Silica nanoparticle: a potential of non-invasive and as a natural insecticide application for beet armyworm, *Spodoptera exigua* Hubner (Lep.: Noctuidae) control

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Abstract: The application of pesticides for controlling crop pests produces a deleterious effect on natural enemies, humans, and the environment. Therefore, the use of non-invasive and safe alternative methods is essential. Nanotechnology is a promising field of interdisciplinary research, and its practical applications in agriculture are receiving attention nowadays due to the potential benefits that nanomaterials (NMs) can guarantee for pests management. In this study, a potency of silica nanoparticles (SNPs) in controlling the second larval instar of beet armyworm, *Spodoptera exigua* in laboratory and field conditions, damage of pest, and effect on total chlorophyll contents were evaluated. The LC_{50} and LT_{50} values of SNPs in three methods of application including dust spray, leaf dipping and solution spray were determined. The LC_{50} value of SNPs against the 2nd larval stage after 24, 48, and 72 hours in dust spray, leaf dipping, and solution spray were (660.40, 431.35, 893.10), (460.44, 833.31, 690.12) and (279.28, 565.59, 323.96) mg L^{-1}, respectively. The LT_{50} value of SNPs against the 2nd larval stage of *S. exigua* by three methods showed that dust application can cause 50% mortality in a shorter time in comparison to leaf dipping and solution spray methods. In the field trial, the result of mortality and damage assessment showed that dust SNPs had significant differences with control treatment ($p \leq 0.05$). Total chlorophyll contents in dust SNPs treatment had no significant differences with control treatment ($p \geq 0.05$). In summary, it can be noted that SNPs could be a new alternative to chemical insecticides and could be used in dust spray without using water in the development of new natural insecticides in integrated pest management programs.

Key words: nanotechnology, non-chemical control, beet armyworm, bioassay

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1 Introduction

The beet armyworm, *Spodoptera exigua* Hübner (Lep.: Noctuidae) is an outbreak herbivore and results in serious economic losses in many areas of the world (Mardani-Talaei et al., 2014; Hafeez et al., 2019). Beet armyworm is considered as one of the most serious and destructive pests not only for beet plants, but also for other vegetables, ornamental and field crops (Taylor and Riley, 2008). Extensive use of chemical insecticides to control pests has led to the development of resistance and pollution of the environment (Yadav, 2010; Ditta, 2012). In addition, pesticide use reduces biodiversity, and nitrogen fixation (Lin et al., 2013), contributes to pollinator decline

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(Goulson, 2013), destroys habitat (Palmer et al., 2007), and threatens endangered species (Miller, 2004). Therefore, recent investigations have been aimed to reduce dependency on chemical pesticides and to use safe alternatives in pest control programs. In recent years, consumer awareness of health hazards from residual toxicity of insecticides which are commonly used to control pests and the growing problem of insect resistance to these conventional insecticides have led researchers to look for alternate strategies (Debnath et al., 2011). More recently, materials including diatomaceous earth (DE) and silica nanoparticles (SNPs) have been increasingly finding use in commercial storage in the developed world, replacing conventional chemicals (Golob, 1997). Nano-particles technology when exploited in the right way has a strong potential of being used in agricultural pest control (Panacek et al., 2011; Biswal et al., 2012; Al-Samarrai, 2012; El-bendary and El-Helaly, 2013). The application of nanomaterials in the area of plant sciences (i.e., nutrients and/or pest control) has been extensively investigated to overcome the expected increases in the global population without negative impacts on the environment and/or public health (Gogos et al., 2012; Raliya et al., 2016; Wang et al., 2016). Therefore, it is meaningful to investigate the pesticidal behaviors of the inorganic NPs such as Ag, CuO, MgO, SiO2, and ZnO nanoparticles or their formulations to reduce harmful organic pesticide usage (Xiang et al., 2013; Osman et al., 2015 . The working mechanism by which SNPs control pests was speculated to be breaking the protective lipid water barrier by physisorption of SNPs, which resulted in the death of targeted organisms (Ulrichs et al., 2005; Rai and Ingle, 2012). Many previous studies have confirmed that NPs, whether metal or nonmetal, can be used to control plant and animal pathogens and protect the economic crops and stored grains from insect attack (Gajbhiye et al., 2009; Goswami et al., 2010; Debnath et al., 2011; Rouhani et al., 2012; Arumugam et al., 2016). The nanopesticides of biological origin named as bio- nanopesticide could be fabricated using any metal such as Ag, Cu, SiO2, or ZnO with broad-spectrum pest protection efficiency (Barik et al., 2008; Stadler et al., 2010).

