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Comparative insecticidal activity of nano and coarse silica on the Chinese beetle *Callosobruchus Chinensis* (L) (Coleoptera: Bruchidae)

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ABSTRACT

The insecticidal activity of two types of silica (normal or coarse [CS] [150mm] and nano-silica particles [NSPs] [35 nm]) was determined using *Callosobruchus chinensis* (L) as the test insect. When the unsexed adults were exposed to broad bean seeds admixed with different concentrations of both tested silica (0.5, 1.0 and 2.0 g/100 g seeds) for different periods (48, 96, 144 and 192 hrs), it was found that NPS had more insecticidal activity against the bruchid beetle C. *chinensis.* Moreover, it was found that as the exposure time and concentration increased, the mortality of the adults increased. For each concentration of the tested silica with different exposure time, the LT_{50} of the bioassay tests showed that the calculated LT_{50} values of NSPs (83.44, 72.23 and 67.52 hrs) were less than those of CS (140.00, 131.44 and 120.15 hrs at the three tested concentrations of 0.5, 1.0 and 2.0 g/100g seeds, respectively). Therefore NSPs are more biologically active than CS against *C. Chinensis*. The adults survived treatment were left in the same bioassay jars to reproduce the first generation (F_1) to determine the delayed effect of such tested concentration of both silica types. The results showed that there were no emerged adult insects at the concentrations of 1.0 and 2.0 g NSPs /100 g broad bean seeds. The numbers of the emerged insects after treatment with coarse silica was decreased as the concentration increased giving a range of emerged adults of 2.3-10.0 as compared with untreated check (control), which produced 24.7 adults. The pattern of picking-up silica particles of both tested silica types was also illustrated showing more pick-up of particles in case of using NSPs concentrations. Nano-silica particles (NSPs) were found to be effective against the bruchid beetle C. *chinensis* and could be effectively used in the Integrated Pest Management (IPM) program for C. *chinesis* and for other stored grain insect-pests.

Keyword: ***Callosobruchus Chinensis*, nano-silica particles, LT_{50}, Pick-up**

INTRODUCTION

Legumes (pulses) are important components in our daily diet as they considered being rich as a source of proteins, minerals and vitamins. They are excellent sources of proteins (20-40 %), carbohydrates (50 -60 %) and are fairly good sources of thiamine, niacin, calcium and iron. Because they are known for their high protein content of good quality, they are invaded by a series of beetles and these beetles can cause serious damage and seed weight loss. Infestation commonly begins in the field, where eggs are laid on mature pods (Singh, 1997; Nahdy *et al*., 1999). The pulse crops are attacked by more than 150 insect pests. Among the insects which infest various pulses are *Callosobruchus chinensis* (Linn.), *C maculatus* (Fab.) and *C. analis* (Fab.).

The cowpea seed beetle (Chinese beetle), *Callosobruchus maculatus* F. (Coleoptera: Bruchidae), is the most important storage insect-pest of cowpea throughout the tropics. Moreover, *C. chinensis* is a major insect-pest of chickpeas (Pandey and Singh, 1997), lentils, green gram, broad beans, soybean (Srinivasacharyulu and Yadav, 1997; Yongxue *et al*., 1998) adzuki bean and cowpeas in various tropical regions. The eggs are cemented to the surface of pulses and are smooth, domed structures with oval and flat bases. The larvae and pupae are normally only found in cells bored within the seeds of pulses (Chavan *et al*., 1997).

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). In addition, 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 (Taukder and Howse, 2000; Isman, 2006). Therefore, environmentally safe and convenient methods such as the use of inert dusts, plant extracts, oils, leaf powders and pressurized carbon dioxide and temperature management techniques (low and high temperature) are the growing interest to replace synthetic pesticides (Yuya *et al.,* 2009). There are a number of reviews on inert dusts (Korunic, 1998; Subramanyam and Roesli, 2000; Fields and Korunic,

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2002). The admixture of such desiccant dusts or ash (Chiranjeevi and Sudhakar, 1996; Mohamed, 1996) can be useful for controlling the Chinese bruchid, *Callosobruchus chinensis*. Also, plants insecticidal allelochemicals appear to be toxic to adults and also inhibit reproduction (Roger, 1997).

