Aphicidal and biochemical effects of emulsifiable concentrate and nanoemulsion of two selected essential oils against black bean aphid, *Aphis fabae* (Scop.)

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Background and objectives
Aphids are cosmopolitan pests that feed on a wide range of host plants from different botanical families. Aphids have developed resistance to several groups of synthetic insecticides. Because of their antimicrobial, antiviral, and insect-repellent properties, essential oils extracted from medicinal plants are excellent sources of various bioactive compounds. Formulation of essential oils as emulsifiable concentrate (EC) and nanoemulsion (NE) could help to enhance their bioavailability.
Materials and methods
The insecticidal activity of essential oils derived from two medicinal plants, namely, *Proserpinaca palustris* L. and *Terminalia chebula* Retz., was evaluated against black bean aphid, *Aphis fabae* (Scop.), under laboratory and semifield conditions. The essential oils from both plants were synthesized as EC and NE formulations to enhance their insecticidal efficacy. The stability of ECs and droplet size of NEs were assessed. The toxicity of ECs in comparison with NEs was evaluated against *A. fabae* adults. Moreover, the biochemical efficacy of the two essential oils on the activity of acetylcholinesterase and glutathione S-transferase enzymes of *A. fabae* was studied.
Results and conclusion
In laboratory bioassay, both ECs and NEs of selected oils displayed significant toxicity in controlling *A. fabae*, with lethal concentration values (LC_{50}) for *P. palustris* EC and NE being 0.59 and 0.50%, respectively. Moreover, LC_{50} for *T. chebula* EC and NE was 0.65 and 0.78%, respectively. The bulk essential oils showed less toxic activity against *A. fabae* adults, with LC_{50} of 0.68 and 1.16% for *P. palustris* and *T. chebula* bulk forms, respectively. Under semifield conditions, EC of *P. palustris* and *T. chebula* at LC_{90} and LC_{90}x3 exhibited greatly lethal effects for aphid adults compared with NE formulations. Both formulations (ECs and NEs) significantly increased the reduction percent of acetylcholinesterase and glutathione S-transferase enzymes of the treated aphid adults. Our results suggest that EC and NE formulations from *P. palustris* and *T. chebula* enhanced the insecticidal toxicity of the selected oils and could be used to effectively control *A. fabae* adults.

Keywords:
aphicidal activity, *Aphis fabae*, enzymatic activity, *Proserpinaca palustris*, *Terminalia chebula*

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Introduction

Black bean aphid, *Aphis fabae* (Scop.) (Homoptera: Aphididae), is a serious and cosmopolitan pest attacking and causing crop loss in many agronomical, horticultural, and medicinal plants [1]. In Egypt, 26 medicinal and aromatic plants are infested by more than nine aphid species [2]. This pest has a host-alternating life cycle [3] and is able to multiply at an immense rate, thus producing a dense population on their host plants under favorable environmental conditions. High densities of the pest on the secondary host plant lead to the production of specialist-winged virginoparae [4], which migrate to other summer host plants to start new colonies. Aphids ingest phloem sap from their hosts through narrow piercing-sucking stylets [5]. *A. fabae* is in addition one of the main vectors for different plant viruses [6]. Black bean aphids have several enzymatic proteins such as phenol oxidases, peroxidases, hydrolases, acetylcholinesterase (AChE), and glucosidases [7].

Aphid control is mainly achieved through the use of various classes of insecticides, often with multiple applications throughout the year [8]. However, other studies revealed no significant differences between

insecticide-treated and untreated plots [9]. The overuse of such nonselective synthetic pesticides has resulted in environmental pollution, pesticide residue in food, and destruction of many beneficials, including natural enemies. Moreover, several aphid species have developed resistance to several groups of insecticides [10]. These drawbacks have forced the development of eco-friendly alternative methods as a growing interest in aphid control. One of these alternatives could be the use of essential oils, which exhibited an insecticidal action against a broad range of insect pests including aphids [11]. Essential oils are among the most common plant products in agriculture, due to their antioxidant, antiviral, antifungal, antibacterial, anticancer, and insect-repellent properties [12,13]. Essential oils extracted from medicinal and aromatic plants can be used as good substances for pest control [7]. They strongly revealed efficiency, multiple mechanisms of action, more selectivity, and low toxicity on nontarget organisms [14]. However, poor physical stability, low water solubility, and rapid degradation in the environment are serious problems limiting the usage of essential oils as pesticides [15]. Therefore, formulation of essential oils as emulsifiable concentrate (EC) and nanoemulsion (NE) could help to enhance their physical stability, water solubility, and bioavailability [16,17]. Biological nanomaterials have played a significant role in the field of pest management [18].

