Journal of Agricultural Science and Technology (JAST), 28(5)
In Press, Pre-Proof Version

Urea-Formaldehyde Nanoencapsulation Enhances *Bunium persicum* Essential Oil's Toxicity Against *Brevicoryne brassicae*

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ABSTRACT

Brevicoryne brassicae L. (Hemiptera: Aphididae), a highly destructive cabbage aphid native to Europe, has become a globally distributed pest of significant agricultural concern. This species currently poses significant challenges for cabbage production, resulting in substantial crop losses. The fresh consumption of cabbage necessitates the development of non-chemical control methods to ensure food safety while effectively managing pest populations. However, this study investigated the aphicidal activity of both pure and nanoencapsulated *Bunium persicum* (Boiss.) Fedtsch essential oils against *B. brassicae*. The essential oil (EO), obtained through hydrodistillation and analyzed by gas chromatography and mass spectrophotometry (GC-MS), contained γ-Terpinen (36.62%), ρ-Cymene (18.41%), Carvacrol (13.60%), and Cuminaldehyde (13.50%) as its major components. Nanocapsules were synthesized via in situ polymerization of an oil-in-water emulsion and characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM), revealing spherical particles with a median diameter of 10.88 nm and a low polydispersity index (PDI) of 0.057. Bioassays demonstrated that the nanoencapsulated formulation exhibited significantly higher toxicity than the pure EO, with lower contact LC_{50} and LC_{90} values (365.43 and 1908.46 µL/L water, respectively) compared to the pure EO (1030.40 and 3977.08 µL/L water). Similarly, the fumigant LC_{50} and LC_{90} values for the nanocapsules (23.15 and 59.49 µL/L air) were significantly lower than those of the pure EO (35.07 and 79.59 µL/L air). The findings suggest the potential of nanoencapsulated *B. persicum* EO in integrated pest management for controlling cabbage aphid.

Keywords: Aphid, Biocompatible insecticide, Efficacy, Encapsulated, Nanoparticle.

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INTRODUCTION

The Brassicaceae (or Cruciferae) family, the largest within the order Brassicales, comprises 12–15 tribes, 338–360 genera, and approximately 3709 species distributed globally (excluding Antarctica). This family includes economically significant species cultivated for food, fodder, medicine, oil production, and ornamental purposes (Raza *et al.* 2020). However, brassica crops face substantial yield losses due to insect pests, particularly sap-sucking insects. The cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae), is one of the most serious insect pests of cruciferous crops throughout the world. This insect pest species inflicts direct damage by feeding on plant sap and indirectly by transmitting viral pathogens, posing a significant threat to cruciferous crops (Canassa *et al.* 2020; Patel *et al.* 2024).

Conventional management of *B. brassicae* relies heavily on synthetic insecticides, which, despite their efficacy in boosting crop yields, have led to the development of widespread resistance in aphid populations and significant environmental contamination (Ahmad and Akhtar, 2013; Nematollahi *et al.* 2014). Documented resistance includes key insecticide groups such as neonicotinoids (e.g., imidacloprid, thiamethoxam), pyrethroids (e.g., cypermethrin, deltamethrin, bifenthrin), and organophosphates (e.g., profenofos, chlorpyrifos) (Ahmad and Aslam, 2005; Ahmad and Akhtar, 2013). Given these challenges, there is an urgent need for sustainable solutions, such as biopesticides, to address the environmental and resistance concerns linked to synthetic chemicals (Moharramipour and Negahban, 2014; da Silva *et al.* 2023).

Plant-derived essential oils (EOs) have emerged as promising alternatives to synthetic insecticides for pest management due to their broad-spectrum bioactivity and eco-friendly properties (Abdelgaleil *et al.* 2009; Campolo *et al.* 2018; Rezaei *et al.* 2019). These complex volatile mixtures, typically obtained through hydrodistillation or steam extraction of aromatic plants, contain diverse bioactive constituents including monoterpenes, sesquiterpenes, and oxygenated derivatives such as alcohols, aldehydes, ketones, esters, phenols, and lactones (Abbassy *et al.* 2009; Abdelgaleil *et al.* 2009; Lopez and Pascual-Villalobos, 2010; Campolo *et al.* 2018). EOs exhibit multiple modes of action against insect pests, demonstrating contact toxicity, fumigant activity, repellency, and antifeedant effects when applied through conventional application methods (Ahmadi *et al.* 2018; Rezaei *et al.* 2019). Their biological activity stems from both vapor-phase toxicity and cuticular penetration upon topical application (Abbassy *et al.* 2009; Khanavi *et al.* 2017; Campolo *et al.* 2018). Particularly against *B. brassicae*, numerous studies have documented the efficacy of various EOs and their constituent

compounds through these mechanisms (Jahan *et al.* 2016; Heidary *et al.* 2022; da Silva *et al.* 2023). For instance, EO from *Schinus terebinthifolius* (Raddi) (Brazilian pepper tree) demonstrated superior insecticidal and repellent activity against *B. brassicae* compared to *Eucalyptus citriodora* (Hook) and *Cymbopogon winterianus* (Jowitt). Notably, while effectively controlling the aphid pest, the *S. terebinthifolius* EO simultaneously attracted *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae), a key parasitoid of *B. brassicae* (da Silva *et al.* 2023). However, it is reported that EO of *Elettaria cardamomum* (L.) Maton is an appropriate compound against *B. brassicae* than other tested EOs, including *Cinnamomum zeylanicum* Blume, *Citrus sinensis* (L.) Osbeck, *Foeniculum vulgare* Mill., and *Thymus carmanicus* Jalas (Jahan *et al.* 2016).