For this aim, in this study silica nanoparticle as the physical control agent in 2nd larval stage of beet armyworm, S. exigua (Lep.: Noctuidae) was evaluated.

2 Material and methods

The present study was carried out in the laboratory and farm of Agriculture Faculty, Higher Education Center of Shahid Bakery, Miandoab, Iran, at a longitude and altitude of 37.009885, 46.071573, from spring to summer of 2019.

2.1 Insect colonies

The second larval stage of beet armyworm was used in the present experiments. A colony of beet armyworm was obtained from a sugar beet farm near Miandoab city at a longitude and altitude of 37.009885, 46.071573. They were reared under, an artificial diet containing 120 g mung bean powder, 10 g dried brewer yeast, 3 g methyl parahydroxy benzoate, 2 g sorbic acid, 2.5 g ascorbic acid, 12.5 g agar, 2 mL of 40 formalin, 30 mL of vitamin stock and 900 mL distilled water to make about 1080 mL diet at laboratory conditions 25℃±2℃, with Light: Dark 16: 8 and 54%±10%RH (Elvira et al., 2010).

2.2 Material

Silicon dioxide nano powder 40-50 nm particle size with a purity of 99.99% (Pishgamannano® www.Irannanotech.com, Iran) was used in bioassay experiments (Figure 1).

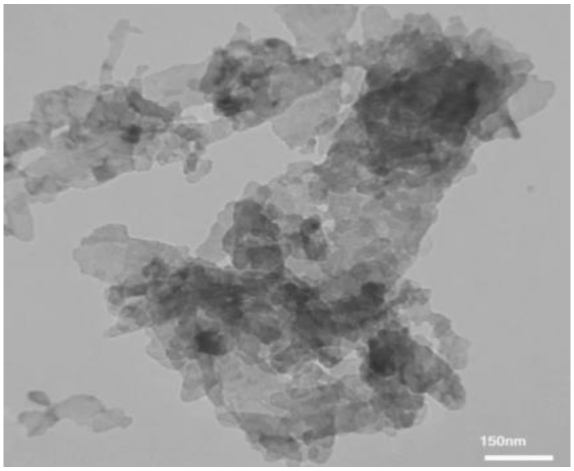

Figure 1 The transmission electron microscope (TEM) images of silica nanoparticles (SNPs)

2.3 Laboratory bioassays

The laboratory bioassays for the determination of median lethal concentration (LC50) and median lethal time (LT50) were performed at 25℃±4℃ with 65%±5% RH and a light–dark cycle of 16:8 h using dust spray, leaf dipping, and solution spray methods on second instar larvae. Three replicates for each concentration were performed, and 10 larvae were utilized for each replicate. Insect mortality was recorded at 24, 48, and 72 h after the larvae were exposed to SNPs.

2.4 Median lethal concentration (LC_{50}):

Median lethal concentration (LC_{50}) was determined by measuring minimum and maximum concentrations of SNPs against 2nd larval stage in pretest experiments that caused 20% to 80% mortality in dust spray, leaf dipping and solution spray methods then three concentrations between a minimum and maximum concentrations were calculated by the logarithmic method (Pourmirza, 2005). Thus, five concentrations (0.125, 0.25, 0.5, 0.75, and 1 mg cm^{-2} equal to 96, 284.5, 673, 961.1, and 1250 mg L^{-1}) and control, each concentration in three replicates on sugar beet leaves, which were enclosed with net cover cage 10×8×5 cm containers, on ten 2nd instar larvae in each replication were performed. Larval mortality was recorded after 24, 48, and 72 hours and analyzed by the probit program.

2.5 Median lethal time (LT_{50})

For obtaining LT_{50} value, five concentrations of SNPs including (0.125, 0.25, 0.5, 0.75, and 1 mg cm^{-2} equal to 96, 284.5, 673, 961.5, and 1250 mg L^{-1}) in three methods on sugar beet leaves, which were enclosed with net cover cage 10×8×5 cm containers with ten 2nd instar larvae on each replication in three replicates were applied. The mortality rate was recorded every 6 hours until 72 hours (Pourmirza, 2005).