Therefore, the present work aimed to examine the toxicity of two different formulations (ECs and NEs) of eco-friendly essential oils derived from two medicinal plants, *Proserpinaca palustris* L. (Family: Haloragaceae) and *Terminalia chebula* Retz. (Family: Combretaceae) against *A. fabae* adults. The LC_{25}, LC_{50}, and LC_{90} values of the tested formulations were estimated under laboratory conditions. The persistence of the prepared formulations (ECs and NEs) of both oils in a semifield experiment was evaluated. Moreover, the effect of ECs and NEs from *P. palustris* and *T. chebula* on AChE and glutathione S-transferase (GST) enzymes activity of *A. fabae* was studied.

Materials and methods

Insect rearing

For all laboratory assays, a stock culture of *A. fabae* was collected from naturally infested broad bean *Vicia faba* L. plant. The plant had received no chemical treatment during the growing phase. Aphids from the same source were similarly used to establish a colony on another batch of potted broad bean plants. The aphids were reared on broad bean plants grown in small plastic pots containing a mixture of soil, sand, and vermiculite substrate (1 : 2 : 1) at 15–20°C and light : dark 16 : 8 h photoperiod.

Extraction of essential oils

The aerial parts of two medicinal plants, *P. palustris* L. (Marsh mermaid-weed) (F: Haloragaceae) and *T. chebula* Retz. (Black myrobalan) (F: Combretaceae), were dried and pulverized. The essential oils were isolated after hydrodistillation for 4 h in a steam distillation unit using a Clevenger apparatus. Two types of formulations were naturally prepared as follows:

Emulsifiable concentrates

The EC formulation was prepared by mixing each of the tested oils with an appropriate amount of different emulsifiers and natural solvents (mineral and vegetable oils) according to Abdel-Aziz *et al.* [19].

Essential oil nanoemulsion

Ultrasonication method was used to formulate oil-in-water NEs of two essential oils: *P. palustris* and *T. chebula*. In this method, two phases were prepared: an organic phase and an aqueous phase. Both phases were combined to formulate the oil emulsions. *P. palustris* and *T. chebula* essential oils at 5.0% were used as the organic phase, whereas the aqueous phase consisted of Tween 80 as a nonionic surfactant and deionized water. The oil phase was added slowly to the aqueous phase at 25°C with magnetic stirring (500 rpm) for 20 min. Then, the resulted coarse emulsion was subjected to ultrasonication. The selected ratio of oil : Tween 80 was established at 1 : 1.5 (w/w), and the sonication time was 10 min using Ultrasonic (Sonics & Materials Inc., Newtown, CT, USA) with a probe diameter of 13 mm at a high frequency of 20 kHz and power output of 750 W. Ice was used to reduce the resulted heat, and the produced NEs were kept at 4°C for further analysis and bioassays.

The physical and chemical properties of the prepared formulations

Emulsifiable concentrate emulsion stability and foam test

The test was performed according to WHO specifications [20], with 75–80 ml of distilled water poured into a 250-ml beaker and brought to a temperature of 30±1°C. Then, the EC was added with continuous stirring with a glass rod of about 4 revolutions/s. While stirring, distilled water was added to the beaker to bring the total volume to 100 ml. The

contents were then poured into a clean, dry, graduated 100-ml cylinder. The stirring time was 3 min from the beginning of the addition of the EC until the emulsion was poured into the 100-ml cylinder. The cylinder was then kept at 30–31°C for 1 h and examined for creaming or phase separation. Furthermore, EC emulsion foam test was carried out to measure the amounts of foam formed on the emulsion surface in the cylinder after 5 min.

Droplet size of nanoemulsions

The average size of *P. palustris* and *T. chebula* essential oils NEs was determined by the dynamic light scattering technique using a Santa Barbara (CA, USA), instrument. Measurements were performed at 23°C, using the 632-nm line of a HeNe laser as the incident light with an angle of 90°.

