

Control of *Cassida vittata* (Vill) (Coleoptera: Chrysomelidae) using chitosan and nano chitosan

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ABSTRACT

Sugar beet is considered one of the most important crops in Egypt because it was used in the production of sugar as it contains 15-20% sugar. *Cassida vittata* (Vill) (Coleoptera: Chrysomelidae); harmful insect pest causing a lot of damage of the sugar crop. Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles, which are well dispersed and stable in aqueous solution have been prepared by template polymerization of acrylic acid in chitosan solution which have an insecticidal effect on insect pests. The usage of chitosan and nano chitosan test against *C. vittata*. Results showed that, the LC50 obtained 150 and 110 ppm after *C. vittata* treated with different concentrations of chitosan and nano chitosan. Also, under field conditions when *C. vittata* treated with the chitosan and nano chitosan, the number of eggs significantly 22 ± 1.7 and 3 ± 8.9 eggs/ female as compared to 266 ± 8.7 eggs /female in the control. The percentage of egg hatching, larval mortality, malformed pupae and malformed adults significantly decreased in case of chitosan treatments and almost reduced after nano chitosan treatments. The weight of sugar beet significantly increased to 2397 ± 66.11 and 2499 ± 54.98 kg/ feddan as compared to 1780 ± 55.43 and 1220 ± 45.09 kg/ feddan in the control during season 2016 and 2017 respectively.

Key words: Nano, chitosan, *Cassida vittata*, sugar beet, control.

Introduction

Sugar beet is considered one of the most important crops in Egypt because it was used in the production of sugar as it contains 15-20% sugar. The sugar beet has currently been infested with many insect pests which cause high losses in the crop yield and decrease its sugar content (Bassyouny, 1993). Among these insects, *Cassida vittata* (Vill) (Coleoptera: Chrysomelidae). Chemical insecticides were used to control these insect pests, but they were always causing a lot of pollution to the environment (Goodwin *et al.*, 2007). Thereafter microbial control agents were advocated to be used against such pests. Chitosan (CS)-g-poly (acrylic acid) (PAA) nanoparticles, which are well dispersed and stable in aqueous solution have been prepared by template polymerization of acrylic acid in chitosan solution (Sahab *et al.*, 2014). The prepared CS-PAA had a white powder shape and was insoluble in water and diluted acid. Chitosan nanorod with minimum particle size of <100 nm was prepared by crosslinking low molecular weight chitosan with polyanion sodium tripolyphosphate and physicochemically characterized. Chitosan is a natural polysaccharide prepared by the N - deacetylation of chitin. It has been widely used in food and bioengineering industries, including the encapsulation of active food ingredients, in enzyme immobilization, and as a carrier for controlled drug delivery, due to its significant biological and chemical properties such as biodegradability, biocompatibility, bioactivity, and polycationicity.

The aim of this work to evaluate the effectiveness of chitosan and nano chitosan against *Cassida vittata* (Vill) (Coleoptera: Chrysomelidae).

Materials and Methods

Laboratory studies.

The sugar beet insects *C. vittata*; was reared under laboratory conditions (26 ± 2 C° and 60 ± 5 %RH) in cages 50X 50X 60 cm per each. The third larval stage was used in the experimental work.

Preparation of Nano-Chitosan.

Chitosan Nanoparticles were synthesized by hydrolyzing titanium tetra isopropoxide in a mixture of 1:1 anhydrous ethanol and water. 9 ml of titanium tetra isopropoxide is mixed with 41ml of anhydrous ethanol (A). 1:1 ethanol and water mixture is prepared. (B) Solution A is added in drop wise to solute ion B and stirred vigorously for 2hrs. At room temperature hydrolysis and condensation are performed, using 1M sulphuric acid and stirred for 2 hrs. Then the ageing was undertaken for 12hrs. The gel was transferred into an autoclave and tightly closed, and the mixture was subjected to hydrothermal treatment at 353K for 24hrs. After filtration the solid residue was washed thoroughly with water and ethanol mixture, dried at 373K in an oven and calcined at 773K.

Nano encapsulation

The Nano encapsulation is a process through which a chemical is slowly but efficiently released to the particular host for insect pests control. "Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific pH" (Vidhyalakshmi *et al.*, 2009). Encapsulated of the Chitosan nanoemulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets which that increase the retention of the oil and cause a slow release of the nano materials. The release rate depends upon the protection time; consequently a decrease in release rate can prolong insect pests protection time (Nurruzzmzn *et al.*, 2016).

