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Toxicity of *Foeniculum vulgare* essential oil, its main component and nanoformulation against *Phthorimaea absoluta* and the generalist predator *Macrolophus pygmaeus*

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The encapsulation of essential oils (EOs) in nanostructured lipid carriers (NLCs) represents an innovative and advanced approach to improve their stability and efficacy. This study evaluated, for the first time, the toxicity of *Foeniculum vulgare* EO (FVO), its main component, and nanoformulation (FVO-NLC) on *Phthorimaea absoluta* and their side effects on generalist predator, *Macrolophus pygmaeus*. The obtained FVO-NLC had spherical and small (86.32 nm) particle sizes, and its encapsulation efficacy was greater than 98%. FTIR analysis revealed no significant chemical interaction between EO and NLC components. Trans-anethole (TAL) (66.22%) was identified as the key FVO component. The leaf dip method was used for bioassays. FVO-NLC and FVO showed a similar level of contact toxicity against *P. absoluta* with LC_{50} values of 5.57 and 5.44 µL/mL, respectively, while TAL had an LC_{50} value of 3.93 µL/mL. The lethality test revealed the slow and persistent release of FVO from FVO-NLC. Furthermore, the life table data were analyzed using the TWO SEX-MSChart program. Results indicated that all selected bio-insecticides at their LC_{50} values reduced fecundity and negatively affected biological and population growth parameters in target pest, and usage of them was proved to be harmless for the predator according to IOBC protocols.

Keywords Bioinsecticide, *Phthorimaea absoluta*, Nanotechnology, Side effect, Two-sex life table, Miridae

Tomato (*Solanum lycopersicum* L.) is the second most valuable vegetable crop in the world after potato. The world's tomato production was estimated at 190 million tons in 2023[1]. This crop is affected by many pests in greenhouse and open field conditions. Among the tomato pests, the tomato leaf miner, *Phthorimaea* (= *Tuta*) *absoluta* Meyrick (Lepidoptera: Gelechiidae), is one of the most economically important pests of the crop in the world. The larvae penetrate plants and create mines in leaf mesophyll, young stems, and fruits, reducing plant photosynthetic capacity and high yield reduction ranging from 50 to 100%[2,3]. In addition to quantitative damage, feeding inside the fruit paves the way for secondary pathogens to enter the fruit, thereby causing fruit rot and remarkable qualitative crop loss[4,5].

Control of *P. absoluta* traditionally relies on synthetic insecticides[4]. However, they have low to moderate efficiency due to *T. absoluta* developing resistance to many insecticides from different groups and the endophytic habitat of its larvae[6]. Biological control can supply optimal pest control while reducing the negative impacts of synthetic pesticides. Among natural enemies of pests, generalist predators such as mirid bugs play critical roles in biological control programs against *P. absoluta*. Adult and juvenile instars of *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) feed on lepidopteran pest eggs/young larvae, whiteflies, thrips, aphids, and other soft-bodied insect pests[7].

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However, overreliance on insecticides has led to several disturbances including soil and groundwater contamination, negative impacts on biocontrol agents and human health, the resurgence of secondary pests, and resistance development of target organisms[8,9]. The awareness of the injurious impacts of chemical insecticides has prompted researchers to find safer alternatives to conventional insecticides[10,11].

Plant-based insecticides constitute a significant group of these alternatives, displaying a variety of insecticidal mechanisms such as insect repellent, insect attractant, anti-feedant, ovicide, larvicide, etc. Plant extracts and essential oils (EOs) have extremely complex components that give them unique modes of action, hence acquiring resistance by pests against plant-based insecticides is very difficult[10]. *Foeniculum vulgare* Miller (Apiaceae), commonly called fennel, is a well-known and important medicinal plant that is cultivated widely in most parts of temperate Europe and the Mediterranean due to its high antioxidant, nutritional, and pharmaceutical properties[12,13]. The major constituent of *F. vulgare* is trans-anethole[14] which has strong insecticidal activity[15]. Combinations among bioinsecticides and natural enemies of pests can play essential roles in integrated pest management programs and environmental protection. To optimize such strategies, the potentially lethal and sublethal effects of bioinsecticides on natural enemies must be evaluated[16].

Nevertheless, due to volatile compounds in plant EOs, in their pure forms, they are unstable and are easily degraded by environmental factors (such as sunlight, ultraviolet rays, heat, humidity, and oxygen), which can reduce their efficacy[17]. Encapsulation of EOs in nano-systems is a useful approach to overcome such problems[18]. Nanostructured lipid carriers (NLCs) are the newer generation of lipid nanoparticles introduced after solid lipid nanoparticles (SLNs)[19]. This new technology not only reduces the concentration of EO at which it causes potential efficacy properties due to their small size and high surface reactive area but also improves the physical and colloidal stability of the formulations and their resistance to ultraviolet light, evaporation, and oxidation[20]. Many studies have revealed significant results for EOs encapsulation in NLCs in the pharmaceutical, food, and cosmetic industries[17,20].

This study reports for the first time the toxicity and long-term insecticidal activity of pure *F. vulgare* EO, its main component, and NLC formulation against *P. absoluta* as well as their side effects on omnivore mirid predator. Our findings provide important insights into the combination of biocontrol agents and bioinsecticides, thereby contributing to *P. absolute* integrated management.

Materials and methods

Insect rearing

Tomato leaves containing immature stages of *P. absoluta* were collected from infested tomato plant fields and greenhouses in West Azarbaijan Province, Urmia, Iran. Infested leaves were transferred to 40 × 60 × 60 cm mesh net-rearing cages with pots of tomato plants, and adults were fed with a 10% honey solution. The colony of pest was reared for at least three generations to avoid any contamination at 25 ± 2 °C, 65 ± 5% relative humidity, and 16: 8 h (light: dark) photoperiod.

The initial colony of *M. pygmaeus* was obtained from a mass production company (Farmerz, Lorestan, Iran) and maintained in rearing cages in greenhouse conditions for several generations. Potted tomato plants and *Ephestia kuehniella* Zeller eggs, as a reference diet and a protein- rich source were offered to the predatory mirid bug in the cages[21].

The original colony of *E. kuehniella* was obtained from the insectarium of the Agricultural Research, Education, and Extension Organization of Tehran, Tehran province, Iran, and reared on an artificial diet consisting of wheat flour, wheat bran, and glycerol in plastic dishes (25 × 15 × 10). The colony was kept in a rearing room at 27 ± 2 °C, 65 ± 5% relative humidity, and 14:10 h (light: dark) photoperiod. Then, the newly emerged *E. kuehniella* adults were released into a funnel-shaped plastic container (15 cm diameter) as an oviposition container with its lid covered with a fine mesh net. A wax paper was positioned at the bottom of the funnel to collect the eggs, which was removed and replaced daily[22].

EO isolation and chemical characterization

Seeds of fennel were obtained from growing and harvesting local genotype in research farms of Urmia University (37°40′ N, 44°55′ E). The plant material was identified by Dr. Mozhgan Larti and deposited at the herbarium at Agricultural Research, Education and Extension Organization (AREEO), West Azerbaijan, Urmia, Iran with voucher specimen 11,074. Essential oil from the dried and powdered fennel seeds (100 g) and deionized water (1 L) was extracted using the hydro-distillation method in a Clevenger-type apparatus for over 4 h. The extracted EO was dehydrated using anhydrous sodium sulfate. Oil without water was stored at 4 °C in airtight dark vials before use. The GC/MS analysis of the EO was performed using a gas chromatography (GC Agilent, 7890 USA) coupled with a mass selective detector (Agilent 5975 A, USA) and a capillary column (BP-5 MS, length of 30 m, inner diameter of 0.25 mm, thin-film of 0.25 µm). The initial oven temperature was kept at 80 °C for 3 min and increased at the rate of 8 °C min^{-1} up to 180 °C. The sample was maintained at this temperature for 10 min. Helium with a flow rate of 1 mL/min was the carrier gas. All mass spectra were recorded in the electron impact ionization at 70 eV and were scanned in the range of 40–500 m/z. The constituents of EO were identified by comparing their mass spectra and retention indices with those described in the instrument libraries[23].

Chemicals

Precirol® ATO5 and Poloxamer® 407 were obtained from Gattefossé, (Saint-Priest, Cedex, France) and Sigma Alderich (Chemie GmbH, Germany), respectively. Miglyol® 812 was purchased from Sasal, (Hamburg, Germany). Trans-anethole was purchased from Sigma-Aldrich (St. Louis, USA).

Foeniculum vulgare EO-NLC preparation

For the production of *F. vulgare* EO-NLC (FVO-NLC), in a hot water bath, 200 mg FVO was dissolved in 200 mg Miglyol and was then added to 1.8 g melted Precirol. Meanwhile, about 25 mL aqueous surfactant solution (containing 1.5 g Poloxamer) was prepared separately at the same temperature and added dropwise into the melted lipid phase and homogenized using a high shear homogenizer (Silent Crusher M, Heidolph, Nuremberg, Germany), at 20,000 rpm for 20 min. The sample was then stored at a refrigerated temperature (4–6 °C) for 15 min for crystallization and finally, FVO-NLC was formed. Moreover, a control formulation without *F. vulgare* EO was prepared using the same method[18].

Characterization of FVO-NLC

FVO-NLC particle size, zeta potential, morphology assessment and FTIR analysis

The mean particle size, polydispersity index (PDI), and zeta potential of FVO-NLC formulation were characterized by dynamic light scattering (DLS) and a Zetasizer (Nano ZetaSizer, Zen 3600, Malvern Instruments Ltd., United Kingdom) at 25 °C after 24 h of preparation. FVO-NLC morphology (shape and surface characteristics) was evaluated by scanning electron microscope (SEM, KYKY-EM3200, Beijing, China).

FTIR spectrometry (Shimadzu Corp., Kyoto, Japan) was used to investigate the type of intramolecular interactions among different compounds. For this purpose, freeze-dried FVO-NLC sample and native lipid were mixed with potassium bromide (KBr) and pressed with a hydraulic press instrument to prepare pellets. The FTIR spectra of the samples were recorded in 400–4000 cm^{-1} wavelength region with an optical resolution of 4 cm^{-1} for 100 scans[24].

