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Persistence and Efficacy of Two Nanosilica Formulations on *Callosobruchus maculatus* in Different Pulses

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ABSTRACT

The persistence effects of Silica Nanoparticles (SNPs), namely, Nanosav and Aerosil®, were evaluated on several pulses for controlling *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). For this purpose, one kg of each pulse was treated with four concentrations (50, 100, 200, and 300 mg kg-1) of each SNPs. The sampling was tested after 0, 2, 4, and 6 months' storage period following the treatment. Adults were introduced to these samples and the number of dead adults was counted 1, 2, and 4 days after the exposure to the treated pulses. The percentage decrease in F1 progeny was calculated 42 days later. The mortality was 100% in black gram, cowpea, green gram, and chickpea when treated at a concentration of 300 mg.kg-1 of both SNP formulations in 0-month post-treatment four days after the exposure. No progeny was observed in lentil treated with 300 mg kg-1 of Nanosav in 0-month post-treatment. Another experiment was conducted to calculate SNPs adherence to the seeds. The highest adherence was on the black gram with 86 and 99.5%, in Nanosav and Aerosil®, respectively. Our results indicated that two SNPs had insecticidal activity against *C. maculatus* and can be used effectively in integrated pest management program of *C. maculatus* in stored pulses.

Keywords: Aerosil nanosilica, Infestation, Integrated pest management, Nanosav nanosilica, Weevil.

INTRODUCTION

Due to the adverse effects of synthetic insecticides and fumigants, like ethyl formate plus methyl isothiocyanate (Ren *et al*., 2012) and Phosphine (Kostyukovsky *et al*., 2010), that have been applied for controlling *C. maculatus*, it is essential to find a promising alternative against infestations of *C. maculatus* in storage.

Nanoparticles can be used effectively against weeds, plant pathogens, and insect pests and can also be included in new formulations of pesticides and insect repellents (Barik *et al*. 2008) without the dangers of conventional chemical pesticides (Thabet *et al*. 2021). Nanopesticides have been a topic of interest in recent years and they can be applied as an appropriate alternative for the application of conventional insecticides for protecting stored grains (Athanassiou *et al*., 2018). Among nanopesticides, SNPs (silica nanoparticles) can satisfactorily protect stored products against Coleoptera stora pests, including *Sitophilus oryzae* (L.) (Curculionidae) (Debnath *et al*., 2011), *Tribolium castaneum* Herbst (Tenebrionidae) (Debnath *et al*., 2012; Sabbour and Abd El-Aziz, 2015), *Rhyzopertha dominica* (F.) (Bostrichidae) and *Tribolium confusum* du Val. (Tenebrionidae) (Ziaee and Ganji, 2016), *C. maculatus* (Arumugam *et al*., 2016; Rouhani *et al*., 2012), and *Callosobruchus chinensis* (L.) (Bruchinae) (Mesbah *et al*., 2017) on different commodities. The active ingredient of SNP is SiO_2. SNP acts the same as Diatomaceous Earth (DE), which absorbs insects' protective

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wax layer and causes death through desiccation and, to some extent, by abrasion (Debnath *et al*., 2011; Ziaee and Ganji, 2016). *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchina) is a cosmopolitan insect pest (Hill, 2002). Many species of this genus are feeding on stored, dry postharvest beans of the tribe Phaseoleae and are adapted to arid climate (Tuda *et al*. 2006).

The effects of two nanosilica formulations has been documented on *C. maculatus* oviposition deterrence, egg hatching rate, adult emergence, progeny reduction as well as weight loss of different pulses due to insect damage (Yousefnezhad Irani *et al*., 2018). To the best of our knowledge, the persistence of these formulations has not been studied against *C. maculatus.* Therefore, the present research was conducted to: (1) Evaluate the persistence of two SNP formulations on adults of *C. maculatus* on different pulse seeds for six months storage period, (2) Assess the efficacy of SNP formulations on *C. maculatus* F_1 progeny on different pulses, and (3) Evaluate the adhesion rate of SNPs to the pulse seeds.

MATERIALS AND METHODS

Insects

The adult insects were collected from the stock, then kept in the Entomology Laboratory of Plant Protection Department, Urmia University, Urmia, Iran. The insects were reared on cowpea (var. Mashhad) at 28°C, 55% RH, and 12:12 L:D conditions (Radha and Susheela, 2014). In all experiments, 1-day-old male and female adults were used (equal sex ratio), sex differentiation was performed according to Bandra and Saxena (1995).

Pulse Seeds

The pulses tested in the bioassays were cowpea (var. Mashhad), chickpea (var. Adel), lentil, green gram, and black gram. Clean, infestation- and pesticide-free pulse seeds were stored at –20°C for two days to ensure that all lived stages were died (Obeng-Ofori *et al*., 1997).

Nanosilica Formulations

Two Silica Nanoparticles (SNPs) were utilized in the bioassays. Nanosav SNP was obtained from Nanosav Co., with the average particle diameter was 20–30 nm. Nanosav SNP contains SiO_2> 98%, 0.328% Na as Na_2O, 0.393% Ca as CaO, 0.294% Fe as Fe_2O_3, 0.185% sulfate as SO_3, and loss on ignition < 2%. Aerosil® was purchased from Evonik Industries AG, Germany, with average particle diameter of 12 nm. Aerosil® contains SiO_2> 99%, Ti< 120 ppm, Ca< 70 ppm, Na< 50 ppm, and Fe< 20 ppm.

Bioassays

Insecticidal Efficacy and Persistence of SNPs against *C. maculatus* Adults

The insecticidal efficacy and persistence of SNPs were tested as reported by Vayias *et al*. (2010) with some changes. Each pulse variety was treated with SNP formulations at conc. 50, 100, 200, and 300 mg kg^{-1} in 2.4 L glass jars. The jars were sealed and shaken by hands for five minutes to achieve equal dispersion and they were allowed to stand for 30 minutes to settle down the particles. The untreated lots of pulses were served as control. Pulse samples were taken on the same day of storage and 2, 4, and 6-month post-treatment at 28°C and 55% RH. Three samples of 80 g (sub-replications) were removed from each jar and poured into glass vials (300 mL volume). Then, ten pairs of 1-day-old adults (equal sex ratio) were introduced to each vial. The vials were enclosed with lace fabric for adequate ventilation, andthey were incubated at 28°C, 55% RH, and 12:12 L:D conditions. The mortality (insects with no movement) was recorded 1, 2, and 4 days after releasing adults in the vials. The entire experiment was repeated three times (true replications) and the bioassays were repeated over a 6-month storage period.