Aphicidal potential of tested formulations

Toxicity study

Toxicity study was performed using different concentrations of *P. palustris* and *T. chebula* essential oils and their prepared formulations (ECs and NEs) using the leaf dipping procedure. After the preparation of the stock solution of EC and NE formulations (5.0%), dilution was done to obtain desired concentrations. For essential oil bulk form, EC, and NE formulations, each plant oil solution was prepared at five concentrations (0.5, 1.0, 1.25, 1.5, and 2.0%) to be used in this study using water (0.1% v/w Tween 80). Fresh leaves of broad bean plant were collected, and each leaf was dipped into the working solutions of essential oils for 10 s, and the leaves were then air-dried at room temperature. After the treatment, each leaf was placed individually on a water-saturated cotton wool pad to remain vigorously flourishing in covered glass Petri dishes (9 cm diameter) [21]. Five healthy aphid adults were isolated and transferred with a fine brush to the surface of treated leaves and then incubated at 25°C and 16 : 8 h light : dark. Untreated leaves were dipped into water (0.1% v/w Tween 80) and maintained as the control group. Inspection and recording of the dead and living individuals of *A. fabae* adults were carried out using microscopic examination after 24 and 48 h for each treatment. Insects were considered as dead when no movements appeared after touching them with a fine brush. The percentage mortality was recorded to calculate LC_{25}, LC_{50}, and LC_{90}. The corrected mortality percentage was calculated according to the Abbott's formula [22]:

$$\%\ \text{Corrected} = \left[1 - \left(\text{n in T after treatment} / \text{n in C after treatment}\right)\right] * 100.$$

Where: n=Insect number in the sample, T=treated, C=control.

Persistence and semifield bioassay

This study was carried out to evaluate the toxicity and persistence of the nano-oils in comparison with EC oil formulations in a semifield bioassay environment against *A. fabae* adults. Young potted broad bean plants were used, and each pot was covered with filter paper before the treatment to protect the soil from being subjected to the tested oils and to observe any fallen died individuals. Broad bean plant leaves were sprayed from all sides with selected lethal concentration (LC_{90}) values of 1.088 and 1.032% for *P. palustris* EC and NE, respectively, and 1.342 and 1.145% for *T. chebula* EC and NE, respectively, and LC_{90}x3, whereas control plants were sprayed with water (0.1% v/w Tween 80) only. Then, 10 newly apterous adults were released on each plant of treated and control groups. All treatments and controls were replicated five times. Insects were examined using the previously mentioned technique, and mortality was recorded after 24, 48, and 72 h of exposure to the tested formulations.

Enzymatic study

Aphids were sampled from untreated and treated groups with EC and NE of essential oils from *P. palustris* and *T. chebula* using LC_{25} (0.339 and 0.229 for *P. palustris* EC and NE, respectively, and 0.287 and 0.590% for *T. chebula* EC and NE, respectively). The leaf dipping method was used as previously mentioned, and after 48 h of treatment, pools of aphid adults (1 g) were homogenized in phosphate buffer (pH 7) using a Teflon tissue homogenizer surrounded by crushed ice. The homogenates were then centrifuged (8000 rpm) for 20 min at 4°C. The supernatant was used directly to determine the activity of AChE according to the method described by Simpson *et al.* [23] and GST as described by the method of Habig *et al.* [24]. Total protein was determined by the method of Bradford [25]. Three replicates were performed for each assay.

Statistical analysis

The LC_{25}, LC_{50}, and LC_{90} values were calculated by Ldp line software using the Probit analysis method of Finney [26]. The statistical difference between multiple groups was analyzed by one-way analysis of variance followed by Duncan's test as a post-hoc test. Means were statistically different at P value less than 0.05. The statistical analysis was performed using the SPSS, version 14.0 statistical software package (SPSS version 14.0; SPSS Inc., Chicago, Illinois, USA).

Results and discussion

The physical and chemical properties of synthesized formulations

As illustrated in Table 1, all tested formulations passed the stability and foam test, where the creaming layers appeared less than or equal to 2 ml for both essential oil EC formulations. To pass the emulsion stability test, creaming and/or oily layer should not exceed 3 ml. Furthermore, the tested formulations passed the foam formation test, and the foam layer should not be larger than 5 ml in order to pass the test. As shown in Table 1, the measured foam layers were 0.0 and 1.0 ml for *P. palustris* and *T. chebula* essential oils, respectively.

In this work, oil-in-water NEs of *P. palustris* and *T. chebula* essential oils at 5.0% concentration were formulated by the ultrasonication method. In Fig. 1, the mean droplet size diameters measured for *P. palustris* and *T. chebula* NEs were 211.9±0.619 and 108.7±0.658 nm, respectively.

