Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

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Synthesis and efficacy of cinnamon oil formulations and their sustainable release against common house mosquito larvae

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Abstract

Background Control of mosquitoes is considered an essential public health priority. This study was designed to estimate the larvicidal activity of two formulations of *Cinnamomum zeylanicum* EO for controlling *Culex pipiens* larvae.

Results The prepared formulations were a nanoemulsion of cinnamon (CNE), cinnamon (CN) alone and ordinary cinnamon essential oil mixed with sesame oil (CSO). The cinnamon + sesame oil (CSO) was added as one part cinnamon to 3 parts SO. Different concentrations were prepared and applied following the WHO larvicidal bioassay protocol. Our findings revealed that the LC_{50} of the CNE form ranged from 85.3 µg/mL to 28.30 µg/mL. The LC_{50} of SO alone was 1265 µg/mL but when mixed with CNE to form the CSO mixture, this decreased to 159.00 µg/mL. In terms of residual effect, the ordinary form of cinnamon had a residual effect in water for 72 h at a dose of 1000 µg/ml, but this extended to 120 h at the same dose when the CNE form was used. However CSO did not have a residual effect, however.

Conclusion The nanoemulsion form significantly improved the efficacy and residual effect of cinnamon against *Culex pipiens* larvae. Additionally, mixing cinnamon with sesame oil had a synergistic effect. This may assist control strategies against the house mosquito, *Culex pipiens*.

Keywords *Culex pipiens*, Cinnamon oil, Sesame oil, Nanoemulsion, Synergism, Residual effect

1 Background

Mosquitoes cause annoyance, and many diseases resulting from their bites, such as malaria, dengue fever, yellow fever, West Nile, chikungunya, Zika, and filariasis, makes them one of the globe's deadliest pests [1]. Control of mosquitoes is therefore considered an essential public health priority. Malaria is transmitted by Anopheline mosquitos and causes an estimated 219 million cases worldwide, with over 400,000 deaths each year. Furthermore, Aedes mosquitos carry dengue illness. More than 3.9 billion individuals are at risk of infection, with an estimated 96 million symptomatic cases and 40,000 deaths per year [2].

Although manufactured insecticides are widely used to control mosquitoes, these can have negative impacts on the environment, and on human and animal health. In particular, insecticides can leave highly toxic and long-lasting residues in the environment and encourage the development of resistance in the target insects [3, 4].

Plant essential oils are secondary metabolites and are recognized as a safe and natural alternative source of bioactive substances suitable for the control of mosquitoes [5]. The essential oils act as nerve agents disrupting insects' respiratory systems [6]. They can also be used as

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fumigants [7], repellents [8], antifeedants [9] toxins [10], and to interfere with insect growth, development, metamorphosis, and reproduction [11].

Cinnamon is a tropical tree related to the Lauraceae family; it is a tropical tree growing Sri Lanka, East and Middle Asia [12]. Cinnamon extract contains a range of bioactive phytochemicals, includes cinnamaldehyde, cinnamic acid, cinnamate, cinnamyl acetate, trans-cinnamaldehyde, and eugenol [13]. Various studies have reported that cinnamon essential oils have larvicidal, ovicidal, adulticidal, and repellent activities against mosquitoes [14].

Sesame (*Sesamum indicum* L.), meanwhile has been grown by humans for thousands of years. The essential oil crop is derived mainly from plants in the Pedaliaceae family, genus Sesamum, which is cultivated largely in Africa and, to a lesser extent, India [15]. *Sesamum indicum* peel extract has been shown to have larvicidal and repellent activities against *Culex pipiens* [16], Sesame oil has been identified as a good antioxidant that works synergistically with pesticides against *Spodoptera littoralis* [17]. Furthermore, an 8:2 blend of clove oil andsesame oil has been shown to possess a synergistic effect when applied against *Callosobruchus maculatus* (a common agricultural pest) [18].