Encapsulation efficacy

A volume of 0.5 mL NLC formulation was added to 3.5 mL ethanol and stirred for 15 min. Formulation of NLC was added to an Amicon ultrafilter tube and centrifuged at 5000 rpm for 5 min (Hettich EBA 20, Tuttlingen, Germany). Then, the filtered sample was exposed to a UV–VIS spectrophotometer (Ultrospec 2000, Scinteck; UK) and its absorbance was measured at λ_{max} = 300 nm. Finally, the encapsulation efficacy percentage (EE %) was estimated by Eq. (1)[24,25]:

$$EE\,(\%) = \frac{C_2 - C_1}{C_2} \times 100 \tag{1}$$

where C_1 is the amount of free EO (filtered) (mg) and C_2 demonstrates the total EO (mg) added to the nanostructured lipid carrier.

Insecticidal assay

Contact toxicity bioassay

For *P. absoluta* bioassay, the tomato leaves (6 cm diameter) were prepared and dipped in desired concentrations of TAL (3.00, 3.47, 3.98, 4.57, and 5.27 µL/mL), FVO (3.45, 4.46, 5.62, 7.08, and 8.98 µL/mL) and FVO-NLC (3.30, 4.36, 5.49, 6.92, and 8.68 µL/mL) for 15 s. The solvent for the dilution of compounds was ethanol 5% (Merck, Germany). After drying, each leaf was placed in a Petri dish (6 cm diameter). Then, 20 s instar larvae of *P. absoluta* were transferred onto each leaf. The Petri dishes were kept under greenhouse conditions. Larval mortality was recorded after 72 h. The NLC sample prepared without FVO loading and distilled water–ethanol mixture (95:5 v/v) were used as controls. The bioassay for each treatment was replicated four times[26].

In the sublethal study, 200 *P. absoluta* second instar larvae (< one day old) were exposed to LC_{50} values of TAL, FVO, and FVO-NLC. After 24 h, 75 live larvae were randomly selected and individually transferred to Petri dishes provided with an untreated leaf tomato that was replaced by a new one every two days. Petri dishes were checked daily and observations (developmental duration and survivorship of different biological stages) were recorded using a stereoscopic microscope (40 ×) until adults emerged. After the adults' emergence, 20 newly emerged pairs of each treatment were picked randomly, and each pair was transferred to a mating plastic cage (14 × 11 × 5 cm) provided with 10% honey solution to lay eggs on tomato leaves. The number of eggs laid per female was recorded daily until the death of the females[27].

Non-target effects assay

To evaluate the side effects of bio-insecticides on predator, tomato leaves were treated with FVO, TAL, and FVO-NLC at LC_{50} concentrations obtained from contact toxicity bioassay for *P. absoluta* and both controls. Each treatment was replicated four times. For each treatment and control sample, 20 fourth instar nymphs of predators were released in plastic Petri dishes provided with treated leaflets tomato and *E. kuehniella* eggs. Nymphal mortality was assessed 72 h after exposure. Afterward, for each treatment, groups of 10 one-day-old females (that have emerged from the aforementioned experiment) were held in 2 L plastic jars supplied with *E. kuehniella* eggs and untreated leaflet tomato plant as an oviposition substrate. Ten males from laboratory colonies were also added to the jars. The jars were kept under greenhouse conditions until the eggs hatched. Fecundity (the number of neonate predator nymphs) was determined over a total period of 2 weeks[28].

Persistence assessment of contact toxicity

Persistence toxicities of TAL, FVO, and FVO-NLC against *P. absoluta* were assessed by dipping tomato leaves in the LC_{95} concentration of TAL, FVO, and FVO-NLC. From the date of the treatment, every 24 h, 20 *P. absoluta* second instar larvae were exposed to the tested leaves. Then, the mortality rates were recorded. The conditions for the treated leaves complied with those for the contact toxicity assay (section contact toxicity bioassay). Each assay was repeated four times[29,30].

Statistical analysis

Probit analysis (SPSS v. 17.0. software) was performed to analyze the data obtained from bioassay and determine lethal concentrations and 95% confidence limits[31]. The life history data of *P. absoluta* were statistically analyzed using TWOSEX-MS Chart software[32] and age-stage two-sex life table theory[33,34]. The variance and standard errors of the biological and population parameters were estimated via 100,000 bootstrap replicates[35]. All treatment variations were compared using a paired bootstrap test at a 5% significance level based on the confidence interval of differences. Sigma Plot v. 12.0 software was used to create the figures.

The toxicity effects of TAL, FVO, and FVO-NLC against predator were also calculated according to Eq. (2).

$$E\,(\%) = 100 - (100 - M) \times R \tag{2}$$

where E is percentage of toxicity, M is the percentage of predator mortality, and R is the ratio between the mean number of offspring of TAL, FVO, and FVO-NLC treated females and offspring of females in the control group. The resulting values were classified according to the International Organization for Biological Control (IOBC) toxicity categories as: harmless (E < 30%); slightly harmful (30% < E < 80%); moderately harmful (80% < E < 99%) and harmful (E > 99%)[36].

Results

Chemical characterization of Foeniculum vulgare EO

Hydrodistillation of fennel seeds afforded light-yellow EO with a yield of 2.91%. EO from *F. vulgare* seeds components was identified by GC–MS (Table 1). The constituents with the highest concentrations were trans-anethole (66.22%), limonene (10.74%), fenchone (5.54%), carvone (3.26%), and estragole (2.70%). Their retention time were obtained 13.14, 7.09, 8.45, 12.06, and 11.79 min, respectively (Fig. 1).

FVO-NLC characterization

After 24 h from the preparation, the obtained FVO-NLC was characterized in terms of mean particle size, polydispersity index, encapsulation efficacy, and zeta potential (Table 2). DLS analysis of optimized FVO-NLC exhibited the existence of homogeneous nanoparticles of about 86 nm in size, with only a single peak in particle size distribution (Fig. 2) as confirmed by the value of polydispersity index (PDI = 0.222, lower than 0.3). Moreover, zeta potential measurement showed the presence of a negative superficial charge (−15.35 mV) in the FVO-NLC sample. In addition, the optimized formulation of FVO-NLC had an encapsulation efficacy of 98.57 ± 0.45%.

The shape of FVO-NLC nanoparticles was investigated by SEM technique and the obtained results illustrated that FVO-NLC nanoparticles had spherical shapes with small particle sizes ranging from 50 to 78 nm (Fig. 2). Slight differences in the sizes of FVO-NLC nanoparticles obtained by DLS and SEM techniques could be attributed to the aggregation of nanoparticles in solution[29].

Components	Retention index	Retention time (min)	Composition (%)
α-Pinene	926	5.12	0.73
Sabinene	965	5.90	0.22
β-Pinene	970	5.98	0.64
β-Myrcene	982	6.22	0.21
L-phellandrene	997	6.54	0.27
O-cymene	1019	6.99	1.41
Limonene	1022	7.09	10.74
β-Ocimene	1028	7.23	0.54
γ-Terpinene	1051	7.74	0.92
Fenchone	1083	8.45	5.54
trans-Limonene-Oxide	1133	9.58	0.12
Camphor	1140	9.76	0.14
Estragole	1192	11.00	2.70
Dihydrocarvone	1201	11.15	0.36
Fenchyl acetate	1228	11.79	2.55
Cuminaldehyde	1237	11.98	1.06
Carvone	1240	12.06	3.26
trans-Anethole	1285	13.14	66.22
α-Copaene	1335	14.19	0.12
Germacrene D	1479	17.24	0.11
Total	-	-	97.86
%Yield (mL/100 g dry seeds)	-	-	2.91

Table 1. Eseential oil chemical composition of *Foeniculum vulgare* seeds.

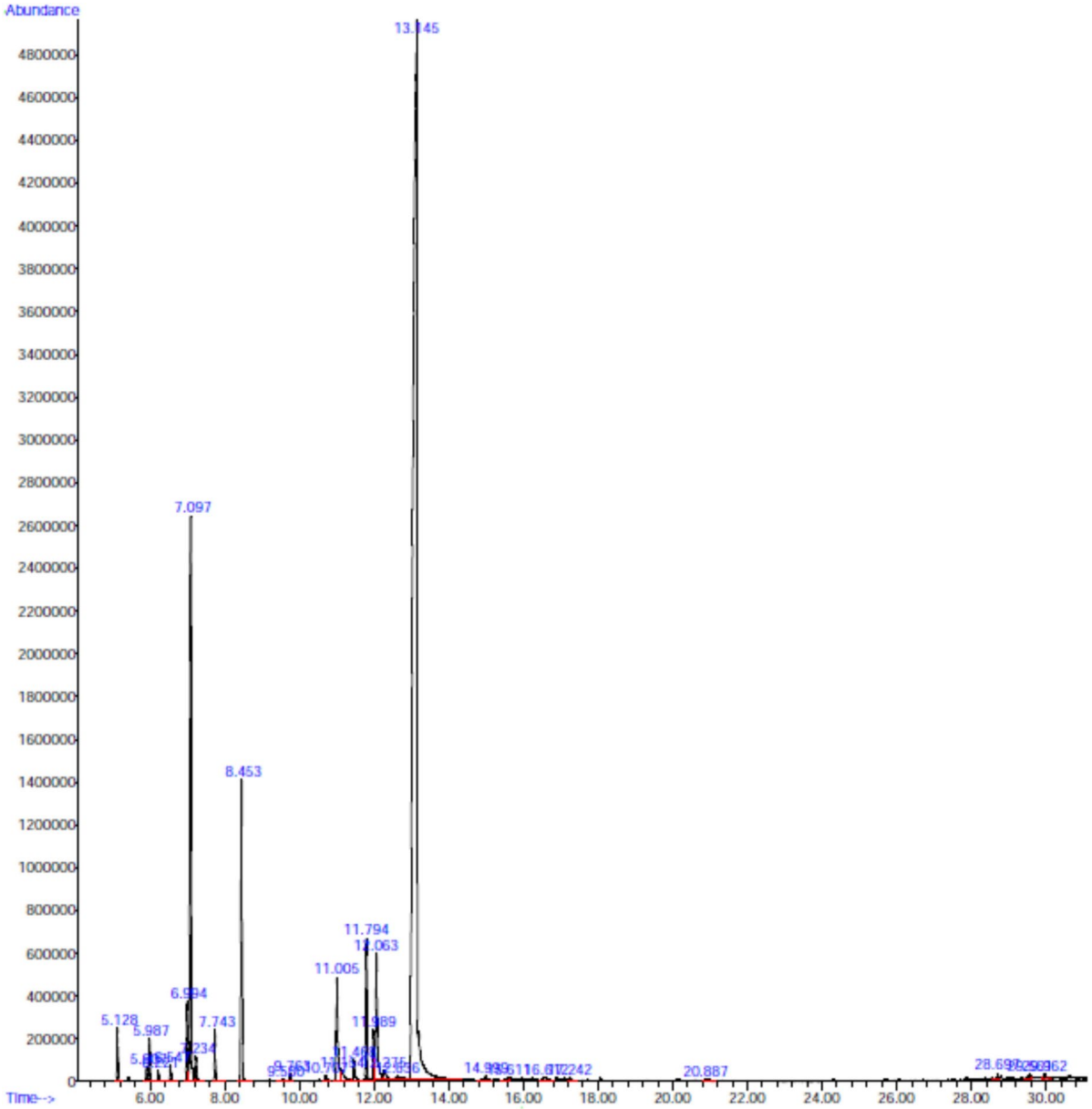

Fig. 1. GC–MS chromatogram of *Foeniculum vulgare* seeds essential oil with identified peaks listed in Table 1.

