

Acaricidal Activity of Nano-Abamectin Against the Two-Spotted Spider Mite; *Tetranychus Urticae* Koch (Acari: Tetranychidae)

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Abstract

The efficiency of prepared nano-abamectin was assayed against two-spotted spider mite, *Tetranychus urticae* (Koch). Prepared formulation showed spherical particles ranged from 66 to 133 nm under examination of Transmission Electron Microscope (TEM) and same pattern of Fourier Transform Infrared (FTIR) spectrum in comparison of conventional acaricide (Vertimec®). In laboratory trail, toxicity index at LC50 level confirmed that nano-abamectin was greater toxic to adult female, *T. urticae* (Koch) with value; 30 than Vertimec® 1.8% EC. Similarly, nano-abamectin reduced the mite fecundity at levels greater than those of Vertimec®. Regarding field trail, nano-abamectin at rate of 60 ml/ha showed very toxic effects after 3 day of spraying followed by 7 and 14 days on soybean plants achieving % of mortality; 89.98, 83.80 and 76.90%, respectively. These findings showed that, bioactivity of nano-acaricide were many folds higher than conventional acaricide against mite, *T. urticae* (Koch). However, the biosafety issues may be also addressed.

Keywords: Nano-emulsion; Abamectin; *Tetranychus urticae* (Koch); Nanotechnology.



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1. Introduction

Pesticides play an important role in agriculture through prevention of crop loss caused by major diseases and pests. The effective amounts of conventional pesticide formulations did not exceed than 30% due to losses through poor dispersion, droplet drift, and biodegradation. However, application of synthetic pesticides caused threat to non-target organisms and the environment due to their overuse [1]. Thus, it is important to improve the used rate of pesticides and extend their duration of activity in the environment [2-5]. Nano-pesticides hold promise to reduce the environmental footprint left by conventional pesticides. These products may allow for more effective targeting pests, use of smaller quantities of pesticide and minimize the frequency of spray-applied surface disinfection. These findings mostly improve human and environmental safety and minimize pest control costs. While using a new technology, safety of the user and its effect on environment has to be considered [6]. In particular, design of nano-formulation of different pesticides has emerged at high speed. Compared to bulk substances, nano-insecticides have many added advantages e.g. (a) less environmental contamination through reduction in application rates; (b) enhance efficiency of chemical and natural insecticides by controlled release; (c) renders insecticides more susceptible to photodegradation; (d) easy/safe handling with reduced risk to animals and (e) less toxicity towards non-target organisms [7].

Most of family tetranychidae are considered phytophagous pests. They attack various vegetables and other crops causing direct damage to greenhouse and field crops [8]. In addition, they make indirect damage by transmission of some micro-organisms such as viral and fungal pathogens. *Tetranychus cucurbitacearum* (Sayed), is considered as one of the major pests attacks different crops, vegetables, fruits and ornamental plants [9]. Two-spotted spider mite, *Tetranychus urticae* (Koch) is a broad-based pest of many field, horticultural and greenhouse crops [10, 11]. It lives inside complex three-dimensional webbings in a colonial microhabitat on the bottom surface of the plant, protecting its life stages from abiotic and biotic factors [12].

The intensive use of synthetic pesticides in the last years does not meet the modern criteria of integrated pest management programs, leading to an increasing interest for natural pesticides, derived from plants and microorganisms, because they are safer than synthetics [13]. Avermectins are known as acaricidal compounds and consist of a group of naturally occurring secondary metabolites produced by the soil actinomycete, *Streptomyces avermitilis*. These compounds have proven to be globally important animal health agents and indispensable human medications [14]. In general, they consist of four closely related major components, A1a, A2a, B1a and B2a and four minor components; A1b, A2b, B1b and B2b, which are lower homologs of the corresponding major components [15]. However, continued use of avermectins may lead to the development of resistance in the target species. Thus, the study aims to evaluate the efficiency of nano-emulsion of abamectin 1% EC against *T. urticae* Koch, in comparison with conventional insecticide; Vertimec® 1.8% EC.