Nanotechnology has recently attracted considerable attention as a method to maximize the effectiveness of botanical-based pesticides. [19, 20]. Benefits include the ability to focus drug delivery more directly to its place of action, lower doses, improved efficacy, and reduced side effects. Nanoemulsions can also preserve active compounds from degradation and deactivation, consequently prolonging the pesticide's half-life [20]. Furthermore, the physicochemical qualities of nanoemulsions are better adapted to overcome the target organism's physiological barriers and can have better affinity with the target tissue. All in all, nanoemulsion techniques can double the efficacy of essential oils compared to their ordinary form [21].

In the above context, the main objective of this research was to assess the toxicity of two formulations of cinnamon essential oil against *Culex pipiens* larvae, with reference to its residual effect.

2 Methods

2.1 Source of the work compounds and their prepared concentrations

The work compounds *Cinnamomum zeylanicum* and sesame oils were obtained from local market in Jazan, Saudi Arabia. The following seven concentrations (15.37 µg/mL, 31.75 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL, and) of cinnamon oil were prepared in ethyl alcohol 70%. In addition, five concentrations of sesame oil were prepared, also in ethyl alcohol 70%; 3000, 95, 187, 375, 750, and 1500, µg/mL. These concentrations were dependent on our pilot work for the activity of each oil when used alone. The binary mixtures between cinnamon and sesame oils were done at a ratio of (1:3); one part cinnamon oil and three parts sesame oil as follows; (32 + 95), (62.5 + 185), (125 + 375), and (250 + 750), µg/mL.

2.2 GC–MS of the essential oils

The cinnamon and sesame oils were analyzed using GC–MS and TRACE GC Ultra Gas Chromatographs at an Educational Research Center, Nawah Scientific, in Egypt (https://nawah-scientific.com/) (THERMO Scientific Corp., USA).

2.3 Essential oil nanoemulsions preparation

Nanoemulsions were created using the Nerimela et al. [22] technique. Briefly, the essential oils were mixed with Tween 80 as a surfactant (1:3; one part oil to three parts T80) before being combined with water to achieve a concentration of 2.50%. Then, this was stirred with a magnetic stirrer for 10 min, at 500 rpm. An ultrasonicator (750 W, Branson Probe sonicator-Advanced model, 20 kHz) was used to sonicate the created macroemulsion for 5 min to create a nanoemulsion.

2.4 Characterization of nanoemulsions

A UV–visible spectrophotometer (UV-2600, Shimadz, Japan) set to 345 nm was used to analyze the produced nanoemulsions. Using a zeta sizer device (dynamic light scattering technique) (Nano-ZS90, Malvern, UK), the distribution of the droplet size (d, nm) and polydispersity index (PDI) of the produced nanoemulsion were measured. To lessen the impact of various scattering effects, all samples were diluted by 10% with deionized water.

2.5 Preparation of *C. pipiens* larvae

C. pipiens colony that was raised in a lab had its egg rafts sieved into water-filled plastic containers. In enamel dishes with 1 L of unchlorinated water and 0.15 g of 50:50 lactalbumin (brewer's yeast), the acquired larvae were put. Every other day, the water was changed, and every day, food was added. In aluminum cages (0.51 m^3 in size) with screens, adult mosquitoes were housed and fed on 10% cotton wicks soaked in a sucrose solution. The bug female was fed blood by a restrained quail. The deposited egg rafts were collected in a 400 cc plastic container. The colony was maintained at 26 °C, 75% relative humidity, and a 16 L: 8 D photoperiod. The bioassays utilized the third and fourth larval instars [23].

2.6 Larvicidal bioassay

This experiment followed the guidelines established by the World Health Organization [24]. The 250 mL plastic cups used for this test were used. The working solution was created by adding an aliquot of one mL of these dilutions to 99 mL of distilled water after the essential oils had been dissolved in ethyl alcohol at the specified quantities. Twenty third-instar/fourth-instar *C. pipiens* larvae were placed in groups of five for each concentration in plastic cups. One mL of the solvent that had been dissolved was utilized in the negative control. To determine the average death rate, motionless larvae were recorded after 24 h and presumed dead [25].