Time	Size (nm) ± SE	PDI ± SE	Zeta potential (mV) ± SE	Encapsulation efficacy (%) ± SE
24 h	86.32 ± 0.33	0.222 ± 0.0006	−15.35 ± 0.33	98.57 ± 0.45

Table 2. Characterization of *Foeniculum vulgare* essential oil-loaded nanostructured lipid carrier (FVO-NLC), PDI: Polydispersity index; SE: standard error.

FTIR spectra of blank NLC suggested that the absorption peak at 2919 cm^{-1} (C-H stretching, alkanes group), 1739 cm^{-1} (C = O stretching esterified carboxylic acid), and 1470 cm^{-1} (-CH_2 bending vibrations). The peak at 1113 indicates C-O band vibration. FTIR analysis of FVO-NLC ascertained no new significant chemical interaction between FVO and the NLC components, which means that FVO-loaded NLC is just physical mixtures (Fig. 2). As shown in Fig. 2. (d_{ii} and d_{iii}), the expanded form of FTIR spectra of FVO-NLC in the range 1600–1000 cm^{-1} and 1800–1600 cm^{-1} showed no discernible new peaks in comparison to FTIR spectra of blank NLC.

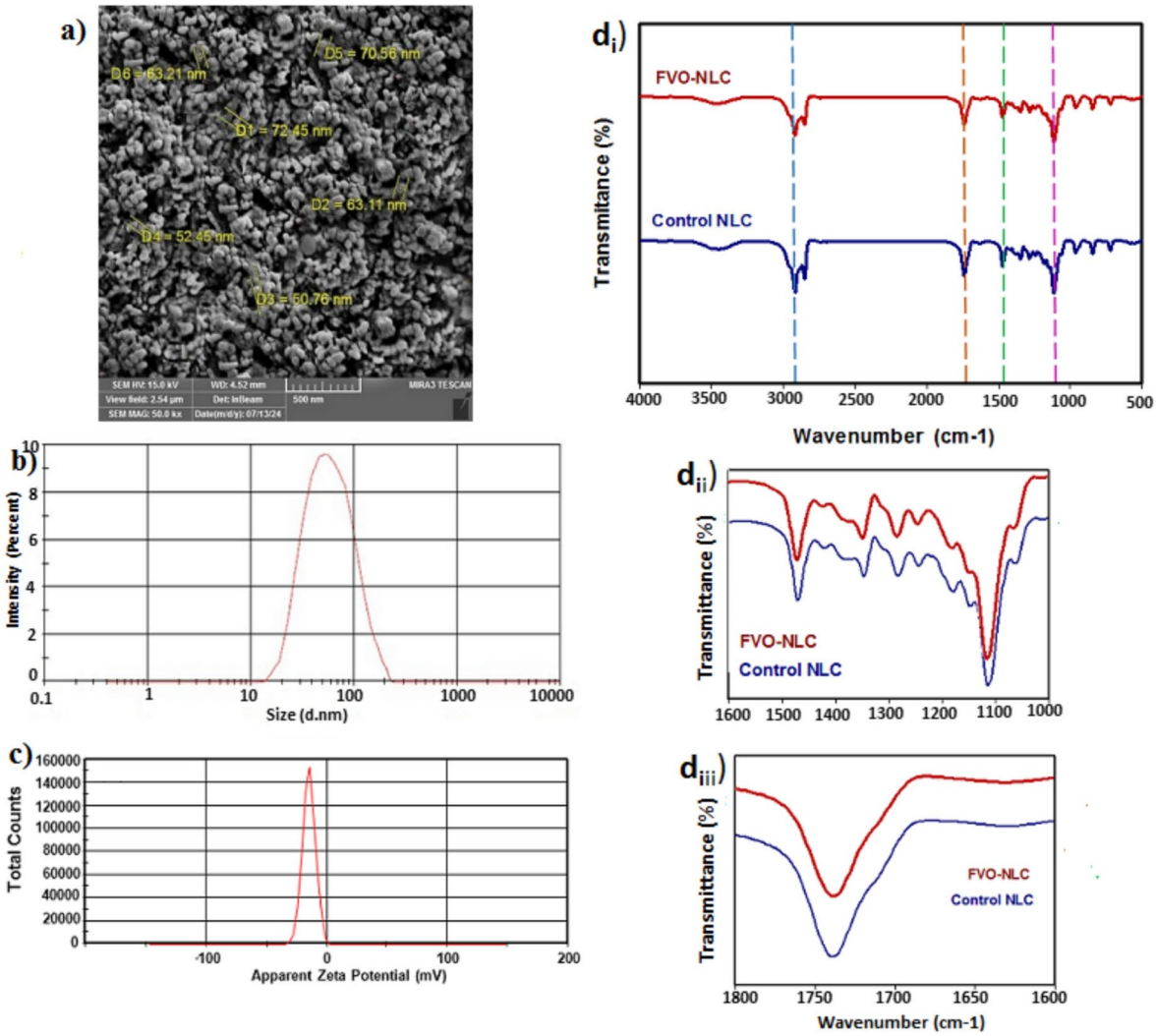

Fig. 2. Physical properties of *Foeniculum vulgare* essential oil-loaded nanostructured lipid carrier (FVO-NLC): (**a**) scanning electron microscope (SEM) micrograph, (**b**) particle size, (**c**) zeta potential distributions profile, ($\mathbf{d_i}$) FTIR spectra of FVO-NLC and nanostructured lipid carrier without FVO (Control NLC), ($\mathbf{d_{ii}}$) FTIR spectra of FVO-NLC and Control NLC in expanded form in the range 1600–1000 cm^{-1}, and ($\mathbf{d_{iii}}$) in the range 1800–1600.

Test material	LC_{50} (µL/mL) (95% CL)	LC_{95} (µL/mL) (95% CL)	χ^2(df)	Slope ± SE
FVO	5.44 (4.94–5.96)	17.29 (13.36–26.99)	0.26(3)	3.27 ± 0.46
FVO-NLC	5.57 (5.10–6.09)	16.51 (12.98–24.66)	0.74(3)	3.48 ± 0.47
TAL	3.93 (3.70–4.16)	8.11 (6.85–10.96)	0.36(3)	4.66 ± 0.77

Table 3. Contact toxicity of trans-anethole (TAL), *Foeniculum vulgare* pure essential oil (FVO), and FVO-loaded nanostructured lipid carrier (FVO-NLC) against second instar larvae of *Phthorimaea absoluta* 72 h after treatment, *LC* lethal concentration, *CL* confidence limits, χ^2 Chi-square value, *df* degree of freedom.

Contact insecticidal toxicity

In the current study, it was found that the major constituent of fennel EO was TAl. Therefore, to understand its contribution to the overall insecticidal activity of crude fennel EO and to compare its efficacy with EO nanoformulation, a comparative evaluation was performed among them against *P. absoluta*. The findings of these assays demonstrated that TAL, FVO, and FVO-NLC had contact toxicity against *P. absoluta* (Table 3). For *P. absoluta* second instar larvae, TAL had the highest contact toxicity with LC_{50} value of 3.93 µL/mL, while the

toxicity of FVO and FVO-NLC were not significantly different based on the overlapping of their 95% confidence intervals.

P. absoluta F1 lineage showed a significant increase in larval developmental periods in comparison with control. Moreover, adult life history parameters such as female and male adult longevity were significantly reduced in treated insects in comparison with control (Table 4). TAL, FVO, and FVO-NLC significantly decreased female fecundity by 50%, 38%, and 60%, respectively. The total oviposition period was decreased from 12.02 days in control to 6.08, 8.21, and 5.31 days for TAL, FVO, and FVO-NLC treatments, respectively.

Age-stage specific survival rate (S*xj*) demonstrates the probability that a newly born *P. absoluta* egg will survive to age *x* and develop to stage *j*. *P. absoluta* larval stages had the lowest rate (Fig. 3). The application of TAL, FVO, and FVO-NLC resulted in shortened life cycles of different biological stages, leading to reduced female longevity. For example, decreases were observed in the survival of the *P. absoluta* second instar larvae with FVO (73.26%), FVO-NLC (75.93%), and TAL (85.18%) when compared to the control (92.53%).When compared to treatments, adults of *P. absoluta* lived for a prolonged duration in the control group (Fig. 3).

In the control, FVO, TAL, and FVO-NLC treatments, the highest age-stage specific life expectancy (*Exj*) was perceived for the egg phase (37.31, 31.50, 33.47, and 30.40 days on day 1, respectively). These results showed that the life expectancy of the tomato leaf miner decreased with exposure to bioinsecticide treatments (Fig. 4).

The curve of age-specific survival rate (l_x) demonstrated that *P. absoluta* treated with FVO-NLC had a more pronounced survivorship decline in the initial stages of pest development in comparison with control, TAL, and FVO treatments. Age-stage specific fecundity (f_{xj}) parameter depicts key information about the average number of eggs per female for a given number of days at age x and stage j. Following the peak of this parameter curve, fecundity levels in *P. absoluta* population treated with TAL, FVO, and FVO-NLC were lower than that of the control. Indeed, the highest recorded f_{xj} peak in a control population was 17.71 eggs per female per day laid over 26 days, while in TAL, FVO, and FVO-NLC treatment groups the corresponding values were 12.78, 13.37, and 10.65 eggs per female per day laid over 28, 28, and 30 days, respectively (Fig. 5).

