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Research Article
Effect of Chitosan and Nano-chitosan on *Saissetia oleae* (Hemiptera: Coccidae)

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Abstract

Background and Objective: Olive trees important economically attack by many harmful pests. *Saissetia oleae* (Hemiptera: Coccidae); among these pests, which causing a lot of damage to the trees. The objective of this study is to decrease the amount of insecticide using nano-materials. **Materials and Methods:** Chitosan (CS)-g-poly (acrylic acid) (PAA) nano-particles, 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 *Saissetia oleae.* **Results:** Results showed that, the LC_{50} obtained 128 and 37 ppm after *Saissetia oleae* treated with different concentrations of chitosan and nano-chitosan. Also, under field conditions when *S. oleae* treated with the chitosan and nano-chitosan, the number of eggs significantly 54±1.1 and 5±7.3 eggs/female as compared to 289±8.9 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 olive fruits significantly increased to 2498±66.91 and 2528±51.98 kg/feddan as compared to 1779±55.43 and 1210±41.09 kg/feddan in the control during season 2017 and 2018, respectively. **Conclusion:** The usage of nano-chitosan decreased the infestation of *S. oleae* under laboratory and field conditions.

Key words: Nano-chitosan, *Saissetia oleae*, olive, control

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Saissetia oleae (Hemiptera: Coccidae) harmful insect attack olive trees. Mature adult *S. oleae* appear as sessile dark grey or brown-to-black lumps attached to leaf undersides and stems. The limbs of each insect are short and are hidden beneath the body and eyes are only visible in younger specimens with pale bodies. Black scales feed by attaching to the leaves and branches of their host plant and sucking the sap from inside the plant tissue. Depending on the severity of the scale infestation, the resulting damage to the plant may vary. As the scales feed, they exude a sticky, sugary substance, called honeydew, as a waste product. The honeydew falls from the feeding site and coats the leaves and fruit of the host plant or nearby surfaces, which encourages growth of sooty mold[1-2].

Early instars are difficult to distinguish from those of other species of soft scale. First-instar crawlers (0.35 mm long) and intermediate immature instars are translucent light brown, with two black eyes placed anterolaterally. Adult females lack wings; they are 2-5 mm across, approximately circular in outline, fairly flat, yellow or grey and granular in appearance initially, becoming hemispherical and dark grey or brown to black and matt with age[3]. Adult females develop an egg-filled hollow under the body as they become increasingly convex in shape. The small, winged males are rare. It is considered one of the three main phytophagous parasites of *Olea europaea*.

Female black scales deposit eggs from April-September and like other species in the genus *Saissetia*, protect them beneath the body until they hatch. Each female can lay from a few hundred to over 2,500 eggs[4]. Chemical insecticides were used to control these insect pests but they were always causing a lot of pollution to the environment[4]. Thereafter microbial control agents were advocated to be used against such pests. Chitosan (CS)-g-poly (acrylic acid) (PAA) nano-particles, which are well dispersed and stable in aqueous solution have been prepared by template polymerization of acrylic acid in chitosan solution[5]. The prepared CS-PAA had a white powder shape and was insoluble in water and diluted acid. Chitosan nano-rod 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[6]. The objective of this study to decrease the amount used of bioinsecticides by using the nano-methods with the biopesticides against the target pests under laboratory and field conditions. The aim of this work to evaluate the effectiveness of chitosan and nano-chitosan against *Saissetia oleae*.

MATERIALS AND METHODS

Laboratory studies: The insects of *Saissetia oleae* (Hemiptera: Coccidae) was reared under laboratory conditions (26±2°C and 60±5% RH) in cages 50×50×60 cm per each. The third larval stage was used in the experimental work.

Preparation of nano-chitosan: Chitosan nano-particles were synthesized by hydrolyzing titanium tetra isopropoxide in a mixture of 1:1 anhydrous ethanol and water. About 9 mL of titanium tetra isopropoxide is mixed with 41 mL 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 2 h. At room temperature hydrolysis and condensation are performed, using 1 M sulphuric acid and stirred for 2 h. Then the ageing was undertaken for 12 h. The gel was transferred into an autoclave and tightly closed and the mixture was subjected to hydrothermal treatment at 353 K for 24 h. After filtration the solid residue was washed thoroughly with water and ethanol mixture, dried at 373 K in an oven and calcined at 773 K.

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[7] with specific pH. Encapsulated of the Chitosan 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 time[8].

Efficacy of chitosan against the target: The insecticide chitosan and nano-chitosan were tested at the 6 concentrations: 6, 5, 4, 3, 2 and 1 ppm. The insecticide, prepared 6 concentrations. Percentages of mortality were calculated according to Abbott's formula, while the LC_{50} values were calculated throughout probit analysis[9]. The experiment was carried out under laboratory conditions at 26±2°C and 60-70% RH.

Field experiments: The field experiments were executed, at national research Centre far in El-Nobaryia (Ibn Malek farm) starting from the first of July to end of August. Three random patches of olive trees were selected; each consisted of 12 trees for chitosan application, 12 trees for nano-chitosan application and 12 trees for control. Both chitosan and nano-chitosan were applied at the rate of 2.00 and 0.12 mg L^{-1}, respectively. Three applications were made at 1 week interval at the commencement of the experiment. Treatments were performed at sunset using a ten liter sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days of application. Each treatment was replicated four times. Four plots were treated with water and used as control. Random samples of olive leaves and fruits were weekly collected from each treatment and transferred to laboratory for examination. The infestation percentage of *S. oleae* was estimated in each case. After harvesting olive fruits, the yield of each treatment was weighed and expressed as kg/Feddan.

