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The silica-nano particles treatment of squash foliage and survival and development of *Spodoptera littoralis* (Bosid.) larvae

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Abstract
The study was conducted throughout the period extended from October, 2014 to January, 2015. The main objective was to study the effect of nano-silica in comparison with Silica & Diazinon as a recommended insecticide, applied as foliar spray on squash plants in the greenhouse and fed to newly hatched larvae of *Spodoptera littoralis* (Bosid.) for both foliar and semi-synthetic diet applications. Squash leaves were treated with 4 different concentrations 200,300,400, and 500 ppm of the three tested compounds. In bioassays, the neonate, second and fourth instars test larvae were fed on treated leaves and monitored for larval mortality as well as certain biological parameters *e.g.*, larval duration, pupal duration, pupal weight, pupation percentage, the rate of adult emergence and adult longevity in both treatments in comparison with untreated control foliage. Obtained results showed that generally hydrophilic nano-silica caused higher toxic action values than with the other treatments. Mortality rate among larvae in any of the treatments was directly correlated with the increase in concentration. Also, the newly hatched larvae were more susceptible to treatments than the other tested instars, where mortality was 73.07, 79, 72, 87.88 and 89.82% in concentration treatments in their ascending order, in comparison with Diazinon which caused 95.95% mortality. The observed developmental stages among survivals of test insects were also affected by the treatments. This investigation recommends the application of nanosilica at 500 ppm concentration for the suppression of *S. littoralis* pest.
Keywords: biological parameters, larval mortality, nano-insecticide, nano-silica, silica nanocide,
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Introduction
The cotton leaf worm, *Spodoptera littoralis* (Boisd.) is one of the most destructive insect pests which attack certain vegetable and field crops such as cotton, tomatoes, cabbage and squash in Egypt and other countries across the region. Extensive use of chemical insecticides to control the insect has led to the development of resistance and pollution of the environment (EPPO, 2008, Bulmer *et al* 2009, Yadav, 2010 and Ditta, 2012) [12, 43, 10]. Therefore, recent investigations have been aimed to reduce dependency on chemical pesticides and to use safe alternatives in pest control programs. Currently, there is a growing need to using environmentally friendly nanoparticles in the field of plant protection. Nano-particles technology when exploited in the right way has a strong potential of being used in agricultural pest control (Biswal *et al.*, 2012, Brennan 2012 and (Elbendary and El-Helaly 2013) [5, 6]. Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers. Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. (Banerjee and Santra 2009, Jeong *et al.* 2010 and Kim *et al.* 201) [3, 22, 23]. Recently Silica nano particles have shown promising potential in the field of biotechnology. The chemistry of silica provides the opportunity for a variety of surface fictionalization with hydroxyl/ amine/ thiol/ carboxyl groups (Sasson *et al*, 2009, Han *et al*. 2009, Perez and Hermosin, 2013) [36, 17, 28]. However, the potential of the nano particles in agriculture remains unexplored.

Materials and methods
For the foliar investigation, squash plants were grown in the greenhouse. *Cucurbita pepo* C.V. Eskandarani seed were sown in plastic pots (15 cm diameter) on the 1 st October.

Seedling height attained 15 cm and had about 5-7 leaves, were sprayed with the 4 different concentrations (200, 300, 400 & 500 ppm) of the 3 tested compounds (nano-silica, silica and recommended pesticide). Treatments of foliar spray included different concentrations of nano-silica in comparison with silica and Diazinon insecticide. The control plants were sprayed with distilled water, three replicates plus control were carried out and each replicate contained five plants. The plants had a second spray after one week from the first one. One type of nanosilica was tested, namely hydrophilic nano-silica, supplied by Nano Tech. Egypt. For the semi synthetic diet investigation, semi synthetic diet (Shorey and Hale 1956) [38] were prepared at 25±1 °C temperature, 70±10% RH and 12h photo.