When a *P. absoluta* individual reaches a certain age x and physiological stage j, its contribution to the future population is predicted using the particular age-specific reproductive value (v_{xj}). As shown in Fig. 6, *P. absoluta* females in the control group were more fertile than those in FVO, TAL, and FVO-NLC treatment groups. In the control treatment, population growth phase spanned from the 19th to 39th day, when the highest values of *vxj* occurred between the 22nd and 27th day, with a reproductive peak on the 24th day. The peak of *vxj* was occurred much later in FVO-NLC treatment followed by FVO and TAL treatments, respectively (Fig. 6).

LC_{50} values of TAL, FVO, and FVO-NLC severely affected the population growth parameters of *P. absoluta* (Table 5). Our results showed that the control had a maximum (0.151 day^{-1}) intrinsic rate of increase (r) and finite growth rate (λ). The obtained net reproductive rate (R_0) and gross reproductive rate (GRR) demonstrated that TAL, FVO, and FVO-NLC treatments reduced *P. absoluta* female population. The median generation time (T) for control was significantly shorter than those of TAL, FVO, and FVO-NLC treatments (Table 5). This result suggests that the progeny from parents treated with FVO-NLC, FVO, and TAL, respectively, requires2.52, 1.19, and 0.94 days more than the progeny from the parents of the control to complete a generation.

Biological parameters	Control*	TAL	FVO	FVO-NLC
Egg (days)	4.05 ± 0.05^{a}	4.08 ± 0.03^{a}	4.04 ± 0.05^{a}	4.03 ± 0.04^{a}
L1 (days)	3.14 ± 0.04^{a}	3.07 ± 0.03^{a}	3.12 ± 0.04^{a}	3.12 ± 0.08^{a}
L2 (days)	2.12 ± 0.04^{a}	1.55 ± 0.05^{b}	1.66 ± 0.06^{b}	1.38 ± 0.06^{c}
L3 (days)	2.92 ± 0.04^{c}	3.82 ± 0.04^{b}	3.68 ± 0.05^{b}	4.21 ± 0.05^{a}
L4 (days)	3.09 ± 0.05^{c}	3.83 ± 0.06^{b}	3.72 ± 0.07^{b}	4.35 ± 0.07^{a}
Pupa (days)	7.94 ± 0.06^{a}	7.67 ± 0.06^{a}	7.90 ± 0.07^{a}	7.95 ± 0.09^{a}
Egg-pupa (days)	23.24 ± 0.13^{c}	24.02 ± 0.15^{b}	24.14 ± 0.15^{b}	25.11 ± 0.17^{a}
Female longevity (days)	17.52 ± 0.09^{a}	12.98 ± 0.02^{c}	13.72 ± 0.10^{b}	11.44 ± 0.09^{d}
Male longevity (days)	15.79 ± 0.08^{a}	11.00 ± 0.01^{c}	12.05 ± 0.17^{b}	10.21 ± 0.10^{d}
Oviposition period (days)	12.02 ± 0.11^{a}	6.08 ± 0.06^{c}	8.21 ± 0.10^{b}	5.31 ± 0.07^{d}
Fecundity (eggs/female)	140.12 ± 1.13^{a}	70.20 ± 0.34^{c}	86.63 ± 1.21^{b}	55.66 ± 0.67^{d}
Egg-adult (days)	40.07 ± 0.19^{a}	36.73 ± 0.17^{c}	37.29 ± 0.19^{b}	36.15 ± 0.21^{d}
TPOP (days)	24.71 ± 0.16^{c}	26.88 ± 0.18^{b}	26.86 ± 0.20^{b}	28.98 ± 0.22^{a}
APOP (days)	1.45 ± 0.08^{d}	3.02 ± 0.02^{b}	2.70 ± 0.09^{c}	3.76 ± 0.08^{a}

Table 4. Mean values (± SE) of developmental period of different stages and other biological parameters of *Phthorimaea absoluta* after treatment with LC_{50} concentration of trans-anethole (TAL), *Foeniculum vulgare* pure essential oil (FVO), and FVO-loaded nanostructured lipid carrier (FVO-NLC) in comparison with control treatment. *=There was no significant difference between NLC sample prepared without FVO loading and distilled water-ethanol mixture, as controls. The means followed by different letters in each row are significantly different (paired bootstrap at 5% significance level). Standard errors were measured by 100,000 bootstrap resampling. L1-L4: larval instar. TPOP: total preovipositional period. APOP: adult preovipositional period.

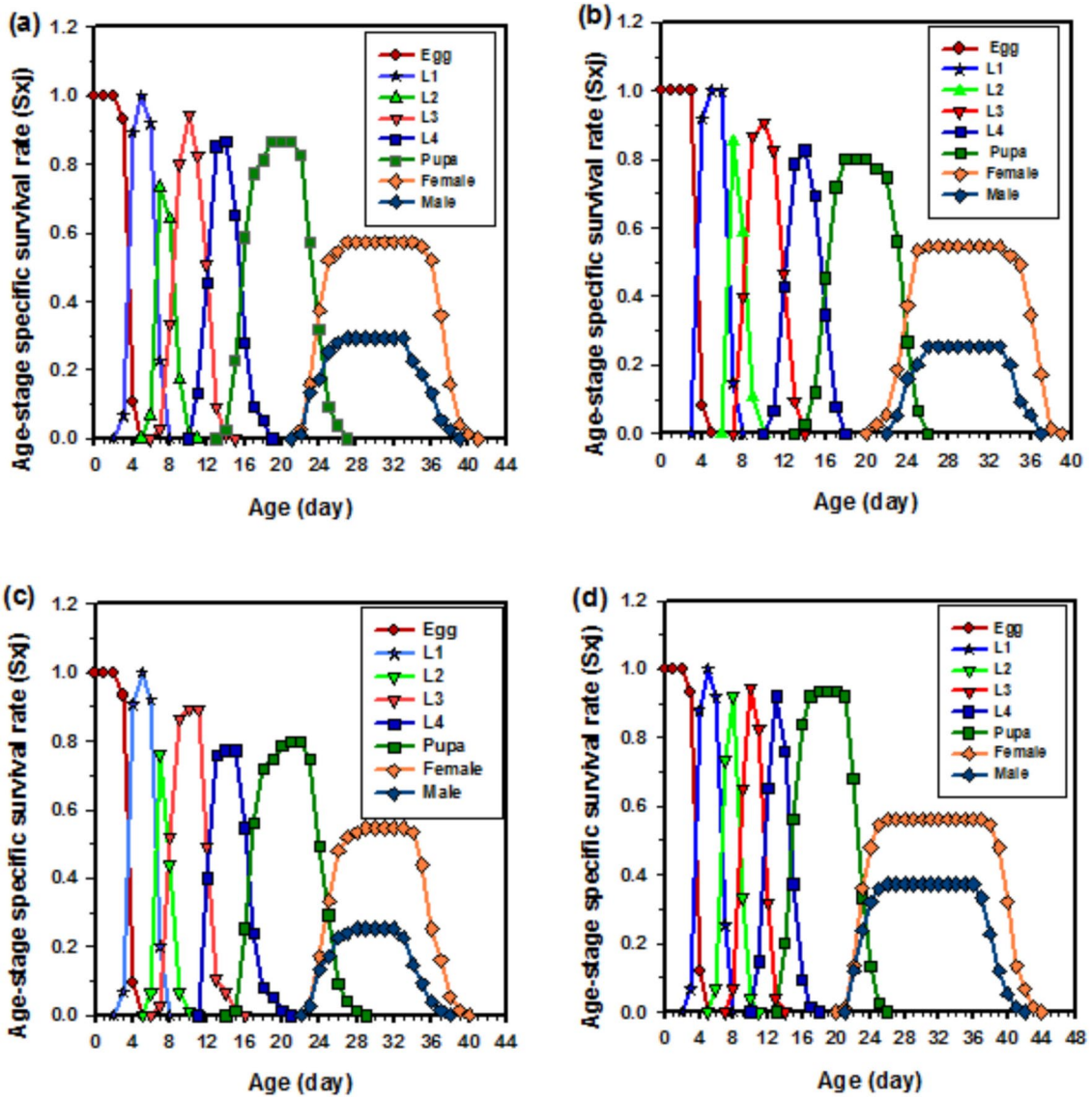

Fig. 3. Survival rate by age-specific stage (*Sxj*) of *Phthorimaea absoluta* in (**a**) *Foeniculum vulgare* pure essential oil (FVO), (**b**) trans-anethol (TAL), (**c**) FVO-loaded nanostructured lipid carrier (FVO-NLC), and (**d**) control (There was no significant difference between NLC sample prepared without FVO loading and distilled water–ethanol mixture, as controls).

Persistence assessment of contact toxicity

The persistence of EOs loaded NLCs contact toxicity has not been previously described. In this perspective, FVO, TAL, and FVO-NLC were assayed in this study for the first time for their persistence toxicity, and the results are summarized in Fig. 7. Contact lethality test of FVO-NLC exhibited the slow and persistent release of *F. vulgare* EO, proving the effectiveness of NLC in prolonging *P. absoluta* insecticidal effects. During experimental periods, the lethal effects of FVO-NLC were continuous over long periods, while the mortality percentage of TAL and pure FVO displayed a significant decrease, implying the improved efficacy of FVO in NLC.

Non-target effects assay

The non-target effects of EOs after encapsulation into NLCs are missing so far. In this context, we assessed the side effects of TAL, FVO, and FVO-NLC on *M. pygmaeus*. 72 h after the application of all selected bio-insecticides in the LC_{50} for *P. absoluta* the mortality rate of fourth instar nymphs of predator and fecundity of its treated females were evaluated. As can be observed in Table 6, when we combined the effects on mortality and reproduction, all three selected bio-insecticides were ranked as harmless according to IOBC categories.