Iran, with its rich biodiversity of over 8200 vascular plant species, approximately 2300 of which are medicinal or aromatic, provides a valuable resource for such bioactive compounds (Mozaffarian, 2012). The Apiaceae family is particularly noteworthy for producing secondary metabolites with demonstrated insecticidal properties (Thiviya *et al.* 2022). Among these, *Bunium persicum* (Boiss.) Fedtsch. (Persian cumin), a perennial aromatic herb indigenous to the Irano-Turanian region, produces schizocarpic fruits containing potent EOs (Nickavar *et al.* 2014). These characteristically aromatic brown seeds serve dual roles as both a traditional culinary spice and a source of bioactive compounds, with their EO profile exhibiting notable insecticidal activity against various agricultural and medicinal pests, including *Sitotroga cerealella* Olivier (Nouri Ganbalani *et al.* 2021), *Tribolium castaneum* (Herbst) (Moravvej *et al.* 2011), *Trichoplusia ni* (Hübner) (Khanavi *et al.* 2017), *Anopheles stephensi* Liston (Sanei-Dehkordi *et al.* 2016; Vatandoost *et al.* 2018), *Culex quinquefasciatus* Say (Perinelli *et al.* 2022), and *Culex pipiens* L. (Sanei-Dehkordi *et al.* 2016). Despite their pesticidal potential, EOs face practical limitations including high volatility, poor water solubility, and rapid environmental degradation (Abdelgaleil *et al.* 2009; Heidary *et al.* 2022). Nanoencapsulation technology addresses these challenges by enhancing compound stability, improving bioavailability, and enabling the controlled release of active ingredients (Nuruzzaman *et al.* 2016; Granata *et al.* 2018; Kumar *et al.* 2019). Although several studies have investigated the efficacy of pure EOs from various plant species against *B. brassicae* (e.g., Jahan *et al.* 2016; da Silva *et al.* 2023), research on nanoformulated EOs for aphid control remains limited. To bridge this knowledge gap, the present study aimed to: (1) characterize the chemical composition of *B. persicum* EO, (2) develop and optimize a nanoencapsulated formulation, (3) analyze nanocapsule morphology and particle size distribution, and (4)

conduct a comparative evaluation of the aphicidal efficacy between pure and nanoencapsulated EOs against *B. brassicae*.

MATERIALS AND METHODS

Plant and insect rearing

Flowering kale, *Brassica oleracea* var. *acephala*, cv. Pigeon F_1 Red FHB 511 (Takii Europe B.V., De Kwakel, Netherlands) was cultivated in clay pots (12 cm diameter ×12 cm height) filled with a sterilized growth medium consisting of soil, leaf mold, manure, peat moss, and humic acid (30:10:10:3:1, v/v). Plants were maintained in a greenhouse at 25 ± 5 °C, 60 ± 5% relative humidity (RH), and a 16:8 h light:dark (L:D) photoperiod, with no pesticide application. The original population of *B. brassicae* was collected from canola fields in PirBakran region (32°28′ N, 51°33′ E; 1610 m altitude), Isfahan Province, central Iran. Culture of apterae *B. brassicae* was maintained on 5-week-old *B. oleracea* in ventilated cages (40 × 40 × 40 cm) under constant environmental conditions (25±1 °C, 65±5% RH, and 16:8 h L:D photoperiod). The plants were watered regularly and replaced with new ones as needed. The aphids were subsequently reared for more than five generations on *B. oleracea* before treatment.

Bunium persicum essential oil

The fruits of *B. persicum* were collected in May 2018 from cultivated fields in Khur and Biabanak County (33°46´N, 55°05´E, Isfahan Province, Iran). A voucher specimen (#16059) was identified and deposited in the Herbarium of Isfahan Research and Education Center for Agriculture and Natural Resources (Isfahan, Iran). The plant material was air-dried at room temperature (23-25 °C) for five days and stored in darkness until distillation. The EO was extracted from dried plant samples (50 g each) via hydrodistillation (material-to-water ratio of 1:10 w/v; 4 h distillation) using a modified Clevenger-Type apparatus. Anhydrous sodium sulfate was used to remove water after extraction. The EO yield was 4% (w/w) on a dry weight basis. The resulting EO was transferred into sealed glass vials and stored at 4 °C in a refrigerator until bioassay analysis (Rezaei *et al.* 2019). For experimental use, the pure EO was diluted in distilled water with Tween 80 (0.5% v/v) as an emulsifier.

Chemical analysis of essential oil

The chemical composition of *B. persicum* EO was analyzed using gas chromatography-mass spectrometry (GC/MS) (Agilent 7890A gas chromatograph coupled with an Agilent 5975C

mass selective detector; Agilent Technologies, Palo Alto, CA, USA). Separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) with helium as the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was initially held at 60 °C for 2 min, then increased to 280 °C at a rate of 4 °C/min. A split injection mode (split ratio 1:50) was used with a manual injection volume of 0.1 µL. Individual compounds were identified by comparing their retention indices (RI) and mass spectra with those reported in the literature (Adams, 2007).

Nanocapsule preparation of essential oil

Nanocapsules were prepared via in-situ polymerization using an oil-in-water (O/W) emulsion method (Rochmadi *et al.* 2010). The core material consisted of *B. persicum* EO, while the nanocapsule shell was formed from urea and formaldehyde (37%). Tween 80 (2%) served as the emulsifier. Also, castor oil (10%) and potassium silicate were used as synergists. Initially, a urea-formaldehyde (U-F) pre-polymer solution was prepared, homogenized, and then mixed with Tween 80 and the EO. To facilitate the encapsulation of EO droplets within the U-F shell, the solution pH was adjusted to 3.0. Stable nanocapsules formed after 4 h of reaction. The nanocapsule formulation containing *B. persicum* EO was prepared with the active ingredient at an 8% concentration. The resulting suspension was cooled to room temperature, filtered, and finally dehydrated by freeze-drying (Ting *et al.* 2010; Zhang *et al.* 2016). The yield and efficiency of nanoencapsulation and oil loading content were calculated according to the following equations (Khoee and Yaghoobian, 2009).

$$Nanoencapsulation\ yield\ \% = \left(\frac{N}{C}\right) * 100$$

where C is the weight of the produced nanocapsules (g) and N is the weight of the initial materials (g).

$$Nanoencapsulation\ efficiency\ \% = \left(\frac{A}{B}\right) * 100$$

where A is the weight of the loaded EO (g) within the nanocapsules and B is the weight of the EO used in the experiment (g).

$$Oil\ loeading\ content\ \% = \left(\frac{A}{D}\right) * 100$$

where A is the weight of the loaded EO (g) within the nanocapsules and B is the weight of the nanocapsules in the experiment (g).

