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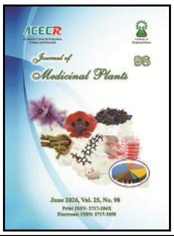

**Research Article**

# Acaricidal activity of a botanical formulation based on neem and cinnamon oils against the two-spotted spider mite (*Tetranychus urticae* Koch)

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ABSTRACT

**Background:** The hazards of chemical pesticides to human health and the environment have driven interest in botanical pesticides as safer alternatives. The two-spotted spider mite (*Tetranychus urticae* Koch) is among the most economically damaging pests in farms and greenhouses. **Objective:** This study aimed to develop and evaluate a botanical acaricide formulation, AzaMite, prepared from neem and cinnamon oils against *T. urticae*. **Methods:** AzaMite was formulated using neem and cinnamon oils, and its physicochemical properties were characterized using standard analytical techniques, including HPLC for azadirachtin quantification and GC-MS for cinnamon oil components. Laboratory bioassays and greenhouse trials were conducted against *T. urticae* across multiple concentrations. Mortality data were analyzed using probit regression and analysis of variance. **Results:** The median lethal concentration $LC_{50}$ and $LC_{90}$ of AzaMite were calculated at 1.46 and 4.09 %, respectively. AzaMite significantly increased mortality in *T. urticae*, indicating its potential for sustainable pest management. Physicochemical analysis further highlighted the time-dependent variation in azadirachtin stability. **Conclusion:** AzaMite demonstrates considerable potential as an environmentally friendly alternative for pest control. Further research is required to determine optimal application rates and timing and to minimize potential unintended ecological consequences.

## 1. Introduction

Pesticides play a critical role in agriculture by controlling pests that can have adverse effects on crop yields and quality. Despite the effectiveness of synthetic pesticides, concerns about their potential negative impact on human health and the environment have prompted the search for safer and more sustainable alternatives [1]. Promising alternatives for effective and environmentally friendly pest control are natural pesticides derived from plant sources, such as neem oil [2] and cinnamon oil [3].

Neem oil, which is derived from the seeds of the *Azadirachta indica* tree, has been utilized for centuries in traditional practices due to its pesticidal properties [4]. Comprising a range of

*Abbreviations:* GC-MS, Gas chromatography–mass spectrometry; HPLC, High-Performance Liquid Chromatography

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biologically active compounds, neem oil's most extensively researched constituent is Azadirachtin, which exhibits a broad spectrum of insecticidal activity. Neem oil has been demonstrated to be effective against various pests, including insects [5], mites, and nematodes [6], while also being relatively benign to humans and the environment. As a result, it is an attractive option for integrated pest management strategies.

Cinnamon oil, extracted from the bark and leaves of cinnamon trees (Cinnamomum spp.), has insecticidal and repellent properties that make it a noteworthy natural pesticide. It contains cinnamaldehyde, eugenol, linalool, and several other active compounds. Cinnamon oil has demonstrated effectiveness against pests such as mosquitoes, ants, and stored grain insects, and is generally regarded as safe with minimal environmental persistence and the environment [7].

The use of neem oil and cinnamon oil as natural pesticides offers several advantages over synthetic pesticides. First, they provide an alternative that reduces the potential risks associated with chemical residues in food and environmental contamination. Moreover, their ability to biodegrade reduces their impact on the environment, and finally, they have the potential for integrated pest management, offering multiple modes of action and reducing the likelihood of pest resistance development [8].

This study aims to evaluate the potency of neem oil and cinnamon oil formulation as eco-friendly pesticides for pest control in agricultural contexts .This study aims to evaluate the efficacy of the formulated botanical product against target pests in agricultural contexts. Furthermore, it will explore their compatibility with other pest management strategies and investigate optimal application methods and concentrations for maximum effectiveness.

