

Entomotoxicity assay of Nanoparticle 4-(silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500) Against Sitophilus oryzae Under Laboratory and Store Conditions in Egypt

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ABSTRACT: Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 were tested against the rice weevil *Sitophilus oryzae* under laboratory and store conditions. Results showed that under laboratory conditions, the number of mortality of *S. oryzae* were significantly increased to 49.4 ± 4.4 , 57.8 ± 5.3 and 50.6 ± 3.2 individuals after treated with 3% of silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 after 7, 21 and 45 days, as compared to 1.0 ± 2.8 , 1.0 ± 5.1 and 2.0 ± 3.1 , respectively in the control. The number of mortality scored a higher mortality reached to 37.1 ± 3.2 , 40.4 ± 3.4 and 44.7 ± 5.1 individuals after treated with 1% of Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 nanoparticles as compared to 2.0 ± 3.8 , 1.0 ± 5.1 and 2.0 ± 3.1 individuals, respectively. The effect of the nanoparticles (Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500) against *Sitophilus oryzae* under store conditions. In 0.2% treatments with nano Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500, and the number mortality of *S. oryzae* were significantly increased to 14.1 ± 2.3 , 28.2 ± 8.8 and 29.8 ± 3.9 individuals after nano Silica gel Cab-O-Sil-750, and silica gel Cab-O-Sil-500 treatments as compared individuals in the control. The number of eggs laid /female were significantly reduced. After 100 and 120 days the number of eggs laid/ female were significantly decreased to 6 ± 1.0 and 11 ± 0.51 as compared to 99.1 ± 1.43 and 97.2 ± 1.82 in the control. The % reduction values in the number of laid eggs and adult emergence after 120 days were 91 and 90%, respectively in case of Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 application on foam covering gunny bags provided promising oviposition deterrence, toxicity and suppressing *S. oryzae* infestation, persistence and protecting rice seeds from beetles' infestation for 120 days during storage.

Key words: *Sitophilus oryzae*, silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500, nanoparticles

Introduction

Insect pests cause extensive damage to stored grains, both qualitatively and quantitatively (Hall, 1970; Semple et al., 1992). Many grain storage managers use synthetic insecticides to reduce losses of stored grain to insect pests. Nanoparticles can be arranged or assembled into ordered layers, or mine layers (Ulrich et al., 2006).

Nanotechnology, a promising field of research opens up in the present decade a wide array of opportunities in the present decade and is expected to give major impulses to technical innovations in a variety of industrial sectors in the future. The potential uses and benefits of nanotechnology are enormous. These include agricultural productivity enhancement involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery and vector and pest management and nanosensors for pest detection. The atom by atom arrangement allows the manipulation of nanoparticles thus influencing their size, shape and orientation for reaction with the targeted tissues. It is now known that many insects possess ferromagnetic materials in the head, thorax and abdomen, which act as geomagnetic sensors. In this paper, our discussion is focused on nanoparticles in insects and their potential for use in insect pest management. (Bhattacharyya et al., 2010). They reported that the potential uses and benefits of nanotechnology are enormous. These include agricultural productivity enhancement

involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery and vector and pest management and nanosensors for pest detection. Thus nanotechnology will revolutionize agriculture including pest management in the near future. Over the next two decades, the Green Revolution would be accelerated by means of nanotechnology. Debnath et al. (2010) reported that the diatomaceous earth was used to design amorphous nano sized hydrophilic, hydrophobic, and lipophilic, surface functionalized silica nanoparticle (SNP) was tested against rice weevil *S. oryzae* and its efficacy was compared with bulk sized silica, application of hydrophilic SNP at 1 g kg⁻¹ could kill more than 80% of the insects, after 7 days of exposure, 95 and 86% mortality were obtained with hydrophilic and hydrophobic SNPs at 1 g kg⁻¹, respectively. Insect mortality due to silica nanoparticles treatment was obtained at dose rates almost comparable with those of commercially available DE formulations ranging from 500 to 5000 mg kg⁻¹ (Subramanyam and Roesli, 2000; Vardeman et al., 2007).