Effect of SNPs on F_1 Progeny of *C. maculatus* on Different Pulses

To evaluate F_1 progeny, after assessing the mortality, glass vials were kept undisturbed for 15 days for egg-laying. Afterward, all adults were discarded and vials were maintained under experimental conditions for 42 days to assess F1 progeny production.

Adherence of SNPs to Different Pulses

The adherence of SNPs to different pulses was determined by the method described by Korunic (1997). Lots of 1 kg of each pulse type were sieved for one min through a 2 mm mesh sieve (Damavand Co.) to remove any finer particles. Then, three samples of each pulse (250 g) were treated with 300 mg kg-1 of SNP formulations in glass vials, and the vials were manually shaken for five min and kept undisturbed for 30 min to settle down the particles. The treated seeds were sieved for five min. The particles collected from the bottom paper were weighed using an analytical balance (Sartorius CPA 224S, Germany) with 0.1 mg precision, as the second weight of the SNPs and subtracted from the first weight. Subsequently, the percentage of SNPs adherence to the seeds was calculated.

Statistical Analysis

Percentage data were transformed using arcsin and progeny number was logarithmically [log(x+1)] transformed. Abbott's formula (1925) was used to correct mortality. The means were compared using the Tukey-Kramer's test (HSD) at $P < 0.05$. For each pulse, the mortality was submitted to Repeated Measures MANOVA with two repeated factors (post-treatment time with four levels and exposure time with three levels) and two main effects (formulation and concentration). Progeny data were submitted to Repeated Measures MANOVA with the mean number of emerged adults as the response variable and the post-treatment time as the repeated factor. At the same time, different pulse seeds, formulation, and concentration were the main effects. The associated interactions of the main effects were included in the analysis. The adherence test results were analyzed using one-way ANOVA with different pulse seeds as the main effect; also, the mean comparison between two SNP formulations for each pulse seed was performed by independent sample t-Test at P< 0.05. SPSS version 22 was used for all analyses (SPSS, 2013).

RESULTS

Insecticidal Efficacy and Persistence of SNPs against *C. maculatus* Adults

According to data shown in Table 1, the main effects and the interactions were significant for *C. maculatus* adults, except for exposure time×SNP on cowpea and post-treatment time×SNP on lentil. Aerosil® caused significantly higher mortality in black gram in comparison with Nanosav. In all exposures of 0-month post-treatment, 300 mg kg-1 for both formulations caused complete insect mortality. However, there was no mortality when adults were exposed to 50 mg kg-1 of Nanosav for one day at the 6-month storage period (Table 2).

For cowpea treated with both SNPs, the insect mortality reached 100% after two days exposure to the highest concentration of 300 mg kg-1 at 0-month post-treatment. The mortality decreased to 86 and 99% for Nanosav and Aerosil® after four days of exposure, respectively (Table 3).

For green gram-treated with SNPs, there was complete mortality when adults were exposed to 300 mg kg-1 of both formulations 2 and 4 days after exposure of 0-month post- treatment. After four days of 6-month post-treatment period, Nanosav and Aerosil® provided 90.6 and 96% mortality, respectively (Table 4).

The insect mortality on chickpea treated with 200 and 300 mg kg-1 of both formulations was 100% after four days

Table 1. MANOVA parameters for mortality levels of *C. maculatus* adults (Repeated factors: Post-treatment time and exposure time).

Source of variation	df	Black gram		Cowpea		Green gram		Chickpea		Lentil	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
SNP	1	176.5	< 0.001	351.2	< 0.001	256.9	< 0.001	266.1	< 0.001	926.2	< 0.001
Concentration	3	1314.0	< 0.001	1982.0	< 0.001	1861.7	< 0.001	789.5	< 0.001	2508.4	< 0.001
SNP × Concentration	2	11.5	< 0.001	3.1	0.049	4.7	0.01	4.6	0.01	15.5	< 0.001
Post-treatment time	3	3274.3	< 0.001	1749.6	< 0.001	2141.8	< 0.001	1357.5	< 0.001	1920.9	< 0.001
Post-treatment time×SNP	3	6.9	< 0.001	10.5	< 0.001	9.8	< 0.001	12.3	< 0.001	1.8	0.1
Post-treatment time×Concentration	9	51.2	< 0.001	38.2	< 0.001	16.9	< 0.001	29.4	< 0.001	22.4	< 0.001
Exposure time	2	6185.1	< 0.001	6503.3	< 0.001	11491.3	< 0.001	6121.9	< 0.001	7587.5	< 0.001
Exposure time×SNP	2	14.7	< 0.001	1.0	0.4	17.5	< 0.001	20.7	< 0.001	164.4	< 0.001
Exposure time×Concentration	6	143.0	< 0.001	84.5	< 0.001	187.0	< 0.001	54.4	< 0.001	44.5	< 0.001
Post-treatment time×Exposure time	6	34.9	< 0.001	44.2	< 0.001	25.1	< 0.001	26.2	< 0.001	47.5	< 0.001

Table 2. Percent mortality (Mean±SE) of *C. maculatus* adults on black gram treated with different concentrations of Nanosav and Aerosil®.[a]

