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# Formulation of abamectin and plant oil-based nanoemulsions with efficacy against the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) under laboratory and field conditions

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**Abstract**

Due to the harmful effects of synthetic chemical acaricides on ecosystems and human health, there is increasing interest in the use of nanotechnology to fabricate eco-friendly nanoemulsions based on plant oils in the field of spider mite control. In this study, nanoemulsions of abamectin, garlic, and neem oils were prepared by a high-energy approach and characterized by transmission electron microscopy. The droplet sizes of all tested nanoemulsions were less than 100 nm. The acaricidal activities of the prepared nanoemulsions compared to abamectin were evaluated against a susceptible laboratory strain of *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory and field conditions. The results showed that abamectin nanoemulsion was the most toxic compound against adult females of *T. urticae* followed by abamectin emulsion. Neem nanoemulsion had moderate toxicity and garlic nanoemulsion had the lowest toxicity. The effects of tested compound residues on egg deposition and egg hatching in descending order were as follows: abamectin nanoemulsion > abamectin emulsion > neem oil nanoemulsion < garlic oil nanoemulsion. In the field experiment, all tested compounds were effective in reducing the population density of *T. urticae* in the motile stage, with mean reductions ranging between 66.08% and 95.24% for all compounds. The most effective compound was abamectin nanoemulsion. The results of the present study demonstrate that nanoemulsion enhanced the biological activity of abamectin. Further, neem and garlic oil nanoemulsions have potential utility as environmentally friendly acaricides in integrated pest management programs.

**Keywords:** Abamectin, Garlic oil, Neem oil, Nanoemulsion, *Tetranychus urticae*, Field experiment

## Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is considered one of the major crop pests, affecting field crops, vegetables, and ornamental plants, and causes more agricultural economic losses than any other arthropod pest [1]. *T. urticae* is highly polyphagous and has been recorded to feed on over 1100 plant species [2].

Approximately 4 million tons of pesticides are utilized annually in pest control. Only small amounts of applied pesticides (1–25%) reach the target pests, while considerable amounts are released into the environment as potential environmental hazards [3]. With recent studies having investigated methods for reducing the side effects of conventional pesticides by developing novel formulations or introducing eco-friendly alternatives to pesticides in pest control [4], the intensive use of synthetic

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pesticides is associated with a range of environmental issues including pest resistance, pollution, and adverse health effects on humans and animals.

Abamectin is a member of the avermectin group and is produced from fermentation of the soil bacteria, *Streptomyces avermitilis,* as a mixture of avermectin B1a and B1b. Abamectin is one of the most important pesticides used worldwide and has a broad spectrum of activity as an insecticide and acaricide with high efficiency and relatively low toxicity to mammals [5]. However, the water insolubility of abamectin and non-uniform spread over applied surfaces may lead to increased release of toxic organic solvents and high concentrations of abamectin into the environment [6].

Recently, there has been growing demand for botanical pesticides as an alternative strategy for pest management. Several studies have reported the use of plant oils, particularly essential oils, as biopesticides. Interest in the use of plant oils in pest control has increased due to the drawbacks of synthetic pesticides including pesticide resistance, toxic residues, and the high cost of pesticide production [7, 8]. Despite this promise, the highly volatile and water-insoluble properties of plant oils limit their use under field conditions. [9, 10]. Among the plant oils, neem oil and garlic oil have been extensively used in pest control. The neem tree, *Azadirachta indica*, is considered to have some of the most benefical pesticidal and medicinal properties of any plant on Earth [11]. Neem oil-based pesticides, with anti repellant, anti feedant, growth inhibition, and insectidal effects, with neem oil being a broad-spectrum botanical pesticide that contains biologically active components including azadirachtin, salannin, and nimbin, have repellent, antifeedant, growth inhibition, and insecticidal effects against numerous species of arthropods without any adverse effects on non-target organisms [12]. Garlic, or *Allium sativum* L., is a rich source of organosulfur substances which confer flavor, aroma, and potential health benefits, and is an important aromatic plant that has multiple uses in food and medicine industries. [13]. Garlic essential oil reportedly provides significant protection against phytophagous mites. The acaricidal activity of garlic is posited to be due to the presence of diallyl sulfide, diallyl disulfide, dimethyl tetrasulfide, trisulfide, di-2-propenyl, and tetrasulfide, di-2-propenyl [14].