2. Materials and Methods

2.1. Formation of Nano-Emulsion

Nano-emulsion of abamectin 1% was prepared under high energy mode using sonication techniques [16]. The active ingredient of acaricide was dissolved in an oil and employed to dispersion of liquid (water) with surfactant and co-surfactant to generate homogenous solution (o/w).

2.2. Evaluation Parameters of Nano-Emulsion

2.2.1. Thermodynamic Stability

Nano-emulsion was subjected to various storage conditions of temperature and humidity to assess the stability according to ICH guidelines Q1A [17]. The prepared formulation was centrifuged at 3500 rpm for 30 min to observe any phase separation. Ten ml of prepared formulation were diluted to 100 ml with distilled water in graduated cylinder. The solution was employed for shaken at 30 times from top to bottom continuously. At the end, the jar was allowed for 10 min and observed for oil separation, creaming and sedimentation.

On the other hand, an aliquot of formulation was taken for heating and cooling cycle. Six cycles between refrigerator temperature of 4 °C and 48 °C for 48 hr were done. So, they were employed to freeze-thaw cycle test. Three cycles were done between -21 °C and +25 °C.

2.2.2. Transmission Electron Microscopy (TEM)

Morphology and structure of prepared formulation was determined by transmission electron microscopy (TEM) (JOEL 1400 Plus, Japan) at filament 120 Kev to reveal the form and size of nano-formulation. So, an aliquot of formulation was diluted with deionized water (1/100) and sonicated for enough time. A drop of the diluted solution was deposited on the film grid, dried and observed [18, 19]. A combination of bright-field imaging at increasing magnification with diffraction modes was used.

2.2.3. Fourier Transform Infrared (FTIR)

The conventional formulation and non-formulation were achieved on TENSOR 27 Buker, Germany-FTIR L203/1 2887. The spectrometer ranged from 4000 to 400 cm⁻¹. The run was conducted with sensitivity range 50 and absolute threshold level of 6.07.

2.3. Rearing of *T. Urticae* (Koch)

Samples of beans plants, *Phaseolus vulgaris* L., heavily infected with *T. cucubitarum* were collected from Hehhyia district, Sharkeia governorate, Egypt. Individuals of mite were initiated by transfer using a fine hair brush to fresh disks of mulberry leaves in Petri-dish (10 cm in diameter). Each leaf was put on a pad of cotton saturated with water to prevent mite escaping under laboratory conditions (27±2 °C and 75±5% R.H.).

2.4. Toxicity Test

Eight concentrations; 40, 50, 100, 150, 200, 250, 500 and 1000 ppm were used for conventional abamectin (Vertimec® 1.8% EC) and nine concentrations; 10, 20, 30, 40, 50, 100, 150, 200 and 250 ppm were used for nano-emulsion of abamectin against adult females of *T. urticae* Koch. Five replicates were used for each concentration with ten adult for each. The individuals in treatments were sprayed with acaricide solutions, but control groups were sprayed with distilled water. The individuals of mites were confined on the lower surface of mulberry leaf disks (3 cm in diameter). Mortality was calculated after 72 hr of treatment and corrected according to Abbott's formula [20].

2.5. Ovicide Bioassay (Direct Toxicity to Newly Laid Eggs)

To provide eggs for this test, four adult females were transferred to each leaf disks and then removed. Disks with deposited eggs (24 hr old) were individually immersed in the same concentrations as described above for 30-60 sec. So, the excess of acaricide solutions were dried. Three replicates were used for each concentration (100 eggs per each). Disks were kept under laboratory conditions as described above. All disks were examined daily for seven days. Egg hatching rate and offspring development were stated daily by counting the numbers of hatched eggs and larvae on the disks.

2.6. Field Trail

Soybean plants were cultivated in Hehhyia district, Sharkeia governorate, Egypt during two seasons 2017/2018. The cultivated area for each treatment was 160 m² divided into 4 replicates (40 m² for each). The population density of mite was recorded before spraying and 3 times; 3, 7 and 14 day after spraying. The sample size was 40 leaves for each treatment. The lower surface of leaves was used for counting the individuals by using Stereo binocular microscopy. The reduction percentages of mite were calculated according to Henderson and Tilton [21].

