Efficacy of Chitosan Nano-particles Against Two Tetranychid Mites and Two Associated Predaceous Mites (Acari: Tetranychidae: Phytoseiidae).

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Abstract: Acaricidal activity of Chitosan Nano-Particles (C.N.Ps.) was tested against different stages of *Tetranychus urticae* (Koch), *Tetranychus cinnabarinus* (Boisduval), and two of its associated predator mites, *Amblyseius californicus* (Mc Gregor) and *Phytoseiulus persimilis* (Evans). The obtained results demonstrated that there were considerable differences between the mortally percentages of different stages of mite pests. C.N.Ps had a lower efficacy against the tow predators' *A. californicus* and *P. persimilis*. The LC_{50} values of C.N.Ps on *T.urticae*, *T. cinnabarinus*, and its immature stages, were 22.08, 159.47, 50.834, and 142.731 ppm, respectively after 72 hrs. of exposure. *T. cinnabarinus* was more tolerant than *T. urticae* to C.N.Ps. Data showed that field treatment with LC_{90} value induced 94.35% mortality of *T. urticae* after three days of treatment. Chitosan Nano-Particles seems to be promising for controlling *T. urticae*, and it supports the need to overcome resistance issues with currently registered acaricides.

Keywords: Chitosan Nano-particles, Tetranchidae, Phytoseiidae, Biological control.

1.Introduction

In recent years, the sustainable development concept has obtained important attention to achieve green technologies and the use of these products over traditional ones contribute to sustainability through decreases in environmental degradation **Gomes *et al.* (2017)**.

Chitin is one of the most abundant natural biopolymer acquired from the waste of industrial fishing activities. By deacetylation of Chitin, Chitosan was obtained and characterized using the Fourier transform infrared and X-ray diffraction **Marei *et al.* (2015)**. Due to its biodegradability and bioactivity, it has received more importance for possible applications in agriculture and biotechnology. As a consequence, Chitosan may serve as a good alternative for broad-spectrum and highly persistent pesticides. It is non-toxic to vertebrates and humans, biodegradable, and may possess insecticidal and microbicidal properties **Badawy and El-Aswad (2012)**.

Nanotechnology has the potential to produce new insecticide ingredients and innovative products, with considerable benefits to control agricultural pests. The notable character of nanoparticles for their small size and quantum size effect could make Chitosan Nano-Particles (C.N.Ps) exhibit superior activities.
Ali and Joshi (2013).

The two-spotted spider mite, *Tetranychus urticae*, is an important pest of a variety of crops. It is a highly polyphagous pest spreading rapidly and it is notorious for developing resistance to pesticides. Carmine spider mite, *Tetranychus cinnabarinus*, is widely distributed in several temperate zones and is harmful to most species of plants **Yan Li (2016)**.

It is important to test the efficacy of newer pesticides on both the pests and their natural enemies to determine if outbreaks are likely to occur and to define potential mechanisms of material selectivity. This is done to understand the non-target effects of acaricides on both tetranychids and their predators, especially phytoseiid, which results in disruption of biological control allowing producers to choose acaricides that do not selectively favor spider mites over their natural enemies **Rebecca *et al.* (2018)**.

The present study was conducted to evaluate the use of Chitosan Nano-Particles as alternative control methods that are safe and environment-friendly, against different stages of *T.urticae*, *T. cinnabarinus* and their predators, *A. californicus* and *P. persimilis*.

2.Materials and Methods

2.1. Preparation of Chitosan Nano-Particles:

Chitosan was purchased from the Oxford lab. India. Chitosan Nano-Particles were prepared according to **Gan *et al.* (2005)** at NAQAA Nanotechnology Network- Giza- Egypt (https://nakaanetwork.webs.com).

2.2. Characterization:

A high-resolution transmission electron microscope

(HR-TEM, Tecnai G20, FEI, Netherland) was used for imaging. Particle size distribution and the zeta potential of C.N.Ps were determined by a Zetasizer Nano ZS (Malvern, UK). The morphologies and distribution of the Chitosan Nano-Particles were measured at Nanotechnology and Advanced Materials Central Lab, Agricultural Research Center. Giza. Egypt. (Fig. 1&2).

