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Efficacy of (Aerosil®) fumed silica nanoparticles against *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) adults on wheat and paddy rice

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Abstract
The toxic effect and persistence of (Aerosil®) silica nanoparticles were conducted against *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) adults at the laboratory condition (26 ± 2°C and 60 ± 5 RH). (Aerosil®) were applied at 4, 1, 2, 0.5, and 0.25 g/kg on wheat and paddy rice seeds. Results revealed that Aerosil® has high toxicity on both species, Aerosil® at 4 g/kg on wheat and paddy rice gave complete mortality (100.0%) after 7days exposure period against tested insect species. Meanwhile, complete reduction in F1-progeny of *S. granarius* was observed at the two highest used concentrations and *R. dominica* at the three highest concentrations on wheat, also complete inhibition in F1-progeny of both tested species was achieved at the three highest concentrations on paddy rice. Serum samples of albino rats results showed that, Aerosil® did not alter activity of (TNF-α), (MDA) and (CEA) in rats fed on treated wheat grains.

Keywords: *Sitophilus granarius*- *Rhyzopertha dominica* - Aerosil® - Germination- Toxicity-Albino rats

1. Introduction
Stored grains are infested by many insect pests causing extensive loss in weight and quality. Insecticides or inert dust are being used as protectant during storage against stored product insects till now. Several insects now resistant to organophosphorus insecticides, the lesser grain borer *R. dominica* (F.) adults were resistant to both malathion, pirimiphos-methyl (El-Lakwah *et al.*, 2004) [6]. Therefore, alternative insecticides or pest management strategies are urgently needed to replace traditional insecticides. Diatomaceous earth and silica gel are used in various physical formulations with or without added pesticide.
Recently, Nano particles help to produce new pesticides, insecticides and insect repellant (Owolad *et al.*, 2008) [13]. (Bhattacharyya *et al.*, 2010) [4], focused on the potential of nanoparticles on in insects for use in insect pest management. Silica nanoparticles does not affect the looseness and bulk density of grain mass like DE even with the highest dose used.
The main chemical composition of silica nanoparticles and DEs is silica which may cause their common properties. However, DEs are of micron particle size which seems to reduce their insecticidal effect more than the silica nanoparticles (Debnath *et al.*, 2011) [5].
MDA is a reactive highly toxic aldehyde resulting from lipid peroxidation and prostaglandin biosynthesis that is mutagenic and carcinogenic (Vaca *et al.*, 1998) [14]. CEA is a glycoprotein found normallyin fetal gastrointestina l tissueandin adult plasma at very low concentrations. It incr eased in the presence of many tumors, particularly colorecta l cancers (NIH Conference: CEA 1981) [10]. Tumor Necrosis Factor Alpha (TNF-α) is a pro- inflammatory cytokine produced mainly by monocytes and macrophage. It is locally produced in acute situation that increase the expression of adhesion molecules on the vascular endothelium to allow immune cells, mainly neutrophils and macrophages, to traffic to sites of tissue damage and infection. It acts as an immunomodulatory agent, It has cytotoxic and proinflammatory activities (Barbara *et al.*, 1996) [3].
The aims of this study to evaluate the efficacy and persistent effect of Aerosil® silica nanoparticles against *Sitophilus granarius* **(**L.) and *Rhyzopertha dominica* (F.) on wheat and paddy rice seeds, as well as the effect of tested Aerosil® on Tumor Necrosis Factor Alpha (TNF-α), Malondialdehyde (MDA) and Carcinogenic Embryonic Antigen (CEA) of Albino rats.

2. Material and Methods
2.1. Insects
Adults of the grain weevil *S. granarius* (L.), the lesser grain borer *R. dominica* (F.) were used for investigations. All species were reared and maintained under laboratory conditions for several generations at 26 ± 2°C and 60 ± 5% RH.

2.2. Aerosil 200
Hydrophilic fumed silica purchased from Taiba Company for scientific services, Egypt.

2.3. Structural of Aerosil 200
The shape and size of Aerosil 200 were checked by Transmission Electron Microscope (TEM). Figure (1) indicates that the original morphology of particles approximately spherical with the diameter varying between 5 to 20 nm (Nilly and Doaa 2019) [11].

2.4. Bioassay test
Fifty grams of wheat or paddy rice grains were poured into 125 ml glass jars and treated with 4, 1, 2, 0.5, and 0.25 g/kg of Aerosil with 3 replications, and group (without Aerosil) for the control. The jars were shaken for 1min. to achieve

equal distribution in the entire grain mass. Then, 30 adults (1weeks old) of *S. granarius* and *R. dominica* were introduced to each jar. The jars were covered with muslin cloth and placed in an incubator set at 26 ± 2°C and 60 ± 5% RH. Mortalities were recorded after 2, 3, 5, 7 and 14 days from treatment. Mortalities were corrected using Abbot's formula (1925) [1]. Number of F1-progeny was inspected after 75 days from treatment and reduction percentages in F1-progeny were calculated according to the following equation:

Reduction % = N_0 - N_1/ N_0 .100

N_0 = No. of adults emerged in control. N_1 = No. of adults emerged in treatment.