2.6 Methods of SNPs application

2.6.1 Dust spray bioassay

For dust spray bioassay, five application rates at 0.125, 0.25, 0.5, 0.75, and 1 mg cm^{-2} were used for the experiment. These rates were chosen according to the pretest experiment. Thirty early second instar larvae were placed in a plastic cup for each concentration in three replicates and then exposed to SNPs powder through a mini air compressor under pressure of 2 kg/cm^2 (Shoaib et al., 2018). After exposure, the larvae and leaves with SNPs powder were kept in containers that were covered by a perforated cover for aeration. The mortality was recorded 24, 48, and 72 h after exposure to SNPs.

2.6.2 Leaf dipping

In the leaf dipping method SNPs solutions with different concentrations were prepared using distilled water, the concentrations were 96, 284.5, 673, 961.1, and 1250 mg L^{-1} and the total volume was 100 mL for each concentration. Leaf dipping bioassay was utilized to test the stomach toxicity of the SNPs to the second larval instar of *S. exigua* larvae. Sugar beet leaf was cut into discs (2.5 cm in diameter) and dipped into the test solution for 30 s with gentle agitation. Those leaf discs dipped in distilled water served as control. Thirty minutes later, the surface of leaf discs was dried through dry air, one leaf disc with 30 second instar larvae was placed in a plastic container with a perforated cover for aeration. The mortality was recorded after 24, 48, and 72 h (Shoaib et al., 2018).

2.6.3 Solution spray

Thirty-second instar larvae were induced into a plastic container and sprayed by a mini air compressor under pressure of 2 kg cm^{-2} with different concentrations of SNPs, and the concentrations were 96, 284.5, 673, 961.1 and 1250 mg L^{-1} (selected SNPs concentrations after pretest); each replicate (container) sprayed with 2 mL SNPs solution. The control larvae were sprayed with 2 mL distilled water. After spray, the larvae were immediately transferred into a clean plastic container and reared with one fresh sugar beet leaf disc (5 cm in diameter) without any SNPs and covered by a perforated cover for aeration. The death was judged from the larval response to gentle prodding with a small writing brush. The larvae that were not exposed to SNPs powder served as control. The mortality was recorded after 24, 48, and 72 h (Thabet et al., 2021).

2.6.4 Field trials

The field trails were carried out at two sugar beet fields at intervals of 500 m from each other as blocks including SNPs (1 mg cm^{-2} equal with 1250 mg L^{-1}), and control (without SNPs) with dimensions of 40×40 m^2, in three replicate per treatment, in representative for commercial sugar beet cultivation with normal distribution infected by beet armyworm without insecticide treatments. All plots were planted in silty clay loam, high fertility, good water retention, long-term annual average temperature is 8.9℃, long-term total annual precipitation is 238.2 mm, in the density of 100.000 seeds ha^{-1} (4.8 kg seed ha^{-1}) (100 seed per 10 m^2), with four rows wide (56 cm row spacing) and 10.4 m long and was managed using standard crop production. The population of live larvae of the *S. exigua* was counted by sampling in blocks and replicate. After that, the SNPs in recommended dosage (1000 mg L^{-1}) were prepared and sprayed in control without SNPs. Then, after 14 days after treatment (DAT), live larvae were counted and estimated in blocks. The Henderson-Tilton formula was used to determine the percentage of efficiency in the application of SNPs due to the heterogeneity of the population in different treatments and also the population count (Henderson and Tilton, 1955).

$$Percentage\ of\ treatments\ efficiency\ (\%) = \left(1 - \frac{T_a \times C_b}{C_a \times T_b}\right) \times 100 \tag{1}$$

Where, T is the number of live larvae per 10 plants in treatment after (T_a) or before (T_b) application, and C is the number of live larvae per 10 plants in control after (C_a) or before (C_b) application. Efficacy of treatment value was calculated for each of the three blocks of each treatment, and these values were subjected to GLM (general linear model) analysis with SPSS statistical analysis software (Ver. 22.0) (SPSS, 2013).

2.6.5 Damage assessment

Damage of *S. exigua* on sugar beet plants that were treated via SNPs and controlled after 14 days in each plot by zigzag direction randomly were selected and estimated.