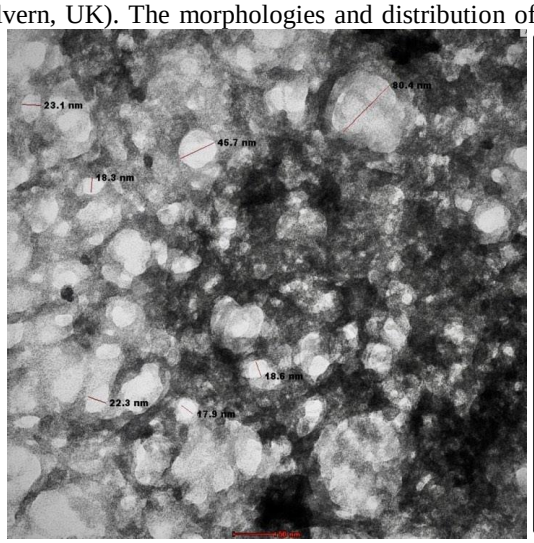

Fig. (1) TEM micrograph of chitosan-nano particles

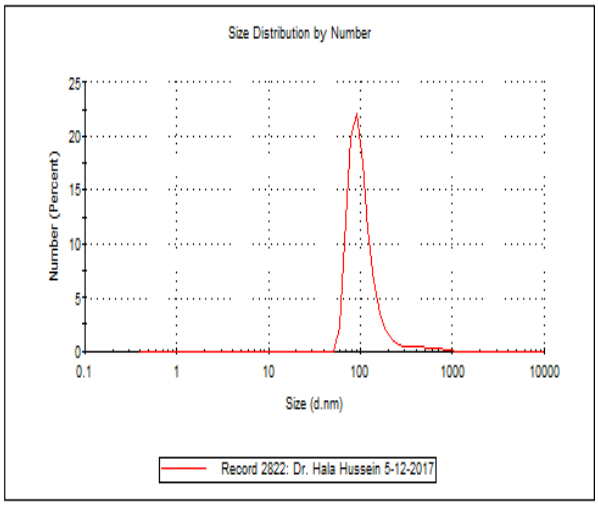

Fig. (2) Size distribution by intensity of Chitosan-Nano-Particles using a Malvern Zetasizer Nano ZS.

2.3. Mites Culturing:

T. urticae and *T. cinnabarinus* were collected from unsprayed green beans (*Phaseolus vulgaris* L.). Mite's identification was carried out at Acarology Laboratory, Plant Protection Research Institute (P.P.R.I), Agricultural Research Center (A.R.C.) using detailed descriptions recorded by (**Zhang (2003), Zhang and Jacobson 2000**).

Collected mites were put on green beans leaves upside down on dampening cotton in Petri-dishes (12 cm in diameter), and cotton was moistened daily. Petri-dishes were kept under controlled conditions at 27±2°C and 16:8 h (L:D) in the Acarology Lab. Green beans leaves were replaced twice a week.

2.4. Rearing of Predators:

Predatory mites, *Amblyseius californicus* and *Phytoseiulus persimilis*, were obtained commercially from predator mite's production unit, P.P.R.I., A.R.C.

2.5. Efficacy of C.N.Ps Against Adult Females:

The acaricidal effect of seven selected concentrations of C.N.Ps. was tested; control was treated with 1% glacial acetic acid. The bioassay arena consisted of a disk 3-cm in diameter cut from a green bean leaf. The disk was placed in 12-cm diameter Petri dishes with moist polyethylene foam surrounded by wet hydrophilic cotton in water to avoid the escape of mites. The experiments were proved in 4 replicates. Mites were treated by spraying on the leaflets with the prepared C.N.Ps. using hand sprayer at the concentrations 800, 400, 200, 100, 50, 25 and 12.5 ppm. After treatment, females were allowed to feed and oviposit for three days, then evaluated as either alive or dead and the mortality percentage were counted.

2.6. Efficacy of C.N.Ps against Immature Stages:

Ten females of each *T. urticae* and *T. cinnabarinus* were placed on green bean leaflets. After four days, females were removed from the leaflets. Eggs were kept under controlled laboratory conditions till all eggs hatched. Different immature stages were transferred to new leaves and sprayed by C.N.Ps. with the selected concentrations.

2.7. Efficacy of C.N.Ps against Egg Hatching of spider mite, *T. urticae*:

Green bean leaf discs 3 cm in diameter were used. Five adult females of the same age were transferred to each disc and left for one day to lay eggs, and then all females were removed. Eggs were sprayed by C.N.Ps., control variants were sprayed with distilled water. After the majority of the eggs in the control hatched, the number of hatched, unhatched eggs, live and dead immature were recorded. Arenas were held in the incubator during the experiment till all live larvae reach the adult stage.

