



Observations of the effect of Chitosan and its nano compositions against the locust *Schistocerca gregaria* (Orthoptera: Acrididae)

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Abstract : The effect of Chitosan and nano Chitosan tested on the target insect pest *Schistocerca gregaria*. Results obtained showed that, under laboratory conditions, the LC50 of the newly hatched is recorded, 278, 244, 233, 247 and 241 ppm for newly hatched, nymphs, Last nymphal stage Adult ♀ and Adult ♂ respectively. Also, when *S. gregaria* treated with nano- Chitosan, the LC50s obtained 268, 204, 213, 231, and 132 ppm for Newly hatched, nymph, Last nymphal stage, Adult ♀ and Adult ♂, respectively after treated with nano-Chitosan. Also, results recorded that, the Number of egg laid/female recorded, 158 ± 2.2 and 88 ± 0.01 eggs/female after treated with Chitosan and nano- Chitosan compared to 258 ± 3.11 eggs/female in the control. The % of Adult ♀ and % of Adult ♂ significantly decreased to 18 and 10 after nano-Chitosan treatments as compared to 99 and 99% in the control. Our results showed, under semi field conditions, the number of *S. gregaria* were significantly decreased after the Chitosan and nano-Chitosan treatment, the number of infestations with *S. gregaria* decreased to 29 ± 3.6 and 8 ± 1.1 individuals after 120 days of treatments.

Key words : locust, *Schistocerca gregaria*, Chitosan, Nano.

1.Introduction

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. The prepared CS-PAA had a white powder shape and was insoluble in water and diluted acid¹. The mean particles size were found to be around 50nm. FTIR spectra of CS-PAA nanoparticles for CS, the intensities of the amide band were observed clearly. The broad peak appeared at 2500cm^{-1} , which confirmed the presence of NH_3^+ in the CS-PAA nanoparticles. Nanoparticles synthesis is currently intensively researched due to its wide variety of potential applications². As an alternative to chemical manufactured pesticides, use of nanoparticles as an antimicrobial agents has become more common as technological advances have made their production more economical^{3,4}. Numerous studies on the antimicrobial activity of chitosan and its derivatives against most economic plant pathogens have been investigated^{5,6,7,8}. Therefore, these compounds are considered as useful pesticides in the control of plant diseases.

The locust, *S. gregaria* is considered one of the most harmful pests to different cultivated crops in Egypt. Its economic importance comes from attacking many cultivated crops, vegetables and even trees, feeding on it and causing great losses in quantity and quality of the attacked crop. In some cases, thousands of cultivated hectares may be attacked by the swarms of grasshoppers leaving it as a divested desert. The economic injury of *S. gregaria* in Egypt had been documented by⁹. Egypt, from 4500 years ago^{10,11,12}. More

than 300 species of locusts and grasshoppers are known to exist in the African continent FAO (1995), but fortunately only a few of them are major pests; most are sedentary, inhabiting a rather confined area throughout their life cycle^{13, 14} showed that range of mortality was between 64-85% based on the end point data after using the bioinsecticides and the nano materials against the locust under laboratory and semifield conditions^{14,16}.

2. Materials and Methods

2.1. Materials.

The raw materials used for ion exchange preparation are:

1. The Chitosan(CS) used in this study was purchased from HAS HMRZEL laboratories LTD (Netherlands)
2. The acrylic acid was purchased from Sd . Finc.Chem. Limited (Laboratory grade for synthesis) and was freshly distilled under reduced pressure to eliminate any inhibitors.
3. Both initiators (ammonium persulfate and sodium bisulfite) and other chemicals were of analytical reagent and used as received

2.2. Insect rearing

S. gregaria was reared under laboratory condition for several generations on semi-artificial diet as mentioned by¹⁰.

2.3. Preparation of nano- Chitozan

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.

2.4. Nanoencapsulation

The Nanoencapsulation 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"¹⁵. Encapsulated of the three oils tested(castor oil, Mustard and *Portulaca oleracea*) nano-emulsion 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 time¹⁶.