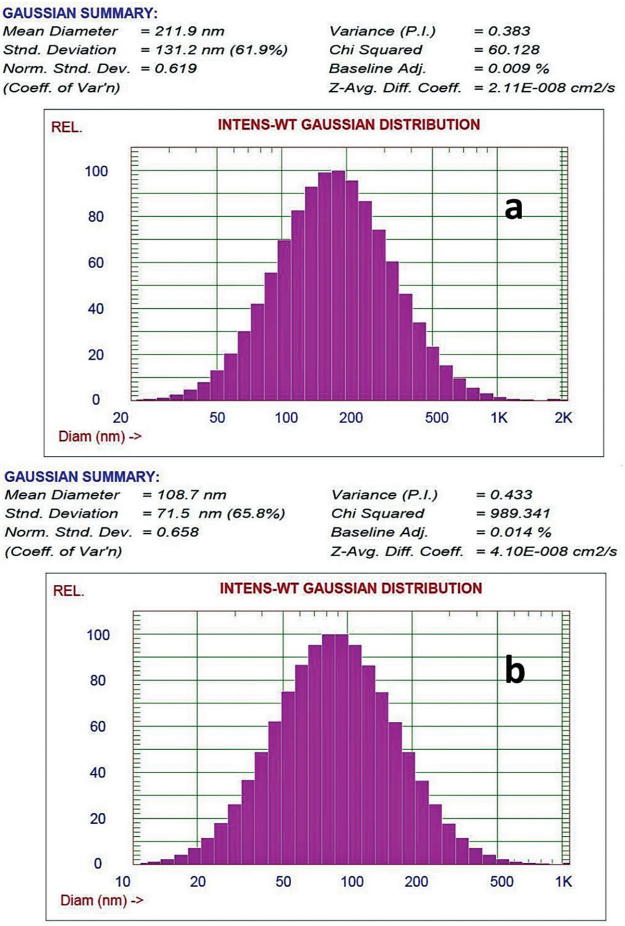
Essential oils have long been recommended as a viable alternative to synthetic chemical pesticides for insect pest control. They are effective, nontoxic to beneficial organisms, biodegradable, and have different mode of actions. Thus, the development of resistance in insect pests is rare [27]. Essential oils of different plants exhibit potent insecticidal activity against several insect orders [15,28]. However, the practical applications of these oils remain rather restricted because of their poor solubility in water and high volatility. These implications entailed an urgent search for more strategies enabling their use without any change of their chemical and biological properties. NEs are currently regarded as promising tools for exploiting the bioactivity of essential oils and their constituents against insects. NE formulations preserve the essential oils from degradation and enhance mobility, solubility, and their residue by reducing evaporation [18].

In this study, essential oils of *P. palustris* and *T. chebula* were synthesized in advanced formulations (ECs and NEs) to enhance their stability and insecticidal activities against *A. fabae*. The EC formulations passed the emulsion stability and foam formation tests, as demonstrated in this work. Our findings are consistent with those of Abdel-Aziz *et al.* [19], who prepared some compounds as essential oil formulations with creaming/oily layers of 1.3, 1.7, and 1.8 ml and foam layers of 1.4, 1.8, and 1.8 ml for their ECs rosacide, sagix, and cura, respectively. Likewise, oil-in-water NEs of both essential oils at 5.0% concentration were successfully formulated by the ultrasonication method using the ratio of oil : Tween 80 (w/w) of 1 : 1.5. Ultrasonication is the most extensively used process for the preparation of NEs; in this technique, intense and disruptive forces reduce the size of NE droplets [29]. These nanocarriers enable the concentration of bioactive compounds within controlled containers, improving the lipophilic molecules' stability and availability. As a result, it is possible to ensure a more controlled release of the active ingredients while minimizing losses during processing and storage [29,30]. A sufficient amount of suitable surfactant should be

Table 1 Emulsion stability and foam formation of the emulsifiable concentrate of essential oils

	Proserpinaca palustris	*Terminalia chebula*
Active ingredient (%)	5.0	5.0
Surfactant (%)	20.0	18.0
Solvents (%)	75.0	77.0
Emulsion stability (Separation ml)	2.0	1.7
Foam formation (ml)	0.0	1.0

Figure 1



used to prepare NEs that are resistant to crystallization, agglomeration, and sedimentation. Tween 80 was used as a surfactant in this study owing to its low toxicity, low irritation, and stability, as well as its small molecules, which can reduce the mean droplet size of NEs [30].