Post-treatment time (Month)	Exposure time (Day)	Nanosilica/Concentration (mg kg^{-1})							
		Nanosav				Aerosil®			
		50	100	200	300	50	100	200	300
0	1	16.7±0.8^{Gfg}	25.0±0.8^{Fg}	85.0±1.9^{Cc}	100.0±0.0^{Aa}	31.7±0.8^{Ed}	66.7±0.8^{Dc}	93.3±0.8^{Bbc}	100.0±0.0^{Aa}
	2	58.3±0.8^{Db}	75.0±1.9^{Bc}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	68.3±0.8^{Cb}	96.7±0.8^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
	4	76.1±0.9^{Ca}	96.3±0.9^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	89.0±2.2^{Ba}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
2	1	8.3±0.8^{Gh}	13.3±0.8^{Fh}	66.7±0.8^{De}	88.3±0.8^{Bb}	16.7±0.8^{EFe}	20.0±1.2^{Ef}	78.3±0.8^{Cd}	96.7±0.8^{Ab}
	2	36.7±0.8^{Fd}	61.7±0.8^{Dd}	91.7±0.8^{Bb}	100.0±0.0^{Aa}	51.7±0.8^{Ec}	80.0±1.2^{Cb}	96.7±0.8^{Aab}	100.0±0.0^{Aa}
	4	60.9±2.8^{Db}	81.4±0.9^{Bb}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	73.9±0.9^{Cb}	96.3±0.9^{Aa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
4	1	5.0±0.0^{Fhi}	10.0±1.2^{Fhi}	50.0±1.9^{Df}	80.0±2.0^{Bc}	8.3±0.8^{Ff}	16.7±0.8^{Efg}	66.7±0.8^{Ce}	88.0±0.8^{Ac}
	2	23.3±0.8^{Ge}	50.0±1.9^{Ee}	75.0±1.9^{Cd}	91.7±0.8^{Bb}	35.0±2.0^{Fd}	65.0±2.0^{Dcd}	88.3±0.8^{Bc}	100.0±0.0^{Aa}
	4	43.0±2.1^{Fc}	62.0±0.9^{Dd}	92.4±0.9^{Bb}	100.0±0.0^{Aa}	54.4±2.3^{Ec}	79.1±0.9^{Cb}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
6	1	0.0±0.0^{Fi}	6.7±0.8^{Ei}	33.3±0.8^{Cg}	55.0±2.0^{Bd}	3.3±0.8^{EFf}	13.3±0.8^{Dg}	50.0±1.9^{Bf}	66.7±0.8^{Ad}
	2	15.0±1.2^{Gf}	33.3±0.8^{Ff}	63.3±0.8^{De}	83.3±0.8^{Bc}	20.0±1.2^{Ge}	48.3±0.8^{Ee}	75.0±2.5^{Cd}	96.7±0.8^{Ab}
	4	22.6±1.0^{Gfe}	45.8±1.0^{Ee}	80.0±0.4^{Ccd}	100.0±0.0^{Aa}	36.1±2.9^{Fd}	61.3±1.0^{Dd}	92.3±1.0^{Bbc}	100.0±0.0^{Aa}

[a] Means followed by the same uppercase letter in each row and lower case letter for each column are not significantly different (Tukey HSD test at P= 0.05).

Table 3. Percent mortality (Mean±SE) of *C. maculatus* adults on cowpea treated with different concentrations of Nanosav and Aerosil®.[a]

Post-treatment time (Month)	Exposure time (Day)	Nanosilica/Concentration (mg kg^{-1})							
		Nanosav				Aerosil®			
		50	100	200	300	50	100	200	300
0	1	6.7±0.8^{Gh}	31.7±0.8^{Eef}	80.0±1.9^{Cb}	90.0±0.2^{Bb}	13.3±0.8^{Ff}	40.6±1.5^{Df}	93.3±0.8^{ABb}	96.7±0.8^{Ab}
	2	33.3±0.8^{Fe}	65.0±2.0^{Dc}	93.3±0.8^{Ba}	100.0±0.0^{Aa}	53.3±0.8^{Ec}	78.3±0.8^{Cb}	98.3±0.8^{Aa}	100.0±0.0^{Aa}
	4	75.0±0.9^{Ea}	89.3±2.0^{Ca}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	82.0±0.1^{Da}	94.0±1.9^{Ba}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
2	1	5.0±0.0^{Fhi}	16.7±0.8^{Egh}	61.7±0.8^{Ce}	75.0±2.0^{Bd}	10.0±0.0^{Ff}	25.0±1.9^{Dg}	75.0±1.9^{Be}	83.3±0.8^{Ad}
	2	21.7±0.8^{Gf}	50.0±1.9^{Ed}	78.3±0.8^{Cbc}	90.0±0.2^{Bb}	31.7±0.8^{Fd}	60.6±1.5^{Dd}	88.0±0.2^{Bc}	100.0±0.0^{Aa}
	4	67.3±2.0^{Db}	78.2±2.0^{Cb}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	76.4±0.9^{Ca}	89.1±2.2^{Ba}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
4	1	1.7±0.8^{Fhi}	10.0±1.2^{Ehi}	35.0±1.9^{Dg}	60.0±1.9^{Be}	8.3±0.8^{Efg}	13.3±0.8^{Eh}	50.0±1.2^{Cg}	75.0±0.2^{Ae}
	2	13.3±0.8^{Gg}	38.3±0.8^{Ee}	66.7±0.8^{Cde}	83.3±0.8^{Bc}	21.7±0.8^{Fe}	51.7±1.7^{De}	80.4±0.2^{Bd}	90.0±0.2^{Ac}
	4	57.4±0.9^{Dc}	66.7±3.5^{Cc}	81.5±0.9^{Bb}	96.3±0.9^{Aa}	66.7±2.8^{Cb}	81.5±0.9^{Bb}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
6	1	0.8±0.0^{Ei}	5.0±1.2^{DEi}	20.0±1.9^{Ch}	33.3±0.8^{Bf}	3.3±0.8^{DEg}	6.7±0.8^{Dh}	30.0±2.0^{Bh}	49.6±0.6^{Af}
	2	6.7±0.8^{Gh}	25.0±2.0^{Efg}	54.0±0.2^{Cf}	75.0±3.1^{Bd}	13.3±0.8^{Ff}	38.3±0.8^{Df}	70.0±0.2^{Bf}	82.0±0.2^{Ad}
	4	42.7±2.3^{Ed}	56.0±0.9^{Dd}	71.3±3.6^{Ccd}	86.0±0.2^{Bbc}	54.1±2.5^{Dc}	69.4±1.0^{Cc}	90.4±1.0^{Bbc}	99.0±0.1^{Aa}

[a] Means followed by the same uppercase letter in each row and lower case letter for each column are not significantly different (Tukey HSD test at $P = 0.05$).

