Original Article

Development of a biocompatible nanoemulsion based on eucalyptus essential oil for the management of *Agonoscena pistaciae*: A novel approach for promoting food safety in the health supply chain

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Abstract

Background: *Agonoscena pistaciae* is the most important pest of Iranian pistachio orchards, and its chemical control raises concerns about pesticide residues in food. This study developed a biocompatible nanoemulsion based on *Eucalyptus globulus* essential oil (Artemizia Eco®) as a plant-based alternative to synthetic pesticides.

Methods: An oil-in-water nanoemulsion was prepared using eucalyptus essential oil, a biocompatible polymer, polyethylene glycol, and suitable surfactants. The chemical profile of the essential oil was investigated by gas chromatography–mass spectrometry (GC-MS). Particle size and distribution were examined by dynamic light scattering (DLS) and transmission electron microscopy (TEM), and stability was assessed for 18 weeks at 30°C. Acute toxicological safety and bioassay were evaluated, efficacy was assessed under field conditions at the Agricultural and Natural Resources Research and Education Centers of Yazd and Kerman Provinces, and the residues of the target chemical pesticides were measured.

Results: 1,8-cineol was the dominant component of the essential oil (44%). The eucalyptus-based formulation (EW 13%) had a particle size of 91 nm, a dispersion index of 0.183, and maintained complete emulsion stability for 18 weeks. The oral and dermal LD_{50} were >2000 and 2309 mg/kg, respectively, with slight eye and skin irritation. The contact LC_{50} of the formulation was 2935 ppm. Under field conditions, application at 3000 ppm resulted in efficacies of 92%, 91%, 88%, and 84% on days 3, 7, 14, and 21 after foliar application, respectively, which was significantly higher than phosalone (EC 35%) (77.8%) on day 21. No residues of the assessed synthetic pesticides were detected on the crop.

Conclusion: The biocompatible nanoemulsion of eucalyptus essential oil (Artemizia Eco®) showed high efficacy and favorable safety in this study, and no residues of the assessed pesticides were detected under the study conditions. These findings suggest that the formulation may serve as a promising plant-based alternative to synthetic pesticides in the integrated management of *Agonoscena pistaciae*, with the potential to reduce dietary exposure to pesticide residues and support food safety in pistachio production.

Keywords: Agonoscena pistaciae; Food Safety; Herbal Medicine; Pistacia; Social Determinants of Health; Toxicology.

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Introduction

As a strategic agricultural crop, pistachio (*Pistacia vera* L.) plays a key role in the economy of producing countries and is a significant component of the global food supply. However, the common pistachio psyllid (*Agonoscena pistaciae*), the most destructive pest of pistachio, is responsible for significant annual quantitative and qualitative losses in pistachio orchards (1). The common strategy for managing this pest is the extensive use of synthetic pesticides. Although they may provide rapid control, they carry potential environmental and health risks, such as the development of pest resistance, the destruction of beneficial insects, and concerns regarding food safety and consumer health (2, 3). Pesticide residues in crops are a relevant factor within the context of social determinants of health, as long-term exposure to these chemical compounds is associated with an increased risk of chronic diseases, including endocrine disruption and cancers (4).

The demand for the development and application of alternative and biocompatible approaches has increased dramatically in response to global concerns about food safety. In this regard, plant essential oils have shown significant insecticidal, repellent, and anti-nutritional properties due to their bioactive compounds such as monoterpenes (5). The essential oil of *Eucalyptus globulus* Labill., owing to its high content of 1,8-cineole, exhibits considerable potential as a botanical pesticide for the management of a wide range of insect pests (6). However, the practical application of essential oils in the field faces inherent limitations such as high volatility, instability in environmental conditions, and low water solubility, which can significantly reduce their effectiveness (7).

Nanotechnology, especially nanoemulsion-based delivery systems, has offered promising avenues for developing green pesticide formulations to overcome these challenges. Nanoemulsions are considered ideal delivery systems for essential oils owing to their thermodynamic stability, optical transparency, ease of preparation, improved bioavailability of active ingredients, and enhanced efficacy of botanical pesticides (8). Development of a biocompatible nanoemulsion can improve the contact efficiency of the essential oil by increasing the permeability of the insect cuticle and preventing its premature degradation by encapsulation. This approach represents a potential solution for the integrated management of the *Agonoscena pistaciae* pest. It also contributes to the broader goal of promoting safe pistachio production and supporting a safer food supply chain by minimizing chemical hazards. The present study was conducted to develop, characterize, and evaluate the efficacy of a biocompatible nanoemulsion formulation based on eucalyptus essential oil to address these challenges.