Nanotechnology has become one of the most promising new approaches for pest control in recent years. 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 and Boopathi, 2009). Silica nanoparticles (SNPs) have been evaluated against the cotton leafworm *Spodoptera littoralis* (El-bendary and El-Helaly, 2013), the tomato borer *Tuta absoluta*, the stored grain insect-pest [the rice moth] *Corcyra cephalonica* (Vani and Brindhaa, 2013), the pink bollworm *Pectinophora gossypiella* (Derbalah *et al*., 2014) and the lesser grain borer beetle *Rhyzopertha dominica* and the red flour beetle *Tribolium castaneum* (El-Samahy *et al*., 2014). The treatment of hydrophobic silica nanoparticles (SNPs) with the pulse seeds against the infestation of stored pulse beetle, *Callosobruchus maculatus* revealed a significant reduction in oviposition, adult emergence and seed damage potential with no effect on seeds germination, growth rate of root and shoot (Arumugam *et al.,* 2016). Therefore, the present investigation was conducted to determine the entomotoxicity (the insecticidal efficacy) of normal (coarse) particles of silica (CS) and nanoparticles (SNPs) against *C. Chinensis* and the delayed effect of both silica types on the number of emerged adults of the first generation post-parent exposure.

MATERIALS AND METHODS

1. Silica types

Two different silicon dioxide (nano particles [SNPs] and Micro or coarse particles [CS] were applied in the experiments. Silica nano-particles (SNPs), named also hydrophilic nano-silica was obtained from Nano Tech Egypt Co., Dream Land City, Wahat Road, 6th October, Egypt. The shape and size of the nanoparticles tested in this study were checked by Scanning Electron Microscope (SEM) at the EM Unit in the Faculty of Science, Alex. Univ. Photo 1 indicates that the original morphology of the silica nano particles is approximately spherical with the diameter mean of 35 mm.

Physical properties of silica particles

The following physical properties were determined: pH and adsorption capacity.

pH

pH of both types of silica have been determined where 5% aqueous solution was prepared for each type of silica and pH was measured with a calibrated pH meter.

Water adsorption capacity of silica

For the water absorption test, the specimens of silica are dried in an oven for 24 hours at 70°C and then placed in a desiccator to cool. Immediately upon cooling the specimens are weighed. Each sample is then tittered with water at lab temperature (25°C) until equilibrium (forming a pasty texture).

Water adsorption is expressed as increase in weight percentage:

Water Adsorption% = [(Wet weight - Dry weight)/ Dry weight] x 100

It could be seen that there were differences in the rate of water adsorbed by the two types of silica. NSPs adsorbed greater weight of water/g of silica dust.

Table 1 lists some physical properties of the tested two types of silica that have been used for toxicity determination.

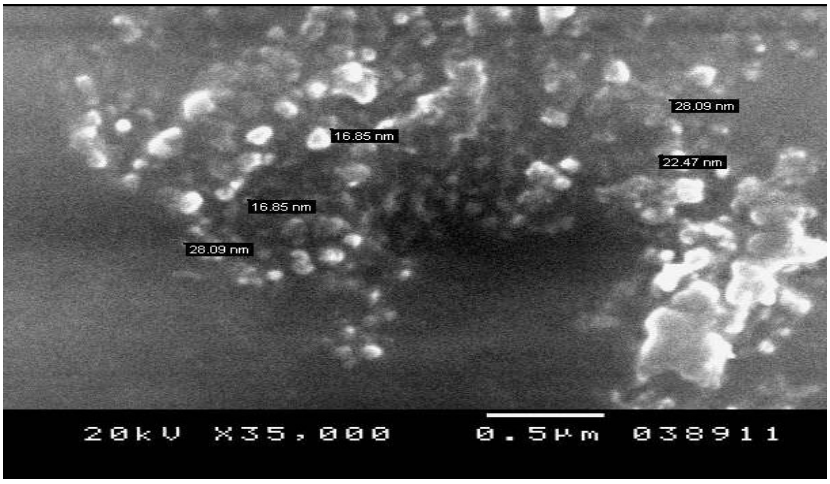


Photo 1. The SEM image of SiO_2 nanoparticles (NSPs)

Table 1. Some physical characteristics of the tested types of silica

Silica type	pH	Mean Particles size	Adsorptio n rate (%)
NSPs (Nano)	11.8	35 nm	40
CS (normal)	11.8	150 mm	25

Insect

The Chinese bruchid, *Callosobruchus chinensis* was obtained from a stock culture maintained in the laboratory of Plant Prtection Dept., Faculty of Agric. (Saba Basha), Alex. Univ. The pure culture of *C. chinensis* was raised on Egyptian broad bean seeds and maintained under controlled conditions of 28 ±2°C and 70±5% R.H. All glass ware used for culture preparation including the culture jars (2 liters) were heat sterilized in an oven at 130±3°C for two hours. *C. chinensis* newly emerged adults (about 200) were introduced into the culture medium. Cuture jars were sealed properly with a piece of muslin fixed tightly with the aid of rubber bands. Age is an important factor determining the susceptibility of insect to insecticides (Sigh, 1981). In the present work, the unsexed adults of *C. chinensis* used in all experiments were 2-4 days old.