Many researchers have focused on the development of more efficient pesticide formulations using nanotechnology to overcome the limitations of synthetic and botanical pesticides [15, 16]. Nanotechnology offers new methods for synthesizing active ingredients with nanoscale dimensions. Pesticides in nanoform have several advantages over their bulk counterparts including: (a) decreased pollution and cost due to reduced pesticide concentrations and hazardous solvents; (b) increased pesticide efficacy due to controlled release and uniform spread over applied surfaces; (c) and easy and safe handling with reduced risk to non-target organisms [17]. Pesticide formulations in nanoforms are considered an effective method of pest management [18]. As the use of nanoemulsion formulations may allow increased efficacy and overcome some of the limitations of synthetic pesticides and plant oils, nanoemulsion is a promising nanoformulation that can be used to increase the persistence, water diffusion, and bioactivity of botanical and synthetic pesticides [19, 20].

Accordingly, the present study was conducted to investigate the formulation of abamectin, neem oil, and garlic oil as nanoemulsions. The acaricidal potentials of these formalations against *T. urticae* was compared to abamectin in emulsion form (recommended acaricide) under laboratory and field conditions.

## Materials and methods

### Culture technique of spider mite

The two-spotted spider mite, *Tetranychaus urticae* (Koch) (Acarina: Tetranitride), was reared according to the Dittrich method [21]. The colonies were obtained from castor bean plants from Kafrelsheikh Governorate and reared under laboratory conditions on the castor bean plant to avoid contamination with pesticide residues. Before starting, the prey culture was kept at 25 ± 2 °C under a 16 h photoperiod and 70 ± 5% relative humidity to encourage plant growth.

### Materials

#### *Abamectin*

Technical grade abamectin (95% w/w) is used as a recommended acaricide by the Agricultural Pesticide Committee, Ministry of Agriculture and Land Reclamation, Egypt [22] and was kindly supplied by KZ Pesticides and Chemicals Company, Egypt.

#### *Preparation of abamectin emulsion and nanoemulsion*

Nanoemulsion of abamectin (10% w/w) was prepared using the O/W emulsion high-energy method. Briefly, abamectin dissolved in methanol was added to Tween 80 (a non-ionic surfactant) at a ratio of 3:1 w/w using a magnetic stirrer at 400 rpm for 30 min to form an organic phase. Abamectin nanoemulsion was prepared through the organic phase being added dropwise under ultrasonic and high shearing physical emulsification processes to distilled water at a ratio of 1:4 w/w at 100 W for 15 min. Abamectin emulsion was formed using the same method described above using a magnetic stirrer instead of an ultrasonic high shear mixer.

#### *Extraction of neem oil*

Fresh leaves of neem, *Azadirachta indica*, were collected from trees that had not been treated with pesticides (Agricultural Research Center, Egypt). Dried at 50 °C for 48 h, neem leaves were ground into a fine powder. Oil extraction from 50 g of neem powder was performed using Soxhlet apparatus and 300 ml ethanol at a temperature of 68 °C. Neem oil was obtained after solvent evaporation according to a previously described method [23].

#### *Extraction of garlic oil*

Fresh cloves of garlic, *Allium sativa* L., were collected from garlic plants that had not been treated with pesticides (Agricultural Research Center, Egypt). Cloves were cut and dried in an oven at 60 °C for 48 h and a powder was formed by milling. Fifty grams of garlic powder was used for extraction with a Soxhlet apparatus using ethanol (20:1) at a temperature of 50 °C. Garlic oil was obtained according to a previously described method [24].

#### *Preparation of neem and garlic oil nanoemulsions*

Both neem and garlic oils (considered as 100% w/w) were prepared as nanoemulsion formulations according to the methods described above for the preparation of abamectin nanoemulsion.

#### *Characterization of prepared nanoemulsions*

Morphology and droplet size were characterized by transmission electron microscopy (TEM; JOEL 1400 Plus, Japan) at 80 keV. The polydispersity index (PDI) was measured using a Brookhaven zeta potential analyzer. The stability of tested nanoemulsions was confirmed by testing under varying heating, cooling, and freezing conditions based on previously described methods [25]. The stability of prepared nanoemulsions was tested by centrifugation at 10,000 rpm for 30 min at 25 °C. Centrifuged nanoemulsions were then stored for 4 weeks at room temperature to monitor for separation or creaming.