Discussion

The chemical characterization of fennel EO revealed that the main components were trans-anethole (TAL) (66.22%), limonene (10.74%), fenchone (5.54%), carvone (3.26%), and estragole (2.70%), among its 97.86%

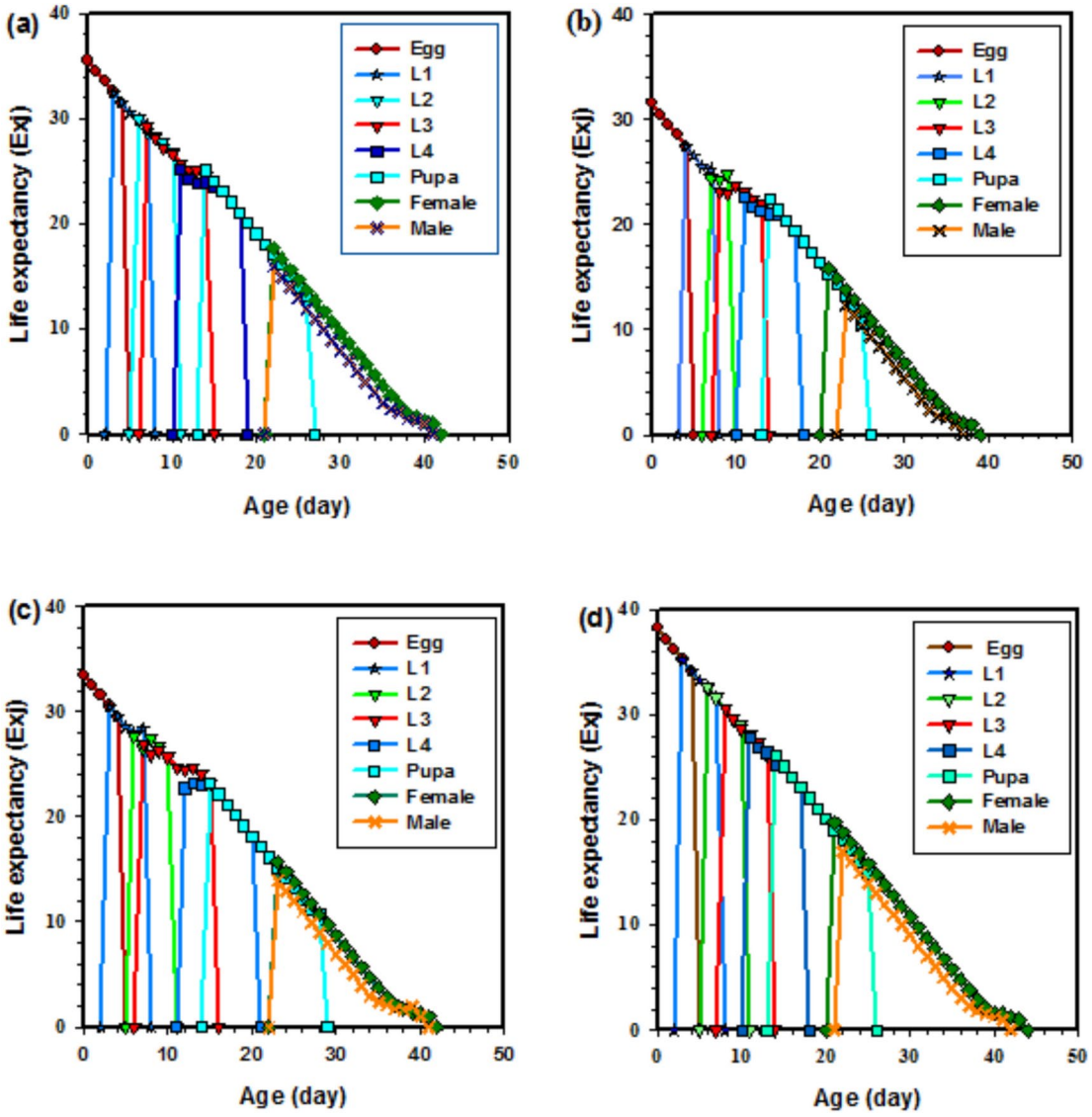

Fig. 4. Life expectancy by age-stage (*Exj*) of *Phthorimaea absoluta* in (**a**) *Foeniculum vulgare* pure essential oil (FVO), (**b**) trans-anethol (TAL), (**c**) FVO-loaded nanostructured lipid carrier (FVO-NLC), and (**d**) control (There was no significant difference between NLC sample prepared without FVO loading and distilled water–ethanol mixture, as controls).

identified compounds. These results agree with Ahmed et al.[37] who reported that the EO of Chinese fennel seeds had the same compounds with different content levels. The high TAL reported in the present study matches well with Durović et al.[38] who found that TAL was the main component in two different geographical fennel seed EOs. The yield of EO in the current study (2.91%) was nearly similar to previous results reported by Pouryousef[39] who found that the EO yield from Iranian fennel seed populations ranged from 2.7–4%. Nevertheless, differences in yield, components and their relative concentrations in EO of fennel seeds were recorded[13,40] that considerably depending on the genotype, phonological state of fennel, the method of EO extraction, geographical origin, and climate condition[41].

Consistent with our results, previous studies reported that NLCs with zeta potential values of lower than −30 mV or greater than 30 mV and PDI value range from 0.1–0.25 are strongly stable and free from aggregation[24,42]. Additionally, the small particle size for FVO-NLC may be associated with the presence of non-ionic surfactant (Poloxamer) on the surface of nanoparticles. Pezeshki et al.[43] revealed that NLCs stabilized with an optimal concentration of Poloxamer (6% w/v) had smaller particle sizes. This agrees with the results obtained by Galvão et al.[44] who reported that the lowest particle sizes were found for Carvacrol-loaded NLCs with the highest surfactant (5%) concentration. Additionally, Múnera-Echeverri et al.[45] suggested that in preparation for EO-NLCs, maintaining a Mygliol: EO ratio of 50:50 or higher achieved smaller particle sizes, which is what happened in the current study. FTIR analysis revealed no significant chemical interaction between EO and NLC components. This was in accordance with some previous findings such as cinnamon essential oil loaded NLC system[46].

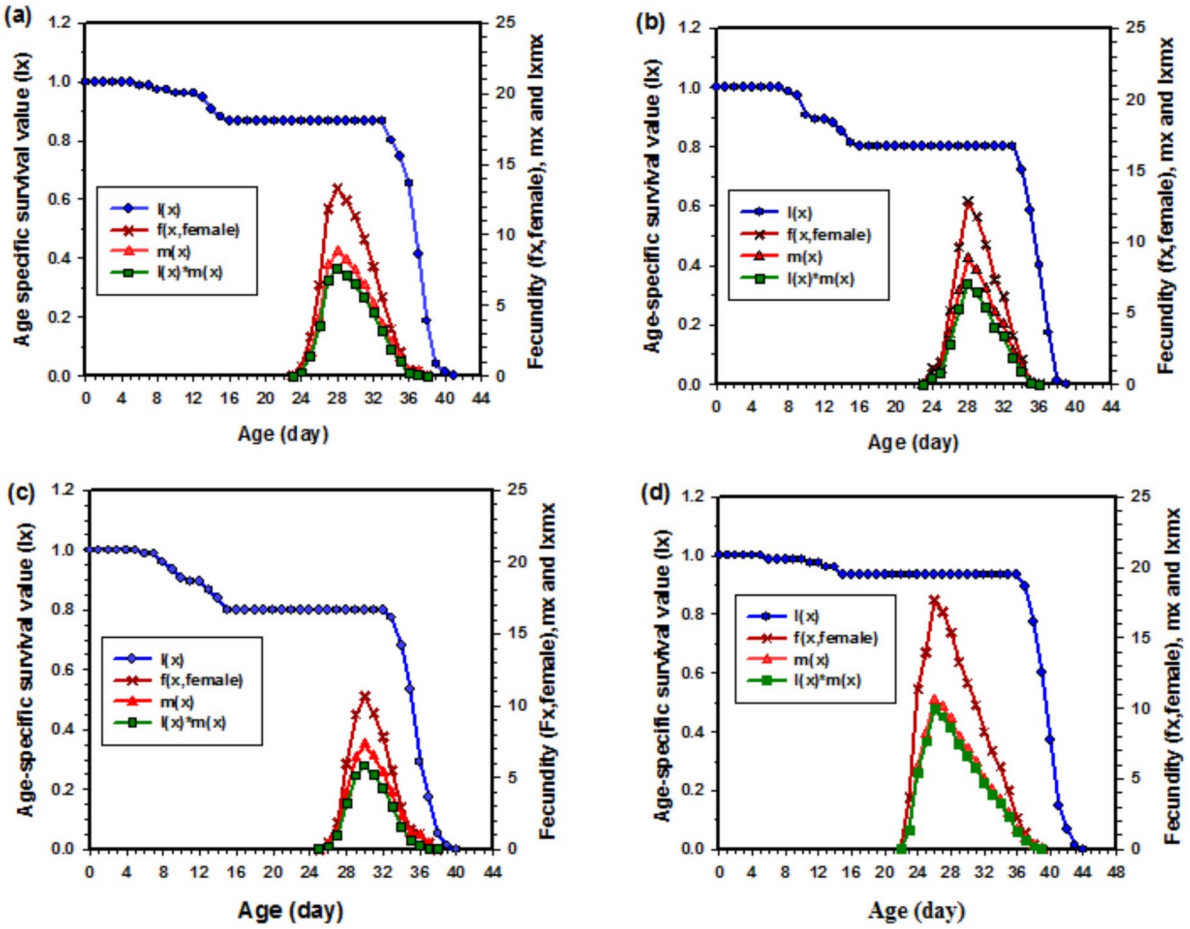

Fig. 5. Survival rate by specific age-stage (lx), fecundity by specific age-stage (Fx, female), fecundity by specific age (mx), and maternity by specific age (lxmx) for *Phthorimaea absoluta* in (**a**) *Foeniculum vulgare* pure essential oil (FVO), (**b**) trans-anethole (TAL), (**c**) FVO-loaded nanostructured lipid carrier (FVO-NLC), and (**d**) control (There was no significant difference between NLC sample prepared without FVO loading and distilled water–ethanol mixture, as controls).

In this study the insecticidal activity of pure fennel EO, its main component, and NLC formulation were evaluated for the first time against *P. absoluta*. The insecticidal activity of fennel EO for other insect pests of the orders Homoptera[12,47], Lepidoptera[48], and Coleoptera[49] has already been verified.

The potential of plant EOs as bio-insecticides depends on several EO components, especially phenylpropanoids and monoterpenoids[50]. As a main phenylpropanoid in fennel EO studied herein, TAL is identified as one of the most toxic compounds against some important pest insects such as *Ephestia kuehniella* Zeller[50], *Spodoptera frugiperda* Smith[51] and *Hyphantria cunea* Drury[52]. Shahriari et al.[50] demonstrated that this compound is a neurotoxic insecticide, and acts as an acetylcholine esterase inhibitor. In current study, TAL showed lower LC_{50} in contact assay on tested pest, compared to the pure EO (FVO). This was in agreement with Zhao et al.[53] and Abdel-Baki et al.[54] who found that TAL exhibited strong toxicity against *Liposcelis bostrychopila* Badonnel. and *Musca domestica* L. in comparison to crude *F. vulgare* EO. Our findings suggest that the insecticidal activity of fennel oil was mainly attributed to the presence of TAL. Another probable reason that might make these results is the existence of antagonistic effects of some components present in pure fennel EO at lower or even trace concentrations.