2.8. Efficacy of C.N.Ps against Predatory Mites:

Seven concentrations were sprayed on green bean leaflets, after drying 10 individuals of predatory mites, *A. californicus* and *P. persimilis*, were transferred to the treated discs. Fifty motile individuals of *T. cinnabarinus* were added for feeding. Mortality percentages were recorded after three days. Additional prey were added

daily until tests were completed.

2.9. Field Experiment: A field experiment was conducted to determine the effectiveness of the C.N.Ps under field conditions. Green beans (*Phaseolus vulgaris* L.), Nebraska variety, plants were used as a host plant. The experimental design was a randomized complete block (RCBD) with three replications.

When the infection was confirmed with *T. urticae*, a pre-spray sample was taken and the treatment was carried out with the LC_{90} value (133.33 ppm) of Chitosan nanoparticles and distilled water was used as a control. Ten leaves were taken from each replicate and the number of mites (immature and adult) was counted before treatment and after three, seven and 14 days post-treatment by the aid of a stereomicroscope.

2.10. Statistical Analysis:

Lethal effect of Chitosan Nano-Particles was evaluated; data were pooled; and mortality was converted to percent mortality. Any mortality in the control was corrected using **Abbott's** formula **(1925)**. Mean lethal concentrations (LC_{50}) were calculated after three days of treatment by Probit Analysis Program **Finney (1971)**, $LC_{50/90}$ values were at p-level < 0.05.

3.Results and Discussion

Laboratory evaluation of Chitosan Nano-Particles against different stages of spider mite, *T. urticae* and *T. cinnabarinus* showed a high mortality percentages through 72 hrs. after treatment. There was no change in mortality percentage after this period without a noticed mortality in control. Acaricidal properties against females of *T. urticae* and *T. cinnabarinus* were investigated at seven concentrations ranged from 800 to 12.5 ppm. According to the obtained results, LC_{50} and LC_{90} values were 22.08 and 133.33 ppm for *T. urticae* while recorded 159.447 and 756.304 ppm for *T. cinnabarinus*, respectively, table (1).

Table 1.Toxicity of C.N.Ps against movable stages of *T. urticae, T. cinnabarinus* and its predators after three days of treatment under laboratory conditions.

Toxicity parameter	LC_{50}	Lower limit	Upper limit	Slope	Toxicity index %	LC_{90}
T. urticae	22.08	15.01	29.24	1.64	100	133.33
***T. urticae* immature**	50.834	37.206	66.818	1.433	44.098	398.36
T. cinnabarinus	159.447	122.805	204.075	1.896	14.059	756.3
***T.cinnabarinus* immature**	142.731	83.688	262.803	1.538	15.706	972.37

Efficacy of the tested concentrations of (C.N.Ps.) against immature stages of *T. urticae* and *T. cinnabarinus* were enhanced with increasing concentrations.

Mortality values caused by C.N.Ps for *T.urticae* immature stages after three days of treatment were 97.5, 92.5, 80, 57.5, 47.5, 35 and 22.5% for the tested concentrations, while they were 82.5, 70, 40, 25, 17.5, 15 and 12.5% for *T. cinnabarinus* immature stages, respectively Fig. (3), whereas immature stages of *T. urticae* and *T. cinnabarinus* showed LC_{50} and LC_{90} values of 50.834, 398.358, 142.731and 972.365 ppm, respectively. Results showed that for all tested concentrations, the motile stages of *T. urticae* were more susceptible to C.N.Ps. than *T. cinnabarinus*.

Table 2. Egg hatchability and adult percentage of treated *T. urticae* eggs.

Conc.	800	400	200	100	50	25	12.5	control
Hatching	74.98± 1.8	77.72±2.4	85.99±2.6	89.96±4.6	95.3± 0.9	95.3± 1.8	97.2± 1.3	100
Larvae	74.98±8.4	77.72±9.9	85.99±2.6	70.02±3.9	43.22±11.9	43.22±1.3	1.1±0.8	0.5±0.5
Protonymph	0	0	1.85±0.4	15.95±5	22.1±2.2	22.1±0.7	58.86±0.5	0
Deotonymph	0	0	0	1.92± 1.3	7.87±2.3	17.87±0.8	23.26± 1.8	1±0.5
Adults	0	0	0	1.92± 1.3	2.18±0.0.8	12.18±0.5	14.28±0.7	98.5±0.7

The treated eggs of *T. urticae* were found to be affected in tits hatchability and ability to complete its life cycle to adults. Table (2) indicated that there was a decrease in egg hatchability compared with control treatment. For high concentrations, hatched larvae were completely dead, while 1.92 -14.88 % of treated eggs succeeded to reach the adult stage for 100 and 12.5 ppm, respectively. Results indicated that C.N.Ps.

has an ovicidal effect to *T. urticae*, while it is ineffectual to *T. cinnabarinus* eggs.