### Bioassay techniques

Acaricidal activity of prepared nanoemulsions against adult females of the two-spotted spider mite *T. urticae*. The leaf disc dip technique was used to evaluate the acaricidal activity of formulated nanoemulsions compared with abamectin in emulsion form against the two-spotted spider mite *T. urticae* [26]. Based on the results of preliminary tests, a range of concentrations that caused 10 to 90% mortality were selected for bioassay tests. Formulated compounds were diluted in distilled water to prepare a series of concentrations of the active ingredient (a.i). The tested concentrations of abamectin in emulsion and nanoemulsion forms were 0.001, 0.01, 0.1, 1, and 10 µg/ml, while the tested concentrations of neem and garlic oils nanoemulsions were 0.1, 1, 10, 100, and 1000 µg/ml. Four discs (35 mm in diameter) of castor bean leaves were dipped in each concentration for 5 s and left to dry. Then, ten adult female mites were transferred to each disc. Discs were placed on moist philtre paper resting on moist cotton wool pads in Petri dishes ($90 \times 105$ mm) and maintained in the same conditions as the breeding room. Mite mortality was measured at 24 h after treatment with abamectin and 48 h after treatment with neem and garlic oils. With data being plotted on log concentration probit paper and $LC_{50}$ values being calculated according to previously described methods [28], each treatment was replicated 4 times, with mortality rates being corrected against controls as previously decribed [27].

#### *Acaricidal activity of prepared nanoemulsions against T. urticae egg deposition and egg hatching*

To measure the residual effect of each tested nanoemulsions on adult female mites, five adult females *T. urticae* of known age were placed on each disc after dipping in one-fifth of the $LC_{50}$ concentration of each tested chemical. Each treatment was replicated 4 times. The numbers of eggs laid were assessed individually on all discs after 24, 48, 72, 96, and 120 h. The number of hatched eggs was also counted 4 days after eggs were laid. This experiment was conducted at $25 \pm 2$ °C with a 16 h photoperiod.

### Field experiments

Performed at the experimental farm of the Faculty of Agriculture, Kafrelsheikh University, Egypt (31°–07 ′N latitude and 30°–57 ′E longitude at 6 m above sea level), field experiments were conducted to evaluate the pesticidal activity of tested formulations against spider mites, *Tetranychs urticae*, infesting soybean plants under field conditions. The experiment was conducted in a randomized block design with four replicates. Plot areas were 10 $m^2$. All tested nanoemulsions of abamectin, neem, and garlic oils, as well as a conventional emulsion of abamectin, were applied using a knapsack sprayer with one nozzle. Plant oil nanoemulsions were applied at concentrations equal to 100 times respective $LC_{50}$ values, while abamectin formulations were applied at concentrations equal to 10 times respective $LC_{50}$ values. The applied concentrations of plant oil nanoemulsions were selected based on the results of preliminary trials. Samples of 10 soybean leaves were randomly collected from each plot before treatment and after 2 days and 1 week of treatment. Reductions in mite numbers were calculated for each treatment according to the method of Henderson and Tilton [29].

#### Statistical analyses and equations

The Henderson and Tilton formula [29] was used to evaluate population changes as follows:

$$\text{Corrected }[\%] = \frac{\text{N in Co before treatment} \times \text{N in T after treatment}}{\text{N in Co after treatment} \times \text{N in T before treatment}} \times 100$$

Where N = Insect population, T = treated, Co=control.

Abbott's formula [27] was used to correct the observed mortality for natural mortality:

$$\text{Corrected }[\%] = 1 - \frac{\text{N in T after treatment}}{\text{N in Co after treatment}} \times 100$$

Where N = Insect population, T = treated, Co = control.

Toxicity indexes of tested compounds were determined according to a previously described method [30] as follows:

$$\text{Toxicity index} = \frac{\text{LC50 of the most effective compound}}{\text{LC50 of the tested compound}} \times 100$$

ANOVA and the post-hoc Tukey test at the 5% level were used to compare treatments. All statistical analyses were performed using SPSS v.18 (SPSS Inc, Chicago, USA).

## Results and discussion

#### Characterization of prepared nanoemulsions

Prepared nanoemulsions were characterized using TEM as shown in Fig. 1. TEM images demonstrated that all prepared nanoemulsion compounds comprised nano-sized, spherical droplets. The droplet sizes of abamectin, neem, and garlic nanoemulsions were approximately 30, 36, and 40 nm, respectively.