2.7 Residual effect bioassay against larvae

Four concentrations (125, 250, 500, and 1000, µg/mL) were prepared for each form of cinnamon essential oil. These concentrations caused at least 75% mortality of larvae (concentrations were higher than the LC_{50} of each cinnamon formulation). Meanwhile, three concentrations of sesame oil were selected (750, 1500, and 3000, µg/mL). For the binary mixture of C-SO, only two concentrations were tested: 1000C + 3000SO, and 500C + 1500SO. These concentrations were applied to the larvae, with exchange the treated larvae (dead and live) from each concentration after 24 h and replaced by new ones. This exchange was done every 24 h up to 144 h. A double lethal dose of deltamethrin double was applied as a comparative positive control.

2.8 Statistical analysis

For each treatment, five replicates were performed, and mean and SE values were computed. ANOVA was used to analyze larval mortality, followed by Duncan's multiple range tests (p 0.05). The LC50 and LC90 values, together with their respective 95% confidence intervals, were calculated using probit analysis. SPSS for Windows (version 22.0) was used for all statistical analysis. The Synergistic Factor (SF) was calculated by dividing the LC_{50} value of the individual test insecticide with the corresponding LC_{50} value of the test insecticide + synergist mixture [26].

$$\text{Synergistic ratio} = \frac{LC_{50} \text{ of insecticide alone}}{LC_{50} \text{ of } (\text{insecticide} + \text{synergists})}$$

Synergistic ratios of less than one are described as antagonistic effect, ratios greater than one are described as synergistic, and the ratios equal to one are described as have no effect.

3 Results

3.1 GC–MS phytochemical composition of cinnamon essential oil

Analysis of the chemical composition of cinnamon essential oil revealed that the chief compotent is cinnamaldehyde, (E)- (63.42%), and cinnamaldehyde dimethyl acetal was (13.16%), 2-propenoic acid, 3 phenyl-methyl ester (6.09%), cinnamaldehyde à-hexyl- (11.75%), and traces of other constituents (Table 1).

3.2 Characterization of cinnamon essential oil nanoemulsion

The hydrodynamic particle size showed a mean droplet size of around = 391.7 d, n, with a PDI = 0.979. This low PDI value indicates that the droplets had a homogeneous size distribution (Fig. 1 A, B).

3.3 Larvicidal activity of cinnamon formulations

The LC_{50} of cinnamon nanoemulsion against *Culex pipiens* larvae was 33.8 µg/mL, While the LC_{50} of the the ordinary form was 85.3 µg/mL for. Similarly, the LC_{90} for the nanoemulsion form was 65.1 µg/mL compared to 179.00 µg/mL for the crude form (Table 2, 3). For sesame oil,, the LC_{50} of the oil itself was 1265 µg/mL, but this decreased to 159.00 µg/mL when mixed at a ratio of one part cinnamon oil to three parts sesame oil (Table 2, 4). The larvicidal activity of sesame oil was

Table 1 The phytochemical composition of cinnamon by GC–MS

Peak	R.t*	Name	Area %	Molecular Weight	Molecular formula	MF**
1	5.20	Benzaldehyde	3.23	106	C7H6O	971
2	6.89	Benzeneacetaldehyde	0.72	120	C8H8O	958
3	12.60	Cinnamaldehyde, (E)-	63.42	132	C9H8O	909
4	15.28	2-Propenoic acid 3 phyenyl,—methyl ester	6.09	162	C10H10O2	836
5	15.41	Cinnamaldehyde dimethyl acetal	13.16	178	C11H14O2	937
6	19.29	n-Butyl cinnamate	0.63	204	C13H16O2	890
7	21.48	Cinnamaldehyde, à-hexyl-	11.75	216	C15H20O	933
8	21.68	1-Hexen-3-ol, 5-nitro-1-phenyl-,(R*,R*)-	1.00	221	C12H15NO3	743