We hypothesized that combining neem fixed oil with cinnamon essential oil in an optimized Emulsifiable Concentrate (EC) could exert a complementary or enhanced acaricidal effect against *T. urticae*. To test this hypothesis, a systematic experimental design was developed to evaluate both the physicochemical stability of the binary formulation and its sequential efficacy through laboratory leaf-disc bioassays and whole-plant greenhouse trials.

## 2. Materials and methods

### *2.1. Plant materials*

The kernels of the neem tree were collected from Chabahar, a city in the Sistan & Baluchestan province in the south of Iran. Then fixed oil was extracted by cold press (in May 2020). The essential oil of Cinnamon was purchased from the Zardband Company in Iran. The castor oil was purchased from the Salamat Yar Company. Azadirachtin-A standard from XOChem and all other chemicals were purchased from the Nasr Company in Iran.

To prepare the Emulsifiable Concentrate (EC) formulation, neem oil (7% w/w; containing 0.7% Azadirachtin) and cinnamon essential oil (3% w/w; containing 75% Cinnamaldehyde) were used as active ingredients. Butylated hydroxyl toluene was added to the oil phase to prevent oxidation and enhance the chemical stability of the components during storage. To formulate a physically stable emulsion and achieve the target hydrophilic-lipophilic balance (HLB), a blend of agriculturally approved nonionic surfactants, an optimized co-solvent, and castor oil (as the carrier) was utilized. The surfactant ratio was adjusted to yield a final HLB value ranging from 10 to 13. Finally, the mixture was





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brought to the desired volume and homogenized using a mechanical stirrer. The optimum concentrations of active ingredients and the final HLB value were determined experimentally based on preliminary physical stability tests.

*2.2. Insect rearing*

The initial population of *T. urticae* collected from cucumber leaves in the greenhouse in Varamin, Tehran province (25-30 ºC, 16/8 h of light/dark photoperiod) since June 2020. Common bean plants (*Phaseolus vulgaris* L.) were used as hosts. Potted bean plants infested with *T. urticae* were kept inside wooden frame cages (0.73 × 0.69 × 0.68 m) covered with organza to isolate the rearing and to avoid contamination by other arthropods. Cages were maintained inside a germinator [12].

*2.3. Toxicity bioassay*

Toxicity tests of AzaMite against *Tetranychus urticae* were carried out in the laboratory according to the methods described by Venzon et al. 2020 [9]. AzaMite was sprayed at different concentrations on cucumber leaf discs (3.0 cm diameter) fixed on Petri dishes containing a 1 % agar-water solution. Formulation spraying was carried out with a volume of 2.5 mL. Sprayed leaf discs were left to dry for one hour. Fifteen *T. urticae* adult females (7 days old) were then transferred to each treated leaf disc (n = 3 leaf discs per treatment), and the discs were kept in a climate chamber (25 ± 2 °C, 60 ± 10 % RH, LD 13:11 h photo cycle). The mortality of individuals was assessed after 48 hours. Negative control (distilled water) and positive control (Dichlorvos 0.1) were used to correct the data for natural mortality [9]. Dichlorvos was used as a conventional reference compound with known activity against spider mites under the same experimental conditions. The concentrations tested for the AzaMite were: 0.25, 0.5, 0.8, 1.5, 2, 3 and 4 percent. For each concentration of AzaMite as well as the control, three replicates are considered.

Furthermore, toxicity tests of AzaMite against *T. urticae* were carried out under greenhouse conditions. Cucumber plants, 30 days post-germination, were used for the experiment. Each plant was artificially infested with *T. urticae* individuals transferred from a bean leaf rearing colony. Seven days post-infestation, the botanical formulation, a negative control (distilled water), and a positive control (Dichlorvos 0.1) were applied to the cucumber plants using a hand-held pressure sprayer at a standardized volume of 15 mL per plant to ensure complete foliar coverage until run-off.