Obtained results revealed that NLC sample prepared without FVO loading did not affect larval mortality against *P. absoluta*. Similar results were obtained by Lucia et al.[55] who evaluated the toxicity of EOs encapsulated in the hydrophobic core of Poloxamer and the base formulation containing only the Polaxamer against *Pediculus humanus* L.

In the current study, contact toxicities of FVO and FVO-NLC against *P. absoluta* were not significantly different. The low mortality of *P. absoluta* might be explained by its larvae feeding strategy. Larvae inside their mines feed on mesophyll tissues of leaves and were less susceptible to the selected bio-insecticides.

In the case of insecticidal toxicity of EOs loaded NLCs, Radwan et al.[56] showed that the *F. vulgare* EO encapsulation in NLC had a higher effect (97.4%) on *Culex pipienis* L. larval reduction than pure EO (89.8%). In another study, NLCs loaded with Rosemary (156 nm), Lavander (198 nm), and Peppermint (179 nm) did not cause a significant effect on the survival of *P. absoluta* second instar larvae[57]. In our study, FVO-NLC formulation

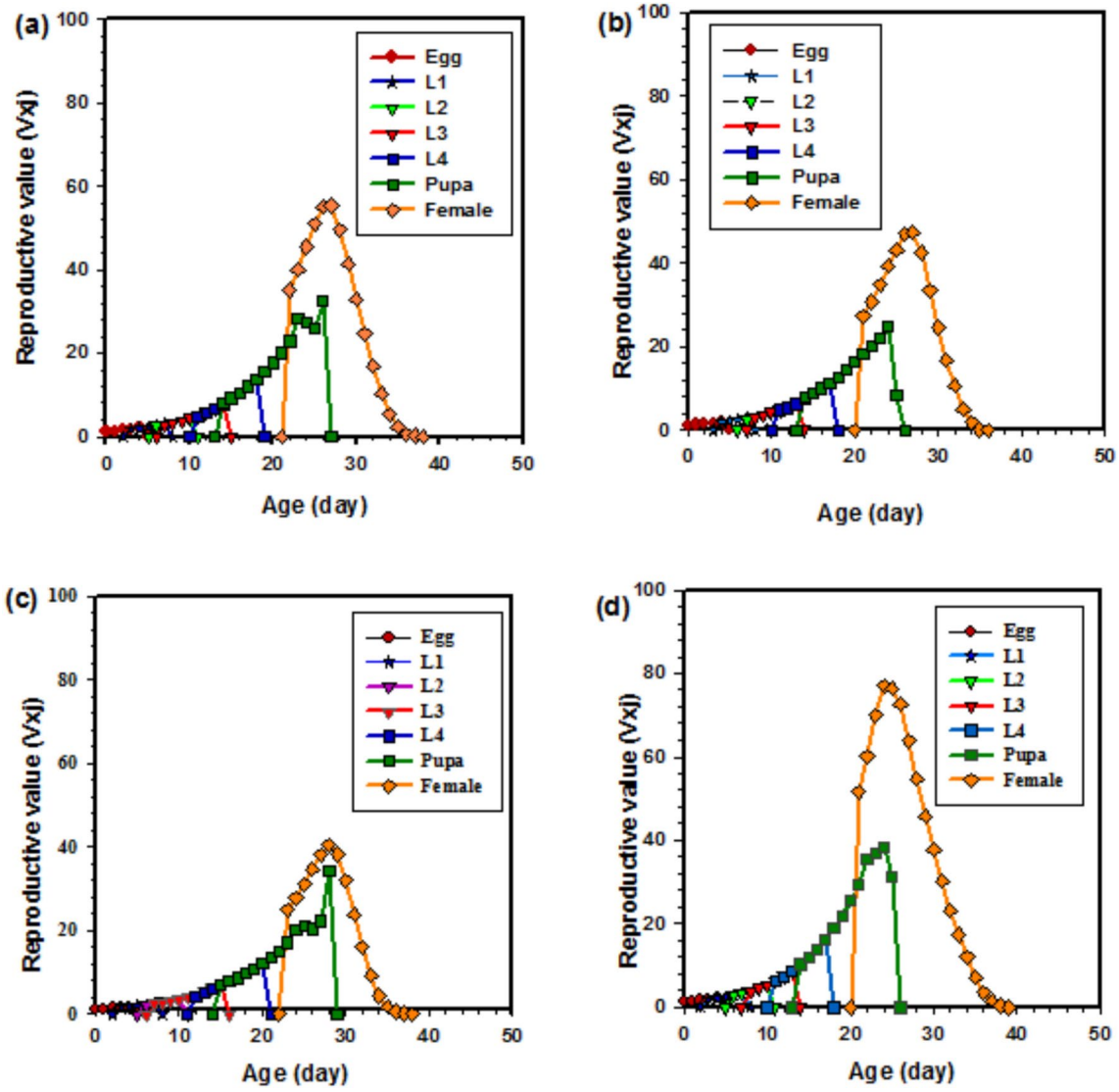

Fig. 6. Reproductive value by age-stage (vxj) of *Phthorimaea absoluta* in (**a**) *Foeniculum vulgare* pure essential oil (FVO), (**b**) trans-anethole (TAL), (**c**) FVO-loaded nanostructured lipid carrier (FVO-NLC), and (**d**) control (There was no significant difference between NLC sample prepared without FVO loading and distilled water–ethanol mixture, as controls).

Population growth parameters	Control*	FVO	FVO-NLC	TAL
Intrinsic rate of increase (r) (day^{-1})	0.151 ± 0.004^{a}	0.129 ± 0.003^{b}	0.109 ± 0.004^{c}	0.122 ± 0.003^{b}
Net reproductive rate (R_0) (offspring/individual)	78.467 ± 8.054^{a}	49.667 ± 4.981^{b}	30.427 ± 3.229^{d}	38.373 ± 4.0459^{c}
Finite rate of population increase (λ) (day^{-1})	1.163 ± 0.004^{a}	1.139 ± 0.004^{b}	1.115 ± 0.003^{c}	1.130 ± 0.004^{b}
Growth reproductive rate (GRR) (offspring)	84.150 ± 8.228^{a}	57.91 ± 5.099^{b}	39.860 ± 3.442^{d}	48.130 ± 4.236^{c}
Mean generation time (T) (day)	28.887 ± 0.167^{c}	30.079 ± 0.192^{b}	31.411 ± 0.222^{a}	29.830 ± 0.1999^{b}

Table 5. Population growth parameters (Means ± SE) of the first generation produced by *Phthorimaea absoluta* treated with LC_{50} concentration of trans-anethole (TAL), *Foeniculum vulgare* pure essential oil (FVO), and FVO-loaded nanostructured lipid carrier (FVO-NLC) in comparison with control. Means within a row followed by different letters are significantly different (paired bootstrap at 5% significance level). * = There was no significant difference between NLC sample prepared without FVO loading and distilled water–ethanol mixture, as controls.

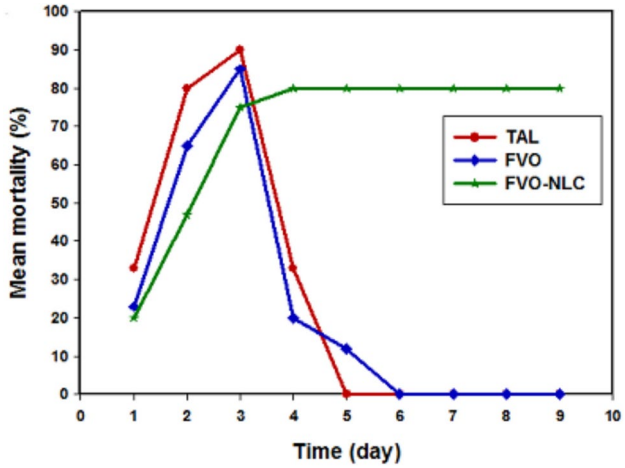

Fig. 7. The persistence of contact toxicity of trans-anethole (TAL), *Foeniculum vulgare* pure essential oil (FVO), and FVO-loaded nanostructured lipid carrier (FVO-NLC) against *Phthorimaea absoluta* second instar larvae at the highest concentration (LC_{95}) for different periods.

Treatment	Mortality (%)	Fecundity (Mean ± SE)	E (%)	IOBC category
FVO	$26.67^{b} \pm 1.11$	$39.36^{c} \pm 0.40$	19.61^{a}	harmless
FVO-NLC	$18.33^{c} \pm 1.70$	$40.67^{b} \pm 0.67$	13.93^{c}	harmless
TAL	$35.00^{a} \pm 0.88$	$27.01^{d} \pm 0.65$	17.85^{b}	harmless
Control*	$3.34^{d} \pm 1.60$	$53.34^{a} \pm 1.02$	-	-

Table 6. Percentage of toxicity (E) for fourth instar nymphs of *Macrolophus pygmaeus* resulting from the application of trans-anethole (TAL), *Foeniculum vulgare* pure essential oil (FVO), and FVO-loaded nanostructured lipid carrier (FVO-NLC) at the LC_{50} estimated for the target pest, *Phthorimaea absoluta*. * = There was no significant difference between NLC sample prepared without FVO loading and distilled water–ethanol mixture, as controls. Mean followed by same letters within each column are not significant (One way ANOVA, Tukey's test, $P < 0.05$).

with a smaller nanoparticle size (86 nm) was able relatively solve this problem. According to Nguyen et al.[58], small sizes of nanocarriers (nanoemulsion (NE), NLC, and SLN) and the physical state of their lipid matrices significantly affected their penetration into plants. Their results revealed that among three nano-formulations with similar characteristics (particle size, PDI, and zeta potential), NE had the fastest penetration, followed by NLC and SLN. Furthermore, EOs, chemical composition is highly important in their insecticidal activity. The high TAL content in fennel EO likely contributed to its enhanced toxicity.

The obtained results from the sublethal effect study corroborated those of de Figueiredo et al.[27] and de Paiva Silva et al.[59] who perceived that EOs of Lauracea family and citrus genus at LD_{50} concentration, negatively affected the life cycle and demographic parameters of *P. absoluta*, in addition to having adverse effects on the fecundity of this pest. Decreased female fecundity might be affected by poor nutrition before adult emergence and anti-feedant activity of EOs[60,61].