Nanoparticles of oxides like SiO₂ produced and characterized in our laboratory were tested against insect pests and pathogens. Nanosilica against insect pests shows nearly 100% mortality (Debnath et al. (2010). (Arthur, 1996). However, there are several reasons to search for alternatives to synthetic insecticides: consumer preference for food without insecticide residues, worker safety concerns, resistant insect populations, and deregistration of current synthetic insecticides. Higher plants are a good source of novel insecticides (Prakash & Rao, 1997). Rice is the most important food crop for more than half of the world's population. Losses in rice storage due to insect pests affect food availability for a large number of people. Milled rice is attacked by various insect pests during storage (Cogburn, 1980). Storage and upkeep of agricultural products are very important post harvest activities. Considerable amount of rice grains is being spoiled after harvest due to chemical insecticides compounds (Larsson et al., 1992). Nano Al₂O₃ and amorphous nano SiO₂ were found to be highly effective and nano Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 was moderately effective against *S. oryzae*. But nano Al₂O₃ has deleterious effects on seeds, whereas non crystalline nano SiO₂ has no such adverse effect on rice seeds. Here we present the first report showing that nanocides, especially nano SiO₂ can be effectively used to control insect pests (Leiderer and Dekorsy, 2008).

The rice weevil, *S. oryzae* (L). (Coleoptera: Curculionidae) is a major pest of stored rice in Egypt, and has been spread worldwide by commerce. Both the adults and larvae feed on whole grains. They attack wheat, corn, oats, rye, barley, sorghum, dried beans and cereal. It causes extensive losses in the quality and quantity of commercial products as well as deterioration of seed viability worldwide (Madrid et al., 1990 and Owolade et al. (2008). Currently, chemical control is the most commonly used strategy against the pests. There are many chemicals that are toxic to stored-grain pests, including insecticides such as organophosphates, pyrethroids and fumigants such as methyl bromide and phosphine (Park et al., 2003; Kljajic and Peric, 2006 and Wadhwa 2009). These chemicals are effective for pest control but have several problems to users (Okonkwo and Okoye, 1996). Nano-pesticides and nano-encapsulated pesticides are expected to reduce the volume of application and slow down the fast release kinetics. (Edibo et al. 2003, Niemeyer and Doz 2001, Leiderer and Dekorsy, 2008). Mode of action occur destruction of the natural water barrier, the waxy layer of the cuticle, results in the desiccation of arthropods, Desiccation follows Fick's law of diffusion., Water absorption by silica particles is not important, Since there is no chemical alteration of the absorbed lipids we can describe the mode of action as physisorption (Leiderer and Dekorsy, 2008). Targeted nanoparticles often exhibit novel characteristics like extra ordinary strength, more chemical reactivity and possess a high electrical conductivity. Thus, nano-technology has become one of the most promising new technologies in the recent decade. Nanoparticles possess distinct physical, biological and chemical properties associated with their atomic strength (Leiderer and Dekorsy, 2008). Nanoparticles (which are 1-100 nm in diameter) are agglomerated atom by atom, and their size (and some-times shape) may be maintained by specific experimental procedure (Roy, 2009). Nanoparticles can be arranged or assembled into ordered layers, or mine layers (Ulrich et al., 2006). Such self-assembly is due to forces such as hydrogen bonding, dipolar forces, hydrophobic interactions, surface tension, gravity and other forces.

Thus nanotechnology deals with the targeted nanoparticles as and when the particles exhibit different physical strength, chemical reactivity, electrical conductance and magnetic properties (Nykypanchuk et al., 2008). Nanotechnology, a promising field of research opens up in the present decade a wide array of opportunities and is expected to give major impulses to technical innovations in a variety of industrial sectors in the future. Nanoencapsulation is currently the most promising technology for protection of host plants against insect pests. Thus nanotechnology will revolutionize agriculture including pest management in the near future. Over the next two decades, the green revolution would be accelerated by means of nanotechnology (Bhattacharyya et al., 2010).