Treatment efficacy was evaluated 48 hours post-application. To accurately assess mortality without disrupting the natural environment, an in situ framing method was employed rather than detaching the leaves. For each replicate, ten standard frames (1 × 1 cm each) were placed on the infested cucumber leaves, and the number of live and dead mites within these defined areas was counted and recorded using a stereomicroscope. Because this sampling method relied on a fixed surface area, the initial mite count was not artificially standardized; thus, the total number of evaluated mites reflected the natural pest density under greenhouse conditions (ranging from 142 to 392 mites per replicate).

The tested concentrations for the botanical formulation were 0.25, 0.5, 0.8, 1.5, 2, 3, and 4 percent. For each concentration, as well as for the negative and positive controls, three



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independent replicates (with each replicate represented by a distinct plant) were considered.

*2.4. Physicochemical analysis*

The amount of active ingredients (AI) in AzaMite was measured by instrumental analysis. High-performance liquid chromatography (HPLC) was used for Azadirachtin A determination. The equipment was Knauer HPLC (Germany) with K1001 pump, UV K2501 detector (Germany) at 214 nm and $C_{18}$ reversed-phase Phenomenex analytical column (250 ×4.6 mm). The isocratic method was carried out with a solvent system composed of water: Acetonitrile (40:60) and a flow rate of 1.0 ml/min.

To determine cinnamaldehyde in AzaMite, Gas chromatography–mass spectrometry was carried out on an Agilent 890 GC gas chromatograph coupled to a 5973N mass selective detector under electron impact ionization (EI) mode at 70 eV. The mass scan range was 50–500 atomic mass units (AMU). BPX5 (30 m, 0.25 mm internal diameter), was employed with helium as carrier gas at a flow rate of 0.5 ml/min. The Injector temperature was 290 ºC. The sample was analyzed with the column held initially at 70 ºC for 5 min, increased to 300 ºC at 10 ºC/min and held for 3 min, the response time was 75 minutes. Peaks were identified by computer searches in commercial reference libraries NIST/ MS search and user-generated reference libraries [10] (Fig. 1).

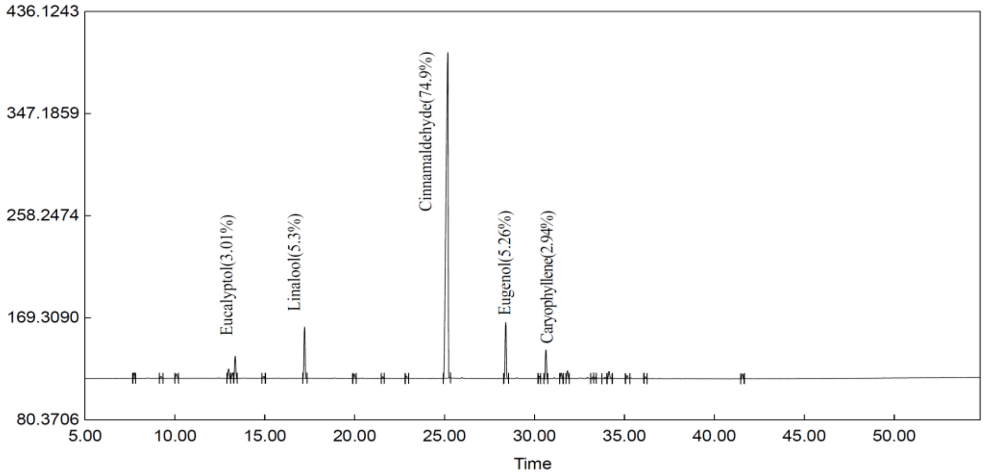

**Fig. 1.** GC/MS chromatogram of the cinnamon essential oil showing the main peak

*2.5. Statistical analysis*

The experiments were set up on a completely randomized design with three replicates per treatment. Statistical differences were assessed based on analysis of variance (ANOVA) using SPSS (version 18, USA). Differences among means were analyzed using the Duncan multiple range test at a probability level of p<0.0۵. The values are expressed as the mean ± standard error (SE).