2.7. Statistical Analysis

The LC₅₀ values and the regression equations were calculated according to Finney [22] using LdPLine® software. All data were presented as mean±SE. The statistical analysis was performed using COSTAT, Costat User Manual, version 3. Cohort Tucson, Arizona, USA [23].

3. Results and Discussion

3.1. Characterization of Nano-Emulsion

The prepared nano-emulsion was stable during freeze-thaw cycle's storage. No creaming or floating phases were made up. In addition, no separated phase was consisted after centrifugation process.

As observed from the TEM image (Figure 1) the abamectin nanoparticles appeared as spherical shapes, and the size was mainly in the range of 66 to 133 nm. The above results demonstrated that this nano-sizing method could effectively construct abamectin as nano-emulsion. Fourier Transformed Infrared (FTIR) was used to verify that abamectin was nanosized emulsion. Figure 2 shows the FTIR spectra of Vertimec® and nano-abamectin. The typical absorption peaks at 1742 cm^{-1} for C=O, 3423 cm^{-1} for OH, and 1618 cm^{-1} for benzene skeleton vibration appear in the spectra of abamectin. Regarding nano-form, 1742 and 1092 cm^{-1} are attributed to the C=O and COC stretching vibration peaks. The broad peak at 3567 cm^{-1} may be attributed for C=O group of used co-surfactant (polymer).

3.2. Bioactivities

Mortality rates of adults; *T. urticae* Koch at the various concentrations tested is shown in Table 1. Probit analysis indicated that, the LC_{50} of Vertimec® and nano-abamectin was 80.70 and 23.90 mg/L, respectively. For Vertimec®, concentration range (40-1000 mg/L) induced mortality rates ranged from 34 to 86%. However, concentration range of nano-abamectin (10-250 mg/L) induced mortality rates ranged from 25 to 96%. Concerning toxicity index at LC_{50} level, the data confirmed that, solution of nano-abamectin was the most toxic compound to adult female of *T. urticae* with index value; 30 compared with conventional acaricide, Vertimec®. This study revealed that, some concentrations of abamectin in nano-formulation affected mite population greater than Vertimec®. This finding, can be attributed to chemical in nano scale has the ability to be more stable, penetrate, and good sticking with treated surfaces resulting in more efficiency and residual effects [24]. For example, Abd El-Rahman [25] stated that, nano-particles of abamectin benzoate were the most toxic compound to adult females of *T. urticae* with LC_{50} (0.006 mg/L) compared with methomyl 90% which exhibited LC_{50} (89.54 mg/L) with toxicity index 0.09.

In fact, prepared insecticides in nano-form enhances their stability on treated plants and become more potent. For example, avermectin-grafted-N, O-carboxymethyl chitosan was more effective against carmine spider mites more than avermectin technical material [26]. The residual rate of the conjugate was 11.22%, while the residual rate of the avermectin technical material was 0.2%. The bounded groups may increase photostability of compound and work as an antioxidant which captures the hydroxyl radical in the aqueous solution [27].

Spraying of these concentrations of Vertimec® and/or nano-abamectin reduced the mite's fecundity compared to the water-sprayed control group which exhibited hatching percent 98.38. Vertimec® treatments reduced the hatching of eggs at percentages ranged from 0.61 to 66.70% with LC_{50} value; 612.8 mg/L (Table 2). On the other hand, nano-abamectin reduced the hatching of eggs at percentages ranged from 4.00 to 66.70% with LC_{50} value; 129.6 mg/L. Several investigations stated that, abamectin's forms or derivatives are more potent to reduced female fecundity of mites. For example, Ismail, *et al.* [28] found that, abamectin significantly reduced female fecundity and killed offspring when applied directly on the egg of *T. urticae*. The data of the present study are accordance with that obtained by Abd El-Rahman [25], where nano particles of abamectin benzoate were the most effective on egg deposition and in reducing mite fecundity by 82.24%. However, common solution of abamectin benzoate was less effective on egg hatchability (35%).