Materials And Methods

Insects rearing

S. oryzae was collected from infested rice obtained from a local market and reared in glass jars under laboratory conditions of $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$, $75 \pm 5\%$ relative humidity (RH) in continuous darkness. The RH was maintained by using saturated solution of sodium chloride (Winston and Bates 1960). After the pupal stage the adults less than 24 hrs old were used for the experiments.

Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 Zinc Oxide Nanorods The silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 nanorods are prepared using the method described (Zhang, et al 2007). The process involves the ultrasonic irradiation of 50 mL of aqueous solution containing 0.05 M of zinc acetate and 0.05 M of triethanolamine, at 80°C for two hours. The resulting product was washed several times with de-ionized water followed by methanol. Filtered and dried at 100°C in air for 2 hours. The structural morphology of the particle was done by scanning electron microscope and presented in Fig. (1 and 2). The particles are found to be rod shaped with 100 to 250 nm dia and 1 to 2 nm length

Repellency test

The experiments were conducted in an arena in choice test. Disc of filter paper (Whatman No. 1) was treated with the tested nanoparticles silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 at 1 % conc. and placed in cell A. While filter paper treated with distilled water and emulsifier only as control was placed in the cell B. Twenty newly emerged beetles were introduced into each arena. After 1, 2, 3, 4, 5, 6 and 7 days, the number of beetles present in the cells A and B was recorded. The percentages of repellency values were calculated using the equation: $D = (1 - T/C) \times 100$ (Lwande et al., 1985) where T and C represent the mean number of beetles in cells A and B (Treated and untreated), respectively.

The insecticidal activity of tested nanoparticles

Experiment was designed to test the initial as well as the persistent effect of the tested nanoparticles silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 on beetles as cumulative mortality during successive intervals (0, 2, 4, and 7 days). Foam granules about 1 cm in diameter were treated at time (zero time) with tested nanoparticles, dried and provided with heat sterilized rice seeds (100g/each) fastened each with a string. Then all treatments were used immediately as non-choice test. The foam granules treated with the tested nanoparticles were mixed with rice seeds (2g foam/100g seeds) according to Abd El-Aziz (2001).

Ovipositional deterrent effect of tested nanoparticles (no choice test)

To evaluate the oviposition deterrent of the tested nanoparticles silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500, a pair of newly emerged beetles was placed with treated or untreated broad seeds in glass jars (250 cc capacity) covered with muslin. The beetles were left to lay eggs, and then the deposited eggs were counted on the seeds in the treated and untreated jars. Each experiment was repeated five times, (Abd El-Aziz and Ismail, 2000). The number of deposited eggs was used as a criterion for the evaluation of reduction percentages.

$$\text{Reduction \%} = \frac{[100 - \text{No. of deposited eggs in treatment}]}{\text{No. of deposited eggs in control}} \times 100$$

The percent reduction is an index of effectiveness of the applied nanoparticles in reducing infestation and was calculated according to, Su (1989).

The persistence of nanoparticles during storage

Experiment was designed to test the persistent effect of silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 on foam as surface protectant at 20 day intervals over 120 days. All gunny sacks (20x20 cm each) were full of heat sterilized rice seeds (100 g each), fastened, each with a string. The foam granules (about 1 cm in diameter) were sprayed with treatments, dried and provided as a layer between sacks. Following exposing to those treatments, two pairs of newly emerged beetles (2–3 day) were placed in a jar (2 l capacity with four gunny sacks) and observed for egg laying. The laid eggs were counted on the seeds in the treated and untreated jars. Each experiment was repeated five times, (Abd El-Aziz 2001).

The number of deposited eggs was used as a criterion for the evaluation of reduction percentages.

$$\text{Reduction \%} = \frac{(100 - \text{no of laid eggs in treatments})}{\text{No of laid eggs in control}} \times 100$$

The percent reduction is an index of effectiveness of the applied nanoparticles in reducing the pest infestation and was calculated according to Su (1989). Dead beetles were removed and the jars were kept

under the same experimental conditions until the emergence of F1 progeny adults occurred. Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as:

$$\%IR = (Cn - Tn) 100 / Cn$$

Where: Cn is the number of newly emerged insects in the untreated (control) jar

TN is the number of insects in the treated jar (Tapondjouet al. 2002).