The toxicity of Chitosan Nano-Particles tested against the two predatory mites was shown in Fig (3). The highest mortality percentages were 17.5 and 27.5 for *A. californicus* and *P. persimilis*, after three days of treatment, respectively.

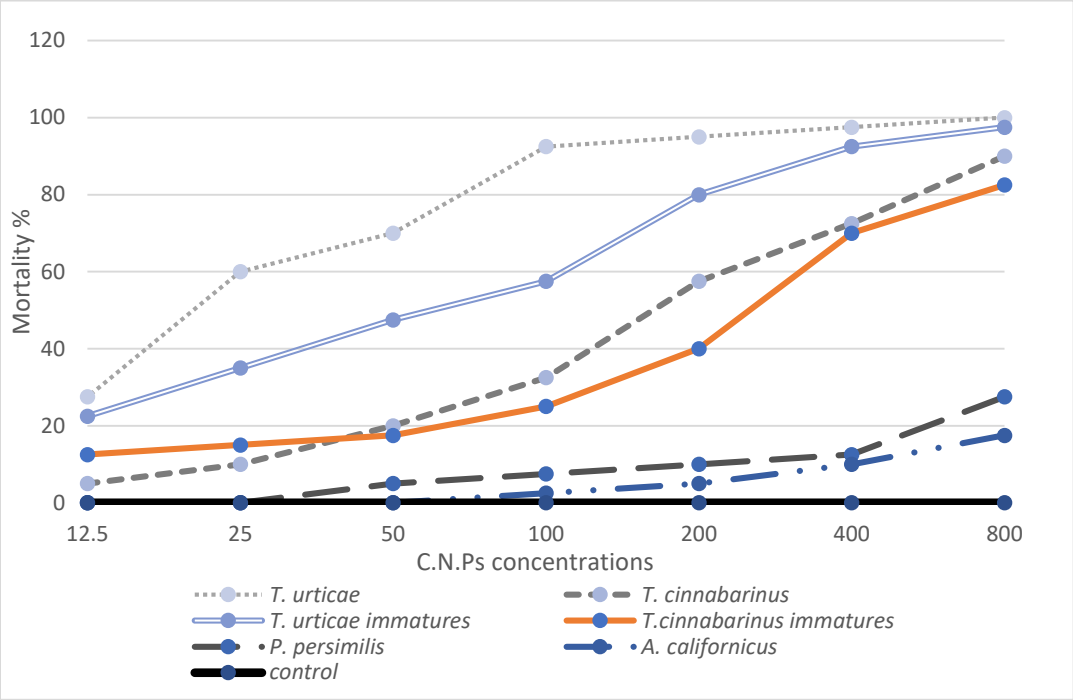

Fig.3: Effect of C.N.Ps against movable stages of *T. urticae, T. cinnabarinus* and its predators after three days of treatment under laboratory conditions.

Table (3) illustrates the effect of the LC_{90} value of C.N.Ps. on *T. urticae* under field conditions on green bean (*Phaseolus vulgaris* L.). It was found to be potent against *T. urticae*, as it caused 94.35, 93.33 and 90.73% reduction percentages for *T. urticae* at 133.33 ppm after three, seven and 14 days of treatment, respectively thus, the post-treatment count was correlated with pre-treatment.

Table 3. The number of *T. urticae* on *P. vulgaris* and efficacy of C.N.Ps. (Ef %) according to Henderson-Tilton formula.

C.N.Ps concentration	Mean number (*) of *T. urticae*				Ef%		
	BT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT
LC_{90} value (133.33 ppm)	33	13	20	25	94.35	93.33	90.73

(*) motile forms; BT = before treatment; DAT = days after treatment.

This experiment confirmed that C.N.Ps. is an effective compound against all motile stages of the two-spotted spider mite, *T. urticae***.** The Green beans plants sprayed with C.N.Ps. recovered and showed better growth than the mite-infested plants (control). No available studies were recorded about the efficacy of C.N.Ps on tetranychid mites and its predators, but it was tested on several insects.