Efficacy of the tested silica types against *C. chinensis*

The efficacy of two types of silica (NSPs and CS) was tested against *C. chinensis* to obtain LT_{50} values for each tested concentration (0.5, 1.0 and 2.0 g/100 g seeds) of each silica type. This was carried out by admixing silica with whole seeds of broad bean at different dust concentrations and then, the insects were introduced. The admixing bioassay technique was followed as tose methods described by Singh (1981) and Tayeb (1988). Modification of their methods as described below was used for the admixture tests.

Broad bean samples (100g) were weighed into 1 lb jam jars. The required silica concentrations (0.5, 1.0 and 2.0 g/100 g seeds) were added to broad bean samples. Each jar was covered with a piece of Clingfilm® and a metal lid and tilted up and dowen for 5 seconds (6 times) and hand rotated for 2 min. with shaking at the following time intervals: 10, 15, 30, 69, 90 and 120 s. After admixing, the jars were kept closed until the dust inside thye jar is settled. Then, *C. chinensis* (20 unsexed adults) were introduced into each jar containg dust and incubated at 28 ±2°C and 70±5% R.H. Mortality counts were made after different exposure times of 48, 96, 144 and 192 hrs. Insects were classified as dead or alive. All tests were done in three replicates. Three broad bean samples (100g each and contain 20 adults) with no dust were used as control. For each concentration of both types of silica, the relationship between mortality and the different exposure times was determined and expressed as LT_{50} values (the time needed for killing 50% of the tested insects at a specific concentration with different exposure times). Also, the slope and the regression coefficient were calculated.

The delayed effect of the tested types of silica

The delayed effect of both types of silica was expressed as the mean number of the emerged adults (first generation F1) post-parents' exposure. The insects survived silica types treatments were left over in their bioassay jars till the emergence of the first generation adults. The adults emerged from each jar containing a specific concentration was monitored for 2 months, where the daily emerged adults were counted and kept away during that period.

RESULTS AND DISCUSSION

The insecticidal activity of the tested silica types

The efficiency of both silica nano particles and coarse silica were assessed against the Chinese bean beetle under laboratory conditions of 28 ±2°C and 70±5% R.H. The results shown in Table (2) cleared the effect of nano-silica on the mortality percentages of C. *Chinese*. When the unsexed adults were exposed to broad bean seeds treated with different concentrations of both tested silica types (0.5, 1.0 and 2.0 g/100 g seeds) for different periods (48, 96, 144 and 192 hrs), it was found that NPS pronounced more insecticidal activity against the bruchid beetle C. *chinesis.*

Moreover, it was found that as the exposure time and concentration increased, the mortality of the adults increased. For each concentration of the tested silica types with different exposure time, the LT_{50} of the bioassay tests showed that the calculated LT_{50} values of NSPs (83.44, 72.23 and 67.52 hrs) were less than those of CS (140.00, 131.44 and 120.15 hrs), therefore NSPs are more biologically active and showed more entomotoxcity than CS did against *C. Chinesis*.

For each concentration of the tested silica types with different exposure time, the LT_{50} of the bioassay tests showed that the calculated LT_{50} values of NSPs (83.44, 72.23 and 67.52 hrs) were less than those of CS (140.00, 131.44 and 120.15 hrs), therefore NSPs are more biologically active and showed more entomotoxcity than CS did against *C. Chinesis*. It could be concluded that the main cause of insect death is the dehydration caused by nano-silica particles that affect or absorb the wax layer of insect cuticle and subsequently lead to loss of water from the body of the insect, and ultimately the death of insects due to drought and that was more or less similar to that description by Ebeling (1971) and Golob (1997).