Although bio-insecticides are generally considered to be safer and more environmentally friendly than conventional insecticides, a number of their detrimental side effects have been reported on natural enemies as biological control agents[16]. In this study, TAL, pure FVO, and FVO-NLC did not reveal any significant side effects toward the non-target predator in laboratory settings according to IOBC guidelines. Still, this aspect should be carefully evaluated under semi-field and field conditions in future research related to this topic and all information on compatibility between biological pest control agents and bio-insecticide use should be declared.

Although there are several limitations to the use of the IOBC approach for assessing insecticide side effects compared to demographic toxicology usage, the use of this method was considered admirable by scientists working in biological control[28]. Regarding IOBC categories, our results were in partial accordance with those of Campolo et al.[62] who found that pure and nanoformulations of citrus EO had negligible impacts on the survival and progeny production of *Nesidiocoris tenuis* Reuter and classified them as harmless. In contrast, our findings were not consistent with the conclusions of de Figueiredo et al.[27] who classified Cinnamomum spp. EOs as harmful to the predator *Macrolophus basicornis* Stal according to their mortality rates. Moreover, the differences in EOs toxicity against non-target organisms might be related to different exposure routes, i.e., the leaf dip method in the present study, while in the latter research topical contact was the exposure route.

Conclusions

This research provided applicable information for developing newer and safer *P. absoluta* control tools, highlighting non-target effects against generalist insect predator. The following conclusions were drawn from this study:

(1) Nanoformulation of fennel EO (FVO-NLC) had similar toxicity with FVO against *P. absoluta*, however, the results of the persistence assessment toxicity indicated that after loading FVO in NLC, this nanocarrier had significant effectiveness in prolonging the insecticidal activity of crude fennel EO. FVO-NLC showed 80% lethality within 9 days, while FVO and TAL indicated lethality less than 30% after 5 days.
(2) The results of sublethal assays indicated that FVO-NLC had a more negative impact on female fecundity compared to other treatments.
(3) In the non-target effect assay, the lower value of Ex obtained for FVO-NLC treatment could be attributed to the effect of nanostructured lipid carrier that improved stability and gradual release of the EO.
(4) Furthermore, the integration of biological and bio-insecticide approaches in *P. absoluta* management strategies should be investigated in field conditions. Further studies on the oviposition deterrent and repellent activities of TAL, pure FVO, and FVO-NLC towards target and non-target organisms are also required.

Data availability

All data supporting this study's findings are included in the article.

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References

1. Koller, J. et al. A parasitoid wasp allied with an entomopathogenic virus to control Tuta absoluta. *Crop Prot.* **179**, 106617. https://doi.org/10.1016/j.cropro.2024.106617 (2024).
2. Bello, A. S. et al. Tomato (Solanum lycopersicum) yield response to drip irrigation and nitrogen application rates in open-field cultivation in arid environments. *Sci. Horti.* **334**, 113298. https://doi.org/10.1016/j.scienta.2024.113298 (2024).
3. Salazar, A. M. et al. Stability of the oil-based nanoemulsion of Laureliopsis philippiana (Looser) and its insecticidal activity against tomato borer (Tuta absoluta Meyrick). *Ind. Crops Prod.* **188**, 115635. https://doi.org/10.1016/j.indcrop.2022.115635 (2022).
4. Yao, J. et al. Sublethal and transgenerational effects of tetraniliprole on the tomato pinworm Phthorimaea (=Tuta) absoluta (Lepidoptera: Gelechiidae). *Crop Prot.* **187**, 106953. https://doi.org/10.1016/j.cropro.2024.106953 (2025).
5. Rostami, E., Madadi, H., Abbasipour, H., Fu, J. & Cuthbertson, A. G. S. Assessment of Tuta absoluta yield loss in Iranian tomato crops. *J. Asia-Pac. Entomol.* **24**, 1017–1023. https://doi.org/10.1016/j.aspen.2021.09.010 (2021).
6. Roditakis, E. et al. A four-year survey on insecticide resistance and likelihood of chemical control failure for tomato leaf miner Tuta absoluta in the European/Asian region. *J. Pest Sci.* **91**, 421–435. https://doi.org/10.1007/s10340-017-0900-x (2017).
7. De Backer, L. et al. Tuta absoluta-induced plant volatiles: attractiveness towards the generalist predator Macrolophus pygmaeus. *Arthropod-Plant Interact.* **9**, 465–476. https://doi.org/10.1007/s11829-015-9388-6 (2015).
8. Biondi, A., Guedes, R. N. C., Wan, F. H. & Desneux, N. Ecology, worldwide spread, and management of the invasive South American tomato pinworm, tuta absoluta: past, present, and future. *Annu. Rev. Entomol.* **63**, 239–258. https://doi.org/10.1146/annurev-ento-031616-034933 (2018).
9. Ahmad, M. F. et al. Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. *Heliyon* **10**, 29128. https://doi.org/10.1016/j.heliyon.2024.e29128 (2024).
10. Khursheed, A. et al. Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. *Microb. Pathog.* **173**, 105854. https://doi.org/10.1016/j.micpath.2022.105854 (2022).
11. Barathi, S., Sabapathi, N., Kandasamy, S. & Lee, J. Present status of insecticide impacts and eco-friendly approaches for remediation-a review. *Environ. Res.* **240**, 117432. https://doi.org/10.1016/j.envres.2023.117432 (2024).
12. Pavela, R. Essential oils from Foeniculum vulgare Miller as a safe environmental insecticide against the aphid Myzus persicae Sulzer. *Environ. Sci. Pollut. Res.* **25**, 10904–10910. https://doi.org/10.1007/s11356-018-1398-3 (2018).
13. Wanna, R. & Khaengkhan, P. Insecticidal Activity of Essential Oil from Seeds of *Foeniculum vulgare* (Apiales: Apiaceae) Against *Sitophilus zeamais* (Coleoptera: Curculionidae) and Its Effects on Crop Seed Germination. *J. Entomol. Sci.* **58**, 104–116. https://doi.org/10.18474/jes22-13 (2023).
14. Abd El-Kareem, M. S. M., Rabbih, M. A., Rashad, A. M. & EL-Hefny, M. Essential oils from fennel plants as valuable chemical products: gas chromatography–mass spectrometry, FTIR, quantum mechanical investigation, and antifungal activity. *Biomass Conv. Bioref.* https://doi.org/10.1007/s13399-024-05675-2 (2024).
15. Cruz, G. S. et al. Effect of trans-anethole, limonene and your combination in nutritional components and their reflection on reproductive parameters and testicular apoptosis in Spodoptera frugiperda (Lepidoptera: Noctuidae). *Chem.-Biol. Interact.* **263**, 74–80. https://doi.org/10.1016/j.cbi.2016.12.013 (2017).
16. Giunti, G. et al. Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates. *Biol. Control* **176**, 105071. https://doi.org/10.1016/j.biocontrol.2022.105071 (2022).
17. Sivalingam, S. et al. Encapsulation of essential oil to prepare environment friendly nano bio-fungicide against Fusarium oxysporum f.sp. lycopersici: An experimental and molecular dynamics approach. *Colloids Surf. A: Physicochem. Eng. Asp.* **681**, 132681. https://doi.org/10.1016/j.colsurfa.2023.132681 (2024).
18. Khezri, K., Farahpour, M. R. & Mounesi Rad, S. Efficacy of Mentha pulegium essential oil encapsulated into nanostructured lipid carriers as an in vitro antibacterial and infected wound healing agent. *Colloids Surf. A: Physicochem. Eng. Asp.* **589**, 124414. https://doi.org/10.1016/j.colsurfa.2020.124414 (2020).
19. Radwan, I. T. et al. Synthesis of eco-friendly lipid-magnetite nanocomposite encapsulated Poinciana extract as promising insecticide against *Culex pipiens*. *Sci. Rep.* **14**, 30456. https://doi.org/10.1038/s41598-024-81078-7 (2024).
20. Nobari Azar, F. A., Pezeshki, A., Ghanbarzadeh, B., Hamishehkar, H. & Mohammadi, M. Nanostructured lipid carriers: Promising delivery systems for encapsulation of food ingredients. *J. Agric. Food Res.* **2**, 100084. https://doi.org/10.1016/j.jafr.2020.100084 (2020).
21. Borges, I. et al. Prey consumption and conversion efficiency in females of two feral populations of Macrolophus pygmaeus, a biocontrol agent of Tuta absoluta. *Phytoparasitica* **52**, 31. https://doi.org/10.1007/s12600-024-01130-0 (2024).
22. Valizadeh, B., Samarfard, S., Sendi, J. J. & Karbanowicz, T. P. Developing an Ephestia kuehniella hemocyte cell line to assess the bio-insecticidal potential of microencapsulated Helicoverpa armigera nucleopolyhedrovirus against cotton bollworm (Lepidoptera: Noctuidae) Larva. *J. Econ. Entomol.* **113**, 2086–2095. https://doi.org/10.1093/jee/toaa148 (2020).