Results

Data in table 1 show that under laboratory conditions, the number of mortality of *S. oryzae* were significantly increased to 49.4 ± 4.4 , 57.8 ± 5.3 and 50.6 ± 3.2 individuals after treated with 3% of silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 after 7, 21 and 45 days, as compared to 1.0 ± 2.8 , 1.0 ± 5.1 and 2.0 ± 3.1 , respectively in the control. When *S. oryzae* were treated with Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 under laboratory conditions, the number of mortality scored a higher mortality reached to 37.1 ± 3.2 , 40.4 ± 3.4 and 44.7 ± 5.1 individuals after treated with 1% of Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 nanoparticles as compared to 2.0 ± 3.8 , 1.0 ± 5.1 and 2.0 ± 3.1 individuals, respectively (Table 1).

The effect of the nanoparticles (Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500) against *Sitophilus oryzae* under store conditions showed in Table 3. In 0.2% treatments with nano Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500, and the number mortality of *S. oryzae* were significantly increased to 14.1 ± 2.3 , 28.2 ± 8.8 and 29.8 ± 3.9 individuals after nano Silica gel Cab-O-Sil-750, and silica gel Cab-O-Sil-500 treatments as compared individuals in the control (Table 2). At higher concentrations of 3% the mortality percent were significantly increased to 89.0 ± 1.1 individuals after 45 days of applications as compared to 3.0 ± 2.6 individuals in the control, (Table 2).

Data in Table (3) indicated that accumulative mortality (%) of *S. oryzae* beetles increased gradually by increasing the period of exposure in case of treated foam with different tested nanoparticles Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500. Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 had the highest cumulative mortality (69.7%) after seven days as compared to (1.1 %) after seven days in the control Table 3. In this respect, Chander and Ahmed (1986) applied different doses of the essential oil of *Acorus calamus* seeds of green gram *Vigna radiata* (Wilcz) to protect them against *Callosobruchus chinensis* and found that 1ml/Kg offered a high degree of protection up to a period of 135 days. Prolonged protection of the seeds was mainly due to a high adult mortality besides reduced oviposition and low hatching. Foam sprayed with clove oil (5%) and placed between sacks caused the highest mortality (66.6%) of *C. maculatus* as compared with treated sacks or foam inside sacks (63.3% and 42%, respectively) after 6 days of storage (Abd El-Aziz, 2001). The same results were obtained by Chander and Ahmed (1986); Saxena et al., (1976), Surabaya et al., (1994) and Sabbour (2012).

The persistence of nanoparticles tested during storage

The persistent effect of nanoparticles with on foam covering gunny bags displayed several different modes of action by reducing oviposition and adult emergence (F1) of *Sitophilus oryzae* (Table 4). The oviposition was completely inhibited when stored rice seeds were treated with Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 during 20, 40, 60 and 80 days of storage. The number of eggs laid /female were significantly reduced. After 100 and 120 days the number of eggs laid/ female were significantly decreased to 6 ± 1.0 and 11 ± 0.51 as compared to 99.1 ± 1.43 and 97.2 ± 1.82 in the control Table 4. The % reduction values in the number of laid eggs and adult emergence after 120 days were 91 and 90%, respectively in case of Silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500 application on foam covering gunny bags provided promising oviposition deterrence, toxicity and suppressing *S. oryzae* infestation, persistence and protecting rice seeds from beetles' infestation for 120 days during storage.