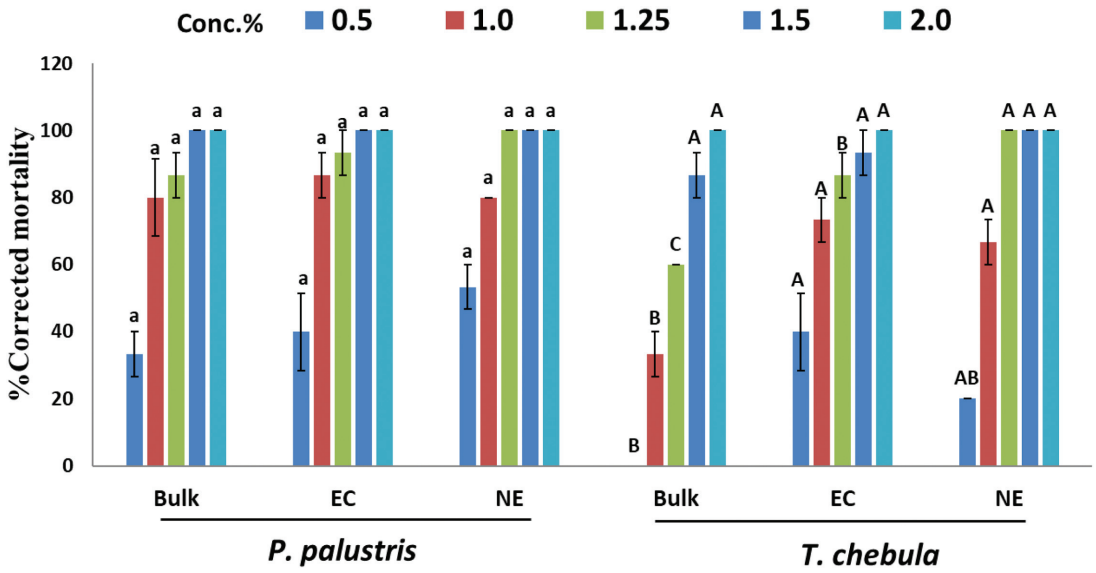
Aphicidal activity of emulsifiable concentrate and NE formulations under laboratory conditions

In this study, essential oils as well as their EC and NEs were evaluated for their insecticidal activities against *A. fabae* adults. Figure 2 shows that all tested formulations of *P. palustris* appeared almost the same in that they all had a high lethal influence on aphid adults at any of the five tested concentrations. The lowest corrected mortality (33.33±6.66) was obtained from *A. fabae* treatments with bulk *P. palustris* at the lowest concentration (0.5%), which increased to 40.00 ±11.54 and 53.33±6.66 after EC and NE treatments, respectively. Figure 2 clearly shows that the mortality of *A. fabae* adults increased by increasing the concentration of all tested formulations. The NE of *P. palustris* and *T. chebula* appeared almost similar to EC formulations in that it was extremely lethal to the treated aphid adults, with mortality rates reaching 100% when tested at the highest concentrations of 1.25, 1.5, and 2.0%. Treatments with *T. chebula* bulk form at the lowest concentration (0.5%) had no toxic effect and resulted in the lowest mortality (0.0) among the aphids tested, which was proportionally increased by increasing the concentration used to reach 100% at the highest concentration assayed.

Table 2 shows the toxicity results against *A. fabae* adults after 48 h of exposure to bean leaves treated with the selected essential oils. The results confirm that all tested essential oil formulations had significant toxicity with LC_{50} ranging from 0.50 to 1.16% after 48 h of treatment. The most toxic oil for the aphid adults was *P. palustris* NE, which had the lowest LC_{50} value (LC_{50}=0.506%), followed by the EC and bulk form formulations (LC_{50}=0.59 and 0.68%, respectively). Compared with bulk form and NE of *T. chebula*, the EC was the most potent with LC_{50}=0.65%. Furthermore, the bulk form of both tested oils was found to be less toxic than the EC and NE formulations. Considering LC_{90} values, NE formulations of both selected oils (*P. palustris* and *T. chebula*) had the highest lethal effects on *A. fabae* adults, with LC_{90}=1.03 and 1.14%, respectively, followed by EC formulations compared with the bulk form of the oils, which had the lowest toxicity against *A. fabae* adults.

Our bioassays revealed that the bulk and tested formulations (ECs and NEs) of both selected essential oils had a high lethal influence on aphid adults, most notably at concentrations of 2.0, 1.5, 1.25, or 1.0%. The mortality of *A. fabae* adults increased as the concentration increased. Importantly, we should refer to the phytochemical studies of Kumar *et al.* [31] and Rizvi *et al.* [32] with medicinal plants. Their research revealed that secondary metabolites from various plants, such as phenols, alkaloids, steroids, glycosides, and

Figure 2



Corrected mortality of *Aphis fabae* after 48 h, as affected by *Proserpinaca palustris* and *Terminalia chebula* essential oils at different formulations using different concentrations. Mean (±SE) values with different letters within the same concentration for each essential oil are significantly different ($P<0.05$) (analysis of variance) (Duncan test).