Additionally, synthesized nanoemulsions of abamectin, neem oil, and garlic oil were found to have uniform size distributions with polydispersity indices (PDI) of approximately 0.21, 0.26, and 0.23, respectively ($PDI < 0.3$). These findings confirm the high monodispersity and homogeneity of synthesized nanoemulsions. Stability tests demonstrated all prepared nanoemulsions were stable with no separation or creaming observed during storage periods.

#### Toxicity of tested formulations against adult females of T. urticae

The present investigation was conducted to evaluate the acaricidal activities of prepared nanoemulsions of abamectin, neem, and garlic oils compared to abamectin emulsion. The results shown in Table 1 demonstrate that abamectin nanoemulsion was the most toxic compound to adult females of *T. urticae* followed by normal abamectin emulsion, with $LC_{50}$ values of 0.35 µg/mL and 0.88 µg/mL, respectively. Neem oil nanoemulsion had moderate toxicity to adult females of *T. urticae* with a $LC_{50}$ value of 70.44 µg/mL and garlic oil nanoemulsion had the least toxicity with a $LC_{50}$ value of 210.37 µg/mL. Regarding the toxicity index at $LC_{50}$ levels, abamectin nanoemulsion was found to be the most toxic compound to adult females of *T. urticae* with a toxicity index of 100% followed by conventional abamectin emulsion with a toxicity index of 39.77%. The toxicity indexes of neem

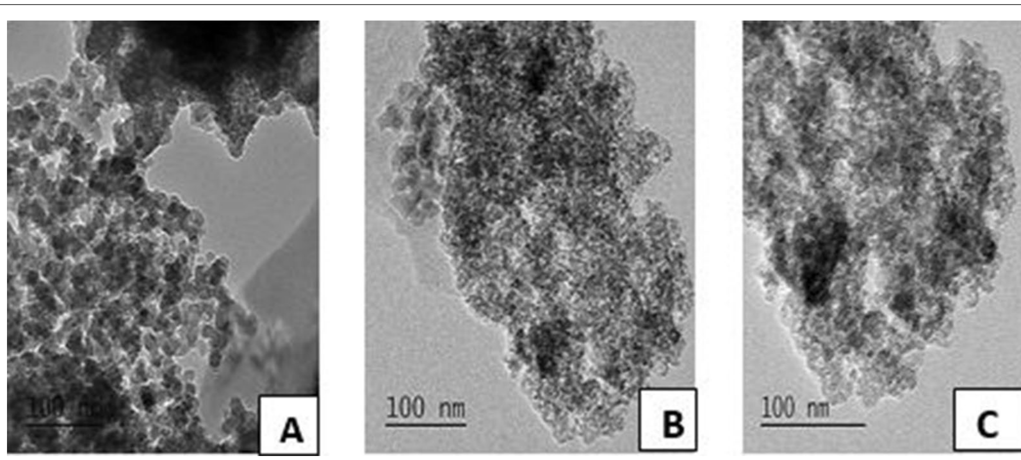


**Fig. 1** TEM images of tested nanoemulsions of abamectin (**A**), neem oil (**B**) and garlic oil (**C**)

**Table 1** Toxicity of prepared formulations against adult females of *T. urticae* by soybean leaf disc technique after 24 h (abamectin) and 48 h (plant oils)

| Compounds | $LC_{50}$ (μg/ml) | Confidence limits | | Toxicity index |
|---|---|---|---|---|
| | | Lower | Upper | |
| Abamectin emulsion | $0.88^{b}$ | 0.73 | 1.29 | 43.8 |
| Abamectin nanoemulsion | $0.35^{a}$ | 0.32 | 0.55 | 100 |
| Neem oil nanoemulsion | $70.44^{c}$ | 65.36 | 75.45 | 0.45 |
| Garlic oil nanoemulsion | $210.37^{d}$ | 202.63 | 225.42 | 0.15 |