Materials and Methods

Insect Colony and Experimental Sites

Second-instar nymphs of the common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae), were used throughout this study. The laboratory colony was established from insects collected from naturally infested pistachio orchards in the Bahadoran region of Yazd Province, Iran. The colony was maintained under controlled environmental conditions (25 ± 2°C, 60 ± 5% relative humidity, and a 14:10 h light/dark cycle) for several generations prior to the experiments. Only healthy and active second-instar nymphs that responded to gentle stimulation with a fine camel-hair brush were used in the laboratory bioassays. Nymphs that died before treatment or failed to establish on the seedling leaves were excluded. One-year-old pistachio seedlings bearing four to five healthy leaves, showing no visible symptoms of pest infestation or disease, were selected for all laboratory experiments.

Field experiments were conducted in commercial pistachio orchards located in Yazd and Kerman provinces, Iran. Orchards with naturally established and relatively uniform infestations of *A. pistaciae* were selected. Before treatment application, the pest population had reached the economic threshold of approximately five second-instar nymphs per leaf. Trees affected by root diseases such as gummosis, trees exhibiting more than 20% branch dieback, and trees treated with any chemical pesticide within 14 days before the experiment were excluded.

Formulation Development and Characterization

The biocompatible nanoemulsion was prepared using *Eucalyptus globulus* essential oil, a biocompatible polymer, polyethylene glycol, and a combination of anionic and nonionic surfactants. The chemical profile of the essential oil was standardized and characterized by gas chromatography–mass spectrometry (GC-MS, Younglin and Agilent). Particle size, morphology, and dispersion index (PDI) were examined by transmission electron microscopy (TEM, Zeiss-EM 10C) and dynamic light scattering (DLS, Nanofax). The physicochemical stability of the formulation was assessed by monitoring particle size and emulsion integrity over 18 weeks at 30°C.

Experimental Design and Bioassays

Laboratory Bioassays

Preliminary range-finding bioassays were conducted to determine the appropriate concentration range for toxicity assessment. Based on the preliminary results, six concentrations with logarithmic intervals were selected for the determination of the median lethal concentration (LC_{50}). Each concentration was evaluated using four independent replicates, with 20 second-instar nymphs per replicate. The nymphs were exposed to the formulation via leaf immersion in a controlled environment to simulate contact toxicity.

Field Evaluation

Field trials were arranged in a randomized complete block design (RCBD) consisting of six treatments and four replicates. To minimize selection bias, experimental plots were assigned to treatments using a computer-generated random number sequence. To ensure objective assessment and eliminate observer bias, a blinded approach was implemented, where investigators performing the nymph density counts were unaware of the specific treatment applied to each branch.

Each experimental plot comprised three pistachio trees of the same cultivar and similar age, resulting in a total of 72 trees per orchard. Four branches representing the four cardinal directions of each tree were selected. Four fully expanded compound leaves (each consisting of three leaflets) were randomly sampled from each branch before treatment and at each evaluation date (3, 7, 14, and 21 days after application) to determine the density of *A. pistaciae* nymphs. Applications were performed using a motorized backpack sprayer, ensuring uniform coverage of the foliage.

Residue Analysis and Toxicology

Acute toxicological safety was evaluated according to OECD guidelines (No. 425 for oral, 402 for dermal, 436 for respiratory, 404 for skin irritation, 406 for sensitization, and 405 for eye irritation) at the Institute of Pharmaceutical Sciences, University of Tehran.

To evaluate the impact on food safety, pesticide residues on harvested pistachio kernels were measured. The QuEChERS extraction method was employed, followed by analysis using an Agilent LC-MS/MS model 6410 Triple Quadrupole equipped with a Zorbax Eclipse SB-C18 column. The analytical method was validated for accuracy and precision. The limits of detection (LOD) and limits of quantification (LOQ) were determined to be (0.01) µg/kg and (0.05) µg/kg, respectively. Recovery rates for the target analytes ranged from (85% to 105%), with intra-day and inter-day precision (CV) within acceptable limits (< 15%, following SANTE guidelines).

Statistical Analysis

All experimental data were subjected to Shapiro-Wilk tests to confirm the assumptions of normality and Levene's test for homogeneity of variance. Laboratory bioassay mortality data were analyzed using probit analysis (SAS 6.12) to calculate LC_{50} and its corresponding 95% confidence intervals. Field efficacy was determined by calculating the percentage of nymph population reduction using the Henderson-Tilton formula. Differences between treatment means were analyzed using one-way analysis of variance (ANOVA), reporting F-statistics and degrees of freedom (df), followed by the Tukey HSD test ($p<0.05$) using IBM SPSS Statistics software.