Statistical analysis: Data were statistically analyzed by F-test; LSD value was estimated using SPSS statistical program software.

RESULTS

Table 1 shows that the LC_{50} obtained 128 and 37 ppm after *S. oleae* treated with different concentrations of chitosan and nano chitosan.

When *S. oleae* treated with the chitosan and nano-chitosan, the number of eggs are significantly decreased to 54±1.1 and 5±7.3 eggs/female as compared to 289±8.9 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 (Table 2).

The weight of olive fruits significantly increased to 2498±66.91 and 2528±51.98 kg/feddan in plots treated with nano-chitosan as compared to 1779±55.43 and 1210±41.09 kg/feddan in the control during season 2017 and 2018, respectively (Table 3). Figure 1 shows the nano-particles re coded by scanning electron microscopy.

DISCUSSION

Our findings meet with Sabbour and Nayera[10], 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[11]. The bioinsecticide decrease *C. vaitta* under laboratory and field conditions. It found that the nano-chitosan have an insecticidal effect against *Aphis gossypii* under laboratory and field conditions[5].

Sabbour[12] reported that Imidacloprid and nano-Imidacloprid reduced the rate of infestation by

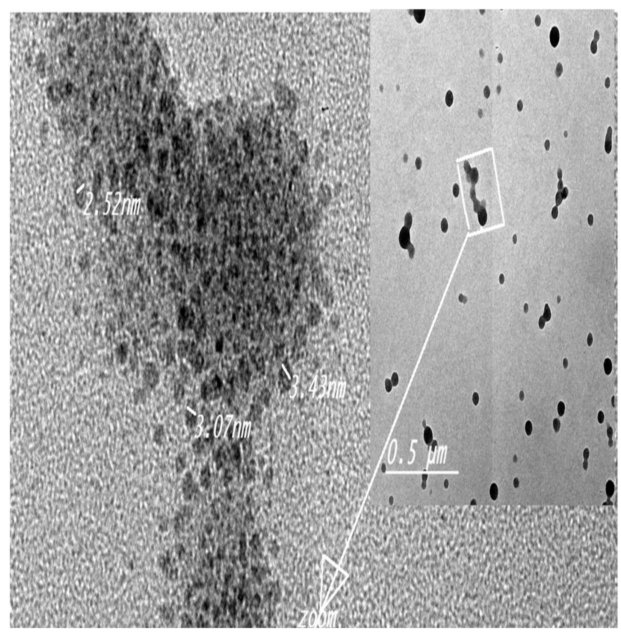

Fig 1. Scanning electron microscopy of chitosan

Table 1: Evaluation of tested chitosan and nano-chitosan on *Saissetia oleae* under laboratory conditions

Treatments	LC_{50} ppm	S	V	95% Confidence limits
Chitosan	128	0.2	1.1	88-163
Nano-chitosan	37	1.1	1.3	20-149

Table 2: Effect of the against the target insects *S. oleae* biology

Treatments	No. of eggs laid/female	Egg hatching (%)	Larval mortality (%)	Malformed larvae (%)	Malformed pupae (%)	Emerged adults (%)	Malformed adults (%)
Chitosan	54±1.1[b]	19	65	71	79	12	70
Nano-chitosan	5±7.3[c]	0	91	97	94	0	0
Control	289±8.9[a]	100	-	-	-	100	-
F-value	30.4	2	5	5	22	20	21
LSD 5%	10.1	1	3	3	10	10	9

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

	Season 2017	Season 2018
Target pest	Wt. of olive (kg/feddan)	Wt. of olive (kg/feddan)
Chitosan	2228±54.60	2567±67.99
Nano-chitosan	2498±66.91	2528±51.98
Control	1779±55.43	1210±41.09

C. capitata and *P. oleae* in olive trees. Sabbour[13] 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[14] against *B. oleae*, *C. capitata* and *P. oleae* in olive trees treated with spinosad, nano-materials used for controlling *S. olae* by nano-materials[15].

These results are in consistence with those obtained by Sabbour and Shaurub[16] 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[13]. Similar results were obtained by Sabbour[12] for spinosad-treated olive trees that were infested by *B. oleae*, *C. capitata* and *P. oleae*. The olive weight increased after bioinsecticide applications[17]. The nano-biopesticides application increase the productivity of the olive fruits under field conditions[17]. Sabbour and Solieman[18-20] control *Tuta absoluta* by nano chitosan and results showed a reduction in the infestation numbers. The nano-chitosan against *Schistocerca gergaria* and found a loss of the pests number after treatments under laboratory and field conditions[21]. The same obtained by Sabbour and Abd El-Aziz[22], Shaurub and Sabbour[23], Sabbour and Abd El-Aziz[24,25].

CONCLUSION

Nano-formulation of chitosan was more effective than chitosan in controlling *Saissetia oleae*. These results encourage the extension in the use of nano-technology for insect pest control.

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