Table 2 Toxicity of *Proserpinaca palustris* and *Terminalia chebula* essential oil formulations against *Aphis fabae* adults after 48 h under laboratory conditions

Essential oil	Tested formulation	LC_{25} (95% confidence limits)	LC_{50} (95% confidence limits)	LC_{90} (95% confidence limits)	Slope ±SE	χ^2
Proserpinaca palustris	Bulk	0.407 (0.109–0.572)	0.683 (0.499–0.808)	1.208 (1.074–1.422)	2.443 ±0.13	111.798
	EC	0.339 (–0.008 to 0.516)	0.597 (0.382–0.732)	1.088 (0.951–1.314)	2.614 ±0.15	124.917
	NE	0.229 (–0.003 to 0.373)	0.506 (0.359–0.607)	1.032 (0.937–1.163)	2.438 ±0.15	47.534
Terminalia chebula	Bulk	0.968 (0.884–1.032)	1.162 (1.107–1.215)	1.531 (1.456–1.638)	3.475 ±0.18	46.222
	EC	0.287 (–0.188 to 0.519)	0.650 (0.372–0.814)	1.342 (1.173–1.637)	1.854 ±0.10	122.274
	NE	0.590 (0.480–0.670)	0.781 (0.705–0.849)	1.145 (1.065–1.253)	3.519 ±0.16	58.344

EC, emulsifiable concentrate; NE, nanoemulsion.

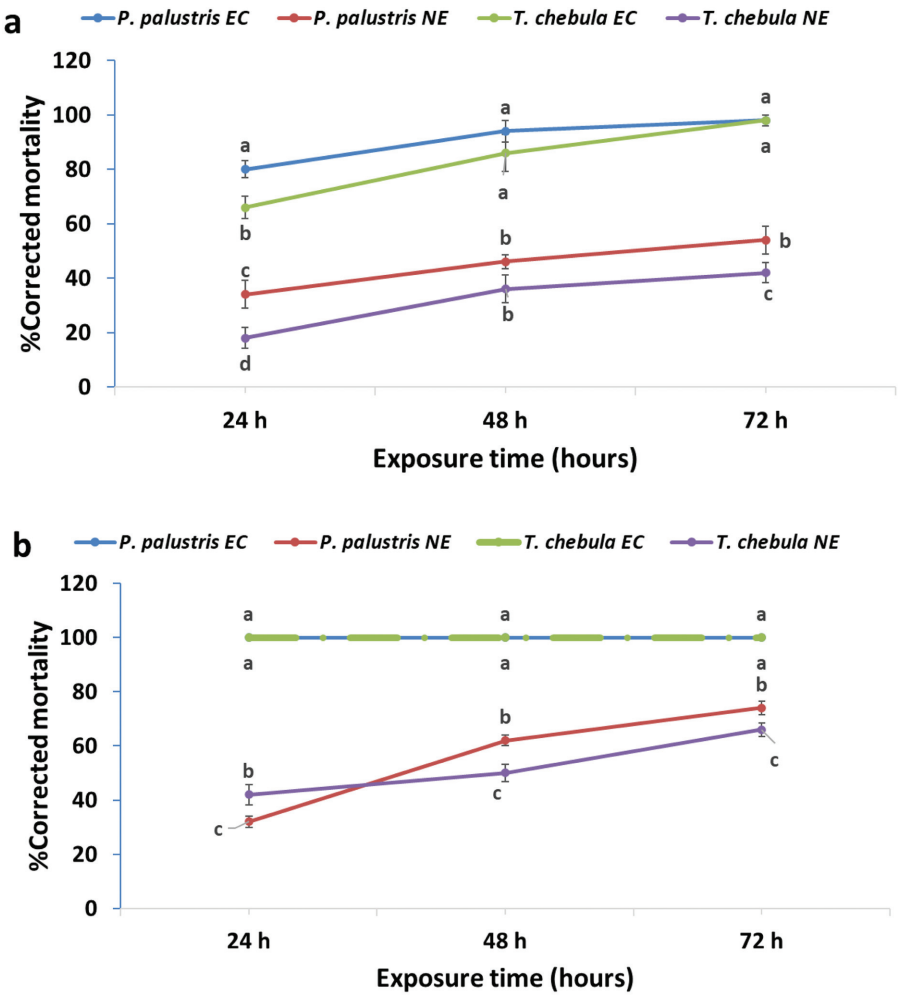
flavonoids, interfere with the growth and behavior of many herbivores. Moreover, plants belonging to Haloragaceae family, such as *P. palustris*, contain extremely high levels of phenolics [33], which act as herbivore deterrents in terrestrial plants [34]. About 148.6 Tannic acid equivalent (mg/g dry mass) was measured in *P. palustris* tested samples as reported by Choi *et al.* [35]. Likewise, in *T. chebula*, (Family: Combretaceae) about 33% of the total phytoconstituents are hydrolysable tannins (20–50%) [36]. The toxicity of both *P. palustris* and *T. chebula* essential oils against *A. fabae* adults may be attributed to their phytochemicals. Phenolic compounds especially hydrolysable and condensed tannins (polyphenols) are known to interfere with the growth of herbivorous insects [35] and are widely recognized as effective herbivore deterrents [34]. In fact, essential oils' toxicity to aphids is primarily due to their repellent, sucking-inhibiting, and locomotor-stimulating properties. Such impacts, particularly under field conditions, may lengthen the time spent seeking for food sources, resulting in an increase in insect mortality [37]. This is the first study regarding the aphicidal activity of *P. palustris* and *T. chebula* essential oils particularly on black bean aphid. However, Sathya-Narayanan *et al.* [38] and Thanigaivel *et al.* [39] have reported that the plant extract derived from *T. chebula* could be used to control *Culex quinquefasciatus* and *Aedes aegypti* with no harmful effects on nontarget beneficial insects.