Table 4. Percent mortality (Mean±SE) of *C. maculatus* adults on green gram treated with different concentrations of Nanosav and Aerosil®.[a]

Post-treatment time (Month)	Exposure time (Day)	Nanosilica/Concentration (mg kg^{-1})							
		Nanosav				Aerosil®			
		50	100	200	300	50	100	200	300
0	1	5.0±1.2^{Ffg}	8.3±0.8^{EFgh}	31.7±0.8^{Dg}	73.3±0.8^{Bd}	8.3±0.8^{EFg}	11.7±0.8^{Ei}	55.0±1.9^{Ch}	83.3±0.8^{Ad}
	2	25.0±2.0^{Ge}	58.3±0.8^{Ed}	83.3±0.8^{Cc}	100.0±0.0^{Aa}	33.3±0.8^{Fe}	71.7±0.8^{Dd}	91.7±0.8^{Bbc}	100.0±0.0^{Aa}
	4	85.5±0.9^{Da}	94.5±1.8^{BCa}	98.2±0.9^{ABa}	100.0±0.0^{Aa}	90.9±0.9^{Ca}	96.4±0.9^{ABa}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
2	1	3.3±0.8^{Fg}	6.7±0.8^{EFh}	23.3±0.8^{Dh}	48.3±0.8^{Be}	6.7±0.8^{EFg}	8.3±0.8^{Eij}	41.7±0.8^{Ci}	75.0±1.9^{Ae}
	2	10.0±0.0^{Gf}	38.3±0.8^{Ee}	75.0±3.0^{Cd}	91.7±0.8^{Ab}	21.7±0.8^{Ff}	56.7±0.8^{Df}	83.3±0.8^{Bde}	95.0±1.2^{Ab}
	4	63.0±0.9^{Eb}	81.5±0.9^{Cb}	90.7±0.9^{Bb}	100.0±0.0^{Aa}	72.2±2.1^{Db}	90.7±0.9^{Bb}	98.1±0.9^{Aab}	100.0±0.0^{Aa}
4	1	0.0±0.0^{Eg}	3.3±0.8^{DEhi}	16.7±0.8^{Ci}	31.7±0.8^{Bf}	0.0±0.0^{Eh}	5.0±0.8^{Dj}	33.3±0.8^{Bj}	65.0±1.9^{Af}
	2	3.3±0.8^{Gg}	16.7±0.8^{Ff}	58.3±0.8^{De}	83.3±0.8^{Bc}	6.7±0.8^{Gg}	26.7±0.8^{Eg}	75.0±3.1^{Cf}	90.0±1.2^{Ac}
	4	49.4±2.1^{Fc}	68.1±0.9^{Dc}	75.6±0.9^{Cd}	100.0±0.0^{Aa}	60.6±2.1^{Ec}	77.5±2.3^{Cc}	88.7±1.3^{Bcd}	100.0±0.0^{Aa}
6	1	0.0±0.0^{Dg}	0.0±0.0^{Di}	13.3±0.8^{Ci}	23.3±0.8^{Bg}	0.0±0.0^{Dh}	0.0±0.0^{Dk}	21.7±0.8^{Bk}	56.7±0.8^{Ag}
	2	3.3±0.8^{Gg}	13.3±0.8^{Ffg}	50.0±1.9^{Df}	75.0±2.0^{Bd}	5.0±0.0^{Ggh}	21.7±0.8^{Eh}	66.7±0.8^{Cg}	83.3±0.8^{Ad}
	4	39.6±0.9^{Fd}	43.4±2.1^{EFe}	70.2±0.7^{Cd}	90.6±0.9^{Ab}	49.0±2.1^{Ed}	62.3±0.9^{De}	78.6±1.8^{Bef}	96.2±0.9^{Aab}

[a] Means followed by the same uppercase letter in each row and lower case letter for each column are not significantly different (Tukey HSD test at $P= 0.05$).

exposure of 0-month post-treatment, and it decreased to 78% for Nanosav in the 6-month post-treatment period (Table 5).

The insecticidal efficacy of Nanosav formulation was significantly higher in lentil than the corresponding mortality caused by Aerosil®. The concentrations of 200 and 300 mg kg-1 of Nanosav caused complete mortality after four days exposure of 0-month post-treatment period and it decreased to 69 and 98% after six months of storage for lentil (Table 6).

Effect of SNPs on F_1 Progeny of *C. maculatus* on Different Pulses

In all pulses, the highest number of F1 progeny was reported in the control when compared with other treatments. In all pulses, except for lentil, Aerosil® significantly decreased the number of *C. maculatus* progeny, in comparison with Nanosav (Table 7).

Adherence of SNPs on Different Pulses

In all pulses, Aerosil® demonstrated higher adherence on seeds, in comparison with Nanosav. The highest adherence was found in black gram treated with Aerosil® and Nanosav with the mean of 99.5 and 86%, respectively, while the lowest adherence was found in Nanosav-treated chickpea with the mean of 66.9% (Table 8).

DISCUSSION

The results of the present study showed that, among all tested pulses, the mortality of adults was increased by increasing the concentration and time exposure to each concentration. Debnath *et al*. (2011) reported that toxicity of different SNPs was increased by increasing the application rate against *S. oryzae*. Besides, Rouhani *et al*. (2012) illustrated that prolonged exposure of *C. maculatus* to the higher rates (2 g kg-1) of SNPs led to satisfactory protection of cowpea seeds. Also, Badii *et al*. (2013) found that the mortality of *C. maculatus* adults in peanut treated with DEs formulations: Diatomenerde, Probe-A, Fossilshield, and Damol-D1 was increased by increasing the concentration. This may result from the fact that in higher concentrations and more prolonged exposure, the body of insects has more contact with the particles and, consequently, picks up more particles, which results in more absorption of wax layer, and mortality (Shah and Khan, 2014).