Essential oil nanocapsule size and morphology

The morphology and surface characteristics of the nanocapsules were analyzed using transmission electron microscopy (TEM) (Carl Zeiss-EM10C, 100 kV, Carl Zeiss AG, Oberkochen, Germany). The mean diameter (z-average) and poly-dispersity index (PDI) were determined by dynamic light scattering (DLS) using a Nanophox 90-246 V nanoparticle size analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany), which employs photon cross-correlation spectroscopy (PCCS) (Moghimi *et al.* 2018).

Bioassay

Contact toxicity

The contact toxicity of pure and nanocapsulated formulations of *B. persicum* EO against *B. brassicae* adults was evaluated using the leaf-dipping method (Rezaei and Moharramipour, 2019). To obtain synchronized aphids for experiments, apterous females were individually placed on flowering kale leaf discs (5 cm diameter) in ventilated Petri dishes (9 cm diameter) containing a 5 mL layer of 2% agar solution to produce offspring. After 24 h, the adults were removed, and the nymphs were allowed to develop into adults. The presence of exuviae was used to determine when molting happened.

Preliminary tests were conducted to determine appropriate concentrations (inducing 10–90% mortality at logarithmic intervals) of pure and nanocapsulated *B. persicum* EOs using 2-day-old *B. brassicae* adults. Based on these tests, the selected concentrations for the main experiment were 103, 272, 717, 1886, and 4964 ppm for the pure EO and 33, 104, 326, 1026, and 3226 ppm for the nanocapsulated EO. The different solutions of both raw and nanocapsulated *B. persicum* EO were prepared by dispersing a known quantity of each in a volume of distilled water.

For bioassays, twenty 2-day-old *B. brassicae* adults were placed on a flowering kale leaf disc (5 cm diameter). After 15–30 min (allowing aphids to settle), the leaf discs were dipped into the respective EO concentrations for 5 seconds. The treated leaf discs were then placed in ventilated Petri dishes (9 cm diameter) with a 5 mL layer of 2% agar solution. Parafilm was used to seal the dishes and prevent aphid escape. Controls consisted of distilled water (with 0.5% Tween 80 for pure EO and without Tween 80 for nanocapsulated EO).

Mortality was assessed after 72 h (Heidary *et al.* 2022), with aphids considered dead if they showed no movement after gentle prodding with a fine brush (Khoobdel *et al.* 2017; Rezaei and Moharramipour, 2019). Each concentration was replicated five times, and all bioassays

were conducted under controlled conditions (25 ± 1 °C, 65 ± 5% RH, and a 16:8 h L:D photoperiod).

Fumigant toxicity

The fumigant toxicity of pure and nanocapsulated *B. persicum* EO against apterous *B. brassicae* females was evaluated using sealed 400 mL glass vials. Similar to the contact toxicity bioassays, preliminary tests were conducted with 2-day-old adults to determine effective concentrations.

For the experiments, flowering kale leaf discs (5 cm diameter) were placed on a 7.5 mL layer of 2% agar solution. Synchronized *B. brassicae* females were carefully transferred onto the leaf discs using a soft paintbrush. Filter paper strips (Whatman No. 1, 5 × 2 cm) were treated with the pure or nanocapsulated EOs at designated concentrations and attached to the inner vial walls to prevent direct insect-pesticide contact.

In the main bioassay, the tested concentrations were 12.47, 20.80, 34.65, 57.72, and 96.17 µL/L air for the pure EO and 7.65, 13.32, 23.22, 40.45, and 70.50 µL/L air for the nanocapsulated EO. Untreated filter papers served as controls (Ahmadi *et al.* 2018). The vials were sealed with parafilm, and each treatment was replicated five times. The mortality and bioassay conditions were the same as those used for the contact toxicity.

Statistical analysis

The dynamic light scattering (DLS) data were analyzed using the Cumulants analysis (mono-modal and Gaussian distribution). The distribution of particles was calculated by polydispersity index (PDI) as follows:

$$PDI = (\frac{\sigma}{d})^2$$

where δ and *d* denote the standard deviation and mean diameter of particles, respectively (Fang *et al.* 2004). The mortality was evaluated using the Abbott correction formula for the natural mortality in untreated controls (Abbott, 1925). Generalized linear models (GLMs) with the binomial family were applied to the bioassay data. In particular, the dose-response data were analyzed using logistic regression (binomial errors), where regression lines were fitted to dose-mortality data on a log (of concentration)-logit (of mortality) scale, and the estimated lethal concentrations (LC) and associated 95% confidence intervals (CI) were then calculated from the estimated linear regression parameters. Because of non-parallel regression lines, the toxicity ratio test (lethal dose ratios) was used to compare LCs, instead of overlapping CIs,

based on the statistical power of the ratio test and its better type I error rates (Wheeler *et al.* 2006; Crawley, 2013). All statistical analyses were performed in *R 3.6.1* (R Development Core Team).

RESULTS

Chemical analysis of essential oil

The GC-MS analysis identified 28 constituents in *B. persicum* EO, with 18 compounds present at concentrations >0.1% (Table 1). The oil was dominated by terpenes and benzenes, with γ-terpinene (36.62%) being the most abundant constituent, followed by ρ-cymene (18.41%), carvacrol (13.60%), cuminaldehyde (13.50%), limonene (7.12%), β-pinene (3.11%), 2-caren-10-al (1.96%), and α-pinene (1.57%).