Efficacy of Chitosan against the Target

The insecticide Chitosan and Nano chitosan were tested at the 6 concentrations: 6 ppm, 5ppm, 4ppm, 3ppm, 2ppm, 1ppm. The insecticide, prepared 6 concentrations. Percentages of mortality were calculated according to Abbott's formula, while the LC50 values were calculated throughout probit analysis (Abbott, 1925). The experiment was carried out under laboratory conditions at 26 ± 2 °C and 60-70% RH.

Statistical analysis

Data obtained was statistical analysed using Duncan's multiple range tests according to (Finney, 1971) Data were statistically analyzed by *F*-test; LSD value was estimated, using SPSS statistical program software.

Results and Discussion

Table 1 shows that the LC50 obtained 150 and 110 ppm after *C. vittata* treated with different concentrations of chitosan and nano chitosan. Table 2 show that when *C. vittata* treated with the chitosan and nano chitosan, the number of eggs significantly 22 ± 1.7 and 3 ± 8.9 eggs/ female as compared to 266 ± 8.7 eggs /female in the control decreased to 266 ± 8.7 eggs/ female . The percentage of egg hatching, larval mortality, malformed pupae and malformed adults significantly decreased in case of chitosan treatments and almost reduced after nano chitosan treatments (Table 2). The weight of sugar beet significantly increased to 2397 ± 66.11 and 2499 ± 54.98 kg/ feddan as compared to 1780 ± 55.43 and 1220 ± 45.09 kg/ feddan in the control during season 2016 and 2017 respectively (Table 3).

Our findings meet with Sabbour and Nayera Soleiman, (2014) who found that the bioinsecticides control the percentage of the sugar beet pests significantly decreased during both two successive season 2012 and 2013 after fungi treatments. Sabbour and Abd El Rahman, (2007) found the bioinsecticide decrease *C. vittata* under laboratory and field conditions. Sahab *et al.* (2015) found that the nano chitosan have an insecticidal effect against *Aphis gossypii* under laboratory and field conditions.

Similarly, Sabbour, (2015a) reported that Imidacloprid and nano-Imidacloprid reduced the rate of infestation by *C. capitata* and *P. oleae* in olive trees. Again, Sabbour (2015b) recorded decreased infestation rate by potato tuber moth, *Phthorimaea operculella*, in plants treated with nano-fungi *Isaria fumosorosea* and *Metarhizium flavoviride*. Similar findings were also attained by Sabbour (2013) against *B. oleae*, *C. capitata* and *P. oleae* in olive trees treated with spinosad.

These results are in consistence with those obtained by Sabbour, (2015a) for olive trees treated with Imidacloprid and nano-Imidacloprid and infested by *C. capitata* and *P. oleae*. Also, treatment of potato plants, infested by *P. operculella*, with nano-fungi *I. fumosorosea* and *M. flavoviride* increased the yield (Sabbour, 2015b). Similar results were obtained by Sabbour, (2013) for spinosad-treated olive trees that were infested by *B. oleae*, *C. capitata* and *P. oleae*. Sabbour 2017, found that the olive weight increased after bioinsecticid applications. Sabbour and Nayera Solieman, (2017), reported that nano-biopesticides application increase the productivity of the olive fruits under field conditions. Also, Sabbour and Nayera Soleiman, (2016), control Tuta absoluta by nano chitosan and results showed a reduction in the infestation numbers. Sabbour, (2016) use the nano chitosan against schistocerca gergaria and found a loss of the pess number after treatments under laboratory and field conditions.

In conclusion, nano-formulation of chitosan was more effective than chitosan in controlling *C. vittata*. These results encourage the extension in the use of nanotechnology for insect pest control.

Table 1: Evaluation of, tested chitosan and Nano chitosan on *Cassida vittata* under laboratory conditions

Treatments	LC ₅₀ ppm	S	V	95 % Confidence limits
Chitosan	150	0.1	1.4	88-163
Nano chitosan	110	1.1	1.1	90-149

Table 2: Effect of the against the target insects *C. vittata* biology

Treatments	No of eggs laid/female	% of egg hatching	% of larval mortality	% of malformed larvae	% of malformed pupae	% of emerged adults	% of malformed adults
Chitosan	22±1.7 ^b	4	61	66	77	3	78
Nano chitosan	3±8.9 ^c	0	91	97	94	0	0
Control	266±8.7 ^a	99	-	-	-	100	-
F value	33.4	2	5	5	22	21	21
L.S. D. 5%	11.1	2	3	3	11	11	9

Table 3: Assessments of damage caused after treatment with the chitosan Nano chitosan

Target pest	Season 2016	Season 2017
	Wt. of suger beet (kg/ feddan)	Wt. of suger beet (kg/ feddan)
Chitosan	2305± 54.66	2527±67.91
Nano chitosan	2397±66.11	2499±54.98
Control	1780±55.43	1220±45.09

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