Superscript lower-case letters indicate significant differences (based on overlapping confidence limits)

and garlic oil nanoemulsions were 0.49% and 0.166%, respectively (Table 1). The results of adulticide bioassays indicated that the acaricidal activity of synthetic acaricide abamectin toward the adult female of spider mite is greater than that of neem and garlic oils. This effect is likely due to abamectin acting as an agonist of the neurotransmitter, GABA, leading to direct lethal effects [31]. Further, the acaricidal toxicity induced by abamectin nanoemulsion was higher than that of abamectin emulsion. This greater toxicity of abamectin nanaemulsion against mites may be related to the greater surface area, adhesiveness, and penetration of nano-particles [7]. Maybe due to greater concentrations of biological components with acaricidal activities in neem oil compared to garlic oil, the toxicity of neem oil nanoemulsion was higher than that of garlic oil nanoemulsions against adult females of *T. urticae*. Neem oil contains azadirachtin as a major active component, which is responsible for toxicity against most insect pests and affects insect fertility [32, 33]. Azadirachtin in mites acts as a feeding inhibitor by stimulating cells that inhibit the feeding process, leading to weakness and death [34]. Diallyl disulfide, diallyl trisulfide, and diallyl sulfide are three primary components of garlic essential oil that have antagonistic characteristics against a variety of arthropod pests of economic interest, including the red spider mite [35]. Abdel-Halim and Kalmosh [36] stated that nano-abamectin was more toxic to females of *T. urticae* than vertimec 1.8% EC. Badawy et al. [48] found that abamectin was the most toxic agent against the adults of *T. urticae* ($LC_{50} = 5.39$ mg/L) followed by chlorfenapyr ($LC_{50} = 106.51$ mg/L) after 24 h using the slide-dip technique. Mossa et al. [7] found that garlic oil nanoemulsion had high acaricidal activity against *Aceria oleae* and *Tegolophus hassani*, with $LC_{50}$ values of 298.225 μg/mL and 309.634 μg/mL, respectively. Xu et al. [37] demonstrated that microemulsion of neem oil was effective in the control of *Sarcoptes scabie*, with a median lethal time of 81.7463 min.

### The effect of tested formulation residues on egg deposition in adult females of T. urticae

Since spider mites have high fertility and lay a large number of eggs, reducing the number of viable eggs is important for maintaining mite populations below levels that can cause economic damage. Thus, one of the most important issues in integrated pest management is the effects of synthetic and natural pesticides on mite egg deposition and egg hatchability. The effect of prepared nanoformulations, as well as conventional emulsion of abamectin, on egg deposition in adult females of *T. urticae* was evaluated using one-fifth of their respective $LC_{50}$ values.

The results shown in Table 2 demonstrate the mean reduction in the number of eggs deposited by adult female mites following treatment with the tested compounds for 5 days using the leaf disc technique. In general, with abamectin nanoemulsion being the most effective compound in reducing egg deposition (96%), followed by abamectin emulsion, neem oil nanoemulsion, and garlic oil nanoemulsion with reductions of 72.52%, 61.85%, and 48.65%, respectively, all tested formulations had significant oviposition deterrent effects. The ovicidal effects of tested formulations in descending order were as follows: abamectin nanoemulsion < abamectin emulsion > neem oil nanoemulsion < garlic oil nanoemulsion.

**Table 2** Reduction in the egg-laying capacity of *T. urticae* females treated by prepared formulations at 1/5th $LC_{50}$ values

| Compounds | Reduction (%) | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1st day | 2nd day | 3nd day | 4th day | 5th day | |
| Control | 10.75 ± 0.50 | 13.00 ± 0.82 | 22.00 ± 1.41 | 28.75 ± 0.50 | 33.28 ± 1.26 | $21.55 \pm 0.9^{e}$ |
| Abamectin emulsion | 80.5 ± 0.4 | 75.0 ± 1.5 | 69.7 ± 0.7 | 65.8 ± 0.2 | 65.4 ± 0.9 | $72.52 \pm 0.5^{b}$ |
| Abamectin nanoemulsion | 87.7 ± 1.1 | 80.00 ± 0.7 | 77.1 ± 0.6 | 70.9 ± 0.1 | 68.4 ± 0.5 | $96 \pm 0.57^{a}$ |
| Neem oil nanoemulsion | 58.2 ± 1.7 | 53.3 ± 0.9 | 51.8 ± 0.9 | 47.4 ± 0.5 | 36.7 ± 1.1 | $61.85 \pm 0.87^{c}$ |
| Garlic oil nanoemulsion | 55.3 ± 0.5 | 49.9 ± 1.3 | 35.0 ± 0.3 | 33.9 ± 1.1 | 20.5 ± 1.2 | $48.65 \pm 0.40^{d}$ |