Nanocapsule size, morphology, and formulation characteristics

Transmission electron microscopy (TEM) analysis (16700× magnification) revealed that nanocapsulated *B. persicum* EO formed distinct core-shell structures with near-spherical morphology (Fig. 1), confirming successful encapsulation. The nanocapsules exhibited slightly irregular surface topography. Size distribution analysis showed nanoparticle diameters ranging from 8.11 nm (10^{th} percentile) to 14.62 nm (90^{th} percentile) (Fig. 2), with median diameter (x_{50}) of 10.88 nm, volume mean diameter (VMD) of 11.17 nm, and Sauter mean diameter (SMD) of 10.60 nm. The corresponding polydispersity indices were 0.057 (x_{50}), 0.054 (VMD), and 0.060 (SMD), indicating a narrow size distribution.

In the nanocapsule polymerization process, the yield and efficiency of nanoencapsulation and oil loading content were 91, 92, and 88.22%, respectively.

Contact and fumigant toxicity

Logistic regression revealed significant dose-response relationships for both pure and nanocapsulated EO formulations in both contact and fumigant toxicity tests ($P < 0.001$ for all comparisons). For contact toxicity, the models showed strong fits (McFadden's $R^2 = 0.833$ and 0.879 for pure and nanocapsulated EO, respectively) with significant z-values (12.06 and 12.28). Similarly, fumigant toxicity models demonstrated excellent fits ($R^2 = 0.924$ and 0.921) with significant z-values (12.20 and 12.29) (Table 2).

Toxicity ratio tests indicated significantly greater potency of nanocapsulated EO across all lethal concentrations (LC_{10}-LC_{90}) for both test methods (Table 4). For contact toxicity, nanocapsulated EO required substantially lower concentrations (69.97-1908.46 ppm)

compared to pure EO (266.96-3977.08 ppm) to achieve equivalent mortality levels (Table 3). This enhanced efficacy was similarly evident in fumigant tests, where nanocapsulated EO showed lower required concentrations (9.01-59.49 µl/l air) versus pure EO (15.45-79.59 µl/l air) (Table 3).

Dose-response curves (Figs. 3 and 4) consistently demonstrated superior toxicity of nanocapsulated EO across the entire tested range (0.01-99.99% mortality), confirming the enhanced insecticidal efficacy of the encapsulated formulation through both exposure routes.

DISCUSSION

This study demonstrated the insecticidal efficacy of both pure and nanoencapsulated formulations of *B. persicum* EO against *B. brassicae* using standardized contact and fumigant bioassay methods. Our findings align with previous reports of this EO's toxicity against other insect pests (Moravvej *et al.* 2011; Khanavi *et al.* 2017; Perinelli *et al.* 2022). To our knowledge, this work provides the first evidence of *B. persicum* EO's activity against *B. brassicae* and represents the first evaluation of its nanoencapsulated formulation for this pest. The rationale for selecting *B. persicum* EO was based on: (1) documented insecticidal properties against various pests (Sanei-Dehkordi *et al.* 2016; Nouri Ganbalani *et al.* 2021); (2) potential for enhanced efficacy through nanoformulation (Kumar *et al.* 2019; Heidary *et al.* 2022); (3) scalability of production; (4) widespread distribution and cultivation feasibility in Iran (Thiviya *et al.* 2022); and (5) its favorable environmental and human safety profile (Moharramipour and Negahban, 2014; Campolo *et al.* 2018). However, the high volatility of the EO limits their practical application in pest management programs, necessitating the development of stabilization strategies.

Our study successfully developed stable nanocapsules containing *B. persicum* EO with favorable physicochemical properties. The polydispersity index (PDI) values for the nanocapsulated *B. persicum* EO (0.057, 0.054, and 0.060) fall within the mid-range (0.05–0.7), indicating a moderately uniform particle size distribution. PDI values below 0.05 indicate a highly monodisperse system, while those exceeding 0.7 reflect a very broad size distribution, which is unsuitable for dynamic light scattering (DLS) analysis. Mid-range PDI values are compatible with various size distribution algorithms, ensuring reliable characterization (Danaei *et al.* 2018).

Nanoencapsulation enhances pesticide efficacy by improving the availability of active ingredients, through slow release and increased cuticular absorption, to target arthropod pests

(Nuruzzaman *et al.* 2016; Kumar *et al.* 2019). The controlled release of pesticide actives depends on the physicochemical properties of the encapsulation material, including its composition, nanoparticle morphology (shape and size), polymorphism, shell thickness, porosity, encapsulation efficiency, and payload capacity (Kah *et al.* 2013). Notably, nano-pesticides exhibit greater efficiency due to their high surface area-to-volume ratio, which reduces the required dosage (Nuruzzaman *et al.* 2016). Furthermore, nanoencapsulation not only protects the active ingredient but also facilitates its sustained release (Kah *et al.* 2013; Kah and Hofmann, 2014). In this study, nanocapsulated *B. persicum* EO exhibited well-defined core-shell structures with near-spherical morphology and irregular external surfaces. Spherical nanoparticles are optimal for controlled-release mechanisms, as they promote uniform active ingredient diffusion (Perlatti *et al.* 2013).