## 3. Results

*3.1. Toxicity bioassay result*

The effect of the AzaMite formulation on two-spotted spider mite (Tetranychus urticae) mortality was evaluated under laboratory and greenhouse conditions (Fig. 2). The toxicity of AzaMite was compared with a negative control (distilled water) and a positive control (Dichlorvos 0.1 %). AzaMite significantly increased mortality of T.urticae after 48 h in





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both laboratory and greenhouse assays (Fig. 2). Probit analysis showed $LC_{50}$ and $LC_{90}$ values of 1.46 % (95 % CI: 1.01–1.92) and 4.09 % (95 % CI: 2.90–9.62), respectively (Table 1). The chi-square value (6.33, df = 3) indicated an acceptable fit of the Probit model (Table 1).

**Table 1.** Evaluation of the effect of AzaMite on the control of two-spotted mite laboratory in 48 hours
Mortality in the AzaMite treatment was significantly higher than the negative control and comparable to the positive control (Dichlorvos 0.1 %) ($P < 0.05$)

| Treatment | Chi-Square (x2) | df. | Heterogeneity | $LC_{50}$ (%) | $LC_{90}$ (%) |
|---|---|---|---|---|---|
| AzaMite | 6.33 | 3 | 2.1 | 1.46 (1.01-1.92) | 4.09 (2.90-9.62) |

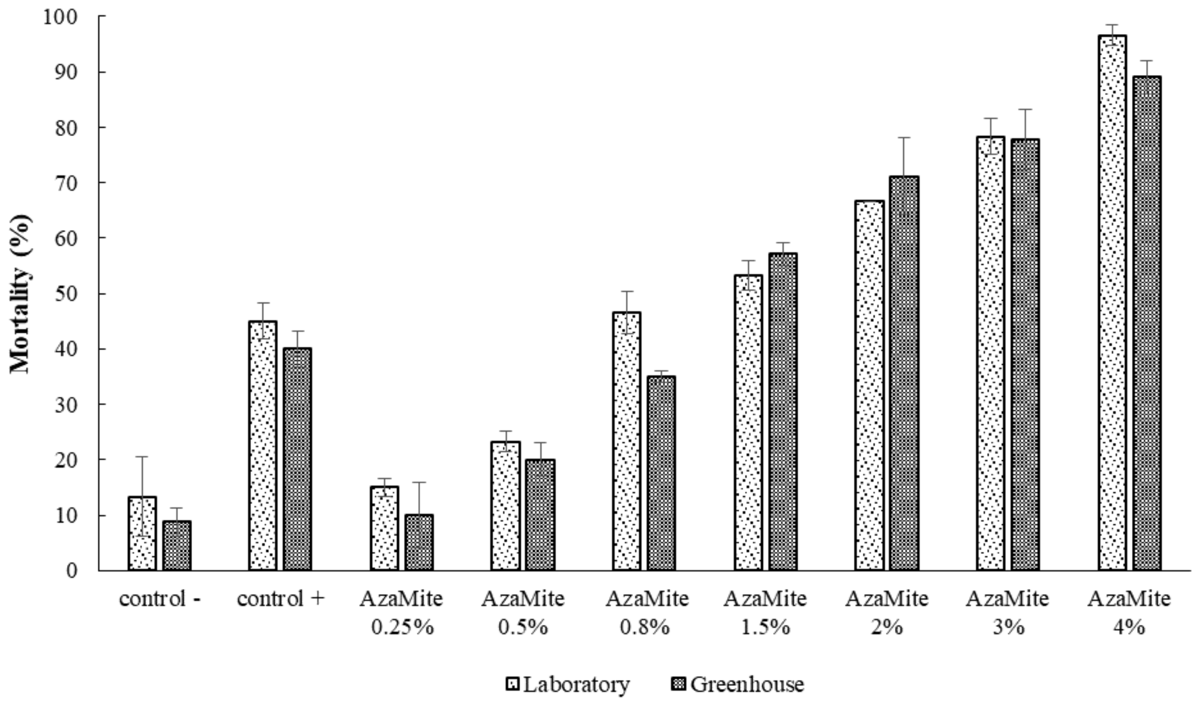


**Fig. 2.** The percent of mortality of *T. urticae* after treatment with AzaMite, controls – (distilled water), and control + (Dichlorvos) in 48 hours in the laboratory and Greenhouse

