et al.* (2010) is also consistent with our results. They demonstrated insecticidal effect of alumina nanoparticles on *S. oryzae* and *Rhyzopertha dominica* (Fabricius). In addition, Rouhani *et al.* (2011) reported that ZnO-TiO_2-Ag nanoparticles has insecticidal activity on *Frankliniella occidentalis* (Pergande) and showed the most mortality effect pertained to 28% ZnO-70% TiO_2-2% Ag (LC_{50}=195.27 mg/L).

For the practical use of SiO_2 nanoparticles and test compounds as novel pesticide to proceed, further research is required on the safety issues of these materials for human health. Other areas requiring attention are its mode of action and development of formulations to improve potency and stability, as well as to reduce cost.

Conclusion

Motivated by the fact that little is known regarding the effects of NPs on stored-grain pests. The present study demonstrated strong toxicity of the tested NPs, nanosilica and nanosilver to *C. maculatus*. Our findings suggest that the use of NPs cause a reduction in density of *C. maculatus*, but among these, nanosilica have more toxic effects than nanosilver on adults and nanosilver is more effective on larvae stage. The NPs can be removed by conventional milling process unlike sprayable formulations of conventional pesticides, leaving residues on the stored grain (Debnath *et al.,* 2011). Therefore, SNP and AgNP have an excellent potential as stored grain as well as seed protecting agent if applied with proper safety measures.

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اثر حشره‌کشی نانو ذرات سیلیکا و نقره روی سوسک چهار نقطه‌ای حبوبات
Callosobruchus maculatus **F. (Col: Bruchidae)**

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چکیده

در سال‌های اخیر نانو تکنولوژی به یکی از امیدبخش‌ترین راه‌های کنترل آفات نزدیک شده است. در این پژوهش، اثر نانو ذرات سیلیکا و نقره روی لارو و حشرات کامل *Callosobruchus maculatus* روی توده لوبیا چشم بلبلی بررسی شد. نانو ذرات سیلیکا و نقره به روش سلووترمال تهیه و در پنج غلظت ٠، ١، ١/٥، ٢ و ٢/٥ گرم بر کیلوگرم لوبیای آلوده به *C. maculatus* بررسی شد. در این پژوهش LC_{50} محاسبه شده برای نانو ذرات سیلیکا و نقره به‌ترتیب ٠/٦٨، ٢/٠٦ روی حشرات کامل و ١/٠٣ و ١/٠٠ گرم بر کیلوگرم روی لاروها بود. نتایج نشان داد که نانو ذرات سیلیکا با ١٠٠ درصد کشندگی دارای اثر کشندگی بیشتر روی حشرات کامل و نیز نانو ذرات نقره با کشندگی ٨٣ درصد لاروها موثر بودند. همچنین نتایج نشان دادند که نانو سیلیکا می‌تواند گزینه مناسبی برای مبارزه با این آفت باشد.

واژه‌های کلیدی: آفت انباری، نانو سیلیکا، نانو نقره، *Callosobruchus maculates*

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