* R.t, retention time (min). **M.F. match factor

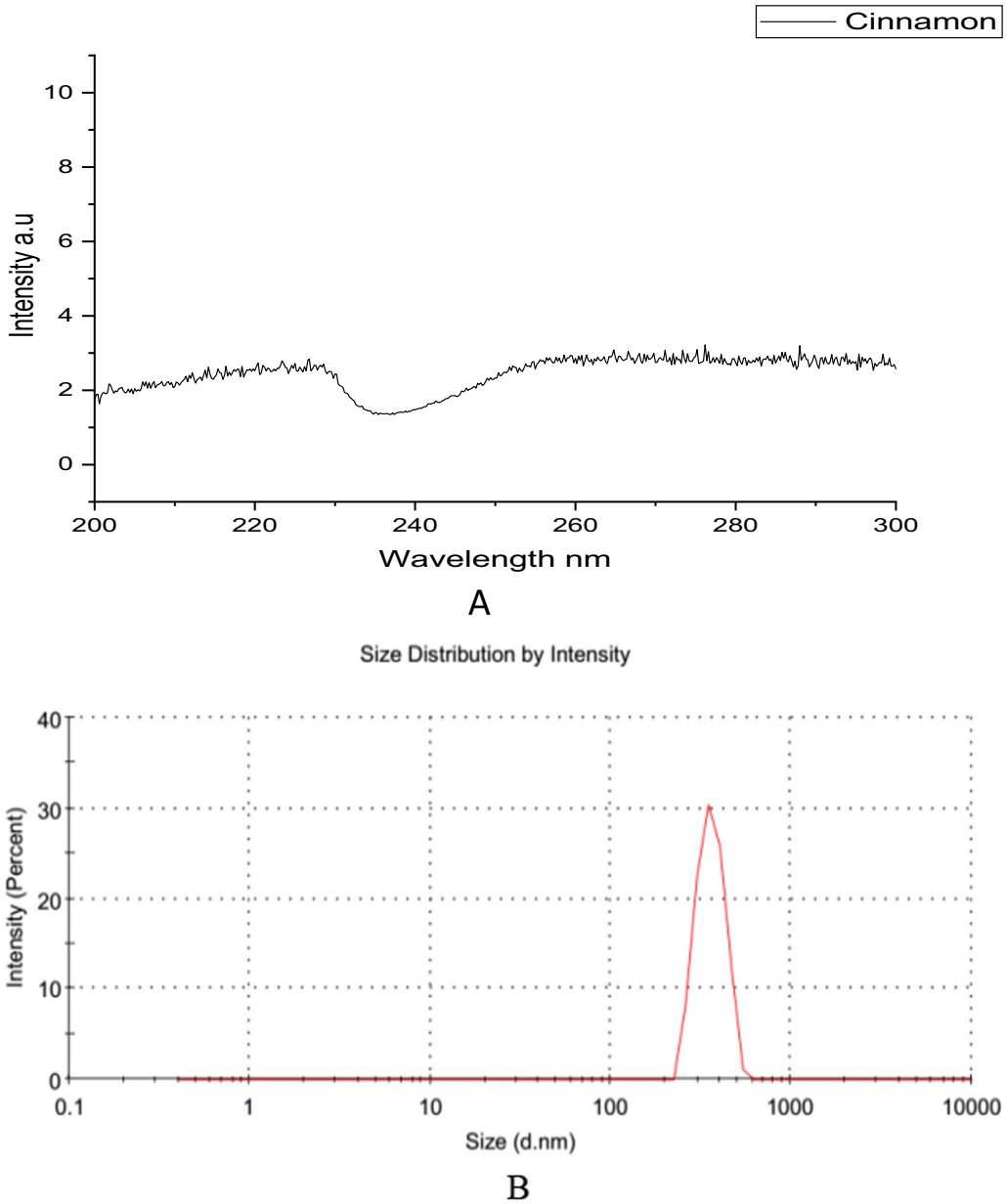

Fig. 1 **A** The wave length of cinnamon nanoemulsion, **B** Size of cinnamon nanoemulsion