It was found that *S. oryzae* adult mortality was significantly increased when the particle size of SNPs was decreased from 100-400 to 15-30 nm (Debnath *et al*., 2011). A similar observation has been found by Arumugam *et al*. (2016) when different pulse seeds were treated with nanostructured silica (with particle size smaller than 12 nm) against *C. maculatus.* The toxicity of nanostructured silica, even in low concentration, was increased by decreasing the particle size. According to our results, in all pulse seeds, except for lentil, Aerosil® provided adequate control against *C. maculatus*, compared to Nanosav. The mean particle size of Aerosil® and Nanosav was 12 and 20-30 nm; respectively. Ziaee and Ganji (2016) reported that the insecticidal efficacy of Aerosil® on wheat and peeled barely was higher in comparison with Nanosav when applied for controlling *R. dominica* and *T. confusum* adults. The increasing surface-to-volume ratio in nano-insecticides facilitates insecticide penetration, increases adhesion of particles to insects' cuticles, and, consequently, increases mortality (Hussein *et al*., 2002).

According to our results, the most effectiveness of both SNPs against *C. maculatus* was observed on the black gram and the lowest was on lentil, followed by chickpea. Kavallieratos *et al*. (2010) assessed the insecticidal activity of three commercial DE formulations, including Protector, SilicoSec, and Insecto, in three wheat cultivars (Athos, Pontos, Sifnos)

Table 5. Percent mortality (Mean±SE) of *C. maculatus* adults on chickpea treated with different concentrations of Nanosav and Aerosil®.^{a}

Post-treatment time (Month)	Exposure time (Day)	Nanosilica/Concentration (mg kg^{-1})							
		Nanosav				Aerosil®			
		50	100	200	300	50	100	200	300
0	1	10.0±1.2^{Fef}	21.7±0.8^{Eg}	41.7±0.8^{Cg}	55.0±1.9^{Be}	13.3±0.8^{Ffg}	33.3±0.8^{Dg}	50.0±2.0^{Be}	66.7±0.8^{Ad}
	2	14.8±0.8^{Gde}	48.9±2.1^{Ed}	74.4±2.1^{Cc}	83.0±0.8^{Bb}	23.3±2.1^{Fe}	62.5±0.8^{Dd}	83.0±0.8^{Bb}	94.9±1.2^{Aab}
	4	63.6±0.9^{Ea}	81.8±0.9^{Ca}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	72.7±3.4^{Da}	90.9±0.9^{Ba}	100.0±0.0^{Aa}	100.0±0.0^{Aa}
2	1	6.7±0.8^{Ffg}	13.3±0.8^{Eh}	33.3±0.8^{Ch}	41.7±0.8^{Bf}	10.0±1.2^{EFfgh}	25.0±2.0^{Dh}	41.7±0.8^{Bf}	55.0±1.9^{Ae}
	2	10.2±0.8^{Fef}	40.7±0.8^{Ee}	66.1±0.8^{Cd}	74.6±2.7^{Bc}	16.9±0.8^{Eef}	52.5±0.8^{De}	74.6±2.7^{Bc}	89.8±2.1^{Ab}
	4	46.4±2.0^{Db}	67.9±2.0^{Cb}	85.7±0.9^{Bb}	96.4±0.9^{Aa}	62.5±2.2^{Cb}	82.1±0.9^{Bb}	96.4±0.9^{Aa}	100.0±0.0^{Aa}
4	1	5.0±1.2^{Eg}	10.0±0.0^{Dhi}	21.7±0.8^{Ci}	33.3±0.8^{Bg}	8.3±0.8^{DEgh}	18.3±0.8^{Ci}	30.0±1.2^{Bg}	41.7±0.8^{Af}
	2	7.3±0.8^{Ffg}	32.6±0.8^{Ef}	49.4±1.2^{Cf}	64.6±2.5^{Bd}	12.4±0.8^{Ffg}	42.7±0.8^{Df}	64.0±0.2^{Bd}	74.7±2.1^{Ac}
	4	35.1±0.9^{Ec}	56.1±0.9^{Dc}	73.7±2.1^{Cc}	84.2±2.0^{Bb}	53.0±0.4^{Dc}	72.0±0.4^{Cc}	88.0±0.4^{Bb}	100.0±0.0^{Aa}
6	1	3.3±0.8^{Dg}	6.7±0.8^{Di}	15.0±1.2^{Cj}	25.0±1.2^{Bh}	5.0±0.0^{Dh}	13.3±0.8^{Cj}	21.7±0.8^{Bh}	35.0±2.0^{Ag}
	2	5.0±0.0^{Eg}	25.0±2.0^{Dg}	33.3±0.8^{Ch}	50.0±2.0^{Be}	8.3±0.8^{Egh}	33.3±0.8^{Cg}	50.0±2.0^{Be}	66.7±0.8^{Ad}
	4	19.0±0.9^{Hd}	39.7±0.9^{Ge}	56.9±0.9^{Ee}	78.0±0.2^{Bbc}	43.0±0.4^{Fd}	62.0±0.4^{Dd}	70.0±0.4^{Ccd}	100.0±0.0^{Aa}

^{a} Means followed by the same uppercase letter in each row and lower case letter for each column are not significantly different (Tukey HSD test at P= 0.05).