Each value is presented as the mean ± SE (standard error). Mean values followed by the same superscript letter indicate no significant difference between coreseponding values at $P < 0.05$ by the Tukey test

The tested compounds may exert toxicity via chemosterilization or inhibition of oviposition by impairing the release of eggs from the ovary [38]. Our findings indicate that nanoemulsions significantly reduce the total number of eggs laid by *T. urticae* compared to conventional emulsions. Moreover, nanoemulsions of neem oil and garlic oil significantly decreased egg laying by *T. urticae*. Azadirachtin, found in neem oil, has adverse effects on oviposition and feeding in over 540 insect species [39]. Badawy et al. [48] stated that abamectin had the highest toxicity toward eggs, while pyridaben had the lowest toxicity. Our findings are in accordance with those of Roy et al. [40], who suggested that the number of eggs laid by red spider mites exposed to jatropha oil and garlic oil was significantly lower compared to controls.

#### The effect of tested formulation residues on the hatching of two-spotted spider mite eggs

The results of Table 3 demonstrate that all tested formulations caused a significant decrease in egg hatchability compared to the control treatment when using one-fifth of their respective $LC_{50}$ values. The ovicidal activities of the tested compounds can be arranged in descending order as follows: abamectin nanoemulsion < abamectin emulsion > neem oil nanoemulsion < garlic oil nanoemulsion. The hatchability following treatment with abamectin nanoemulsion and abamectin emulsion was 21.2% and 39.2%, respectively. Neem oil and garlic oil nanoemulsions had a moderate effect on ovicidal activity (45% and 58.8%, respectively). The proportion of eggs hatching at 5 days indicated that abamectin nanoemulsion was the most potent acaricide in reducing egg hatching. In other words, abamectin nanoemulsion is a good ovicide relative to the other tested materials. Further, neem and garlic oil nanoemulsions possess significant ovicidal deterring effects. With Ismail et al. [42] reporting that abamectin and λ-cyhalothrin were the most effective compounds in reducing oviposition and hatchability in *T. urticae*, while *Allium sativum* plant extract had the least effect, Narahashi and Chambers [41] stated ovicides reduce the hatchability process by indirect effects on oocyte development and protein sequestration, leading to the generation of non-viable eggs. Neem oil contains azadirachtin, a limonoid triterpene, a major bioactive component that causes impaired development, lower fecundity, decreased adult fertility, behavior modifications, cell abnormalities, and increased mortality in eggs, larvae, and adult stages [43].

#### Field studies on soybean plants

Assessing the nanoformulations of tested compounds under field conditions is important to determine the true acaricidal efficacies of these compounds against *T. urticae*. The efficacies of tested compounds in reducing the population density of motile stages of *T. urticae* on soybean crop are shown in Table 4

The results in Table 4 demonstrate that abamectin nanoemulsion was the most potent compound in reducing the population density of motile stages of *T. urticae* mites 2 days after treatment (92.56%) followed by abamectin emulsion (73.87%). Both neem oil and garlic oil nanoemulsions had moderate efficacy in reducing the population density of motile stages of *T. urticae*, with reductions of 57.17% and 56.63%, respectively. The population density of motile stages of *T. urticae* decreased 1 week after the application of all tested compounds. Abamectin nanoemulsion remained the most effective compound in reducing the population density of motile stages of *T. urticae* (98.22%) followed by abamectin emulsion (95.17%) and neem oil nanoemulsion (88.71%), while garlic oil nanoemulsion was the least effective compound (75.52%). These reductions indicate all compounds were effective in reducing the population density of motile stages of *T. urticae*, with mean reductions ranging between 66.08% and 95.24% for all compounds. The acaricidal activities of tested compounds under field conditions in descending order were as follows: abamectin nanoemulsion > abamectin emulsion > neem oil nanoemulsion > garlic oil nanoemulsion. Nanoemulsion improves