100% at concentrations of 3000 µg/mL and 60% at concentrations of 1500 µg/mL and,. The larvicidal activity at lower concentrations was not significant (Table 4). The reference insecticide (1.7 ug/ml deltamethrin) showed 100% mortality (Table 2). The cinnamon sesame oil combination at a ratio of 1:3 exhibited a 100% mortality rate of larvae at all applied concentrations (Table 5). These results suggest that cinnamon and sesame oils have a synergistic larvicidal effect, increasing the potency of sesame oil by tenfold (synergistic factor SF), and the potency of cinnamon oil by twofold (synergistic factor SF), as shown in (Table 3).

3.4 Residual effect of different formulations of cinnamon essential oil

Higher concentrations of cinnamon essential oil resulted in increased residual effect. Specifically, the highest concentration, 4131 ug/mL, exhibited larvicidal effect that lasted up to 96 h (Table 6). Regarding the nanoemulsion form of the cinnamon oil, when used at a concentration

Table 2 Larvicidal mortalities of cinnamon oil and its nanoemulsion against *Culex pipiens* larvae (*N* = 20 larvae)

Treatment	Ordinary form	Nanoemulsion
15.6 µg/mL	1.0 ± 0.3^{b}	5.40 ± 1.14^{b}
31.25 µg/mL	2.4 ± 0.2^{c}	12.0 ± 1.00^{e}
62.50 µg/mL	6.2 ± 0.3^{d}	16.2 ± 0.84^{f}
125 µg/mL	16.4 ± 0.2^{f}	$20.0 \pm .0^{g}$
250 µg/mL	$20.0 \pm .0^{g}$	$20.0 \pm .0^{g}$
500 µg/mL	$20.0 \pm .0^{g}$	$20.0 \pm .0^{g}$
1000 ug/mL	$20.0 \pm .0^{g}$	$20.0 \pm .0^{g}$
Deltamethrin 1.7 µL/L	$20.0 \pm .0^{g}$	$20.0 \pm .0^{g}$
Ethyl alcohol 70%	0.4 ± 0.2^{a}	0.4 ± 0.2^{a}

Superscript of the same letter in cells of the same column indicates a non-significant effect compared to the control. Superscript of different letters in cells of the same column indicates a significant effect compared to the control ($P \leq 0.05$)

of 1122 ug/mL, its efficacy remained stable for up to 120 h (Table 6, 7). Sesame oil showed the shortest period of residual effect (74% after 48 h at the highest concentration 3000 ug/mL which decreased to 27% after 72 h (Table 8). However, the residual effect of the mixture of cinnamon and sesame oil mixture was not significantly different from that of each oil singly (Table 9). AS for the positive control, deltamethrin (3.5 ug/L) demonstrated a prolonged residual effect lasting up to 144 h (Table 9).

4 Discussion

The first line of defense against mosquitoes should be directed to control the larvae. Therefore, larvicidal control has the most significant impact on reducing mosquito populations. Extensive use of chemical insecticides is associated with insecticide resistance, environmental damage, and public health risks [5]. In this context, it is significant that cinnamon essential oil possesses an efficient insecticidal activity [27]. The analysis of the essential oil *C. zeylanicum* in this study revealed Cinnamaldehyde, (E) to be the predominant constituent (63.42%). Other studies have also identified cinnamaldehyde as the main component in cinnamon essential oil, albeit in different concentrations [28, 29]. Previous studies on the insecticidal activity