Regarding field trail, two tested levels of nano-abamectin and recommended rate of Vertimec® reduced the number of mite (Table 3). Nano-abamectin at rate of 60 ml/ha showed very toxic effects after 3 day followed by 7 and 14 days achieving % of mortality; 89.98, 83.80 and 76.90%, respectively. However, level of 40 ml/ha showed % mortality values; 81.93, 78.29 and 65.85% after the same periods. Vertimec® showed moderately toxic effect after 7 and 14 day of application achieving values; 75.52, and 65.5%, respectively. Even at nearly half of the field rate, nano-abamectin was very toxic to *T. urticae* (Koch) after 3 and 7 days. Based on present observations, these effects could be caused by residual toxicity of nano-formulation on survival of the mite. Although low applied concentrations of nano-abamectin induced very toxic effects, further investigations concern them may be demonstrated on predators to provide an adequate indication for selectivity of acaricide which is essential for development of pest management programs [29].

The study revealed the processes for the preparation of nano-pesticide. The bioactivity of nano-pesticide was many folds higher than conventional formulations against mite, *T. urticae* (Koch). Therefore, nanotechnology has potential to provide green and efficient alternatives for the management of pests in agriculture without harming the nature. However, the biosafety issues of these nano-pesticides are also addressed. The legal and registration requirements for nano-pesticides may be modified and also a protocol for handling them has to be developed.

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Figure-1. TEM photograph of insecticide; nano-abamectin visualized at 30000x