2.5. Persistence of Aerosil

Persistence of Aerosil® at 4g/kg was investigated against the adults of above mention insect species for 6 months, 30 adult insects were introduced to 50 g of the treated or untreated samples that were taken monthly from wheat or Paddy rice lots stored at 26 ± 2°C and 60 ± 5 RH and adults mortality was recorded after 7 days of exposure.

2.6. Germination of seeds

Every month, 25seeds of wheat or Paddy rice were cultivated in each of 4 Petri dishes (4 replicates) for 7days and germination% was calculated.

2.7. Albino rats test

12 Sprague dawley albino rats were divided into 2 groups each of 6 rats; the first group fed on wheat only and serves as control, while the second group fed on wheat treated with Aerosil (4mg/kg) for 3months. All rats were sacrificed after the end of the experiment, fresh blood samples were collected into sterile centrifuge tubes for sera collection for detection of Malondialdehyde (MDA) calorimetrically according to method described by (Ohkawa *et al.*, 1979) [12], Tumor Necrosis Factor alpha (TNF-α) using ELISA kits according to (Aggarwal and Natarajan 1996) [2] and Carcinogenic Embryonic Antigen (CEA) as described by (Mansour *et al.*, 2010) [8] using ELISA kits**.**

2.8. Data analysis

The lethal concentrations of Aerosil® to the adults of the two stored grain insects were statistically analyzed according to Finney (1971) [7].

The obtained data were analyzed by ANOVA test and significant means were separated by Duncan's multiple rang test using a computer program of SPSS 14.0.

3. Results and Discussion

3.1. Toxicity of Aerosil

Results concerning effects of Aerosil Nano silica on mortalities and reduction in F1-progeny of *S. granarius* and *R. dominica* adults are illustrated in Tables (1&2). Data revealed that, adult mortality was increased with the rise of concentration and exposure time.

In case of wheat grains treated with different concentrations of Aerosil, the mortality percentages of *S. granarius* adults after 3dayes were 50.0, 41.1, 25.6, 13.3 and 12.2 % for 4, 2, 1, 0.5, and 0.25 g/kg, respectively. Complete mortality was achieved at the highest used concentration (4 g/kg) after 7days. Meanwhile, complete reduction in F1-progeny of *S. granarius* was observed at the two highest used concentrations.

The effect of Aerosil on *R. dominica* was higher than *S. granaries*, at the highest concentration the mortality was 55.6% adult mortality after 3days exposure then increased to reach (100.0%) after 7 days exposure, also the three highest concentrations gave (100.0%) adult mortality after 14 days and a completely hindered its of F1-progeny production.

On paddy rice, at the three highest used concentrations (4, 2 and 1 g/kg) the mortality percentages were 61.1, 48.9 and 43.3; 58.9, 52.2and 43.3% after 3dayes for *S. granarius* and *R. dominica* adults, respectively. After 7 days of exposure (100.0%) was achieved against *S. granaries* adults at (4 and 2 g/kg) and at 1 g/kg only against *R. dominica* adults. Also, a complete inhibition of F1-progeny of both tested insect species the three highest concentrations.

3.1.1. Lethal concentrations of Aerosil to the tested insect adults

The calculated lethal concentrations of Aerosil to the adults of the two stored grain insects at 5days exposure period are summarized in Table (3). The mean values of LC_{50}, LC_{90}, LC_{95} and LC_{99} after 5 days exposure on wheat seeds were 0.9, 6.2, 10.6 and 29.1 ; 0.4, 3.7, 6.9 and 22.3 ppm for *S. granarius* and *R. dominica* adults, respectively.

The corresponding values on paddy rice seeds were 0.2, 1.1, 1.7and 3.8 and 0.2, 1.8, 3.3 and 9.9 for aforementioned insect species, respectively.

Results showed clearly that *S. granarius* adults were the most sensitive to Aerosil® than by *R. dominica* which was the most tolerant species.

All data revealed that, Aerosil silica nanoparticles was more effective against two tested insect species on Paddy rice than wheat grains, but *S. granarius* was more susceptible than *R. dominica* adults on Paddy rice seeds. In this respect, (Masumeh and Zahra 2016) [9] found that, two silicon dioxide nanoparticles Aerosil and Nanosav have a high toxicity on *Rhyzopertha dominica* and *Tribolium confusum* fed on treated wheat or peeled barley seeds.