Bioassay

For the foliar investigation: Leaves from each foliage treatment were offered to 10 *Spodoptera littoralis* neonate larvae in each replicate. Larvae were observed for mortality rate and survived individuals after treatments were maintained on semi synthetic diet (Shorey and Hale 1956) [38] at 25±1 °C temperature, 70±10% RH and 12h photo phase until pupation. All survived individuals were observed for records of the following biological parameters: Larval duration, pupation rate, pupal period, adult emergence rate, longevity, fecundity and hatchability.

For the semi synthetic diet investigation: four different concentrations (200, 300, 400 & 500 ppm) of silica, namely hydrophilic nano-silica, supplied by Nano Tech, silica, Diazinon and distilled water were applied on semi synthetic diet (Shorey and Hale 1956) [38] at 25±1 °C temperature, 70±10% RH and 12h photo, five replicate for each and 10 *Spodoptera littoralis* (Neonate, second and fourth instar) were maintained on till pupation.

Plant parameters

The treated plants were measured in its longevity for the major plant branch and number of leaves in comparison with the untreated plants.

Pesticide

Diazinon (IUPAC name: O, O-Diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate, INN – Dimpylate

Statistical analyses

Data were submitted to ANOVA, and the means were compared by the means-grouping test of Scott and Knott (1974) [37] at $P < 0.05$. And for concentration- mortality response curve for probit analysis Finny, (1952) [13] was used and Abbott, (1925) [2] for control death if any.

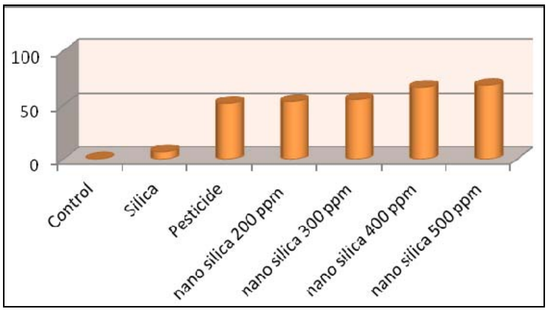
Results

1. The effect of feeding on squash foliage treated with different concentrations of nanosilica on the mortality of *Spodoptera littoralis* neonate

Mortality percentage

Neonate larvae feeding on plants treated with silica showed severe effect in its mortality, it gave 53.33, 55, 66.33 and 68.24 % with 200, 300, 400 and 500 ppm respectively while it gave 51.61% with Diazinon and no mortality with distilled water and only 6.8 % mortality with silicate (Fig 1)

A



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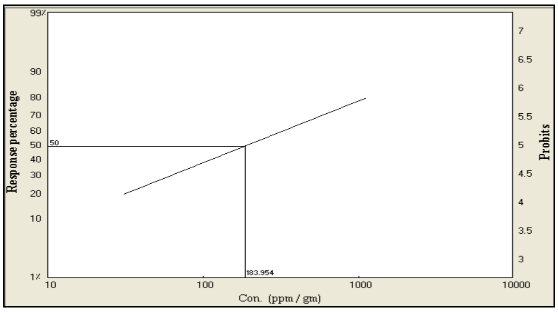

Fig 1. A. Mortality response (%) of *Spodoptera littoralis* neonate larvae exposed to squash leaves treated with four concentrations of nano silica, compared with silica, Diazinon and untreated control. LC_{50} Value.

Biological aspects

different parameters were investigated (Table 1, Fig 2) and the results of these parameters showed significant in both of larval and pupal malformation (Fig 2 b and d) where it gave zero % malformation for control, Diazinon as a recommended pesticide and Silica for both larvae and pupae but it gave 13, 11.9, 14 and 15% malformation in larvae and zero, 5, 3 and 11 % for pupae with 200, 300, 400 and 500 ppm nano silica, respectively. For hatchability pupae and larval duration (fig 2 a, c and f) there were no significant results as it is shown in table 1. for the egg laid / female parameter(fig 2e) only the Diazinon as a recommended pesticide affected this parameter with 244 egg/female while it gave 611, 623, 621, 641, 594 and 550 for Control, silica, 200 nm silica, 300 nm silica, 400 nm silica and 500 nm silica, respectively.