Our results demonstrated significantly greater insecticidal efficacy of nanocapsulated *B. persicum* EO compared to its pure form against *B. brassicae* in both contact and fumigant toxicity bioassays. This observed enhancement aligns with previous reports of superior pesticidal activity in nanoencapsulated EOs from *Allium sativum* L., *Rosmarinus officinalis* L., *Achillea millefolium* L., and *Cuminum cyminum* L. (Yang *et al.* 2009; Ziaee *et al.* 2014; Khoobdel *et al.* 2017; Ahmadi *et al.* 2018), likely attributable to improved nanoparticle mobility facilitating deeper tissue penetration. Nanoformulations address key limitations of pure EOs by enabling controlled release of active compounds (enhancing bioavailability) while reducing non-target toxicity (Khoobdel *et al.* 2017; Granata *et al.* 2018). The urea-formaldehyde polymer matrix used in our nanocapsules offers additional environmental benefits through microbial degradation into plant-available nitrogen (Hayatsu, 2014; Gonzalez-Hurtado *et al.* 2017). However, further validation under different conditions is needed to assess environmental impacts on nanocapsule performance, along with evaluation against other pest species to determine broad-spectrum applicability. Future studies should also investigate lethal time (LT) to assess the long-term stability and efficacy of the nanocapsulated *B. persicum* EO.

The present study identified γ-terpinene as the dominant compound in *B. persicum* EO, followed by ρ-cymene, carvacrol, and cuminaldehyde, collectively representing 82.13% of the total phytochemical profile. These findings align with previous reports by Sanei-Dehkordi *et al.* (2016) and Nouri Ganbalani *et al.* (2021), though with notable compositional variations. Khanavi *et al.* (2017) reported similar major constituents (γ-terpinene: 45%; ρ-cymene: 15%; cuminaldehyde: 18%) along with significant limonene content (11%), while Nickavar *et al.* (2014) identified cuminaldehyde, ρ-cymene, and γ-terpinene as primary components without

detecting limonene as a major constituent. Such variability in EO composition likely stems from differences in plant provenance, growing conditions (climate and season), and extraction methodologies (Ahmadi *et al.* 2018; Campolo *et al.* 2018; Rezaei *et al.* 2019).

γ-Terpinene (4-methyl-1-(1-methylethyl)-1,4-cyclohexadiene), a monoterpene isomer that differs from α- and β-terpinene by its distinctive double bond configuration, is a well-characterized acetylcholinesterase inhibitor that contributes to the insecticidal activity of EOs (Lopez and Pascual-Villalobos 2010). Its bioactivity has been demonstrated across multiple insect species, including larvicidal effects against *Anopheles anthropophagus* Xu & Feng (Diptera: Culicidae) (LC_{50} = 29.21 ppm; Zhu and Tian 2011) and both *Aedes aegypti* (L.) and *A. albopictus* (Skuse) (Diptera: Culicidae) (LC_{50} = 30.7 and 29.8 ppm, respectively; Cheng *et al.* 2009). The compound also shows significant toxicity against lepidopteran (*Spodoptera littoralis* (Boisd.) fourth instar larvae) and hemipteran (*Aphis fabae* Scopoli adults) insect pests (Abbassy *et al.* 2009). Given this broad-spectrum activity, the γ-terpinene-rich EO of *B. persicum* represents a promising botanical insecticide candidate for *B. brassicae* control. Further research under semi-field conditions is warranted to evaluate its practical efficacy and potential integration into integrated pest management (IPM) programs.

In both pure and nanocapsulated *B. persicum* EO bioassays, the fumigant method exhibited significantly higher toxicity than the contact method. Several studies have reported the fumigant activity of *B. persicum* EO against various insect pests (Sanei-Dehkordi *et al.* 2016; Nouri Ganbalani *et al.* 2021), while others have documented both fumigant and contact toxicity (Moravvej *et al.* 2011; Khanavi *et al.* 2017; da Silva *et al.* 2023). Consistent with our findings, Khanavi *et al.* (2017) observed greater fumigation toxicity of *B. persicum* EO compared to contact toxicity in *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). The authors suggested that the contact toxicity of the EO may primarily arise from cuminaldehyde, which showed the weakest activity in fumigation assays. Notably, Abdelgaleil *et al.* (2009) demonstrated the contact activity of cuminaldehyde against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), linking its efficacy to a significant inhibitory effect on acetylcholinesterase (AChE) activity.

CONCLUSIONS

Screening for insecticidal effects of EOs offers an opportunity to develop novel pesticides based on their chemical properties and toxicity, while minimizing the adverse environmental impacts and harm to non-target organisms associated with synthetic insecticides (Abbassy *et*

al. 2009; Ahmadi *et al.* 2018; Campolo *et al.* 2018). Our findings suggest that nanocapsulated *B. persicum* EO has significant potential as a botanical insecticide and could play a valuable role in IPM programs targeting *B. brassicae*, owing to its dual fumigant and contact activity. Additionally, since *B. persicum* has a long history of use in herbal medicine, it likely poses fewer risks to human health compared to conventional chemical insecticides. Nevertheless, further field and semi-field studies are recommended to evaluate the compound's efficacy under real-world conditions. Evaluating the cost-effectiveness of nanocapsulated *B. persicum* EO compared to conventional methods is also recommended for assessing its practical potential.

ACKNOWLEDGEMENTS

We gratefully acknowledge the research staff at the Isfahan Research and Education Center for Agriculture and Natural Resources for generously providing access to their laboratory facilities.

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Table 1. Chemical composition of *Bunium persicum* essential oil.

Compounds	RT[a] (min)	Value (%)	RI[b]	KI[c]
α-Thujene	5.38	0.49	923	924
α-Pinene	5.55	1.57	930	932
β-Pinene	6.47	3.11	969	974
α-Terpinen	7.58	0.26	1014	1017
ρ-Cymene	7.80	18.41	1022	1024
Limonene	7.91	7.12	1027	1029
γ-Terpinen	8.79	36.62	1060	1059
Trans-2-Caren-4-ol	10.43	0.55	1119	-
Borneol	12.19	0.52	1175	1169
γ -Terpineol	13.05	0.41	1202	1199
Thymol methyl ether	14.10	0.16	1227	1235
Cuminaldehyde	14.56	13.50	1239	1239
2-Caren-10-al	16.00	1.96	1274	-
Acetic acid, chloro-, n-decyl ester	16.24	0.12	1280	-
Carvacrol	16.62	13.60	1289	1298
β-Caryophyllene	20.30	0.53	1416	1419
Ledene	22.98	0.12	1499	1490
γ -Bisabolene	23.98	0.24	1538	1533

[a] RT denotes the retention time for each compound.
[b] RI denotes the retention index for each compound.
[c] Kovats retention index.