2.6.6 Chlorophyll content

To evaluate the effect of SNPs on leaf chlorophyll content in field conditions, 10 plants from each replicate and a total of 30 plants from each treatment were selected, and the amount of total chlorophyll was measured at three points of the leaf by using a hand-held chlorophyll meter (Soil–Plant Analysis Development device Minolta Co., Osaka, Japan, SPAD-502) and average chlorophyll content was calculated (Sexton and Carroll, 2002).

2.7 Statically of analysis

The LC_{50} and LT_{50} values (with 95% confidence limits) were calculated by using Probit analysis method (Abbott, 1925).

$$Corrected\ (\%) = \left(1 - \frac{n\ in\ Co\ before\ treatment\ \times\ n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment\ \times\ n\ in\ T\ before\ treatment}\right) \times 100$$

Where, *n* is the insect population, *T* = treated, C_o is the control.

The mortality in different concentrations in three methods was analyzed via a one-way analysis of variance (ANOVA). Mean values were separated through Tukey's HSD test ($p<0.05$). Mortality data, damage assessment, and chlorophyll content in field trails were subjected to independent t-test analyses with SPSS statistical analysis software (Ver. 22.0) (SPSS, 2013).

3 Results and discussion

3.1 Median lethal concentration (LC_{50})

The LC_{25} and LC_{50} values of SNPs against the second instar larvae of *S. exigua* by three methods of application includes dust spray, leaf dipping and solution spray are presented in Table 1. Results of probit analyses showed that LC_{50} value of dust spray with 660.40, 460.44, and 279.28 mg L^{-1} after 24, 48, and 72 hours treatments were effective methods of application SNPs in comparison to leaf dipping and solution spray.

Table 1 The LC$_{50}$ value of SNPs against the second instar larvae of *Spodoptera exigua* by three methods of application includes dust spray, leaf dipping and solution spray

Treatment	Concentration (mg L^{-1})	Time (h)	X^2 (df)	Slope ± SE	Intercept	LC$_{50}$ (LCL–UCL)
Dust spray*	96 284.5	24	2.99 (3)	1.37±0.290	-3.867	660.40 (460.13-1041.79)
	673 961.5	48	2.21 (3)	1.11±0.212	-4.132	460.44 (262.11-1221.77)
	1250 Control (without SNPs)	72	7.13 (3)	1.58±0.284	-3.878	279.28 (6.45.00-691.55)
Leaf dipping	96 284.5	24	1.16 (3)	1.22±0.315	-3.855	431.35 (896.02-2261.29)
	673 961.5	48	1.31 (3)	1.28±0.223	-3.171	833.31 (596.12-1269.09)
	1250 Control (water)	72	1.41 (3)	1.27±280	-3.505	565.59 (379.04-888.54)
Solution spray	96 284.5	24	1.86 (3)	1.37±0.306	-4.046	893.10 (620.17-1603.16)
	673 961.5	48	1.34 (3)	1.31±0.184	-3.802	690.12 (520.15-1863.17)
	1250 Control (water)	72	4.93 (3)	1.26±0.272	-3.166	323.96 (189.91- 477.09)

Note: LCL: lower confidence limit and ULC: upper confidence limit. *In dust spray unit convert mg cm^{-2} to mg L^{-1}

3.2 Median lethal time (LT$_{50}$)

The LT$_{50}$ values of SNPs against the second instar larvae of *S. exigua* by three methods of application including dust spray, leaf dipping, and solution spray are presented in Table 2. The LT$_{50}$ values of SNPs against the second instar larvae of *S. exigua* showed that dust spray was effective application method of SNPs in all concentrations in comparison to leaf dipping and solution spray.

Mortality of different concentration of SNPs in laboratory bioassay on second larval instar of *S. exigus* after 72 hours showed that in all concentration (96, 284.5, 673, 961.5 and 1250 mg L^{-1}) in three application methods significant differences were observed [F (3, 8) =73.33, p=0.001; F (3, 8) =360.60, p=0.001; F (3, 8) =91.02, p=0.001 and F (3, 8) =1386.18, p=0.001] (Figure 2).