Table 1. Data of some biological parameter measurements of *Spodoptera littoralis* neonate larvae exposed to squash leaves treated with four concentrations of nano silica, compared with silica, Diazinon and untreated control.

	Larval duration	Larval malformation%	Pupal duration	Pupal malformation%	No. of eggs laid/female	Hatchability%
Control	16±0.10	0±0.00	9±0.40	0±0.00	611±0.40	73±0.50
Silica	15.9±0.20	0±0.00	9.2±0.80	0±0.00	623±0.10	69±0.70
Diazinone	16.1±0.70	0±0.00	9.1±0.50	0±0.00	244±0.70	40±40
Nano silica 200 ppm	16.1±0.20	13±0.20	8.9±0.80	0±0.00	621±0.40	70±0.80
Nano silica 300 ppm	16.2±0.10	11.9±0.20	9.1±0.70	5±0.70	641±0.40	67±40
Nano silica 400 ppm	16.1±0.90	14±0.70	9.2±0.50	3±0.20	594±0.70	63±0.80
Nano silica 500 ppm	15.8±0.90	15±0.90	9.4±0.20	11±0.90	550±0.40	66±0.70

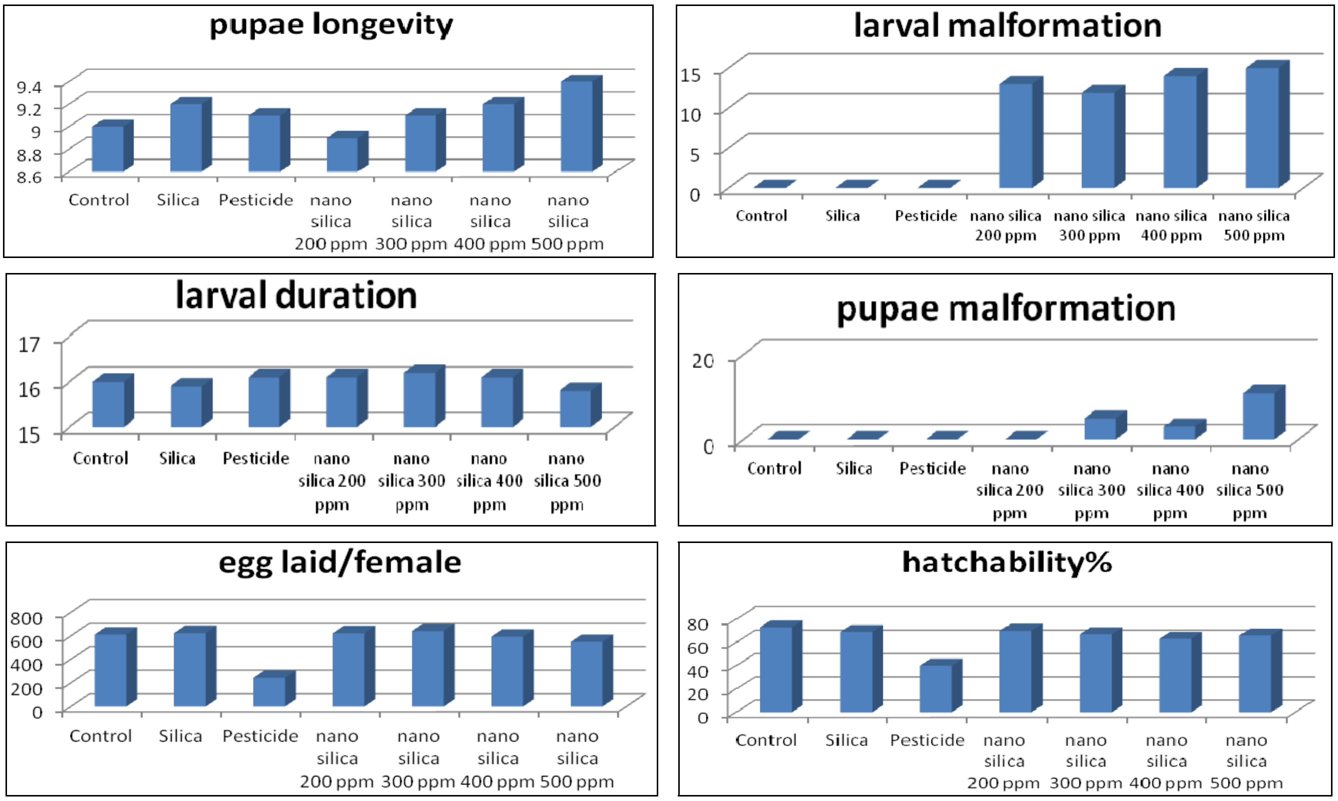

Fig 2: Data of some biological parameter measurements of *Spodoptera littoralis* neonate larvae exposed to squash leaves treated with four concentrations of nano silica, compared with silica, Diazinon and untreated control.

2. The effect of feeding on semi synthetic diet treated with different concentrations of nanosilica on the mortality of *Spodoptera littoralis* (Neonate, second and fourth instar)

Mortality percentage

The effect of four nano silica concentrations (200, 300, 400 and 500 nm) were investigated in three larval stages Neonate, second and fourth in stars as it shown in Tables 2, 3 and 4.

The effect of nano silica was greater in second instar than second and fourth instar where it gave 73.07, 79.722, 87.88 and 89.82% mortality percentage with neonates and 49.74, 56.87, 64.24 and 85.73% with second instar and 41.604, 46.178, 57.7 and 59.99% with fourth instar with 200, 300, 400 and 500 nm, respectively while it gave 95.95, 56.8 and 57.772% with Diazinon as a recommended pesticide and no mortality was recorded with silica or distilled water control.

Table 2: Mortality percentages among neonate larvae of *Spodoptera littoralis* tested with nano-silica after 15 days post emergence.

	Silica	Diazinon	200 ppm silica	300 ppm silica	400 ppm silica	500 ppm silica	control
R1	0	90	66.66	70	82.75	80	0
