

## Entomotoxicity assay of two Nanoparticle Materials 1-(Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub>) Against *Sitophilus oryzae* Under Laboratory and Store Conditions in Egypt

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**ABSTRACT:** Two nano materials Aluminium oxide Al<sub>2</sub>O<sub>3</sub> and Titanium dioxide (TiO<sub>2</sub>) , were tested against rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) under laboratory and store conditions.

Results showed that Nano Al<sub>2</sub>O<sub>3</sub> were found to be highly effective against *S. oryzae* and nano TiO<sub>2</sub> has lower moderately effective against *Sitophilus oryzae*. Under laboratory conditions, the number of mortality of *S. oryzae* were significantly increased to 41.4±4.4, 47.8±5.8 and 50.6±3.6 individuals after treated with 3% of TiO<sub>2</sub> after 7, 21 and 45 days, as compared to 1.0 ± 2.8, 2.0 ± 5.1 and 3.0 ± 3.4, respectively in the control. The effect of nanoparticles Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> under store conditions the results showed that the nanoparticles significantly increased the number of mortality reached to 67.3 ± 1.4 after 45 days of storage as compared to 3.8 ± 3.8 individuals in the control . Accumulative mortality (%) of *S. oryzae* beetles increased gradually by increasing the period of exposure in case of treated foam with different tested nanoparticles Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> Al<sub>2</sub>O<sub>3</sub> had the highest cumulative mortality (73.3%) followed by TiO<sub>2</sub> reached to (59.7 %) after seven days.

**Keywords:** *Sitophilus oryzae*, Titanium dioxide (TiO<sub>2</sub>) , Aluminium oxide Al<sub>2</sub>O<sub>3</sub>.

### INTRODUCTION

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; Kumar et al., 2010). 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 (Subramanyam and Hagstrum, 1995; 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. (Edibol 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).

In particular, in this paper, our discussion is focused on nanoparticles in insects and their potential role in insect pest management

## 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°C ± 1°C, 75 ± 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.

### ***Materials and Methods***

Characterization of the synthesized nanoparticles were prepared according to Abduz Zahir *et al* (2012). Lethal concentration and their associated confidence intervals were estimated from 24 h concentration mortality data using probit analysis (Finney, 1971).

### ***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 Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> 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 Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> on beetles as cumulative mortality during successive intervals (0, 2, 4, and 7 days). Foam granules about 1cm 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 Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub>, 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.

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

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 Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> 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.

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

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:

$$\%IR = (C_n - T_n) 100 / C_n$$

where: C<sub>n</sub> is the number of newly emerged insects in the untreated (control) jar

T<sub>n</sub> is the number of insects in the treated jar (Tapondjou et al. 2002).

## **RESULTS AND DISCUSSION**

Data in table 1 show that under laboratory conditions, the number of mortality of *S. oryzae* were significantly increased to 41.4±4.4, 47.8±5.8 and 50.6±3.6 individuals after treated with 3% of TiO<sub>2</sub> after 7, 21 and 45 days, as compared to 1.0 ± 2.8, 2.0 ± 5.1 and 3.0 ± 3.4, respectively in the control. When *Sitophilus oryzae* were treated with Al<sub>2</sub>O<sub>3</sub> under laboratory conditions, the number of mortality scored a higher mortality reached to 58.4±4.4, 68.8±0.8 and 84.6±0.7 individuals after treated with 3% of Al<sub>2</sub>O<sub>3</sub> nanoparticles as compared to 2.0 ±3.8, 2.3 ±5.2 and 3.1 ±3.6 individuals, respectively (Table 2). The effect of the nanoparticles Titanium dioxide (TiO<sub>2</sub>) against *Sitophilus oryzae* under store conditions showed in Table 3. In all treatments with TiO<sub>2</sub>, the number mortality of *S. oryzae* were significantly increased after the TiO<sub>2</sub> treatments as compared with the control Table 3. The effect of nanoparticles Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> under store conditions in tables 4 and 5, the results showed that the nanoparticles significantly increased the number of mortality reached to 67.3 ±1.4 after 45 days of storage as compared to 3.8 ±3.8 individuals in the control. Table 5 show that in the store the number of mortality of *S. oryzae* were significantly decreased to 17.1±3.6 and 25.0±9.9 after 21 days of treatments with TiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub>, respectively as compared to 2.0±5.9 in the control after 120 days of treatments with the nanoparticles TiO<sub>2</sub> 39.0±5.9 and Al<sub>2</sub>O<sub>3</sub> the 78.6 ±6.8 as compared to 5.9 ±8.9 in the control. Data in Table (6) 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 Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub>. Al<sub>2</sub>O<sub>3</sub> had the highest cumulative mortality (73.3%) followed by TiO<sub>2</sub> reached to (59.7 %) after seven days. In this respect, Chander and Ahmed (1986) applied different doses of the essential oil of *Acorus calamus* to 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).