Table 2. Logistic regression of dose-response data of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults.

Bioassay procedure	Formulation	Fitted model[a]				
		a (SE)	b (SE)	z-value	*P* value <	R^2_{McF}[c]
Contact	Pure EO	-11.29 (0.94)	1.63 (0.13)	12.06	0.001	0.833
	Nanocapsulated EO	-7.84 (0.65)	1.33 (0.11)	12.28	0.001	0.879
Fumigation	Pure EO	-7.08 (0.59)	2.68 (0.22)	12.20	0.001	0.924
	Nanocapsulated EO	-5.03 (0.42)	2.25 (0.18)	12.29	0.001	0.921

[a] $y = \frac{e^{a+bx}}{1+e^{a+bx}}$,where x and y represent natural logarithm of concentration (ppm) and the proportion of dead adult, respectively.
[b] The standard normal deviate
[c] McFadden's R-squared.

Table 3. Contact and fumigant toxicity of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults

Bioassay procedure	Formulation	Lethal concentration (95% CI) in ppm or µl/l air[a] (n = 5)			
		LC_{10}	LC_{25}	LC_{50}	LC_{90}
Contact	Pure EO	266.96 a[b] (205.65 - 346.55)	524.48 a (436.23 - 630.58)	1030.40 a (882.86 - 1202.59)	3977.08 a (3018.89 - 5239.39)
	Nanocapsulated EO	69.97 b (50.86 - 96.27)	159.91 b (127.68 - 200.28)	365.43 b (303.62 - 439.83)	1908.46 b (1378.10 - 2642.93)
Fumigant	Pure EO	15.45 A (13.19 - 18.09)	23.27 A (20.87- 25.95)	35.07 A (32.18 - 38.12)	79.59 A (68.02 - 93.06)
	Nanocapsulated EO	9.01 B (7.55 - 10.76)	14.45 B (12.80 - 16.30)	23.15 B (21.04 - 25.47)	59.49 B (49.74 - 71.08)

[a] Units for contact and fumigant lethal concentrations are ppm and µl/l air, respectively.
[b] Values marked with different small or capital letters within columns are significantly ($P < 0.05$) different (based on ratio test; see Table 4).

Table 4. Relative toxicity ratios of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults.

Bioassay procedure	Lethal dose ratio (95% CI) of Nanocapsulated EO:Pure EO[a]			
	LC_{10}	LC_{25}	LC_{50}	LC_{90}
Contact	0.266 (0.176 - 0.402)	0.305 (0.228 - 0.409)	0.351 (0.275 - 0.447)	0.463 (0.301 - 0.712)
Fumigant	0.584 (0.460 - 0.740)	0.621 (0.527 - 0.730)	0.660 (0.581 - 0.751)	0.747 (0.589 - 0.947)

[a] If the 95% confidence interval of the ratio includes 1, then the lethal doses are not significantly different.