R2	0	100	62.06	93.33	76.66	93.33	0
R3	0	96.66	93.33	58.62	93.33	85.71	0
R4	0	100	70	90	90	93.33	0
R5	0	93.1	73.33	86.66	96.66	96.77	0
mean	0	95.95	73.07	79.722	87.88	89.82	0

Table 3: Mortality percentages among 2nd larvae of *Spodoptera littoralis* tested with nano-silica after 15 days post emergence or till pupation.

	Silica	Diazinon	200 ppm silica	300 ppm silica	400 ppm silica	500 ppm silica	control
R1	0	50	53.33	50	63.33	83.33	0
R2	0	53.33	53.33	51.61	65.51	86.66	0
R3	0	60.71	50	60	72.41	82.75	3.44
R4	0	56.66	62.06	62.06	56.66	86.66	0
R5	0	63.33	30	60.71	63.33	89.28	0
mean	0	56.8	49.74	56.87	64.24	85.73	0.688

Table 4: Mortality percentages among 4th larvae of *Spodoptera littoralis* tested with nano-silica after treatment and till pupation.

	Silica	Diazinon	200 ppm silica	300 ppm silica	400 ppm silica	500 ppm silica	control
R1	0	63.33	46.66	44.82	50	63.33	0
R2	0	50	41.37	37.93	63.33	60	0
R3	0	53.33	40	46.66	60	46.66	0
R4	0	63.33	36.66	44.82	55.17	66.66	0
R5	0	58.62	43.33	56.66	60	63.33	0
mean	0	57.772	41.604	46.178	57.7	59.99	0

Biological aspects

Different parameters were investigated (Table 5, Fig 3) as it is shown, the direct effect was obvious in larval duration where it gave 15.3, 15.4 and 15.2 days with control distilled water, silica and Diazinon as a recommended pesticide without any malformation except Diazinon as a recommended pesticide which gave 5 % while it gave duration 17, 18.1, 18.3 and 19.1 days with 200, 300, 400 and 500 nano silica concentrations, respectively with malformation increased gradually with concentration as follows 11, 13.7, 19.2 and 21%. The parameter of pupal duration also gave 9.1, 9.2 and 9.1 with Distilled water, silica and Diazinon as a recommended pesticide with malformation 6% with Diazinon as a recommended pesticide while it gave 14, 17, 14 and 15 days with 200, 300, 400 and 500% nano silica, respectively. And the malformation increased gradually as follows 7, 8, 12 and 11%. The last parameter was the mean of hatchability (hatched eggs/total laid eggs %) it gave 69, 70 and decreased to 40% with distilled water, silica and pesticide and gave 41, 44, 43 and 31 with silica concentrations.