Table 2. Insecticidal activity of the tested nano-silica particles (NSPs) and coarse silica (CS) against *C. Chinensis* adults

Exposure Time (h)	Mortality percentages of exposed adults to two types of silica					
	Coarse silica (CS) (150 mm)			Nano silica (NSPs) (35 nm)		
	Tested Concentration			Tested Concentration		
	0.5	1.0	2.0	0.5	1.0	2.0
48	8.5	10.0	15	30.0	35	36.5
96	21.5	38.5	43.5	68.5	71.5	73.5
144	76.5	78.5	95.0	100.0	100.0	100.0
192	100.0	100.0	100.0	100.0	100.0	100.0
LT_{50} (hours)	140.00	131.44	120.15	83.44	72.23	67.52
b (slope)	7.83	6.89	5.86	5.26	3.15	3.01
r^2*	0.95	0.90	0.89	0.96	0.83	0.82

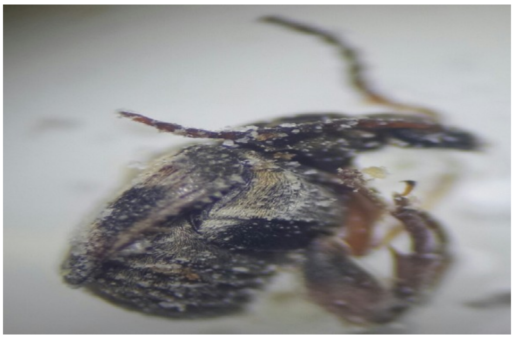
* r^2 = regression coefficient

Mortality effect of nano silica (NSPs) was far more effective on adults and this mortality could be attributed to the impairment of the digestive tract 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.

The pattern of pick-up of silica was cleared in Photo2. The nano particles were more distributed over the insect body affecting more surface area and increasing the effects caused by the drying activity of the insect treated with silica. The effect on the wax layer increased, thus increasing the loss of water and thus insect dehydration which eventually led to death.

(A) Dorsal view

(A) Ventral view



(B)

(C)

Photo 2. Pick-up of both tested types of silica dusts by the pulse beetle *C. chinensis* adult

(A) (Nano) NSPs pick-up (dorsal and ventral view)

(B) Normal or coarse silica (CS) pick-up (C) control (20X)

Table 3. The delayed effect of both tested types of silica expressed as the mean number of the emerged insects (first generation F_1) resulted from parent survived treatment

Silica type	No. of emerged adults (F_1) at different silica concentrations (g/100 g seeds)			
	0.5	**1.0**	**2.0**	**Control**
NSPs (Nano)	1.00 ±1.00	0.00 ±0.00	0.00 ±0.00	24.67 ± 6.03
CS (normal)	10.00 ± 2.65	5.33 ±2.52	2.33 ±1.53	

The results obtained in Table (3) show the effect of both nano- particles and coarse or normal silica on the number of emerged insects of the first generation post parents-treatment. The results showed that there was no egg laying or hatching could be detected for those adult insects exposed to the concentrations of 1.0 and 2.0 g NSPs / 100 g and that was reflected on the number of emerged adults showing no emerged adults. While numbers of the output insects after treatment with coarse silica was decreased as the concentration of dust increased. The lowest level of progeny was achieved with the higher concentration (2.0 g / 100g) which recorded a mean of 2.3 insects compared with check untreated (24.7 emerged insects).

These presented results are in agreement with those reported by Rouhani *et al.* (2012) who determined the effectiveness of silica nano particles (SNP) and silver nano particles (AgNP) on larval stage and adults of *Callosobruchus maculatuso* n cowpea seed. The LC_{50} value for SiO_2 and Ag nano particles were calculated by 0.68 and 2.06 g/kg cowpeas on adults and 1.03 and 1.00 g/kg on larvae, respectively. Result showed that both nano particles (silica and silver) were highly effective on adults and larvae with 100% and 83% mortality, respectively. The results also showed that SiO_2 nano particles can be used as a valuable tool in pest management programs of *C. maculatus.* Also, Sabbour and Abd El- Aziz (2015) evaluated the potential of Nano- Diatomaceous earth (Nano-DE) in comparison with natural Diatomaceous earth (DE) against the red flour beetle *T. castaneum* and the confused flour beetle, *T. confusum* under laboratory and stored conditions. They found that the percentages of larval mortality of tested insects increased as the treatment concentration increased. Larvae of *T. confusum* were more susceptible to the treatments than *T. castaneum* larvae. Nano-DE was more effective than natural-DE. The fecundity of tested insects was highly affected with both DE and nano DE.