*3.2. Physicochemical analysis*

A calibration curve for Azadirachtin-A was constructed using standard solutions at different concentrations (Fig. 3). The curve showed good linearity within the tested range ($R^2 = 0.995$). Based on this calibration, the concentration of Azadirachtin-A in the AzaMite formulation was determined to be 0.05 % (w/w). Gas chromatography–mass spectrometry analysis of the formulation confirmed the presence of cinnamaldehyde at 2 % (w/w) (Fig. 1). The detected concentration of cinnamaldehyde (2 % w/w) represents a minor deviation from the theoretical yield (2.25 % w/w), which is attributed to the inherent volatilization of essential oil components during the mechanical stirring and thermal homogenization process of the EC formulation. The chromatographic profiles indicated that Azadirachtin-A and cinnamaldehyde were the main active constituents in the AzaMite formulation under the tested conditions.



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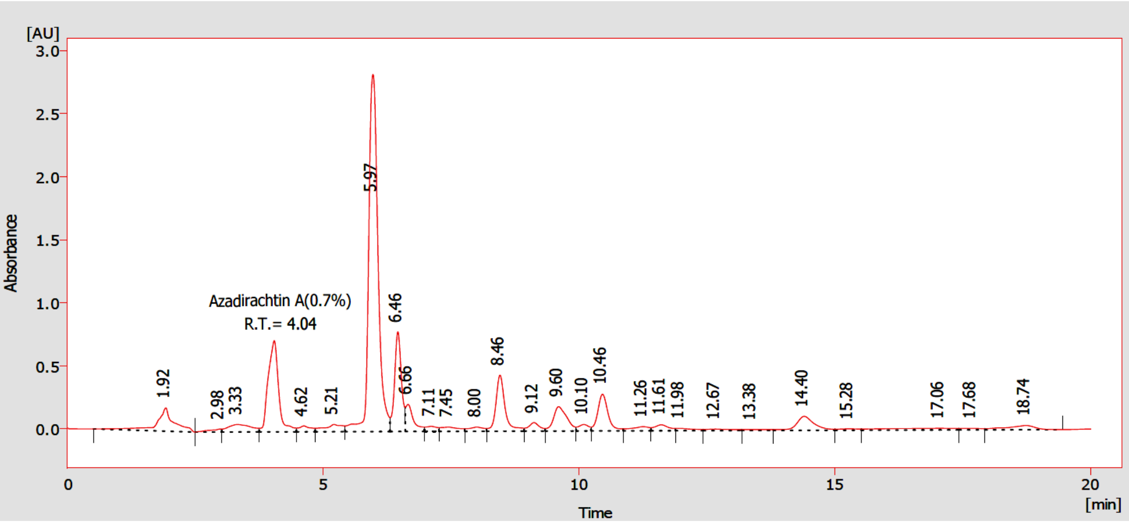


**Fig. 3.** HPLC chromatogram of *Azadirachta indica* fixed oil

## 4. Discussion

The present study demonstrated that the AzaMite formulation, based on neem oil and cinnamon essential oil, has good potential as a botanical pesticide against the two-spotted spider mite, *Tetranychus urticae*. Laboratory bioassays showed that AzaMite caused a marked increase in adult mortality, with $LC_{50}$ and $LC_{90}$ values of 1.46% and 4.09 %, respectively, after 48 h. These values indicate that relatively low concentrations of the formulation can achieve substantial control of *T. urticae* and are within the range reported for effective neem-based products in previous studies [11].