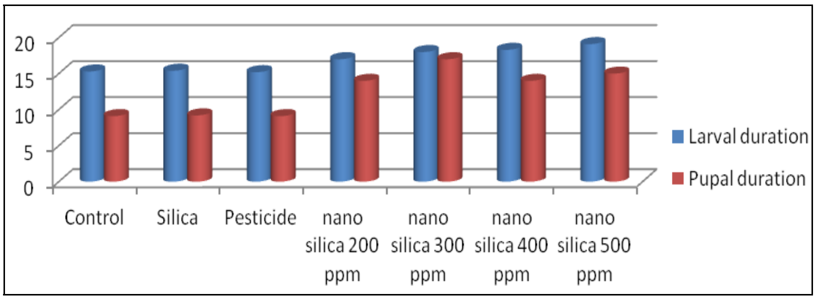

Fig 3: Larval duration and Pupae longevity with direct application of different nano silica concentrations against *Spodoptera littoralis* neonates

Table 5: Duration of the larval and pupal (days), Number of laid eggs and hatchability (%), larval and pupal malformation of treated and non-treated neonates of *Spodoptera littoralis* larvae.

	larval duration	larval malformation%	pupae longevity	pupae malformation%	hatchability%
Control	15.3±0.20	0±0.00	9.1±0.20	0±0.00	69±0.30
Silica	15.4±0.60	0±0.00	9.2±0.30	0±0.00	70±0.20
Diazinon	15.2±0.60	5±0.40	9.1±0.70	6±0.20	40±0.70
nano silica 200 ppm	17±0.30	11±0.20	14±0.30	7±0.30	41±0.60
nano silica 300 ppm	18±0.40	13.7±0.60	17±0.70	8±0.20	44±0.70
nano silica 400 ppm	18.3±0.60	19.2±0.90	14±0.20	12±0.60	43±0.20
nano silica 500 ppm	19.1±0.40	21±0.40	15±0.30	11±0.40	31±0.60

3. The effect of Nanosilica different concentrations on squash plant, The main stem of squash plant affected wit spraying silica nano particles gradually with concentration after two weeks of application as follows 70, 74, 77 and 76 with 200, 300, 400 and 500 silica nano particles in comparison with only 50, 47 and 54 with distilled water, silica and Diazinon as a recommended pesticide as it is shown in Fig 4

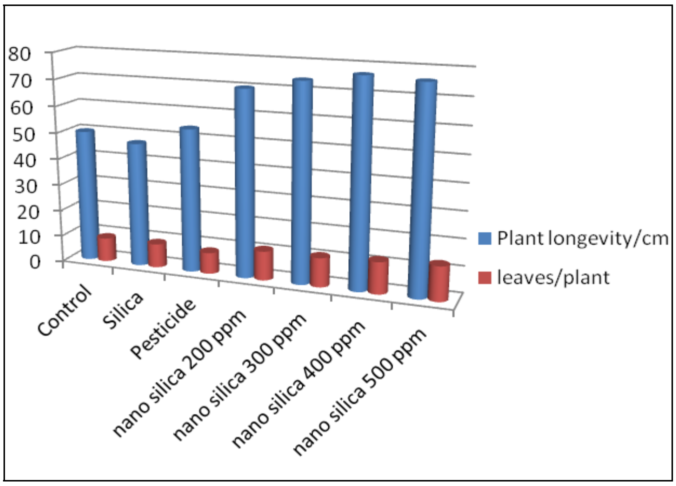

Fig 4: Plants longevity and leaves/plant with direct application of different nano silica concentrations after 15 days post application.