**Table 3** Effect of tested formulations on egg hatching of *T. urticae* at 1/5th $LC_{50}$ values

| Compounds | No. of unhatched eggs out of 25 deposited eggs | | | | | Mean | Hatchability % |
|---|---|---|---|---|---|---|---|
| | 1st day | 2nd day | 3nd day | 4th day | 5th day | | |
| Control | 11.34 ± 1.3 | 9.0 ± 0.83 | 5.73 ± 0.96 | 2.25 ± 0.93 | 1.23 ± 0.50 | $5.51 \pm 0.73^{e}$ | $77.96^{e}$ |
| Abamectin emulsion | 22.5 ± 2.08 | 17.0 ± 1.83 | 15.25 ± 0.9 | 12 ± 1.15 | 9.5 ± 0.58 | $15.2 \pm 0.41^{b}$ | $39.2^{b}$ |
| Abamectin nanoemulsion | 25.0 ± 0.0 | 24.0 ± 0.82 | 19.0 ± 0.82 | 16.0 ± 0.0 | 14.5 ± 1.29 | $19.7 \pm 0.20^{a}$ | $21.2^{a}$ |
| Neem oil nanoemulsion | 20.0 ± 0.82 | 15.50 ± 0.58 | 13.0 ± 0.82 | 10.75 ± 0.9 | 9.5 ± 0.58 | $13.75 \pm 0.53^{c}$ | $45.0^{c}$ |
| Garlic oil nanoemulsion | 18.5 ± 1.29 | 13.25 ± 2.63 | 7.75 ± 1.26 | 7.0 ± 1.15 | 5.0 ± 0.82 | $10.3 \pm 1.10^{d}$ | $58.8^{d}$ |

Each value is presented as the mean ± SE (standard error). Mean values followed by the same superscript letter indicate no significant difference between correseponding values at P < 0.05 by the Tukey test

**Table 4** Effect of tested formulations on the number of *T. urticae* mites at the motile stage on soybean plants

| Compounds | No. of motile stages/10 leaves before treatment | No. of motile stages/10 leaves at the indicated period after treatment | | | | Mean reduction % |
|---|---|---|---|---|---|---|
| | | 2 days | | 7 days | | |
| | | No. of motile stages | Reduction % | No. of motile stages | Reduction % | |
| Control | 82.0 | 105.0 | – | 127.0 | – | – |
| Abamectin emulsion | 92.0 | 49.0 | 73.87$^{b}$ | 10.0 | 95.17$^{a}$ | 84.52$^{b}$ |
| Abamectin nanoemulsion | 99.0 | 15.0 | 92.56$^{a}$ | 4.0 | 98.22$^{a}$ | 95.24$^{a}$ |
| Neem oil nanoemulsion | 63.0 | 55.0 | 57.17$^{c}$ | 16.0 | 88.71$^{b}$ | 72.94$^{c}$ |
| Garlic oil nanoemulsion | 69.0 | 60.0 | 56.63$^{c}$ | 38.0 | 75.52$^{c}$ | 66.08$^{d}$ |

Mean values followed by the same superscript letter indicate no significant difference between corresponding values at $P < 0.05$ by the Tukey test

the biological activities of acaricides by increasing their surface area, water solubility, adhesiveness, and penetration into mites. Babu et al. [44] demonstrated that neem extract at a concentration of 50% has acaricidal activity against red spider mites with no effect on non-target organisms such as natural enemies in the tea field experiment. Mossa et al. [45] reported that garlic oil nanoemulsion has lower toxic effects against the predatory phytoseiid mites, *Neoseiulus californicus* and *Cydnoseius negevi*, in Egypt. Chiasson et al. [49] reported that neem oil reduced egg hatching by more than 98% compared to controls at 5 days after application (water-sprayed), with approximately 22% mortality in adults at 48 h. Besides the short life cycle of *T. urticae*, frequent and intensive use of synthetic acaricides increases the occurrence of acaricide resistance. Accordingly, there is an urgent need for the replacement of synthetic acaricides with natural acaricides with an alternative mode of action. Neem and garlic oils, in addition to being less hazardous to human health and non-target organisms with lower costs of production compared to synthetic pesticides [47], have a broad spectrum of activity as botanical pesticides through multiple modes of action with effects on the nervous, reproductive, and endocrine systems of insects [46]. The comparable efficacy of neem and garlic oil nanoemulsions under laboratory and field experiments indicate these plant oils may represent valid alternatives to synthetic acaricides in integrated pest control of red spider mites, particularly in sustainable and organic cultivation.

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**Author contributions**
Experiment, TI and AK; analysis, TI and LE; writing-original draft. Preparation, TI and AK; writing-review and editing, TI and LE. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Competing interests**
The authors declare that they have no competing interests.

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