Table 2 The LT$_{50}$ value of SNPs against the second instar larvae of *Spodoptera exigua* by three methods of application includes dust spray, leaf dipping and solution spray

Treatment	Concentration (mg L^{-1})	LT$_{50}$ (LCL–UCL)
Dust spray*	96	55.97 (49.08–84.07)
	284.5	43.32 (41.15–59.04)
	673	36.51 (32.55–41.85)
	961.5	25.77 (19.21–31.70)
	1250	20.59 (17.76–23.11)
Leaf dipping	96	67.67 (54.72–98.55)
	284.5	58.47 (49.30–80.87)
	673	43.77 (38.46–52.94)
	961.5	36.79 (32.48–42.86)
	1250	29.51 (25.31–34.09)
Solution spray	96	58.07 (49.55–78.14)
	284.5	46.42 (40.48–57.45)
	673	38.11 (33.51–44.99)
	961.5	32.48 (28.38–37.48)
	1250	23.76 (11.80–32.53)

Note: LCL: lower confidence limit and ULC: upper confidence limit. *In dust spray unit convert mg cm^{-2} to mg L^{-1}

3.3 Mortality of different concentrations of SNPs in three methods

Mortality by three applications in five concentrations showed that in all concentrations dust spray had more effect in comparison to the other two methods.

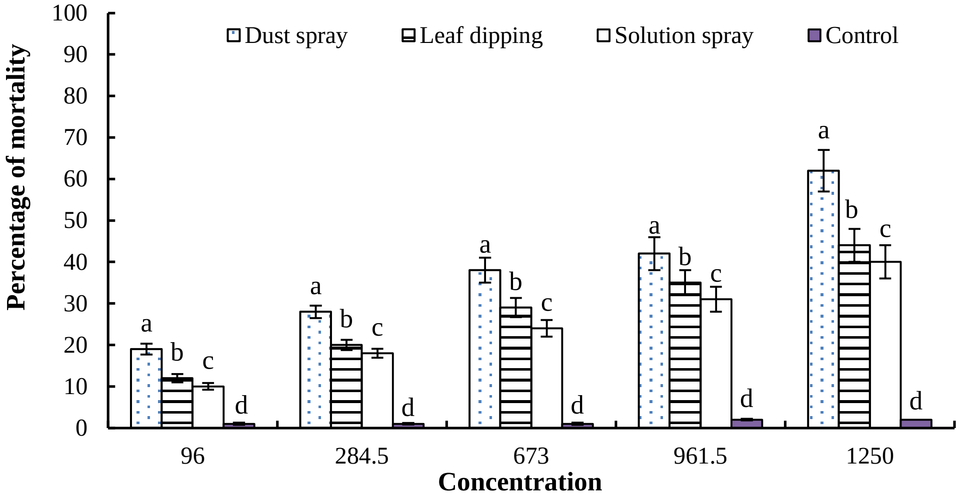

Figure 2 Mean (±SE) efficacy (%) of various concentration SNPs in three methods application against second larval instar of *Spodoptera exigua* after 72 hours in laboratory conditions.

Note: The column marked with different letters within each concentration are significantly different (Tukey's test, $p<0.05$). In dust spray unit convert mg cm^{-2} to mg L^{-1}

3.4 Pest mortality, damage assessment, and total chlorophyll contents

Pest mortality and damage assessment of *S. exigua* on sugar beet plants that were treated via SNPs and control without SNPs after 14 days showed that SNPs had significant differences with control treatments, caused 30.83% mortality and decreased damage until 5.58% (19.25% damage in control) ($p\leq 0.05$). Total chlorophyll contents in SNPs treatment had no significant differences with control treatment after 14 days ($p\geq 0.05$) (Table 3).

Table 3: Mean mortality (±SE) of *Spodoptera exigua*, pest damage (±SE) on plants and total chlorophyll contents (±SE) of sugar beet leaves in field conditions that treated by SNPs and control (Paired *t*-test, 2-tailed).

Variable	Treatment		*t* (df)	*p*
	SNPs	Control		
% Mortality	30.83 ± 4.17^{a}	4.33 ± 1.30^{b}	16.88 (11)	0.001
% Damage	5.58 ± 0.90^{b}	19.25 ± 2.45^{a}	-17.80 (11)	0.001
Total chlorophyll (SPAD value)	39.31 ± 1.42^{a}	38.31 ± 1.13^{a}	1.380 (29)	0.178

Note: Means marked with different letters within the same row are significantly different (Paired *t*-test, 2-tailed, $p<0.05$).