Table 6. Percent mortality (Mean±SE) of *C. maculatus* adults on lentil treated with different concentrations of Nanosav and Aerosil®.^{a}

Post-treatment time (Month)	Exposure time (Day)	Nanosilica/Concentration (mg kg^{-1})							
		Nanosav				Aerosil®			
		50	100	200	300	50	100	200	300
0	1	5.0±0.0^{FGghi}	15.0±0.8^{Eh}	50.0±1.2^{Cf}	88.3±0.8^{Ab}	4.0±0.0^{Ggh}	8.3±0.8^{Fef}	33.3±0.8^{De}	66.7±0.8^{Bd}
	2	16.7±0.8^{Ee}	55.0±1.2^{Cd}	75.0±1.9^{Bd}	91.7±0.8^{Ab}	13.3±0.8^{Ee}	25.0±1.2^{Dd}	58.3±0.8^{Cc}	80.0±1.2^{Bb}
	4	69.0±1.5^{Da}	87.9±0.9^{Ca}	100.0±0.0^{Aa}	100.0±0.0^{Aa}	56.9±0.9^{Ea}	67.2±0.9^{Da}	89.7±1.9^{BCa}	93.1±0.9^{Ba}
2	1	4.0±0.0^{FGhi}	11.7±0.8^{Ehi}	41.7±0.8^{Cg}	80.0±1.2^{Ac}	1.7±0.8^{Ghi}	6.7±0.8^{Ffg}	21.7±0.8^{Df}	58.3±0.8^{Be}
	2	10.0±0.0^{Ff}	33.3±0.8^{Ef}	66.7±0.8^{Ce}	84.0±1.1^{Ac}	8.3±0.8^{Ff}	11.7±0.8^{Fe}	50.0±1.2^{Dd}	71.7±0.8^{Bc}
	4	56.9±0.9^{Eb}	79.3±1.2^{Cb}	91.4±0.9^{Bb}	100.0±0.0^{Aa}	41.4±0.9^{Gb}	50.0±0.9^{Fb}	74.1±1.2^{Db}	82.8±0.9^{Cb}
4	1	3.3±0.8^{Fhi}	8.3±0.8^{Ei}	33.3±0.8^{Ch}	66.7±0.8^{Ae}	0.0±0.0^{Gi}	5.0±0.0^{Ffg}	16.7±0.8^{Dfg}	41.7±0.8^{Bg}
	2	8.3±0.8^{Ffg}	25.0±1.2^{Eg}	50.0±1.2^{Cf}	75.0±0.2^{Ad}	6.7±0.8^{Ffg}	8.3±0.8^{Fef}	31.7±0.8^{De}	58.3±0.8^{Be}
	4	41.4±0.9^{Fc}	62.1±0.9^{Dc}	82.8±0.9^{Bc}	100.0±0.0^{Aa}	27.6±1.2^{Gc}	39.7±0.9^{Fc}	56.9±0.9^{Ec}	70.7±0.9^{Ccd}
6	1	1.7±0.8^{Fi}	6.7±0.8^{Ei}	21.7±0.8^{Ci}	50.0±2.0^{Af}	0.0±0.0^{Fi}	3.3±0.8^{EFg}	13.3±0.8^{Dg}	33.3±0.8^{Bh}
	2	6.7±0.8^{Ffgh}	16.7±0.8^{Eh}	36.7±0.8^{Cgh}	65.0±0.2^{Ae}	5.0±0.0^{Ffgh}	6.7±0.8^{Ffg}	20.0±0.8^{Df}	48.3±0.8^{Bf}
	4	31.0±0.9^{Ed}	48.3±1.9^{De}	69.0±1.9^{Be}	98.3±0.9^{Aa}	19.0±0.9^{Fd}	25.9±0.9^{De}	48.3±1.9^{Dd}	56.9±0.9^{Ce}

^{a} Means followed by the same uppercase letter in each row and lower case letter for each column are not significantly different (Tukey HSD test at P= 0.05).

Table 7. Number of progenies (Mean±SE) of *C. maculatus* exposed on different pulses treated with different concentrations of Nanosav and Aerosil®.[a]