23. Sharifiyan, M., Mehrkhou, F. & Negahban, M. Sublethal effects of nanoformulated Mentha pulegium L. essential oil on the biological and population growth parameters of the greenhouse whitefly, Trialeurodes vaporariorum, (Hemiptera: Aleyrodidae). *Sci. Rep.* **14**, 27357. https://doi.org/10.1038/s41598-024-78249-x (2024).
24. Karimi, N., Ghanbarzadeh, B., Hamishehkar, H., Mehramuz, B. & Kafil, H. S. Antioxidant, antimicrobial and physicochemical properties of Turmeric extract-loaded Nanostructured Lipid Carrier (NLC). *Colloid Interface. Sci. Commun.* **22**, 18–24. https://doi.org/10.1016/j.colcom.2017.11.006 (2018).
25. Khosh Manzar, M., Pirouzifard, M. K., Hamishehkar, H. & Pirsa, S. Cocoa butter and cocoa butter substitute as a lipid carrier of Cuminum cyminum essential L. oil; physicochemical properties, physical stability and controlled release study. *J. Mol. Liq.* **319**, 114303. https://doi.org/10.1016/j.molliq.2020.113638 (2020).
26. Piri, A. et al. Toxicity and physiological effects of ajwain (Carum copticum, Apiaceae) essential oil and its major constituents against Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae). *Chemosphere* **256**, 127103. https://doi.org/10.1016/j.chemosphere.2020.127103 (2020).
27. de Figueiredo, K. G. et al. Toxicity of Cinnamomum spp. essential oil to *Tuta absoluta* and to predatory mirid. *J. Pest Sci.* **97**, 1569–1585. https://doi.org/10.1007/s10340-023-01719-0 (2023).
28. Arnó, J. & Gabarra, R. Side effects of selected insecticides on the Tuta absoluta (Lepidoptera: Gelechiidae) predators Macrolophus pygmaeus and Nesidiocoris tenuis (Hemiptera: Miridae). *J. Pest Sci.* **84**, 513–520. https://doi.org/10.1007/s10340-011-0384-z (2011).
29. Ahmadi, Z., Saber, M., Bagheri, M. & Mahdavinia, G. R. Achillea millefolium essential oil and chitosan nanocapsules with enhanced activity against Tetranychus urticae. *J. Pest Sci.* **91**, 837–848. https://doi.org/10.1007/s10340-017-0912-6 (2017).
30. Ibrahim, S. S. & Sammour, E. A. Evaluating the persistence and insecticidal effects of emulsifiable concentrate containing Cananga odorata essential oil on survival and enzymatic activity of Spodoptera littoralis. *Phytoparasitica* **52**, 85. https://doi.org/10.1007/s12600-024-01199-7 (2024).
31. Finney, D. J. *Probit Analysis*. 3th edition. Cambridge University Press. 333 p. (London, 1971).
32. Chi H. TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. Accessed on 25 May 2005. (2005).
33. Chi, H. S. I. N. & Liu, H. Two new methods for the study of insect population ecology. *Bull. Inst. Zool. Acad. Sin.* **24**(2), 225–240 (1985).
34. Chi, H. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environ. Entomol.* **17**, 26–34. https://doi.org/10.1093/ee/17.1.26 (1988).
35. Effron, B. & Tibshirani RJ. An introduction to the bootstrap. Chapman & Hall/CRC, Monographs on Statistics and Applied Probability, New York. (1993).
36. Angeli, G., Baldessari, M., Maines, R. & Duso, C. Side-effects of pesticides on the predatory bug Orius laevigatus (Heteroptera: Anthocoridae) in the laboratory. *Biocontrol Sci. Technol.* **15**, 745–754. https://doi.org/10.1080/09583150500136345 (2005).
37. Ahmed, A. F., Shi, M., Liu, C. & Kang, W. Comparative analysis of antioxidant activities of essential oils and extracts of fennel *(Foeniculum vulgare* Mill.) seeds from Egypt and China. *Food Sci. Hum. Wellness* **8**, 67–72. https://doi.org/10.1016/j.fshw.2019.03.004 (2019).
38. Đurović, S. D. et al. Fennel (Foeniculum vulgare Mill.) essential oil: The chemical profile, biological activity, and utilization of the commercial products. *Food Biosci.* **61**, 104901. https://doi.org/10.1016/j.fbio.2024.104901 (2024).
39. Pouryousef, M. Variation in the essential oil constituents in indigenous populations of *Foeniculum vulgare* var. *vulgare* from different locations of Iran. *J. Essent. Oil Res.* **26**, 441–445. https://doi.org/10.1080/10412905.2014.956188 (2014).
40. Moustafa, M. A. M. et al. Efficacy of chemical and bio-pesticides on cowpea aphid, Aphis craccivora, and their residues on the productivity of fennel plants *(Foeniculum vulgare). J. King Saud Univ. Sci.* **34**, 101900. https://doi.org/10.1016/j.jksus.2022.101900 (2022).
41. Pavela, R., Žabka, M., Bednář, J., Tříska, J. & Vrchotová, N. New knowledge for yield, composition and insecticidal activity of essential oils obtained from the aerial parts or seeds of fennel (*Foeniculum vulgare* Mill.). *Ind. Crops Prod.* **83**, 275–282. https://doi.org/10.1016/j.indcrop.2015.11.090 (2016).
42. Lacatusu, I. et al. Lipid nanocarriers based on natural compounds: An evolving role in plant extract delivery. *Eur. J. Lipid Sci. Technol.* **116**, 1708–1717. https://doi.org/10.1016/j.indcrop.2015.11.090 (2014).
43. Pezeshki, A., Ghanbarzadeh, B., Mohammadi, M., Fathollahi, I. & Hamishehkar, H. Encapsulation of vitamin A palmitate in nanostructured lipid carrier (NLC)-effect of surfactant concentration on the formulation properties. *Adv. Pharm. Bull.* **4**(2), 563. https://doi.org/10.5681/apb.2014.083 (2014).
44. Galvão, J. G. et al. Carvacrol loaded nanostructured lipid carriers as a promising parenteral formulation for leishmaniasis treatment. *Eur. J. Pharm. Sci.* **150**, 105335. https://doi.org/10.1016/j.ejps.2020.105335 (2020).
45. Múnera-Echeverri, A., Múnera-Echeverri, J. L. & Segura-Sánchez, F. Bio-Pesticidal potential of nanostructured lipid carriers loaded with thyme and rosemary essential oils against common ornamental flower pests. *Colloids Interfaces* **8**, 55. https://doi.org/10.3390/colloids8050055 (2024).
46. Bashiri, S., Ghanbarzadeh, B., Ayaseh, A., Dehghannya, J. & Ehsani, A. Preparation and characterization of chitosan-coated nanostructured lipid carriers (CH-NLC) containing cinnamon essential oil for enriching milk and anti-oxidant activity. *LWT* **119**, 108836. https://doi.org/10.1016/j.lwt.2019.108836 (2020).
47. Benddine, H., Zaid, R., Babaali, D. & Daoudi-Hacini, S. Biological activity of essential oils of *Myrtus communis* (Myrtaceae, Family) and *Foeniculum vulgare* (Apiaceae, Family) on open fields conditions against corn aphids *Rhopalosiphum maidis* (Fitch, 1856) in western Algeria. *J. Saudi Soc. Agric. Sci.* **22**, 78–88. https://doi.org/10.1016/j.jssas.2022.07.001 (2023).
48. Cruz, G. S. et al. Sublethal Effects of Essential Oils From Eucalyptus staigeriana (Myrtales: Myrtaceae), Ocimum gratissimum (Lamiales: Laminaceae), and Foeniculum vulgare (Apiales: Apiaceae) on the Biology of Spodoptera frugiperda (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **109**, 660–666. https://doi.org/10.1093/jee/tow005 (2016).
49. Çiçek, S., et al. A study on insecticidal activity of the fennel (*Foeniculum vulgare*) essential oil and its nanoemulsion against stored product pests and molecular docking evaluation. *Ind. Crops Prod.* **222**, 119859. https://doi.org/10.1016/j.indcrop.2024.119859 (2024).
50. Shahriari, M., Zibaee, A., Sahebzadeh, N. & Shamakhi, L. Effects of α-pinene, trans-anethole, and thymol as the essential oil constituents on antioxidant system and acetylcholine esterase of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). *Pestic. Biochem. Physiol.* **150**, 40–47. https://doi.org/10.1016/j.pestbp.2018.06.015 (2018).
51. Dias, M. L. et al. Insecticidal activity of compounds of plant origin on Mahanarva spectabilis (Hemiptera: Cercopidae). *Insects* **10**, 360 (2019).
52. Pour, S. A. et al. Toxicity, antifeedant and physiological effects of trans-anethole against Hyphantria cunea Drury (Lep: Arctiidae). *Pestic. Biochem. Physiol.* **185**, 105135. https://doi.org/10.1016/j.pestbp.2022.105135 (2022).
53. Zhao, N. N., Zhou, L., Liu, Z. L., Du, S. S. & Deng, Z. W. Evaluation of the toxicity of the essential oils of some common Chinese spices against Liposcelis bostrychophila. *Food Control* **26**, 486–490 (2012).
54. Abdel-Baki, A. S. et al. Larvicidal and pupicidal activities of Foeniculum vulgare essential oil, trans-anethole and fenchone against house fly *Musca* domestica and their inhibitory effect on acetylcholinestrase. *Entomol. Res.* **51**, 568–577. https://doi.org/10.1111/1748-5967.12550 (2021).
55. Lucia, A., Toloza, A. C., Guzmán, E., Ortega, F. & Rubio, R. G. Novel polymeric micelles for insect pest control: Encapsulation of essential oil monoterpenes inside a triblock copolymer shell for head lice control. *PeerJ* **5**, 3171. https://doi.org/10.7717/peerj.3171 (2017).

56. Radwan, I. T., Baz, M. M., Khater, H. & Selim, A. M. Nanostructured Lipid Carriers (NLC) for Biologically Active Green Tea and Fennel Natural Oils Delivery: Larvicidal and Adulticidal Activities against Culex pipiens. *Molecules* **27**, 1939. https://doi.org/10.3390/molecules27061939 (2022).
57. Tortorici, S. et al. Nanostructured lipid carriers of essential oils as potential tools for the sustainable control of insect pests. *Ind. Crops Prod.* **181**, 114766. https://doi.org/10.1016/j.indcrop.2022.114766 (2022).
58. Nguyen, M. H., Nguyen, T. H. N., Hwang, I. C., Bui, C. B. & Park, H. J. Effects of the physical state of nanocarriers on their penetration into the root and upward transportation to the stem of soybean plants using confocal laser scanning microscopy. *Crop Prot.* **87**, 25–30. https://doi.org/10.1016/j.cropro.2016.04.012 (2016).
59. de Paiva Silva, G. T. et al. Survival and demography of the tomato borer (Tuta absoluta) exposed to citrus essential oils and major compounds. *Agriculture* **13**, 538. https://doi.org/10.3390/agriculture13030538 (2023).
60. Goane, L. et al. Antibiotic treatment reduces fecundity and nutrient content in females of Anastrepha fraterculus (Diptera: Tephritidae) in a diet dependent way. *J. Insect Physiol.* **139**, 104396. https://doi.org/10.1016/j.jinsphys.2022.104396 (2022).
61. Ouabou, M. et al. Insecticidal, antifeedant, and repellent effect of Lavandula mairei var. antiatlantica essential oil and its major component carvacrol against Sitophilus oryzae. *J. Stored Prod. Res.* **107**, 102338. https://doi.org/10.1016/j.jspr.2024.102338 (2024).
62. Campolo, O. et al. Side effects of two citrus essential oil formulations on a generalist insect predator, plant and soil enzymatic activities. *Chemosphere* **257**, 127252. https://doi.org/10.1016/j.chemosphere.2020.127252 (2020).

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Declaration

Competing interests

The authors declare that they have no conflict of interest.

Additional information

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