Ethical Considerations

This study did not involve human participants or vertebrate animals. All experiments were conducted exclusively on arthropod pests in accordance with accepted scientific and environmental ethical standards. The study was conducted with the permission of the Agricultural and Natural Resources Research and Education Centers of Yazd and Kerman Provinces, Iran.

Results

Chemical Characterization of Essential Oil

GC-MS analysis of the *Eucalyptus globulus* essential oil identified several monoterpenes as the primary constituents. The dominant component was 1,8-cineole (44.53%), followed by p-cymene (27.26%), limonene (7.12%), α-pinene (5.30%), and γ-terpinene (3.09%) Table 1.

Table 1. Main chemical constituents of *Eucalyptus globulus* essential oil based on GC-MS analysis.

Compound	Percentage (%)	Retention Index (RI)
1,8-cineole	44.53	1039.14
p-cymene	27.26	1030.07
Limonene	7.12	1034.51
α-pinene	5.30	938.23
γ-terpinene	3.09	1062.75

Physicochemical Stability of the Formulation

The stability of the nanoemulsion was evaluated over 18 weeks at 30 ± 2°C. The formulation maintained emulsion stability (5%) throughout the storage period. The pH remained relatively constant, shifting from 7.25 at the start to 7.29 after 18 weeks. Furthermore, the concentration of 1,8-cineole showed minimal fluctuation, decreasing from 44.17% to 43.06% Table 2.

Table 2. Physicochemical properties and storage stability of the eucalyptus essential oil formulation.

Parameter	Test Method	Initial Value	Value after 18 weeks (30°C)
Emulsion stability (5%)	MT36.1.1	-	Stable
pH	MT75	7.25	7.29
1,8-cineole content (% W/V)	USP	44.17	43.06

Table 3. Summary of toxicological results and pesticide residue analysis.

Evaluation Parameter	Standard Method	Result
Acute oral toxicity (LD_{50})	OECD 425	> 2000 mg/kg
Acute dermal toxicity (LD_{50})	OECD 402	2309 mg/kg
Acute respiratory toxicity	OECD 436	No mortality observed
Eye irritation	OECD 405	Slight/Transient
Chemical residue (Pistachios)	LC-MS/MS (QuEChERS)	ND (Not Detected)

Toxicological Safety and Residue Analysis

Acute toxicity assessments in rats indicated that the LD_{50} for oral and dermal exposure were >2000 mg/kg and 2309 mg/kg, respectively (Table 3). No mortality or clinical signs of acute respiratory toxicity were observed. Regarding local effects, slight and transient eye and skin irritations were recorded.

Multiresidue analysis of harvested pistachio samples via LC-MS/MS (QuEChERS) showed no detectable residues (ND) for any of the 30 target pesticides screened, including acetamiprid, imidacloprid, chlorpyrifos, and others Table 3.

Insecticidal Activity against Agonoscena pistaciae

The insecticidal efficacy of the 13% EW formulation against second-instar nymphs was evaluated through laboratory bioassays. Probit analysis yielded an LC50 of 2935 ppm (95% CI: 5618–1431) and an LC90 of 5737 ppm (95% CI: 5983–3970). The slope of the probit regression line was 4.9 ± 0.78, with a $\chi 2$ value of 0.27 (df = 4, p=0.96). The LC_{90}/LC_{50} ratio was calculated at 1.95 Table 4.

Field Efficacy against *A. pistaciae*

The efficacy of the eucalyptus essential oil-based formulation (EW 13%) at two different concentrations (3000 and 7000 ppm) was evaluated in field conditions (Kerman Province) and compared to a positive control (Phosalone 35% EC, 2000 ppm). The percentage of nymph mortality was recorded at 3, 7, 14, and 21 days after foliar application Table 5.

Within the first 3 days post-application, the efficacy of the 3000 ppm formulation (92.03 ± 1.89%) was comparable to that of phosalone (94.4 ± 1.59%) and the 7000 ppm formulation (94.9 ± 1.19%) (Table 5). At day 7, all treatments maintained high efficacy levels >90%), with no significant differences observed between the 3000 ppm formulation, the 7000 ppm formulation, and the positive control. However, by day 21, a significant difference was observed; the 3000 ppm formulation (84.2 ± 3.60%) and

Table 4. Probit analysis of the insecticidal activity of the eucalyptus essential oil-based formulation (13% EW) against *A. pistaciae* nymphs.