Post-treatment time (Month)	Pulses	Control	Nanosilica/Concentration (mg kg^{-1})							
			Nanosav				Aerosil®			
			50	100	200	300	50	100	200	300
0	Blackgram	185.7 ± 0.2^{Ak}	75.0 ± 0.4^{Bm}	66.0 ± 0.5^{Cl}	20.0 ± 0.2^{Fk}	4.0 ± 0.4^{Hlmn}	50.0 ± 0.6^{Dn}	27.3 ± 0.2^{Elm}	11.0 ± 0.2^{Glm}	2.0 ± 0.2^{Ilm}
	Cowpea	219.7 ± 1.3^{Ai}	166.7 ± 1.1^{Bi}	128.3 ± 1.0^{Di}	50.7 ± 1.1^{Fh}	27.0 ± 0.9^{Gi}	139.0 ± 0.9^{Ci}	100.0 ± 1.4^{Ei}	15.0 ± 0.2^{Hjk}	4.0 ± 0.2^{Ikl}
	Greengram	257.3 ± 1.2^{Ag}	173.3 ± 1.5^{Bh}	133.3 ± 1.5^{Dhi}	81.7 ± 0.9^{Fe}	50.0 ± 1.2^{He}	158.3 ± 1.0^{Cgh}	106.7 ± 1.1^{Eh}	69.3 ± 0.7^{Gd}	45.3 ± 1.0^{Hd}
	Chickpea	106.7 ± 1.3^{An}	43.3 ± 0.8^{Bn}	26.7 ± 1.0^{Cn}	13.0 ± 0.2^{El}	3.0 ± 0.2^{Gmno}	30.0 ± 0.9^{Co}	16.7 ± 0.8^{Dn}	9.0 ± 0.2^{Fm}	1.4 ± 0.2^{Glm}
	Lentil	20.0 ± 0.3^{Aq}	11.7 ± 0.5^{Cp}	4.3 ± 0.2^{Ep}	2.0 ± 0.2^{Fn}	0.0 ± 0.0^{Go}	13.3 ± 0.4^{Bq}	9.0 ± 0.2^{Do}	4.0 ± 0.3^{En}	1.0 ± 0.3^{Fm}
2	Blackgram	286.3 ± 1.2^{Af}	199.0 ± 1.2^{Bg}	136.7 ± 1.3^{Dh}	77.0 ± 0.4^{Ff}	32.0 ± 0.7^{Hh}	157.0 ± 0.9^{Ch}	116.7 ± 0.7^{Eg}	40.0 ± 0.4^{Gg}	20.0 ± 0.4^{Ih}
	Cowpea	293.0 ± 0.9^{Ae}	237.0 ± 0.6^{Be}	163.3 ± 0.4^{Df}	91.7 ± 0.2^{Fd}	56.7 ± 0.9^{Gd}	179.0 ± 1.2^{Cf}	133.3 ± 0.2^{Ef}	50.0 ± 0.4^{Hf}	30.0 ± 0.4^{Ig}
	Greengram	329.3 ± 0.7^{Ac}	243.3 ± 1.1^{Bd}	220.7 ± 1.3^{Cd}	130.7 ± 1.6^{Fc}	60.0 ± 0.4^{Hd}	200.7 ± 0.2^{Dd}	156.7 ± 1.6^{Ed}	83.3 ± 1.0^{Gc}	50.0 ± 1.2^{Ic}
	Chickpea	128.3 ± 1.0^{Am}	73.7 ± 0.9^{Bm}	43.3 ± 1.0^{Dm}	24.0 ± 0.2^{Fjk}	13.0 ± 0.4^{Hk}	55.3 ± 1.0^{Cm}	26.7 ± 0.9^{Em}	19.0 ± 0.4^{Gi}	9.0 ± 0.2^{Ij}
	Lentil	33.3 ± 0.9^{Ap}	16.7 ± 0.4^{Dop}	10.0 ± 0.2^{Fo}	6.7 ± 0.3^{Gm}	4.0 ± 0.2^{Hlmn}	23.3 ± 0.7^{Bp}	20.0 ± 0.6^{Cn}	13.0 ± 0.2^{Ekl}	6.0 ± 0.2^{Gk}
4	Blackgram	310.0 ± 1.2^{Ad}	220.0 ± 1.0^{Bf}	155.3 ± 1.0^{Dg}	83.3 ± 1.2^{Fe}	56.7 ± 0.9^{Gd}	162.7 ± 0.9^{Cg}	130.0 ± 0.6^{Ef}	52.7 ± 1.2^{Gf}	40.0 ± 0.4^{He}
	Cowpea	409.3 ± 1.9^{Ab}	360.0 ± 1.5^{Bb}	302.7 ± 1.3^{Cb}	134.0 ± 1.1^{Fc}	70.3 ± 1.2^{Gc}	268.7 ± 1.3^{Db}	210.7 ± 1.3^{Ec}	70.7 ± 0.4^{Gd}	42.0 ± 0.6^{He}
	Greengram	429.0 ± 1.5^{Aa}	370.0 ± 1.2^{Ba}	321.7 ± 1.3^{Ca}	193.0 ± 1.3^{Fa}	96.7 ± 0.9^{Ha}	313.0 ± 1.5^{Da}	260.3 ± 1.5^{Ea}	172.0 ± 1.5^{Ga}	86.7 ± 0.9^{Ia}
	Chickpea	200.0 ± 2.1^{Aj}	130.0 ± 1.5^{Bk}	86.7 ± 0.9^{Dk}	31.7 ± 0.8^{Fi}	20.0 ± 0.4^{Gj}	110.0 ± 1.2^{Ck}	56.7 ± 0.9^{Ek}	26.7 ± 0.8^{Fh}	12.0 ± 0.4^{Hi}
	Lentil	43.0 ± 0.4^{Ao}	22.0 ± 0.4^{Do}	14.0 ± 0.2^{Fo}	9.0 ± 0.2^{Glm}	7.0 ± 0.2^{Hl}	34.0 ± 0.2^{Bo}	28.0 ± 0.2^{Clm}	18.0 ± 0.2^{Eij}	9.0 ± 0.2^{Gj}
6	Blackgram	226.7 ± 0.8^{Ah}	151.7 ± 0.5^{Bj}	100.7 ± 0.8^{Dj}	55.7 ± 0.7^{Fg}	37.3 ± 1.2^{Gg}	133.3 ± 0.7^{Cj}	89.0 ± 0.9^{Ej}	40.0 ± 0.4^{Gg}	30.0 ± 0.4^{Hg}
	Cowpea	306.7 ± 1.3^{Ad}	200.0 ± 2.2^{Bg}	181.7 ± 1.1^{Ce}	81.3 ± 0.9^{Ee}	43.3 ± 0.7^{Gf}	186.7 ± 1.1^{Ce}	150.0 ± 1.2^{De}	60.0 ± 0.4^{Fe}	35.0 ± 0.4^{Hf}
	Greengram	309.3 ± 1.3^{Ad}	263.0 ± 1.5^{Bc}	251.7 ± 1.5^{Cc}	168.3 ± 1.2^{Fb}	76.7 ± 1.3^{Hb}	241.7 ± 1.1^{Dc}	233.7 ± 1.1^{Eb}	145.0 ± 0.6^{Gb}	60.0 ± 1.2^{Ib}
	Chickpea	166.7 ± 1.0^{Al}	106.7 ± 1.0^{Bl}	66.7 ± 0.9^{Dl}	25.0 ± 0.9^{Fj}	6.7 ± 0.6^{Hlm}	73.3 ± 1.0^{Cl}	31.7 ± 1.1^{El}	20.0 ± 0.6^{Gi}	5.0 ± 0.2^{Hk}
	Lentil	28.3 ± 0.4^{Ap}	15.0 ± 0.4^{Dp}	10.0 ± 0.4^{Fo}	5.0 ± 0.2^{Gmn}	2.7 ± 0.2^{Hno}	20.0 ± 0.4^{Bp}	16.7 ± 0.3^{Cn}	13.3 ± 0.4^{Ekl}	3.3 ± 0.2^{Hklm}

Table 8. Percent adherence (Mean±SE) of Nanosav and Aerosil® to different pulses.[a]

SNP	Pulses					$F_{4,10}$, P
	Black gram	Cowpea	Green gram	Chickpea	Lentil	
Nanosav	86.1 ± 0.3^{Ab}	82.0 ± 0.2^{Bb}	80.4 ± 0.2^{Bb}	66.9 ± 0.6^{Db}	73.9 ± 0.3^{Cb}	409.9^{**}
Aerosil®	99.5 ± 0.1^{Aa}	99.1 ± 0.1^{ABa}	97.5 ± 0.3^{Ba}	92.93 ± 0.6^{Ca}	88.7 ± 0.3^{Da}	176.8^{**}
T_4, Sig (2-tailed)	37.3, 0.001	64.0, 0.001	40.5, 0.001	31.6, 0.001	29.7, 0.001	

[a] Means followed by the same uppercase letter in each row and lower case letter for each column are not significantly different (Tukey HSD test at P = 0.05).

against adults of *R. dominica, S. oryzae*, and *T. confusum*. The activity of a DE formulation varied among different grain types, which may be due to the differences in the physicochemical properties of the grain. Since the Pontos variety had longer seeds and more gluten content, causing more serious damage than two other varieties (Kavallieratos *et al*., 2010). Moreover, grain size influences the insecticidal activity of silica-based DE formulations. The more excellent distribution of DE particles is obtained on smaller seed grains than larger ones, which increases the chance of the particles' contact with the insect's body (Debnath *et al*., 2011). Arumugam *et al*. (2016) observed the highest insecticidal activity of SNPs on the black gram, while the lowest mortality was observed on chickpea.