Abd El-Aziz and Sharaby (1997) tested the effects of white mustard oil on egg lying and egg masses viability of *Spodopteralittoralis*. Spraying cotton plants with 2.5% of oil caused reduction in egg laying. The moths laid only 7% of their egg masses and the percentage of repellency was 89.4%. At 2.5% conc., egg masses of different ages (24, 48 and 72 h old) were highly affected and the reductions were 66.6, 45 and 92%, respectively compared to the control. Compared with the investigation of Prakash (1982), white mustard oil was found to protect stored pulses against storage insects' infestation, especially the black gram and the green gram. Regnault-Roger and Hamraoui (1995) reported that eugenol, the main constituent of the essential oil of clove, also produced a strong inhibition of larval penetration of *Acanthoscelides obtectus* (Say) and finally a complete inhibition of emergence. Turcani (2001) experimented combinations of neemazal and Btk products against gypsy moth in oak stands. All combinations gave 100% mortality after three weeks of exposure. Abd El-Aziz (2001) mentioned that the treated foam with clove and eucalyptus oil vapours covering gunny sacks was the most significantly effective against *C. maculatus* infestation after 90 days of storage compared with the other applications (treated sacks or foam inside

sacks). The foregoing results indicate that the mustard and clove essential oils have properties which cause adult mortality, repellency of *B. incarnatus* and this may be correlated to the chemical constituents of these oils. Application of mustard oil formulated with *P. fumosoroseus* on foam covering gunny bags provided promising oviposition deterrence, toxicity and suppressing *B. incarnatus* infestation, persistence and protecting broad bean seeds from beetles' infestation for 120 days during storage (Kohler et al.1987, Maheshwari et al. 1988, Sabbour, 2012 and Madrid et al, 1990).

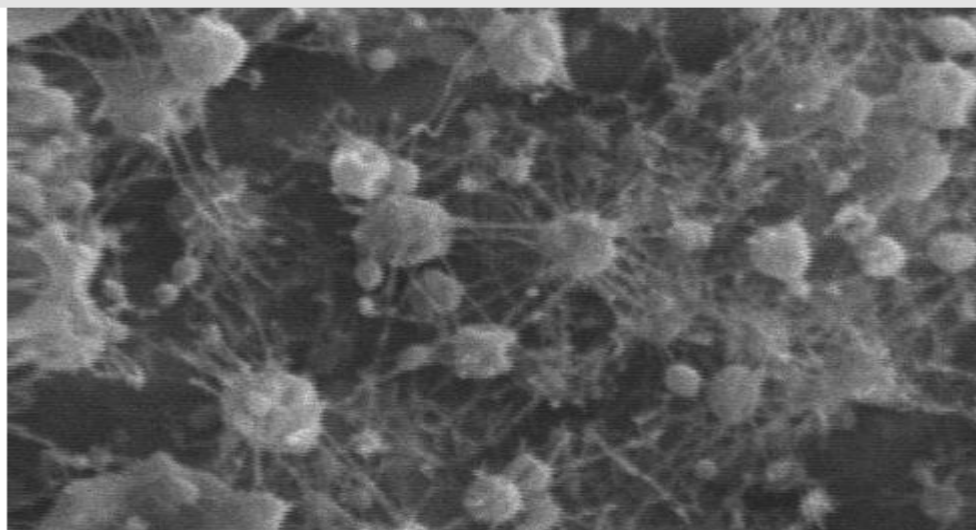


Figure 1. Scanning Electron Microscope Image of nano silica gel Cab-O-Sil-500(200 nm).

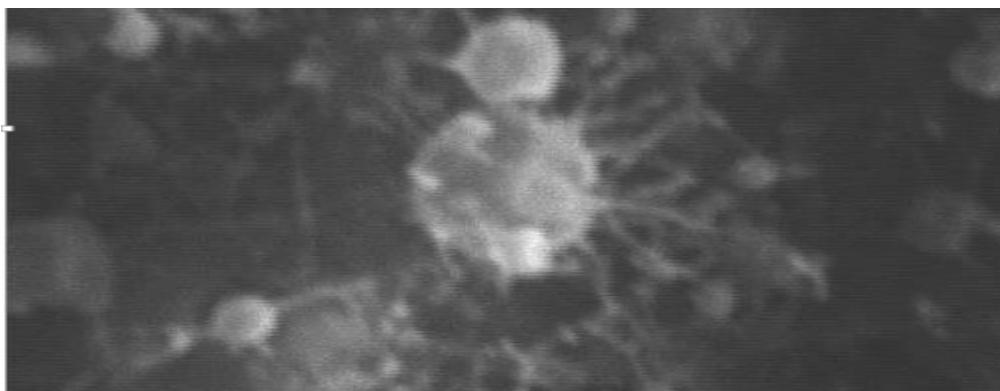


Figure 2. Scanning Electron Microscope Image of nano silica gel Cab-O-Sil-750(200 nm).