Under laboratory conditions, our findings demonstrated that the ECs and NEs of the tested essential oils were nearly equally toxic, with mortality increasing to an average 100% of treated *A. fabae* adults at the highest concentrations (1.25, 1.5, and 2.0%) in comparison to the essential oils in their bulk form. Previous studies have demonstrated the high efficacy of NE of different essential oils against several aphid species and other insect pests compared with their bulk form; for instance, NE of *Basilicum ocimum*, *Cuminum cyminum*, *Origanum marjorana*, and *Matricaria chamomilla* had proved considerable toxic activities against laboratory and field strains of *Aphis craccivora* [40]. The smaller particle size and increased surface area of emulsion droplets in NEs could ensure the contact of formulations with the target pest and enhance penetration through the insect cuticle [41]. Subsequently, the biological activity and insecticidal activity of applied nanoformulations are improved against different pests. Regarding the toxic effect of EC of both tested essential oils against aphid adults, our results are similar to those reported by Wiwattanapatapee *et al.* [42], who indicated that the Derris EC was more efficient than the Derris extract solution against *Spodoptera litura*.

Toxicity and persistence of emulsifiable concentrate and nanoemulsion formulations under semifield conditions

Data obtained in Fig. 3 show the toxicity and persistence of ECs and NEs of *P. palustris* and *T. chebula* oils after 24, 48, and 72 h when tested using LC_{90} and LC_{90}x3 under semifield conditions. It was noticed that EC formulations of both essential oils with LC_{90} values could be considered strongly lethal for *A. fabae* adults after 72 h of exposure, where mortality percentage had reached as high as 98%. However, the NEs of both oils were the least effective on *A. fabae*, where mortality recorded was 54 and 42% after 72 h of exposure to *P. palustris* and *T. chebula*, respectively (Fig. 3a). Similarly, ECs of both tested oils at LC_{90}x3 induced the maximum mortality percentage (100%) after 24 h of treatments as illustrated in Fig. 3b. On the contrary, the NE of tested oils at LC_{90}x3 values were less toxic to *A.*

Figure 3



Corrected mortality of *Aphis fabae* showing the persistence effect of EC and NE of essential oils from *Proserpinaca palustris* and *Terminalia chebula* using concentrations of (a) LC_{90} and (b) LC_{90}x3, at three treatment intervals under semifield conditions. Mean (±SE) values with different letters within the same time interval for all formulations are significantly different ($P<0.05$) (analysis of variance) (Duncan test). EC, emulsifiable concentrate; NE, nanoemulsion.

fabae adults than the EC formulation. In other words, they caused an average increase in mortality gradually during 72 h of oil application from 32.00 to 74.00% and from 42.00 to 66.00% for *P. palustris* and *T. chebula*, respectively.

Under semifield conditions, EC formulations of both oil concentrations of LC_{90} and LC_{90}x3 induced maximum mortality percentages, which had reached as high as 98 and 100%, respectively, of *A. fabae* adults after 72 h. In support of our findings, Rizvi *et al.* [17] observed that EC formulation maintains the persistence of plant extract and improves its efficiency both in laboratory and semifield conditions and significantly controls citrus psyllids with minimal phytotoxic effects. The modest activity of NE formulations of both oils compared with ECs, on the contrary, was a rather surprising aspect of our findings. The insecticidal activity of nanoformulations is believed to be varied with several parameters such as insect species, time of exposure, the concentration of applied formulations, and the application technique [43].