### **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 7). The oviposition was completely inhibited when stored rice seeds were treated with Al<sub>2</sub>O<sub>3</sub> during 20, 40, 60 and 80 days of storage. The

% reduction values in the number of laid eggs and adult emergence after 120 days were 97 and 95 93%, respectively in case of Al<sub>2</sub>O<sub>3</sub> 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 laying and egg masses viability of *Spodoptera littoralis*. 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. incarnates* infestation, persistence and protecting broad bean seeds from beetles' infestation for 120 days during storage (Kohler *et al.* 1987, Maheshwari *et al.* 1988 and Madrid *et al.* 1990).

Table 1. Effect of the nanoparticles Titanium dioxide (TiO<sub>2</sub>) against *Sitophilus oryzae* under laboratory conditions

Concentrations	No. of mortality± S.E after		
	7 Days	21D	45D
0.2%	22.3±3.4	25.3±1.9	35.7±6.4
0.5%	25.5±3.2	33.1±2.2	39.7±3.4
1%	39.1±3.6	41.4±3.8	47.7±5.2
3%	41.4±4.4	47.8±5.8	50.6±3.6
control	1.0±2.8	2.0±5.1	3.0±3.4
F-value		11.3	
LSD 5%		10.6	

Table 2. Effect of nanoparticles aluminum oxide Al<sub>2</sub>O<sub>3</sub> against *Sitophilus oryzae* under laboratory conditions

Concentrations	No. of mortality ± S.E after		
	7 Days	21D	45D
0.2%	29.5±4.5	39.4±4.4	55.4±0.7
0.5%	32.3±3.4	44.4±4.4	65.8±0.8
1%	44.6±5.8	51.2±0.6	7.9±0.8
3%	58.4±4.4	68.8±0.8	84.6±0.7
Control	2.0±3.8	2.3±5.2	3.1±3.6
F-value		11.4	
LSD 5%		10.2	

Table 3. Effect of the nanoparticles Titanium dioxide (TiO<sub>2</sub>) against *Sitophilus oryzae* under store conditions

Concentrations	No. of mortality± S.E after		
	7 Days	21D	45D
0.2%	10.1±2.3	18.2±8.3	19.8±3.4
0.5%	22.0±3.4	30.1±4.8	38.3±5.7
1%	31.1±3.5	35.1±3.9	45.5±1.3
3%	41.22±4.5	451.1±2.5	49.9±1.1
control	1.0±2.8	2.1±3.2	3.0±8.6
F-value		10.9	
LSD 5%		10.2	

Table 4.: Effect of nanoparticles aluminium oxide Al<sub>2</sub>O<sub>3</sub> against *Sitophilus oryzae* under store conditions

Concentrations	No. of mortality± S.E after		
	7 Days	21D	45D
0.2%	26.4±4.7	35.4±3.3	47.6±1.0
0.5%	35.3±4.5	43.3±4.8	58.8±1.4
1%	45.4±5.6	55.6±3.4	67.6±10.1
3%	51.3±4.1	63.5±1.7	87.3±1.4
control	2.2±3.8	3.3±5.6	3.8±3.8
F-value		11.8	
LSD 5%		10.3	

Table 5. Entomotoxicity Assay of the two nanoparticles materials tested against *Sitophilus oryzae* under store conditions

Tested materials	NO. of mortality ± S.E after			
	21 D	45 D	90D	120D
TiO2	17.1±3.6	26.7±5.2	32±7.7	39.0±5.9
Al2O3	25.0±9.9	44.7±4.8	58.6±9.4	78.6 ±6.8
Untreated (control)	2.0±5.9	2.5±9.6	4.0±7.9	5.9 ±8.9
F-test =	14.5			
LSd5%=	11.4			

Table 6. Accumulative mortality of *Sitophilus oryzae* adults during the first week of rice seeds exposed to treated foam with different oils

Treated oils	Time(days)	Accumulative mortality%
TiO2	0	18.3
	2	31.6
	4	41.7
	7	59.7
	0	26.7
Al2O3	2	48.8
	4	62.4
	7	73.3
	0	0
	2	0
untreated	4	0
	7	2.1

Table 7. Effect of nanoparticles on number *Sitophilus oryzae* of laid eggs/female and % of adult emergence (F1) of *B. incarnates* beetles during storage periods of rice seeds

Storage Interval [days]	Control		Al2O3		TiO2	
	no. of eggs /♀±S.E.	% adult emergence(F1)	no. of eggs /♀±S.E.	% adult emergence(F1)	no. of eggs /♀±S.E.	% adult emergence(F1)
20	87.8±1.56	82	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
40	90.2±1.39	83	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
60	85.0±1.84	83	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
80	92.0±1.42	90	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
100	98.0±1.44	88	6±1.0	9	19.4±2.16	14
%of reduction	-	-	95	103	82.2	97
120	91.4±1.81	91	11±0.51	13	22.2±1.43	22
%of reduction	-	-	97	95	85	85

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