Table 1. Effect of the nanoparticlessilica gel Cab-O-Sil-500 against *Sitophilus oryzae* under laboratory conditions

Concentrations	No. of mortality± S.E after		
	7 Days	21D	45D
0.2%	33.2±3.1	38.3±1.7	43.6±6.1
0.5%	39.9±3.4	40.8±4.6	51.9±3.9
1%	49.1±3.8	55.4±3.0	69.7±5.3
3%	69.4±4.4	73.7±5.7	88.6±3.6
Control	1.0±2.1	1.0±2.1	2.0±1.1
F-value	14.5		
LSD 5%	13.1		

Table 2. Effect of the nanoparticlessilica gel Cab-O-Sil-750 against *Sitophilus oryzae* under store conditions

Concentrations	No. of mortality± S.E after		
	7 Days	21D	45D
0.2%	29.1±1.3	36.2±6.4	47.4±3.0
0.5%	40.1±5.1	48.7±4.9	50.3±5.4
1%	50.1±3.1	66.1±1.6	70.5±1.8
3%	72.6±4.3	89.9±9.5	91.2±1.6
Control	1.0±2.1	2.0±1.2	2.0±1.6
F-value	14.2		
LSD 5%	11.3		

Table 3. Accumulative mortality of *Sitophilus oryzae* adults during the first week of rice seeds exposed to treated foam with silica gel Cab-O-Sil-500 and silica gel Cab-O-Sil-750

Treated oils	Time(days)	Accumulative mortality%
silica gel Cab-O-Sil-500	0	16.1
	2	35.6
	4	47.1
	7	69.7
silica gel Cab-O-Sil-750	0	17.9
	2	41.9
	4	75.9
	7	
untreated	0	0
	2	0
	4	0
	7	1.1

Table 4. Effect of nanoparticles on number *Sitophilus oryzae* of laid eggs/female and % of adult emergence (F1) of *S. oryzae* beetles during storage periods of rice seeds

Storage Interval [days]	Control		silica gel Cab-O-Sil-500	
	no. of eggs / $\bar{x} \pm S.E.$	% adult emergence(F1)	no. of eggs / $\bar{x} \pm S.E.$	% adult emergence(F1)
20	79.8 \pm 8.55	92	0.0 \pm 0.0	0
40	89.2 \pm 1.32	93	0.0 \pm 0.0	0
60	91.1 \pm 5.84	94	0.0 \pm 0.0	0
80	95.2 \pm 4.42	94	0.0 \pm 0.0	0
100	99.3 \pm 1.41	97	5 \pm 1.1	5
%of reduction	-	-	94	92
120	97.2 \pm 1.82	91	10 \pm 0.48	12
%of reduction	-	-	91	90

Table 4. Effect of nanoparticles on number *Sitophilus oryzae* of laid eggs/female and % of adult emergence (F1) of *S. oryzae* beetles during storage periods of rice seeds

Storage Interval [days]	Control		silica gel Cab-O-Sil-750	
	no. of eggs / $\bar{x} \pm S.E.$	% adult emergence(F1)	no. of eggs / $\bar{x} \pm S.E.$	% adult emergence(F1)
20	88.8 \pm 5.57	92	0.0 \pm 0.0	0
40	89.2 \pm 1.32	93	0.0 \pm 0.0	0
60	96.1 \pm 4.71	94	0.0 \pm 0.0	0
80	97.6 \pm 1.72	94	0.0 \pm 0.0	0
100	99.1 \pm 1.43	97	6 \pm 1.0	5
%of reduction	-	-	94	92
120	99.8 \pm 1.82	91	14 \pm 1.41	12
%of reduction	-	-	91	90

$$\text{Reduction \%} = \left[\frac{100 - \text{No. of deposited eggs in treatment}}{\text{No. of deposited eggs in control}} \right] \times 100$$

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