Also (fig 6) show that the number of leaves on plant increased with silica nano particles addition as follows 11, 11, 12 and 13 in comparison with 9, 9 and 8 leaves only with distilled water, silica and Diazinon as a recommended pesticide.

Table 6: Plants longevity and leaves/plant with direct application of different nano silica concentrations after 15 days post application

	Plant longevity/cm	leaves/plant
Control	50	9
Silica	47	9
Diazinon	54	8
nano silica 200 ppm	70	11
nano silica 300 ppm	74	11
nano silica 400 ppm	77	12
nano silica 500 ppm	76	13

Discussion

Silica is one of the most abundant materials on earth. It has different raw materials such as clay, quarts sand and rocks (Salleo *et al.* 2003, Che *et al.* 2003, Suzuki *et al.* 2004 and Harper 2010) [34, 8, 42, 18] nano silica has wide range of applications at several branches of science as industries (Iler 1979) [21] pharmacy (Sumper and Brunner 2006), entero-sorbent (Chuiko, 2003) [9] and recently as novel pesticide (Ulrichs *et al.* 2005, Peteu, 2010, Nitai, 2012 and Prasad *et al.* 2014) [32, 29, 27, 30]. Naturally occurring silica is considered to be safe for human consumption by different regulatory agencies worldwide. Silica nano particles could be effectively use as novel drug. The application of higher dose of nano silica was very effective as direct application which agreed with (Ulrichs *et al.* 2006) [33] who found that it deletes the cholesterol lipids which is used by the malarial parasite mainly for their intra erythrocyte growth besides. Besides (Hui-peng *et al.* 2006) [20] discovered that silica nano particles is the only viable option for controlling *BM NPV*. it was evident that silica nano particles based insecticide is physically active i.e. these nanocide cause damage to the cuticle water barrier of the insects mostly by abrasion and to some extent due to adsorption and insect death occurs due to desiccation (Harper, 2010)[18]. Efficacy of SNP as an insecticidal agent was tested on *sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Lipaphis pseudobrassicae* (Kaltenbach) (Hemiptera: Aphididae) and *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) by (Nitai 2012) [27] who found that the higher concentration 500 ppm gave 97.0, 96.00 and 95% Mortality percentage; Moreover, entomotoxicity of SNPs was compared with other oxide NPs like zinc oxide NP (ZNP), Titanium dioxide NP (TNP) and Aluminum oxide NP(ANP). 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, 2004 [40]). This work was compared with Silica which gave no reaction as distilled water this was disagreed with Jamin 1967[11] who found atoxicant role of silica in resistance to Asiatic rice borer, *Chilo suppressalis* (Walker), in rice varieties and (Salim *et al.* 1992) [35] who found the same results working on rice for (Goussain *et al*. 2002, Basagli *et al*. 2003 and Yadav *et al.*, 2010) [15, 4, 34] who found that silica approve and maintain the biotic and a biotic stress of plants and some other investigations have approved the same (Ma 2004, Massey *et al.*, 2006) [25, 26]. But in our investigation as it was shown in results it did not play any role and we refer that to the difference of tested plant and accumulation pathways in each plant. The results of SNPs were also compared with Diazinon pesticide which gave lower effect at LC_{50} level. For Diazinon functions as an acetylcholinesterase (AChE) inhibitor. This enzyme breaks down the neurotransmitter acetylcholine (ACh) into [choline] and an acetate group. The detoxification of diazoxon is processed through the mircosomal mixed function oxidase system. Although not fully known, it is believed that this is the cause for the selectivity of diazinon against insects. After the hydrolysis or oxidation diazinon is further degenerated (Budavari, 1996 and Geller *et al*, 2003). [14, 7]

This investigation recommends application of nanosilica at 500 ppm concentration for the suppression of *S. littoralis* on squash, in Egyptas which is agreed with our brevious report when we applied SNP against *Spodoptera littoralis* on tomato plants (Elbendary and El-Helaly 2013)

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