Formulation	nnn	$\chi 2$ (df)	p-value	Intercept ± SE	Slope ± SE	LC_{50} (ppm) (95% CI)	LC_{90} (ppm) (95% CI)
13% EW	2525	0.27 (4)	0.96	2.53 ± 0.80	4.9 ± 0.78	2935 (5618–1431)	5737 (5983–3970)

Table 5. Field efficacy (percentage of nymph mortality) of different treatments against *A. pistaciae* at various intervals after foliar application.

Treatment	3 days post-app (%)	7 days post-app (%)	14 days post-app (%)	21 days post-app (%)
EW 13% (3000 ppm)	92.03 ± 1.89a	91.0 ± 1.5a	88.02 ± 2.05a	84.2 ± 3.60a
EW 13% (7000 ppm)	94.9 ± 1.19a	91.1 ± 1.6a	90.2 ± 1.7a	88.05 ± 2.3a
Phosalone (2000 ppm)	94.4 ± 1.59a	94.5 ± 1.29a	90.1 ± 1.7a	77.8 ± 3.2b

Values are expressed as Mean ± SD. Different superscript letters within a column indicate significant differences according to Tukey's HSD test (p<0.01p < 0.01p<0.01).

the 7000 ppm formulation (88.05 ± 2.3%) showed significantly higher mortality rates compared to the phosalone treatment (77.8 ± 3.2%, $p<0.01$).

Discussion

The present study developed a biocompatible nanoemulsion-based formulation utilizing eucalyptus essential oil for the management of *Agonoscena pistaciae*, aiming to mitigate chemical hazards in the food supply chain. Our findings demonstrate that the optimized 13% EW formulation exhibits biological efficacy comparable to conventional synthetic pesticides. Given its physicochemical stability and low toxicological profile, this formulation presents a potential practical solution for the sustainable production of pistachio crops.

While the LC_{50} of the eucalyptus-based formulation (2935 ppm) was higher than that of phosalone, it remains within an acceptable range for botanical pesticides and aligns with previous reports. For instance, Ebrahimi et al.,reported a contact LC_{50} of 9515 ppm for emulsified eucalyptus essential oil against leaf aphids (9). This discrepancy in lethality may stem from differences in target species, specific chemical compositions, or the superior bioavailability provided by the nanoemulsion carrier system used in this study (10). Notably, the formulation exhibited rapid knockdown activity, with most mortality occurring within the first 24 hours. This rapid onset of action is likely attributed to the neurotoxic properties of monoterpenes, such as 1,8-cineole, which can induce swift physiological disruptions in the target pest (11).

A significant finding of this research is the sustained efficacy of the formulation under field conditions, where it outperformed phosalone at certain intervals. Specifically, the 3000 ppm formulation maintained higher efficacy than phosalone 21 days after application (84.2% vs. 77.8%). This prolonged residual effect may be attributed to the thermodynamic stability of the nanoemulsion and the formation of a protective film by components such as PEG on the leaf surface, which prevents the rapid evaporation of volatile essential oils (12). This hypothesis is supported by our physicochemical data, which showed that the 1,8-cineole content decreased by only approximately 1% after 18 weeks of storage at 30°C. The maintenance of emulsion stability and a low polydispersity index (PDI = 0.183) further indicate that the polymer matrix successfully encapsulated the volatile constituents, ensuring particle homogeneity and preventing rapid degradation (13). Such stability is a critical prerequisite for the commercial viability of botanical biopesticides.

From the perspective of food safety and environmental health, the eucalyptus-based formulation demonstrated a favorable safety profile. Toxicological assessments indicated low acute toxicity, with an oral $LD_{50}>2000$ mg/kg, consistent with the biocompatible nature of its constituents, such as PEG and eucalyptus essential oil (14). Crucially, multiresidue analysis revealed no detectable residues of the 30 target pesticides on the treated pistachio samples. These findings suggest that the application of this formulation does not introduce additional chemical risks into the food chain, addressing a key concern for organic and export markets that maintain zero-tolerance policies for synthetic residues. Furthermore, the use of botanical alternatives can support Integrated Pest Management (IPM) programs by reducing the chemical load on the environment and minimizing the impact on non-target organisms and natural enemies, which are often sensitive to synthetic insecticides (15-17).