2.5. Efficacy of Chitosan against the target insect pests

The insecticide Chitosan were tested at the 6 concentrations: 6 mg, 5mg, 4mg, 3mg, 2mg, 1 mg. The insecticide, prepared 6 concentrations (prepared according¹⁷ Percentages of mortality were calculated according to Abbott's formula¹⁸, while the LC50 values was calculated throughout probit analysis¹⁹. The experiment was carried out under laboratory conditions at 26±2°C and 60-70% RH

4. Statistical Analysis:

Data obtained was statistical analysed using Duncan's multiple range test according to²⁰.

Efficacy of tested nano- Chitosan applied alone on the mean number of deposited eggs of target insects for conducting the combination tests with Chitosan formulations (0.5 g/kg of grains). The Chitosan alone were used at rate (1.0 g/kg) of grains. Four replicates of 100 g grains for each treatment were used. Each replicate was treated individually with treatments and then shaken manually for 1 min to achieve equal distribution of the dust in the entire formulation quantity and was placed in glass jar. Four replicates jar containing untreated grain

served as control. Subsequently, one paired of newly emerged adults were introduced into each jar. The number of deposited eggs on treated or untreated grains/female was counted. The data was analyzed using analysis of variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the least significant difference (LSD) test at 5% level²¹.

3. Results and Discussion

Table 1 show the effect of Chitosan on the target insect pest *S. gregaria* under laboratory conditions, the LC50 of the newly hatched is recorded, 278, 244, 233, 247 and 241 ppm for newly hatched, nymphs, Last nymphal stage, Adult ♀ and Adult ♂ respectively.

Table 1. Effect of Chitosan against *Schistocerca gregaria* under laboratory conditions.

Stages	LC50	V	S	95% confidence limits
Newly hatched	278	0.01	1.3	198-171
nymphs	244	0.02	0.2	200-237
Last nymphal stage	233	0.04	1.2	201-249
Adult ♀	247	1.01	0.1	210-300
Adult ♂	241	10.1	0.1	220-311

Table 2, show that the under laboratory conditions, the LC50s of locust *S. gregaria* obtained 268, 204, 213, 231, and 132 ppm for Newly hatched, nymph, Last nymphal stage, Adult ♀ and Adult ♂, respectively after treated with nano-Chitosan (Table2).

Table 2. Effect of nano-Chitosan against *Schistocerca gregaria* under laboratory conditions.

Stages	LC50	V	S	95% confidence limits
Newly hatched	268	0.01	1.3	160-301
nymphs	204	0.01	0.2	190-230
Last nymphal stage	213	0.01	1.2	200-247
Adult ♀	231	1.01	0.2	210-305
Adult ♂	232	10.2	0.1	220-301

Under laboratory conditions, the Number of egg laid/female±SE recorded, 158 ± 2.2 and 88±0.01 eggs/female after treated with Chitosan and nano-Chitosan compared to 258±3.11 eggs/female in the control (Table 2). The % of Adult ♀ and % of Adult ♂ significantly decreased to 18 and 10 after nano-Chitosan treatments as compared to 99 and 99% in the control (Table3).

Table 3. Effect of Chitosan and nano-Chitosan against *Schistocerca gregaria* under laboratory conditions.

Stages	Chitosan	Nano -Chitosan	control
Number of egg laid/female±SE	158 ± 2.2	88±0.01	258±3.11
% of Newly hatched nymphs	54	26	98
% of Last nymphal stage	55	24	99
% of Adult ♀	50	18	99
% of Adult ♂	41	10	99

Also, under semi field conditions, the number of *S. gregaria* were significantly decreased after the Chitosan and nano-Chitosan treatment, the number of infestations with *S. gregaria* decreased to 29 ± 3.6 and 8 ± 1.1 individuals after 120 days of treatments (Table 4).