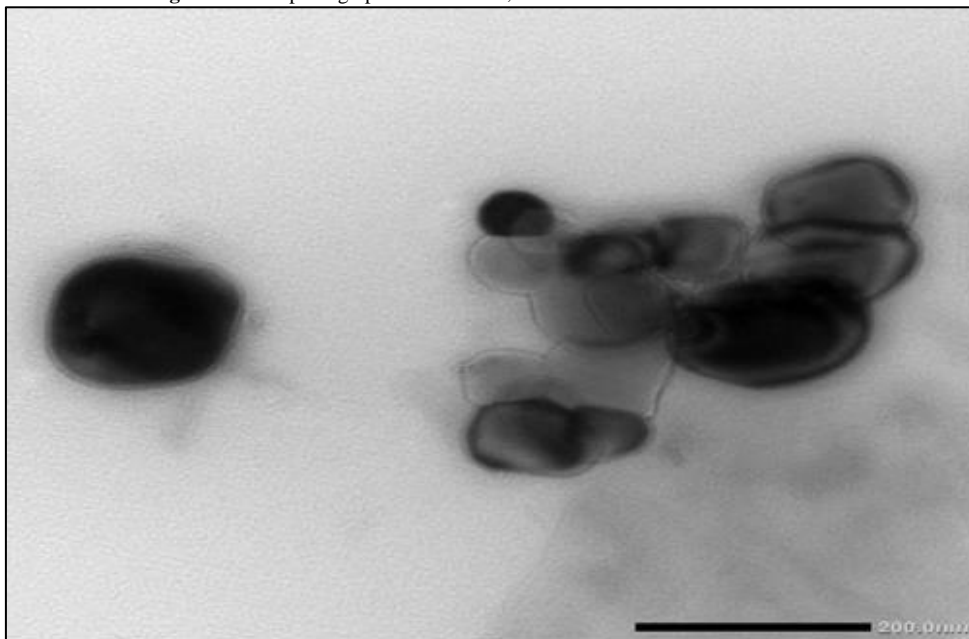


Figure-2. FTIR pattern of insecticides; (a) Vertimec® and (b) nano-abamectin

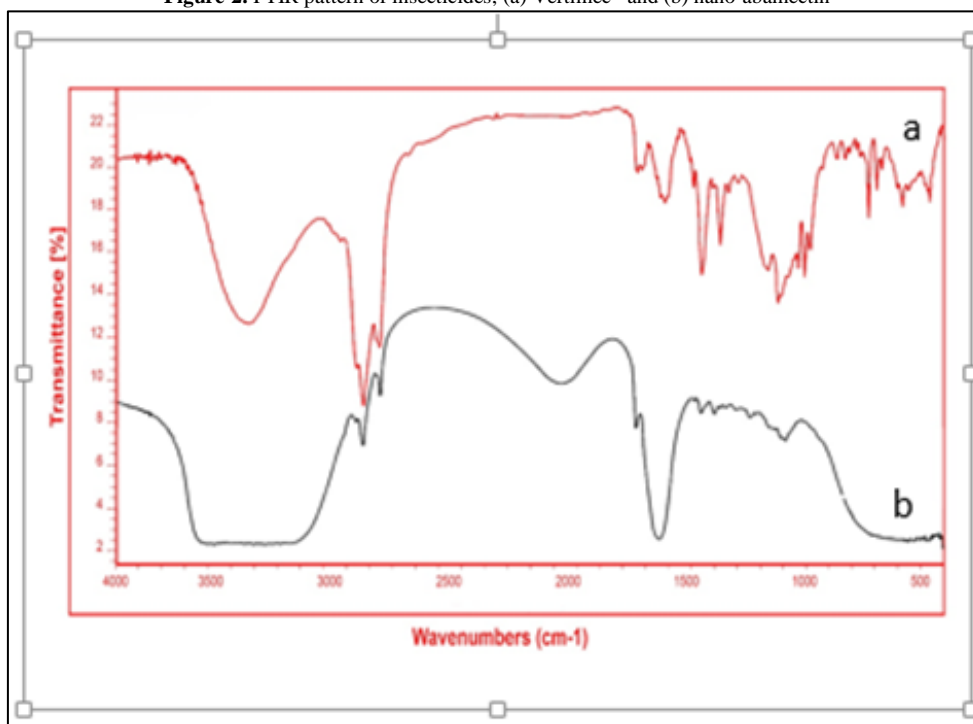


Table-1. Mean mortality rates of *T. urticae* Koch adults after 24 hr laboratorial exposure to Vertimec® and nano-abamectin, calculating LC₅₀ (fiducially limits)

treatment	Concentration used (mg/L)	Mortality rates (mean±SE)	LC ₅₀ (mg/L)
control	Distilled water	01±.67	
Vertimec® 1.8% EC	40	34±1.35	
	50	40±1.18	
	100	54±1.61	
	150	60±0.89	80.7 (63.5-241.8)
	200	74±1.34	
	250	78±0.45	
	500	84±1.79	
	1000	86±0.89	
Nano-abamectin	10	25±2.05	
	20	40±0.89	
	30	52±1.34	
	40	70±1.79	
	50	84±1.34	23.9 (17.4-30.2)
	100	86±1.25	
	150	90±0.89	
	200	92±0.54	
	250	96±0.45	

Table-2. Egg hatching % and offspring development after direct spraying of newly-laid *T. urticae* Koch eggs with Vertimec® and nano-abamectin

Treatment	Concentration used (mg/L)	Hatching %	LC ₅₀ (mg/L)
control	Distilled water	98.48±1.44	
Vertimec® 1.8% EC	40	00.61±0.01	
	50	06.36±0.31	
	100	13.39±1.22	
	150	20.30±1.13	612.8 (481.7-843.6)
	200	36.97±1.23	
	250	43.03±0.38	
	500	51.81±0.94	
	1000	66.70±0.59	
Nano-abamectin	10	4.00±0.60	
	20	9.00±0.89	
	30	15.0±0.83	
	40	27.0±1.18	
	50	30.0±0.89	129.6 (112.9- 151.7)
	100	36.8±0.95	
	150	48.1±1.33	
	200	53.3±0.92	
	250	66.7±0.59	

Table-3. Mortality and toxicity ratings of Vertimec® and nano-abamectin applied at field conditions to *T. urticae* Koch on Soybean plants

Treatment	Applied rate (ml/ha)	% Mortality of mites after application (day)			Mean	LSD (0.05)
		3	7	14		
Vertimec® 1.8% EC	100	81.24 ^d ±0.02	75.52 ^c ±0.02	65.59 ^c ±0.02	74.15 ^c ±0.02	2.286
Nano-abamectin	40	81.93 ^d ±0.02	78.29 ^d ±0.02	65.85 ^c ±0.03	75.36 ^c ±0.02	2.746
Nano-abamectin	60	89.98 ^d ±0.01	83.80 ^d ±0.01	76.90 ^d ±0.01	83.56 ^d ±0.01	2.240

-values followed by the same letter within are not significantly different according to toxicity ratings: ^aNon-toxic (<25% mortality), ^bSlightly toxic (25-50%), ^cModerately toxic (51-75%) and ^dVery toxic (>75%)