3.2. Persistence of Aerosil

The residual toxicity of Aerosil the two tested insect species on wheat and paddy rice grains are given in Table (4). Wheat and paddy rice seeds were treated with at 4 g/kg of Aerosil® stored for 6 months. Data revealed that, this concentration cause a complete protection for the two stored seeds against adults of *S. granarius* and *R. dominica* up to 3months. Also, on paddy rice seeds after 4th month interval the mortality percentages were (92.2 and 87.8%) for *S. granarius* and *R. dominica*, resp. meanwhile, it recorded (85.7and 90.0%) on wheat seeds for the two tested insect species.

Slightly differences obtained in germination% between treated wheat or Paddy rice seeds and control during storage period Table (5). Fumed silica at lower limit LC_{90} can protect wheat seeds from insect infestation until 4th month storage. Also, germination % of seeds stored for 6months after treatment with lower limit LC_{90} of fumed silica slightly affected comparing with untreated seeds (Nilly and Doaa 2019) [11], These results are in harmony with the findings of Silica gel Cab-O-Sil-500 application on foam covering gunny bags provided promising oviposit ion deterrence, toxicity and suppressing *S. oryzae* infestation, persistence and protecting rice seeds from beetles'

infestation for 120 days during storage (Sabbour M. 2015) [14].

Serum samples of albino rats results showed that, no significant differences were achieved between rats group fed on treated wheat grains and other group fed on untreated wheat grains in values of (TNF-α), (MDA) and (CEA), Aerosil® did not alter activity of (TNF-α), (MDA) and (CEA) in treated rats Table (6). According to the International Agency for the Research of Cancer (IARC), amorphous silica belongs to group(3), it is classified as not carcinogenic, United States Department of Agriculture (USDA)has already approved the use of amorphous silica as safe (Stathers *et el.*, 2004) [15].

All results revealed that Aerosil® silica nanoparticles provides a good and safe alternative protectant to wheat and paddy rice seeds during storage against tested insect species infestation.

4. Tables and Figure

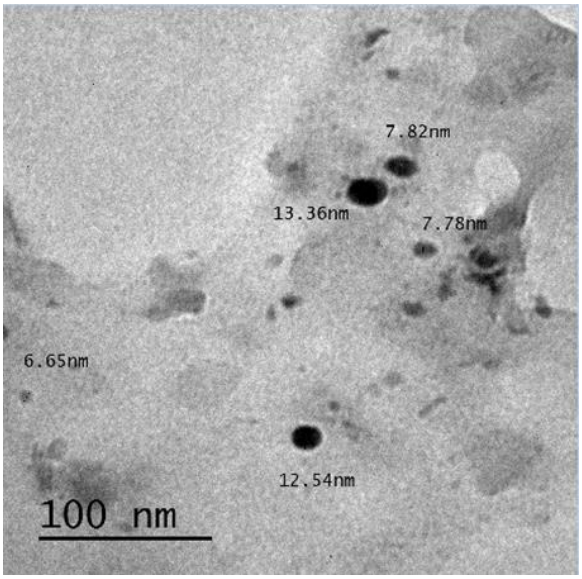

Fig 1: The TEM images of AEROSIL Nano particles (x=40) X: magnification power

Table 1: Adults mortalities and reduction in F_1-progeny of two stored grain insects exposed on wheat treated with Aerosil®.

Conc. g/kg	% Adult mortalities after indicated periods (days) ± S.D.					Reduction% in F1- progeny
	2	3	5	7	14	
S. granarius						
4	17.8±1.6	50.0±2.7	88.9±1.6	100.0±0.0	100.0±0.0	100.0
2	13.3±2.7	41.1±3.2	70.0±2.7	82.2±3.1	97.8±1.6	100.0
1	8.9±1.6	25.6±1.6	41.1±4.2	48.9±4.2	86.7±2.7	97.4
0.5	6.7±0.0	13.3±0.0	33.3±2.7	37.8±1.6	80.0±2.7	97.1
0.25	5. 6±1.6	12.2±1.6	24.5±3.2	26.7±2.7	54.4±1.6	95.8
R. dominica						
4	27.8±1.6	55.6±4.2	92.2±1.6	100.0±0.0	100.0±0.0	100.0
2	23.3±2.7	48.9±1.6	84.4±2.7	96.7±2.7	100.0±0.0	100.0
1	16.7±0.0	40.0±0.0	62.2±4.2	87.8±4.0	100.0±0.0	100.0
0.5	12.2±1.6	26.7±2.7	50.0±2.7	67.8±4.2	92.2±1.6	98.9
0.25	8.9±1.6	18.9±1.6	45.6±1.6	63.3±2.7	74.4±4.2	97.0

Table 2: Adults mortalities and reduction in F_1- progeny of two stored grain insects exposed on paddy rice treated with Aerosil®.