CONCLUSION

Early theories suggested that inert dusts act by either blockage of the spiracles, adsorption of water from cuticle or ingestion. Now, it is generally accepted that inert dusts cause death by promoting cuticular water loss as a result of disruption of the protective lipid layer through the removal of cuticular waxes by either abrasion or adsorption depending upon the physical characteristics such as hardness and particle size which determine the amount adhering to the body of insects. The CS which has the particles size of 150 mm adhere poorly to insects and was found to be less lethal than those silica nano particles (SNP) (35 nm). Specific surface area of powdered materials is depending upon their particle size. It could be concluded that silica nano particles would be effective to be applied for protection of stored grains against those insect-pests attacking grains and can be used at low concentrations considering the moisture content of commodities which affect the activity of silica. However, additional experiments are required to clarify silica nano particles properties, their potential toxicity on different insect species, in various commodities and different environmental conditions.

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الملخص العربي

النشاط الإبادي المقارن للسيلكا النانوية والسيلكا الخشنة علي الخنفساء الصينية

حسن علي عبدالحميد مصباح ، السيد حسن محمد تايب ، أحمد محمد علي كردي ، حيدر حسين غثيث

تم تقدير النشاط الإبادي لنوعين من جزيئات السيلكا هما السيلكا العادية (أو الخشنة) (متوسط قطر الحبيبات١٥٠مم) والسيلكا النانوية (متوسط قطر الحبيبات٣٥نانومتر) وذلك بإستخدام خنفساء البقوليات الصينية كحشرة إختبار. وعند تعرض الحشرات البالغة غير المحددة جنسياً لحبوب الفول البلدي المعامل بتركيزات مختلفة من كلا نوعي السيليكا المختبرة (٠,٥، ١,٠، ٢,٠ جرام/١٠٠ جم حبوب) وذلك لفترات مختلفة (٤٨، ٩٦، ١٤٤، ١٩٢ ساعة) أظهرت النتائج أن جزئيات السيلكا النانوية لديها نشاط إبادي ضد خنفساء البقوليات الصينية أكثر من السليكا الخشنة. علاوة علي ذلك فقد وُجد أن زيادة كل من وقت التعرض وزيادة التركيز المختبر تؤدي الي زيادة التأثير الإبادي وزيادة نسبة موت الحشرات في كلا نوعي السليكا المختبرة. وقد تم تقدير قيمة التركيز اللازم لقتل ٥٠% من الأفراد المختبرة والمعرضة للتركيز (LT_{50}) حيث كانت ٨٣,٤٤ ، ٧٢,٢٣، ٦٧,٥٢ ساعة للتركيزات المختبرة من السليكا النانوية بالمقارنة بـ ١٤٠,٠٠، ١٣١,٤٤، ١٢٠,١٥ ساعة للسيلكا الخشنة مما يوضح أن حبيبات السليكا النانوية كانت أكثر في نشاطها البيولوچي ضد خنفساء البقوليات الصينية.

وتُركت الحشرات التي عاشت بعد المعاملة وإعتبارها آباء لإعطاءها الفرصة لإنتاج أفراد الجيل الأول لتقدير التأثير المتأخر لحبيبات نوعي السليكا المختبرة علي تعداد النسل الناتج. وقد بينت النتائج أنه لم يكن هناك أي نسل أوأفراد ناتجة للجيل الأول عند تركيزي ١,٠، ٢,٠ جرام من السليكا النانوية/١٠٠جرام من حبوب الفول البلدي. وقد وُجد أن النسل الناتج من معاملات السليكا الخشنة يقل بزيادة التركيز المختبر وأعطت هذه التركيزات مدي من الأفراد الناتجة تراوح بين ٢,٣ –١٠ أفراد بالمقارنة بالكنترول الذي كان فيه متوسط تعداد الجيل الأول وصل إلي ٢٤,٧. كما تم توضيح نموذج التقاط الحشرة لكل من نوعي حبيبات السليكا المختبرة (الخشنة والنانوية) حيث التقطت الحشرات جزيئات أكثر بكثير من السليكا النانوية بالمقارن بالسليكا الخشنة. وعلي ما سبق فقد وُجد أن السليكا النانوية كانت أكثر فعالية وكفاءة ضد حشرة خنفساء البقوليات الصينية وعلي هذا يمكن التوصية بفعالية إستخدامها في برنامج المكافحة المتكاملة ضد هذه الحشرة وغيرها من الآفات الحشرية التي تصيب الحبوب المخزونة.