Zhang and Tan (2003) reported that, Chitosan exhibited different insecticidal activity to various aphids ranged between 93 and 99% for *Hyalopterus prun* (Goffroy) on flowers, while **Rabea (2005)** tested the insecticidal activities of Chitosan Nano-Parteciles against larvae of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). The oral feeding bioassay indicated that all the derivatives had a significant insecticidal activity at 5 g kg^{-1} in an artificial diet. The most active was N-(2-chloro-6-fluorobenzyl) Chitosan, which

caused 100% mortality at 0.625 g kg^{-1}, with an estimated Lc_{50} of 0.32 g kg^{-1}.

The same trends were also observed against *Aphis gossypii*; the mean number of eggs/female of *A. gossypi* was significantly decreased to 20.9 and 28.9 eggs/female compared with 97.3 and 90.3 of the non-treated controls, under laboratory and under semi-field conditions, respectively **Sahab *et al.* (2015)**.

The percentage of insect growth significantly decreased from 99% in semi-field control to 22% (77.8% decrease) in treated insects under semi-field conditions. Comparative efficacy of Silica Nano-Particles on mortality percentage of *T. urticae* and *T. cinnabarinus* was found, where SiNPs is more efficient in reducing *T. urticae* than *T. cinnabarinus*. Results showed that the mortality percentages of *T. urticae* adults were 32.5, 35, 67.5 and 85% at the concentrations of 50, 100, 200 and 400 ppm, respectively, while those of *T. cinnabarinus* were 22.5, 27.5, 42.5 and 72.5% for the four used concentration, respectively under laboratory conditions, after 7 days post-treatment. They also reported its effect against 3 spider mite`s predators. Recorded mortality percentages were 32.5, 35 and 97.5% for *Orius insidiosus*, *Phytoseiulus persimilis*, and *Stethorus punctillum* after seven days of treatment, respectively **Alakhdar and Elsamahy (2016**).

Conclusion

The obtained findings suggest that Chitosan Nano-Particles had the potential for biological control of Tetranychid mites -*Tetranychus urticae* and *Tetranychus cinnabarinus*- with a slight effect on its natural enemies *Amblyseius californicus* and *Phytoseiulus persimilis*. All stages of *T. urticae* were found to be more sensitive than *T. cinnabarinus*. The C.N.Ps. have been shown to be a good alternative method for controlling spider mites.

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فاعلية جزيئات الشيتوزان النانوية ضد اثنين من الحلم العنكبوتي واثنين من المفترسات المرتبطة بهما(Acari: Tetranchidae: Phytoseiidae).

هاله حسين الاخضر

قسم بحوث أكاروس القطن و المحاصيل

معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقى - الجيزة - مصر

الملخص العربى:

يعد كلا من الحلم القرمزى العنكبوتى والعنكبوت ذو البقعتين من أهم أنواع الأكاروسات فى مصر وحيث أنهما سريعا الانتشار وبسبب مقاومتهما للمبيدات الأكاروسية كان من الضرورى العمل على ايجاد بعض البدائل لمكافحة هذه الآفات. تم عمل تجارب معملية و حقلية لمعرفة مدى تأثر الاطوار المختلفة لكلا الآفتين و كذلك اثنان من المفترسات الاكاروسية المصاحبة لهما بجزيئات النانو شيتوزان. و قد أظهرت النتائج وجود اختلافات بين النسب المئوية للموت لكلا النوعين في جميع المراحل بينما كانت فاعليته أقل على المفترسات الاكاروسية. و كانت قيم LC_{50} لمادة النانوشيوزان على *T.urticae*، *T. cinnabarinus* ومراحلها أطوارها، 22.08 ، 159.47 ، 50.834 ، و 142.731 جزء في المليون على التوالي بعد 72 ساعة من التعرض.أظهر العنكبوت الأحمر القرمزي تحملا أكثر من العنكبوت الأحمر ذو البقعتين. وبمعاملة بيض ال *T.urticae* بالتركيزات المختلفه للنانوشيتوزان, استطاع 14.28% من اليرقات استكمال دورة حياتهم للوصول للطور البالغ مع أقل تركيز 12.5 جزء في المليون. وأظهرت النتائج أن المعاملات الحقلية للعنكبوت الاحمرذو البقعتين بالتركيز LC_{90} ادى الى خفض نسبة التعداد 94.35% بعد ثلاثة أيام من الرش.