Our findings indicated that at the concentration of 0.8 %, AzaMite caused 46.6 % mortality in adult two-spotted spider mites. Furthermore, when the AzaMite concentration was increased to 1.5 %, mortality exceeded 50 %, a level generally considered acceptable for plant-based pesticides, particularly when applications are repeated. These findings highlight that the formulated botanical product can provide satisfactory efficacy against *T. urticae* using natural active ingredients.

Our results are consistent with reports that Azadirachtin-based formulations effectively reduce feeding, oviposition and survival in *T. urticae*, [11, 18] and can produce mortality levels comparable to conventional acaricides at recommended field rates. In addition to acute lethality, sublethal doses of Azadirachtin have been shown to negatively affect reproduction and population growth of T. urticae, which may contribute to long-term population suppression even when initial mortality is moderate. The performance of AzaMite observed in both laboratory and greenhouse conditions therefore supports the inclusion of neem-derived formulations as key components in integrated mite management programs.

An important advantage of botanical formulations is their relatively lower toxicity to non-target organisms, especially predatory mites and other beneficial arthropods. Previous studies have reported that Azadirachtin causes limited direct mortality in predatory mite species [12], although some sublethal effects on fecundity may occur, which are still less pronounced than those caused by many synthetic pesticides. Although non-target





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organisms were not evaluated in this study, botanical formulations are generally considered more compatible with IPM than broad-spectrum synthetic acaricides.

Physicochemical analysis of the AzaMite formulation confirmed Azadirachtin-A at 0.05 % and cinnamaldehyde at 2 % as the main active constituents. These results are in line with previous work showing that the composition and quality of neem oils and associated essential oils strongly influence their biological activity and stability [14]. Azadirachtin-A is known to be most stable in mildly acidic conditions (pH 4 – 6) and to degrade more rapidly in strongly acidic or alkaline media [13], which can affect its residual efficacy under field conditions. Therefore, the observed stability of the AzaMite formulation under different temperature conditions, as shown in our physicochemical tests, is an important advantage for its practical use.

The presence of cinnamaldehyde, the main component of cinnamon essential oil, may further enhance the acaricidal activity of AzaMite. Cinnamaldehyde has been reported to exhibit insecticidal and acaricidal effects through multiple mechanisms, including interference with neuromuscular function, feeding behavior and oviposition, as well as damage to the cuticle and respiratory system. In combination with Azadirachtin, which primarily affects hormonal regulation, growth and reproduction, cinnamaldehyde may provide additive or even synergistic effects, broadening the spectrum of activity and potentially slowing resistance development. Although the present study did not experimentally evaluate synergy, the satisfactory levels of mortality obtained with AzaMite at relatively low concentrations support the hypothesis that the combination of these two plant-derived compounds is beneficial.

From a practical perspective, the mortality levels achieved by AzaMite demonstrate its viability for integration into IPM programs for *T. urticae* control. To optimize field efficacy and suppress newly emerged populations, a follow-up foliar application is recommended 5–7 days post-initial treatment. Furthermore, alternating this botanical formulation with conventional acaricides possessing distinct modes of action will play a critical role in delaying pest resistance, conserving natural enemies, and promoting sustainable greenhouse production systems [17, 19, 21].

## 5. Conclusion

In conclusion, this study highlights the significant potential of developing binary botanical formulations by pairing fixed and essential oils. The optimized AzaMite formulation offers a physically stable and chemically standardized alternative to synthetic acaricides for managing *T. urticae*. By moving away from single-component biopesticides, this research advances organic plant protection science by demonstrating how surfactant-stabilized botanical blends can maintain residual stability and efficacy. Future field-scale trials will further solidify its role in certified organic farming and pesticide resistance management.

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## Authors contributions

F.N. & D.Y designed and supervised the study and manuscript revision. M.Kh. contributed to data analysis. F.J.Sh. participated





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Conducting laboratory and greenhouse tests and contributed to writing the initial draft.

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**Conflict of interest**

The authors F.N, D.Y, M.Kh, and F.J declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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