Table 4: Effect of Chitosan against *Schistocerca gregaria* under semi field conditions

Treatments	Days after treatment	No .of infestations of the target pests (Means ± S.E.)
Control	20	12.2±3.4
	50	36±3.5
	90	58±1.6
	120	99±8.7
Chitosan	20	7.0±1.1
	50	16±2.5
	90	23±3.1
	120	29±3.6
Nano-Chitosan	20	1±2.1
	50	2. ±2.3
	90	6±2.4
	120	8±1.1

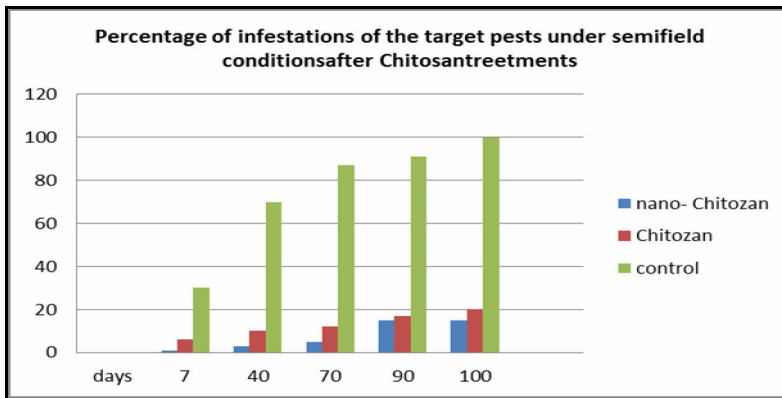
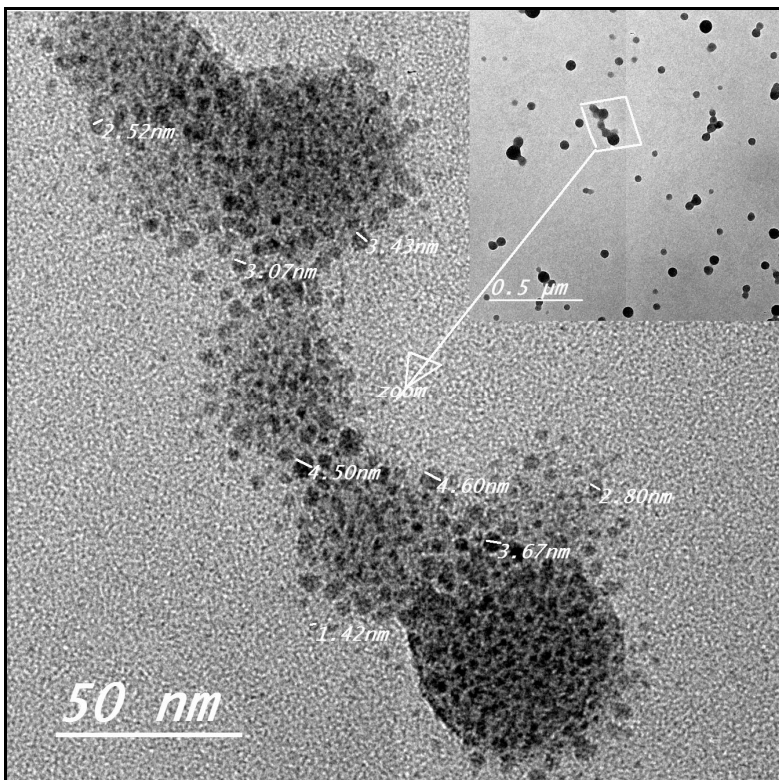
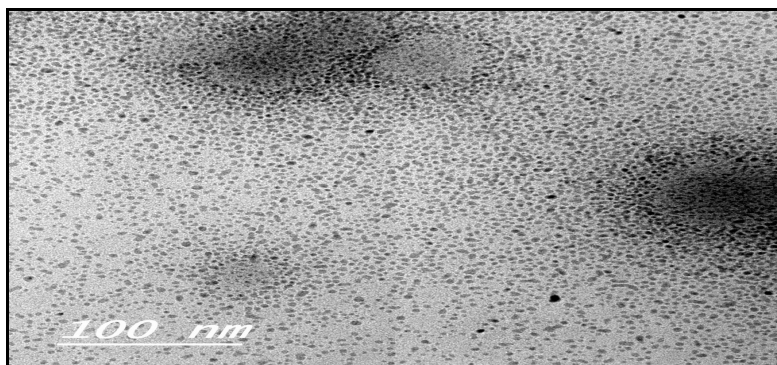