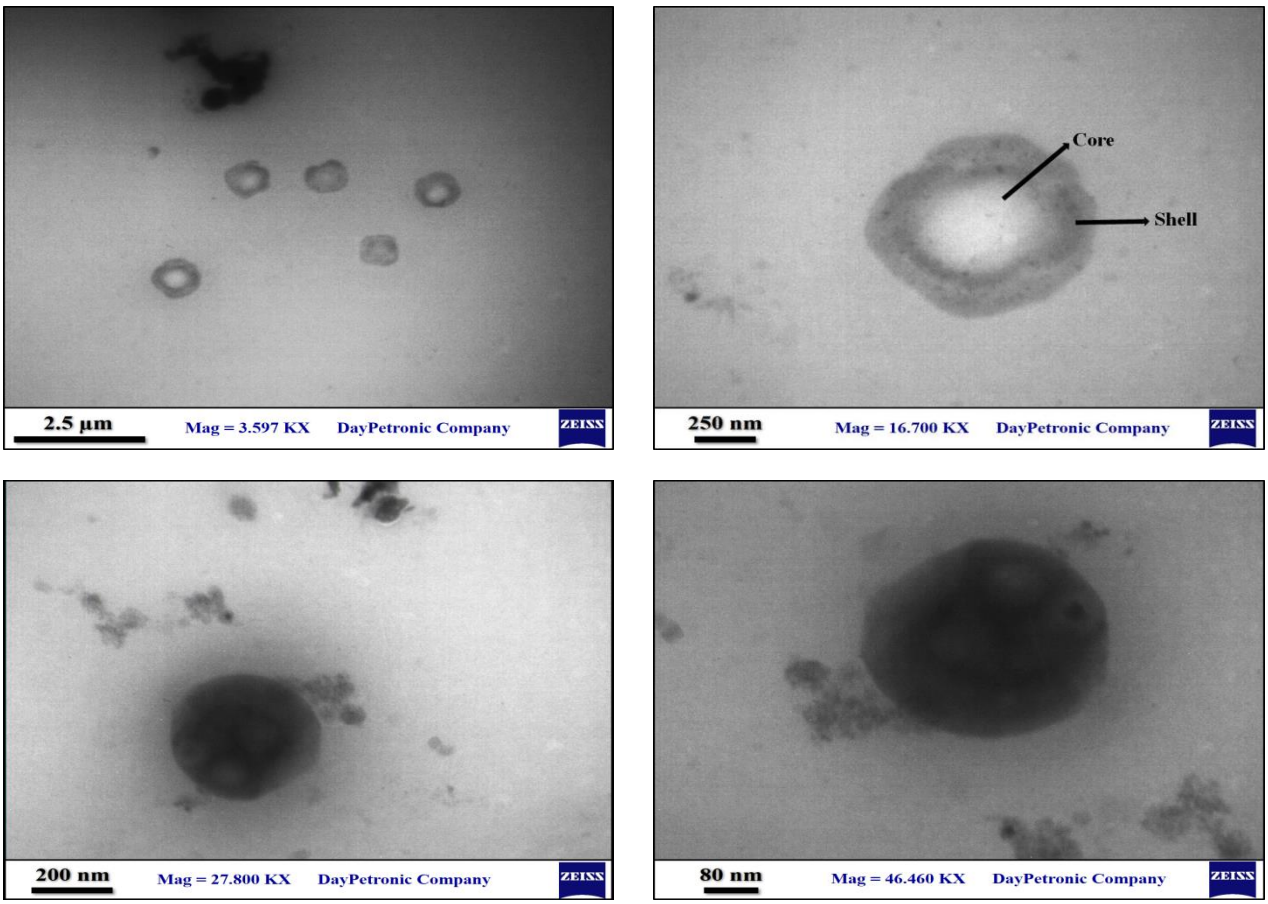

Fig. 1. Transmission electron microscopy images of nanocapsulated essential oil of *Bunium persicum* with different magnifications between 3.597×10^3 and 4.646×10^4.

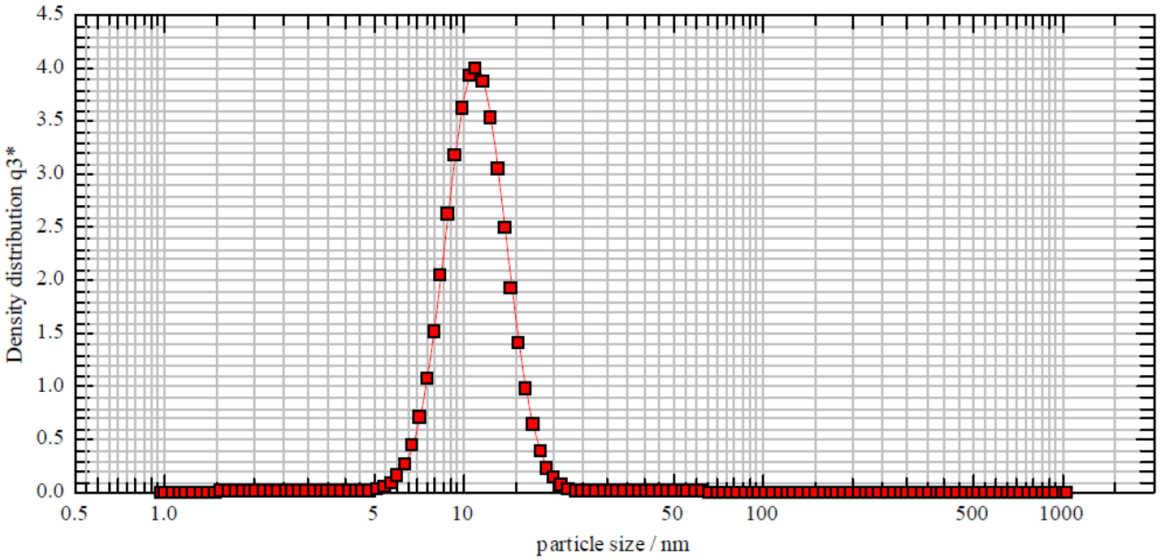

Fig. 2. Size distribution of nanocapsule particles of *Bunium persicum* essential oil.

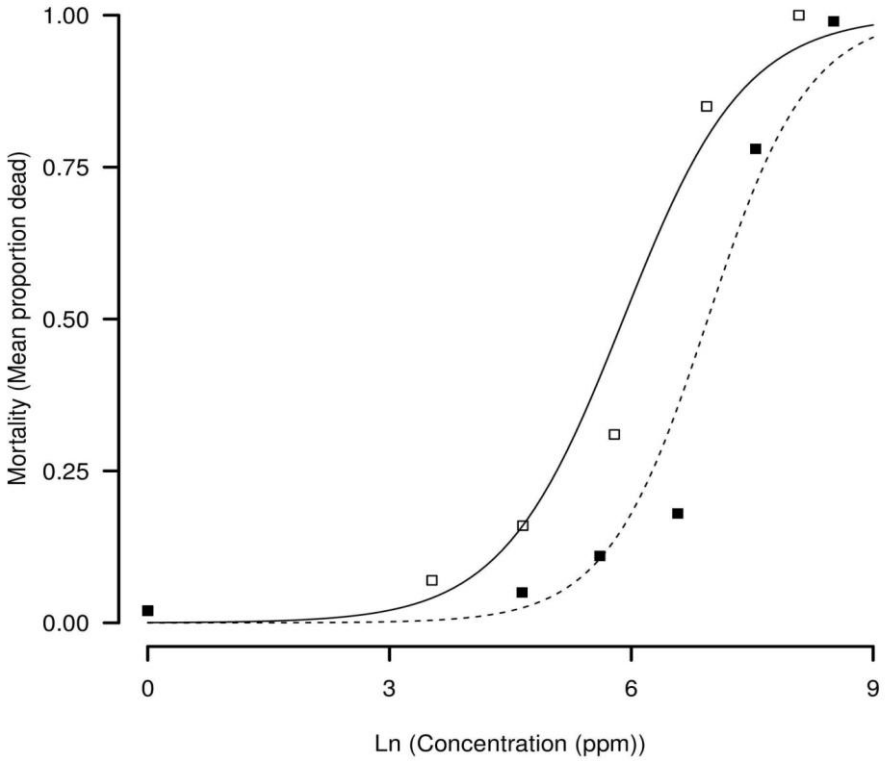

Fig. 3. Dose-response curves of contact toxicity of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults. Dashed (filled-square) and solid (empty-square) lines (points) represent pure and nanocapsuled essential oil of *B. persicum*, respectively.