Both formulations decreased the number of progeny in comparison with the control. These results are inconsistent with other researches (Badii *et al*., 2013; Stathers *et al*., 2004). According to Stathers *et al*. (2004), the number of *C. maculatus* offspring on cowpea treated with DE formulations was decreased by increasing the concentration. The mortality of adults might be achieved during the early days and the insects could not lay eggs. The lowest *C. maculatus* progeny was reported when insects were exposed to the highest concentration of 2 g kg-1 of all DE formulations. This may be due to the change in behavior of *C. maculatus* in the presence of DE (Badii *et al*. 2013). Yousefnezhad Irani *et al*. (2018) showed that on Aerosil®-treated black gram with the highest concentration of 300 mg kg^{-1}, adults produced the least eggs. Aerosil® decreased the percentage of hatched eggs and emerged adults in treated eggs on all pulses when compared to the Nanosav. Mesbah *et al*. (2017) claimed that SNPs on treated broad beans significantly reduced the population of *C. chinensis*, compared to coarse silica; and no offspring were observed in the concentrations of 1 and 2 g NSPs 100 g-1 of seeds. It could be due to the slow mode of action of inert dusts (Korunic, 2013).

Our results demonstrated that the highest adherence of two SNPs was on the black gram and the lowest adherence was recorded on chickpea, followed by lentil-treated with Nanosav and also on lentil followed by chickpea–treated with Aerosil®. The adherence to pulses may be due to SNPs characteristics and the seeds' physical and chemical properties. Athanassiou and Kavallieratos (2005) stated that the adherence of PyriSec (DE) was very low on triticale, peeled barley, and maize out of eight different tested kinds of cereals. It was concluded that there was a positive relationship between adherence and DE toxicity against *R. dominica*. The same findings were reported by Perisic *et al*. (2018), who evaluated three DEs against *R. dominica* in five different cereal grains. They found that the lowest adherence with the mean of 59% was observed for DE s-2 in triticale, while 95% of Protect-It® particles adhered to wheat kernels. Plumier *et al*. (2019) demonstrated that the DE particles with higher surface roughness had a higher adhesion percentage in wheat and corn kernels. The highest adhesion of SNP formulations could be attributed to the rough surface of the particles. The persistence of both SNP formulations declined over 6-month post-treatment storage in all pulse seeds, as the mortality decreased with increasing storage times. It should be noted that even the reduced residue was sufficient for providing satisfactory protection of stored pulse seeds.

Aerosil® was very effective against *C. maculatus* and it successfully eliminated progeny production of this insect pest. According to the present study, both SNP formulations can be used as pulse seeds protectant at a dose rate of 300 mg kg-1. SNP formulations can be considered as an IPM strategy component for the protection of stored pulse seeds.

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دوام و کارایی دو فرمولاسیون نانوسیلیکا برای کنترل *Callosobruchus maculatus* در حبوبات مختلف

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چکیده

دوام دو فرمولاسیون نانوسیلیکا Nanosav و ®Aerosil روی بذرهای حبوبات شامل ماش سیاه، لوبیا چشم-بلبلی، ماش سبز، نخود و عدس برای کنترل سوسک چهار نقطه‌ای حبوبات *Callosobruchus maculatus* (F.) (Col., Chrysomelidae) مورد ارزیابی قرار گرفت. به این منظور، توده های یک کیلوگرمی از هر حبوبات با غلظت‌های (۵۰، ۱۰۰، ۲۰۰ و ۳۰۰ میلی‌گرم بر کیلوگرم) از هر دو فرمولاسیون تیمار شده و در دمای ۲۸ درجه سلسیوس و رطوبت نسبی ۵۵ درصد نگهداری شدند. نمونه‌برداری در دوره‌های انبارداری بلافاصله و سپس هر دو ماه پس از تیمار، تا پایان ۶ ماه انبارداری انجام شد. حشرات بالغ به نمونه‌های تیمار شده اضافه شده و تلفات ۱ ، ۲ و ۴ روز پس از افزودن حشرات شمارش شد. میانگین تعداد نتاج تولید شده نیز ۴۲ روز بعد شمارش گردید. تلفات در ماش سیاه، لوبیا چشم‌بلبلی، ماش سبز و نخود تیمار شده با غلظت ۳۰۰ میلی‌گرم بر کیلوگرم از هر دو فرمولاسیون در دوره بلافاصله پس از تیمار و ۴ روز پس از افزودن حشرات کامل ۱۰۰ درصد بود. در عدس تیمار شده با غلظت ۳۰۰ میلی‌گرم بر کیلوگرم از Nanosav در دوره بلافاصله پس از تیمار نتاجی ظاهر نشد. آزمایش دیگری برای بررسی چسبندگی نانو ذرات سیلیکا روی بذرها انجام شد. بیشترین میزان چسبندگی در ماش سیاه با میانگین ۸۶ و ۹۹/۵ درصد به ترتیب در Nanosav و ®Aerosil بود. نتایج نشان داد هر دو فرمولاسیون نانوسیلیکا توانایی حشره‌کشی خوبی در کنترل سوسک حبوبات دارند و می‌توان از آن‌ها به طور مؤثری در مدیریت تلفیقی سوسک چهار نقطه‌ای حبوبات استفاده کرد.