Conc. g/kg	% Adult mortalities after indicated periods (days) ± S.D.					Reduction% in F1- progeny
	2	**3**	**5**	**7**	**14**	
S. granarius						
4	44.4±1.6	61.1±4.2	98.9±1.6	100.0±0.0	100.0±0.0	100.0
2	41.1±1.6	48.9±3.2	96.7±2.7	100.0±0.0	100.0±0.0	100.0
1	33.3±2.7	43.3±2.7	92.2±1.6	97.8±1.6	100.0±0.0	100.0
0.5	22.2±3.2	35.6±1.6	66.7±2.7	90.0±2.7	98.9±1.6	99.1
0.25	20.0±2.7	24.4±4.2	57.8±3.2	83.3±0.0	86.7±2.7	98.8
R. dominica						
4	33.3±0.0	58.9±3.1	95.6±1.6	100.0±0.0	100.0±0.0	100.0
2	26.7±2.7	52.2±1.6	91.1±3.1	98.9±1.6	100.0±0.0	100.0
1	24.4±1.6	43.3±2.7	86.7±2.7	95.6±4.2	100.0±0.0	100.0
0.5	17.8±4.2	33.3±5.4	60.0±0.0	87.7±1.6	96.7±0.0	98.6
0.25	13.3±0.0	21.1±1.6	55.5±3.2	77.8±3.2	82.2±4.2	96.4

S.D. = Standard deviation

Table 3: Lethal concentrations of Aerosil® to the adults of the two insect species at 5days exposure period

seeds	Insect Species	Lethal concentrations (g/kg) and their 95% confidence limits				Slope ± S.E.	R
		LC_{50}	LC_{90}	LC_{95}	LC_{99}		
wheat	*S. granarius*	0.9(0.5 - 1.6)	6.2(4.6 - 34.9)	10.6(7.9 - 91.1)	29.1(21.1 -564.3)	1.6 ±0.16	0.96
	R. dominica	0.4(0.3 - 0.5)	3.7(2.6 - 6.3)	6.9(4.4 - 14.0)	22.3(11.5 - 64.5)	1.3 ±0.16	0.97
Paddy rice	*S. granarius*	0.2(0.2 - 0.3)	1.1(0.8 - 1.5)	1.7(1.2 - 2.7)	3.8(2.4 - 8.3)	1.9 ±0.27	0.97
	R. dominica	0.2(0.2 - 0.3)	1.8(1.4 - 2.8)	3.3(2.3 - 6.0)	9.9(5.6 - 25.4)	1.4 ±0.18	0.97

R = Correlation coefficient. S.E. = Standard error of regression line.

Table 4: Residual toxicity of Aerosil® at 4 g/kg to the two insect species

seeds	Insect Species	% adult mortality after 7 days from exposure ± S.D. at various intervals (months)						
		0	1	2	3	4	5	6
wheat	*S. granarius*	100.0±0.0	100.0± 0.0	100.0± 0.0	100.0±0.0	85.7±2.1	76.0±2.8	67.8±5.1
	R. dominica	100.0± 0.0	100.0± 0.0	100.0± 0.0	100.0± 0.0	90.0±3.3	82.0±2.0	77.8±1.1
Paddy rice	*S. granarius*	100.0± 0.0	100.0± 0.0	100.0± 0.0	100.0± 0.0	87.8±3.9	86.6±5.8	82.2±5.1
	R. dominica	100.0± 0.0	100.0± 0.0	100.0± 0.0	100.0± 0.0	92.2±5.1	82.7±2.1	74.5±6.9
control	Both insects	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5: Effect of Aerosil® at 4 g/kg on wheat and Paddy rice seeds germination during storage.

seeds	Germination %± S.D. at various intervals (months)						
	0	1	2	3	4	5	6
Treated wheat	87.0±3.3	86.0±4.5	86.0±2.0	85.0±4.4	86.0±2.0	85.0±4.4	86.0±4.5
Control	90.0±2.0	89.0±3.1	88.0±2.8	88.0±4.9	88.0±1.7	86.0±2.0	87.0±3.3
Treated Paddy rice	82.0±2.0	82.0±4.4	81.0±3.3	80.0±2.8	79.0±1.7	74.5±6.9	74.0±2.0
control	84.0±4.5	83.0±5.2	83.0±3.1	83.0±6.6	82.0±6.0	80.0±2.8	76.0±2.8

Table 6: Effect of Aerosil® on (TNF-α) (MDA) (CEA)

Analysis	Mean percentages ± S.D. of each rats group	
	Treated group	control
TNF-α	57.1±0.11[a]	48.5±0.64[a]
MDA	2.31±0.03[b]	2.14±0.01[b]
CEA	1.57±0.05[c]	1.22±0.05[c]

Tumor Necrosis Factor alpha (TNF-α), Malondialdehyde (MDA), Carcinogenic Embryonic Antigen (CEA)

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