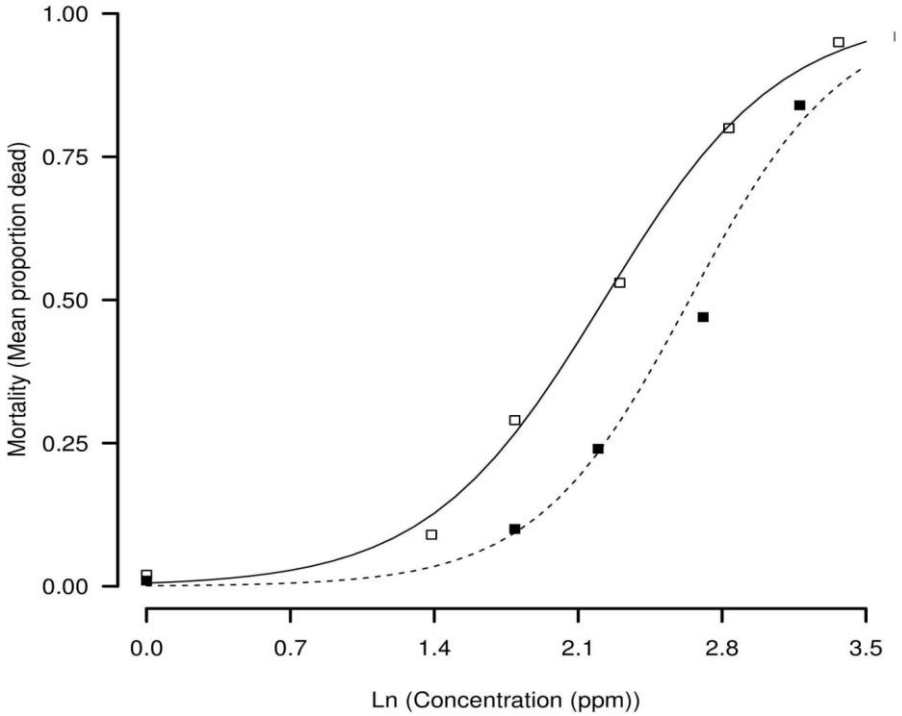

Fig. 4. Dose-response curves of fumigant toxicity of *Bunium persicum* essential oil and its nanocapsule against *Brevicoryne brassicae* adults. Dashed (filled-square) and solid (empty-square) lines (points) represent pure and nanocapsuled essential oil of *B. persicum*, respectively

نانوکپسول اوره-فرمالدهید سمیت اسانس *Bunium persicum* را در برابر *Brevicoryne brassicae* افزایش می‌دهد

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چکیده

شته مومی کلم، *Brevicoryne brassicae* L. (Hemiptera: Aphididae)، آفت بسیار مخرب و بومی اروپا می‌باشد که با پراکنش جهانی به یکی از چالش‌های عمده کشاورزی تبدیل شده است. این آفت در حال حاضر چالش‌های قابل توجهی برای تولید کلم ایجاد نموده است و منجر به خسارت چشمگیری به این محصول می‌شود. مصرف تازه‌خوری کلم، بکارگیری روش‌های کنترل غیرشیمیایی را برای حفظ امنیت غذایی در عین مدیریت مؤثر جمعیت آفت ضروری می‌سازد. این مطالعه، فعالیت حشره‌کشی اسانس‌های خالص و فرمولاسیون نانوکپسول زیره سیاه، *Bunium persicum* (Boiss.) Fedtsch، را روی شته مومی کلم بررسی نمود. اسانس گیاهی از طریق تقطیر با آب جداسازی شد و توسط کروماتوگرافی گازی و طیف‌سنجی جرمی (GC-MS) تجزیه و تحلیل شد. ترکیبات اصلی شامل گاما-ترپینن (۳۶/۶۲ %)، پارا-سایمن (۱۸/۴۱ %)، کارواکرول (۱۳/۶ %) و کومین آلدهید (۱۳/۵ %) بود. نانوکپسول‌ها از طریق پلیمریزاسیون درجا به روش امولسیونی روغن در آب سنتز شدند و با پراکندگی نور دینامیکی (DLS) و میکروسکوپ الکترونی عبوری (TEM)ویژگی‌های آن‌ها شناسایی شد که شکل ذرات کروی با قطر متوسط ۱۰/۸۸ نانومتر و شاخص پراکندگی ذرات (PDI) برای میانه برابر با ۰/۰۵۷ بود. نتایج زیست‌سنجی نشان داد که فرمولاسیون نانوکپسول سمیت به‌مراتب بالاتری نسبت به اسانس خالص نشان می‌دهد. مقادیر LC_{50} و LC_{90} سمیت تماسی فرمولاسیون نانوکپسول (به ترتیب ۳۶۵/۴۳ و ۱۹۰۸/۴۶ میکرولیتر بر لیتر آب) در مقایسه با اسانس خالص (۱۰۳۰/۴۰ و ۳۹۷۷/۰۸ میکرولیتر بر لیتر آب) پایین‌تر بود. به صورت مشابه، مقادیر LC_{50} و LC_{90} سمیت تدخینی برای نانوکپسول (۲۳/۱۵ و ۵۹/۴۹ میکرولیتر بر لیتر هوا) به صورت معنی‌داری پایین‌تر از اسانس خالص (۳۵/۰۷ و ۷۹/۵۹ میکرولیتر بر لیتر هوا) بود. نتایج نشان‌دهنده پتانسیل اسانس نانوکپسول *B. persicum* برای بکارگیری در برنامه‌های مدیریت تلفیقی آفات شته مومی کلم می‌باشد.