The observed efficacy of the 3000 ppm formulation over the 7000 ppm concentration suggests a “less is more” phenomenon, commonly documented in nanopesticide literature. This paradoxical effect likely stems from particle aggregation at higher concentrations, which reduces the effective contact surface area and impairs the bioavailability of the active ingredient (18, 19). Furthermore, increased viscosity at higher concentrations can hinder uniform coverage on the leaf surface. From a practical standpoint, this finding is economically significant, as an optimal concentration of 3000 ppm reduces application costs and minimizes the potential for off-target effects.

The rapid mortality observed within the first 24 hours post-application underscores the potent knockdown effect of this formulation. Given that 1,8-cineole constitutes 44% of the essential oil, the insecticidal activity is primarily attributed to its neurotoxic properties, likely mediated through the inhibition of acetylcholinesterase (AChE) activity (11). This mechanism is consistent with reports by Ebadollahi et al., who identified 1,8-cineole as the key factor in the contact toxicity of eucalyptus against *Rhizopertha dominica* (11).

Recent advancements in Iranian research have increasingly focused on leveraging nanotechnology to overcome the inherent limitations of essential oils, such as high volatility and environmental instability. Studies by Shahinfar et al. and Negahban et al., have established a foundation for the nanoformulation of native essential oils, demonstrating that polymer-based encapsulation, such as nanocapsules, can effectively regulate release rates and enhance insecticidal, repellent, and anti-feeding activities against various pests (20, 21). Our study corroborates these findings, as the use of a PEG-based nanoemulsion matrix achieved long-term stability (18 weeks) and a low polydispersity index (PDI = 0.183). This consistency reinforces the reliability of nanoformulation technology in protecting volatile botanical compounds (21).

The efficiency of our delivery system is enhanced by the synergistic combination of 1,8-cineole and natural adjuvants, such as linoleic acid (51.8%) derived from soybean oil, which is known to facilitate penetration through the insect cuticle (18). By utilizing locally available and indigenous raw materials, this formulation offers a sustainable production cycle. Furthermore, the absence of chemical residues enhances the marketability of the final product, particularly for export sectors with stringent quality requirements. Consequently, this eucalyptus-based formulation provides a multifaceted advantage: ensuring public health, promoting environmental sustainability, and improving economic outcomes for pistachio producers through reduced chemical dependency.

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Conclusion

In conclusion, this study successfully developed a biocompatible nanoemulsion incorporating 13% *Eucalyptus globulus* Labill. essential oil (Artemizia Eco®), which exhibited superior physicochemical properties, including a mean particle size of 91 nm and a sustained stability of 18 weeks. The formulation demonstrated a high safety profile (oral LD50>2000LD mg kg^{-1}) and achieved biological efficacy against *Agonoscena pistaciae* comparable to the conventional insecticide phosalone under orchard conditions.

Crucially, multiresidue analysis confirmed the complete absence of detectable chemical residues in harvested pistachio samples, addressing a major concern in modern pest management. These findings suggest that the developed nanoemulsion serves as a potent, eco-friendly alternative to synthetic insecticides, supporting the transition toward sustainable integrated pest management (IPM) practices. Furthermore, by eliminating chemical residues, this botanical formulation holds significant potential to enhance the marketability and export competitiveness of pistachios, aligning with the global consumer demand for residue-free and environmentally safe agricultural products.

Limitations

This study is subject to several limitations. The field evaluations were limited to a single region and season, which may limit the generalizability of the results to other environments. Additionally, while the formulation showed high efficacy against *A. pistaciae*, further studies are needed to assess its impact on non-target beneficial insects and its performance across different pest life stages. Finally, the economic feasibility of large-scale production and the long-term environmental impact of the PEG-based carrier system warrant further long-term monitoring.

Authors' contribution

Maryam Negahban conceived the study, developed and optimized the botanical pesticide formulation, standardized the formulation, and supervised the toxicological evaluations. Hadi Zohdi and Ghasem Askari Saryazdi designed and conducted the bioassays and evaluated the efficacy of the formulation against the target insect under laboratory and field conditions. Hossein Farazmand and Ahmad Heidari provided scientific consultation on *pistachio psylla* biology, pest management, and pesticide toxicology. Hadi Zohdi and Gholamreza Golmohammadi performed the statistical analyses and interpreted the data. All authors contributed to the scientific discussion, critically revised the manuscript for important intellectual content, and read and approved the final version of the manuscript.

Informed consent

Questionnaires were filled with the participants' satisfaction and written consent was obtained from the participants in this study.

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Conflict of interest

The authors declare that they have no conflict of interests.

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