Inhibitory activity evaluation of emulsifiable concentrate and nanoemulsion formulations on aphid adult enzymes

The EC and NE formulations of essential oils derived from both *P. palustris* and *T. chebula* plants were evaluated for their effects on the activity of selected enzymes within aphid tissues. Results in Table 3 revealed that EC and NE formulations of both essential oils at LC_{25} significantly affected the activity of AChE and GST enzymes in *A. fabae* adults after 48 h of exposure. The treated insects with all four formulated essential oils showed high reduction in the activity of AChE compared with the control group, where the reduction percentages of AChE were 68.42, 59.31, 53.69, and 19.45% for *P. palustris* EC, *P. palustris* NE, *T. chebula* EC, and *T.*

Table 3 Inhibitory activity evaluation of emulsifiable concentrate and nanoemulsion essential oils from *Proserpinaca palustris* and *Terminalia chebula* at LC_{25} on *Aphis fabae* adult enzymes

Tested formulation	AChE[a] (nmol min^{-1} mg of $protein^{-1}$)	AChE % Reduction	GST[b] (nmol min^{-1} mg of $protein^{-1}$)	GST % Reduction
Proserpinaca palustris-EC	189.33±19.22b	68.42	1731.00±188.65 c	65.78
Proserpinaca palustris-NE	224.00±27.00b	59.31	1948.33±341.80c	61.48
Terminalia chebula-EC	227.66±32.83b	53.69	3070.00±220.91b	39.31
Terminalia chebula-NE	483.00±33.51a	19.45	1322.00±184.24c	73.86
Control	599.66±75.04a	–	5059.00±487.11a	–
F value	19.110**	–	24.016**	–

AChE, acetylcholinesterase; EC, emulsifiable concentrate; GST, glutathione S-transferase; NE, nanoemulsion. Mean (±SE) values with different letters within the same column are significantly different ($P<0.05$) (analysis of variance) (Duncan test). **Highly significant.

chebula NE, respectively. Furthermore, all tested formulations resulted in a significant reduction in GST activity after treating *A. fabae* adults. The reduction percentages of GST with respect to control were 73.86, 65.78, 61.48, and 39.31% for *T. chebula* NE, *P. palustris* EC, *P. palustris* NE, and *T. chebula* EC, respectively.

The two formulations (EC and NE) of essential oils significantly exhibited high reduction in the activity of AChE and GST of *A. fabae* adults treated by the leaf dipping method with LC_{25} compared with the control group. Studying the changes that occurred in insect tissues is an important factor to evaluate the efficacy of applied insecticide [44]. In this context, many plant products used to manage insect pests have been shown to affect the enzyme profiles of insects [45,46]. This could substantiate that the phytochemicals of essential oils can penetrate insects and affect their enzymatic activity, leading to physiological dysfunctions [47]. AChE enzyme is a major indicator of the neurophysiological activity of insects; it catalyzes the breakdown of the neurotransmitter acetylcholine, leading to the termination of nerve impulse transmissions [48]. The present study has resulted in an increased reduction percentage of AChE in treated adults. This could simply be attributed to the presence of some terpenoids in tested formulations. Moreover, this reduction could disrupt neuromuscular coordination, eventually leading to paralysis and death [49]. In this context, *Artemisia judaica* plant extract inhibited AChE activity on treated *A. fabae* adults [50]. Furthermore, the inhibition of AChE activity in *Aphis craccivora* treated with different essential oil NEs may indicate that these compounds are highly effective [40]. Moreover, GST plays an important role in the defense system of insects, allowing rapid elimination of ingested toxic substances such as insecticides, allelochemicals, and endogenously activated compounds [51]. In line with our findings, a significant inhibition of GST enzyme activity was also observed in different insects treated with essential oils and plant extracts [52,53]. On the contrary, some studies indicated a significant increase of GST activity in the green peach aphid, *Myzus persicae*, treated with essential oils from different plants [47]. Taking all of the findings into consideration, the formulated oils tested (ECs and NEs) from *P. palustris* and *T. chebula* suppressed AChE and GST enzyme activity and could thus be estimated to be highly lethal to the black bean aphid *A. fabae*.

Conclusion

To the best of our knowledge, this is the first time to clarify the aphicidal activity of essential oils from *P. palustris* and *T. chebula* against the black been aphid. Both essential oils were synthesized as ECs and NEs to enhance their insecticidal activity. The tested formulated oils were found to be toxic to *A. fabae* under laboratory conditions in comparison with the bulk essential oils. In semifield study, EC formulations were more toxic than NEs. Furthermore, EC and NE formulations inhibited the enzymatic activity of AChE and GST enzymes, suggesting that the insecticidal activity of the tested formulations is associated with their neurotoxic and detoxification mode of action.

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Conflicts of interest

There are no conflicts of interest.

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