Fig 1. Percentage of infestations of the target pests under semifield conditions after Chitosan treatments



A. Scanning electron microscopy of nano Chitosan particles size (50 nanometer)



B. Scanning electron microscopy of nano Chitosan particles size (100 nanometer)

Fig 2 . Scanning electron microscopy, the nano chitosan particles 50 nanometer and b. 100 nanometer

Figure 1, show that, the infestation percent significantly decreased especially after nano-chitosan treatments. Figure 2, show that, the nano Chitosan particles by scanning electron microscopy. Figure 2 a and b show that the nano Chitosan at 50 and 100 nanometer.

The obtained by²² who, reported that, under laboratory conditions, the LC_{50} s, were significantly decreased when the adult female of grasshopper *Heteracris littoralis* treated with nano-destruxin and reached to 153×10^4 spores/ml. Also, Under semi-field conditions, the percentage of infestations of *H.littoralis* significantly decreased to 1.0 ± 0.3 , 3 ± 0.1 , 5 ± 3.0 and 10 ± 2.9 individuals after treated with nano-destruxin in 20, 50, 90 and 120 days, respectively as compared to 15.2 ± 2.9 , 39 ± 3.5 , 66 ± 9.6 and 98 ± 6.6 individuals in the control.²³ found LC_{50} s of the locust *S. gregaria* after treatment with destruxin, 210×10^4 , 221×10^4 , 250×10^4 spores/ml, of newly hatched nymphs last nymphal stage and adult stage., respectively The effect of nano-destruxin against *S. gregaria* under semi-field conditions show that after 20 days, the infestations number were significantly decreased to 2.2 ± 1.2 , as compared to 2.4 ± 5.3 , and 12.2 ± 2.2 individuals after treated with destruxin and in the control^{13,14}. the same findings were reported by^{22,23}. Desert locust *Schistocerca gregaria* bioassayed by using the leaves containing early stages larvae and the data were recorded after 1, 2, 3 and 4 days after treatment. Results showed that range of mortality was between 84-65% based on the end point data. The minimum of three days to achieve 60% mortality was proved by probit analysis of time-mortality responses. They found that, the range of mortality was between 88-65% based on the end point data. The minimum of three days to achieve 50% mortality was proved by probit analysis of time-mortality responses. The same results obtained by^{24, 24, 25, 26,27}. Many authors, found the insecticidal activity the nano-chitosan (CS-g-PAA) showed highest effect against the three insect of soybean^{28, 28, 30, 21, 32}. as the means number of eggs deposited /female were significantly decreased. Under laboratory and semifield condition, *Aphis gossypii* were significantly decreased to 20.9 ± 9.1 and 28.9 ± 9.2 eggs/female respectively as compared to 97.3 ± 4.9 and 90.3 ± 4.9 eggs/female in the control, respectively. The same trends were also observed against *Callosobruchus maculatus*.^{33,34,35,36, 37, 38} found that the nano insecticides of Imidacloprid and fungi strains cases a higher mortality for insect infestations. Our results agree with^{39,40,41,42}. also, results agree with,^{43, 27,23} who find that the nano pesticide decrease the infestation percentage of different pests^{43, 27,23}.

Acknowledgments

This research was supported by Agric. Department, National Research Centre, and Cairo, Egypt. Project No (10120601).

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