Nanoscience as a new discipline has a great deal of application in various fields and may also be useful in plant protection areas to control pests (Bhattacharyya et al., 2010; Khot et al., 2012). Until now, nanoparticles were used in the formulation of nano based pesticides and insecticides, encapsulated nanoparticles (Arumugam et al., 2016). Silicon can be taken up by plants in the form of monosilicic acid ($Si(OH)_4$) and transported from the root to the shoot, enhancing plant constitutive defenses against abiotic and biotic stresses, inducing defenses of plants attacked by fungal pathogens (Savvas et al., 2009) and arthropod pests (Gomes et al., 2008), attracting more natural enemies by triggering the production of herbivore-induced plant volatiles (Kvedaras et al., 2009).

Our study demonstrated that SNPs could kill the larvae of *S. exigua*. This result is consistent with earlier reports on other pests, such as *Lipaphis pseudobrassicae* (Goswami et al., 2010), *S. litura* (Goswami et al., 2010; Debnath et al., 2012), and *Plutella xylostella* (Shoaib et al., 2018). In the present study three types of application

SNPs, namely dust spray, leaf dipping, and solution spray to assess their larvicidal properties in second larval stage of *S. exigua* were evaluated and the result showed that dust treatment had a more highly significant effect than other two treatments. However, dust application of the nanosilica was not as effective as that reported by Debnath et al. (2012). Two reasons might contribute to the difference between our and their results. One reason is that our dust different, and the other one is that different insect species were used in the two experiments. Furthermore, the thickness and structure of their cuticle might be different. Debnath et al. (2012) applied SNPs in sol–gel methods against second instar larvae of S. litura, and the results showed that both of these SNPs could effectively kill the insect larvae.

A study was initiated to explore the potential of three nanoparticles including CdS, Nano-Ag, and Nano-TiO2 nanoparticles in causing adverse effects on S. litura (Chakravarthy et al., 2012). In our study reduction in S. exigua populations also increased with concentrations of silica NPs. This result is consistent with a laboratory study on S. littoralis, as well as previous studies that have shown that S. litura is effectively controlled by silica NPs, and that S. littoralis is controlled by silica NPs in a semi-field condition (Borei et al., 2014; El-Bendary and El-Helaly, 2013). In the study by Borei et al. (2014), six concentrations of SNPs (75, 150, 225, 300, 375, and 425 ppm) were examined on neonates of S. littoralis under laboratory conditions. Results showed that the SNPs treatments in the larval test were highly effective at all concentrations, and with the increase of SNPs concentration, adverse effects on the biological aspects of cotton leaf worm increased especially at high concentrations (Borei et al., 2014).

The AgNPs showed potential antifeedant activity of 78.77% and 82.16% against the larvae of S. litura and H. armigera, respectively. The histological examinations showed that the acceleration of the nanomaterial caused severe tissue damage in the epithelial and goblet cells in the larval midgut region of S. litura, H. armigera, A. aegypti, and C. quinquefasciatus (Manimegalai et al., 2020). Shoaib et al. (2018) indicated that the exposure of larvae of P. xylostella to 1 mg cm^{-2} of a siliceous dust formulation resulted in 85% mortality after 72 h. Debnath et al. (2011) showed that the mortality effect of hydrophilic and hydrophobic SiO_2 increased from day 1 to 14, and greater mortality was observed with the highest dose at the end of two weeks. Ziaee and Ganji (2016) conducted a study to assess the effects of two silicon dioxide nanoparticles of Aerosil® and Nanosav® against adults of *Rhyzopertha dominica* F. and *Tribolium confusum* Jacquelin du Val and the results indicated that the SNPs were efficient against tested species and can be used effectively in a stored grain integrated pest management program.

The obtained results by Ma et al. (2011) suggested that treatment of SNPs had a beneficial effect on photosynthesis. Suriyaprabha et al. (2014) reported that treatment of 15 kg ha-1 SNPs from RH in soil showed the better growth promotion of maize in terms of chlorophyll content compared with other treatments and control (Suriyaprabha et al., 2014), but in our study total chlorophyll contents in SNPs treatment had no significant differences with control treatment after 14 days.

4 Conclusions

The use of higher dosage and repeated applications of chemical insecticides have led to the rapid development of insect resistance and adverse effects on human health and the environment. Accordingly, researchers are prompted to identify an alternative entomotoxic agent for crop protection. Nanocides are being considered as alternatives to conventional insecticides because they are expected to lessen the application rate and reduce the chances of resistance development in pests. It can be concluded that SNPs could be applied to protection of damage of beet armyworm in field conditions with dust spray methods without using water and without decreasing the photosynthetic process.

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