Table 4 Larvicidal activity of sesame oil (SO) against *Culex pipiens* larvae

Treatment	Mean Std. Error
Ethyl alcohol 70%	0.4 ± 0.2^{a}
Delta 1.7	$20.0 \pm .0^{f}$
SO 95 µg/mL	$0.4 \pm .2^{a}$
SO 187.5 µg/mL	$1.8 \pm .4^{b}$
SO 375 µg/mL	$2.6 \pm .2^{c}$
SO 750 µg/mL	$6.2 \pm .4^{d}$
SO 1500 µg/mL	$12.4 \pm .2^{e}$

Superscript of the same letter in cells of the same column indicates a non-significant compared to the control. Superscript of different letters in cells of the same column indicates a significant effect compared to the control ($P \leq 0.05$)

Table 5 Larvicidal activity of SO + cinnamon at a rate of three parts SO to one part CIN (3:1) against *Culex pipiens* larvae

Treatment	Larvicidal/20 Mean ± Std. Error (5 replicates)
Ethyl alcohol 70%	$0.8 \pm .2^{a}$
Delta 1.7	$20.0 \pm .0^{c}$
(SO-C) 95 + 32 µg/mL	5.80 ± 0.84^{a}
(SO-C) 185 + 62.5 µg/mL	11.4 ± 1.14^{b}
(SO-C) 375 + 125 µg/mL	18.2 ± 1.30^{c}
(SO-C) 750 + 250 µg/mL	$20.0 \pm .0^{c}$

Superscript of the same letter in cells of the same column indicates a non-significant effect compared to the control. Superscript of different letters in cells of the same column indicates a significant effect compared to the control ($P \leq 0.05$)

of cinnamon/cinnamaldehyde have mainly focused on agricultural insects, demonstrating efficacy against mealworm (*Alphitobius diaperinus*) and *Anopheles gambia* [30], and the larvae and pupae of *Culex quinquefasciatus* [31], against *Culex quinquefasciatus* and *Musca domestica* [32], as well as adults, eggs and larval stages of *Culex quinquefasciatus* [33], and a potent antioxidant effect and activity against piroplasm in *C. cassia* [34–36]. Another study has demonstrated that another competent of cinnamon essential

Table 3 LC_{50} and LC_{90} of cinnamon oil formulations of against *Culex pipiens* larvae

Treatment	LC_{50} (95% CI) (µg/mL)	LC_{90} (95% CI) (µg/mL)	SLOPE ± SE	Synergistic factor
C	85.3 (70.9–104.8)	179 (147–243)	0.02 ± 0.005	–
CNE	33.4 (28.9–37.8)	65.1 (58.8–68.8)	0.13 ± 0.03	2.56
SO	1265 (1037–1610)	2877 (2319–2984)	0.001 ± 0.00	–
SO-C	159 (141–189)	232 (197–411)	0.03 ± 0.01	10.54 for SO 2.13 for CIN

C = cinnamon, CNE = cinnamon nanoemulsion, SO = sesame oil

Table 6 LC_{50} and LC_{99} of cinnamon oil formulations of residual effect against *Culex pipiens* larvae

Time/treatment	LC_{50} (95% CI)	LC_{99} (95% CI)	SLOPE ± SE
C after 24 h	65 (–)	231	0.14 ± 0.01
C after 48 h	323 (263–394)	680 (558–956)	0.007 ± 0.001
C after 72 h	775 (647–962)	1586 (1294–2209)	0.003 ± 0.001
C after 96 h	4131 (–)	8404 (–)	0.01 ± 0.01
C after 120 h	–	–	–
C after 144 h	–	–	–
CNE after 24 h	28.3 (24.3–33.3)	46.2 (38.8–68.8)	0.13 ± 0.03
CNE after 48h	200 (169–232)	337 (287–461)	.017 ± 0.004
CNE after 72 h	386 (316–485)	829 (664–1240)	0.005 ± 0.001
CNE after 96 h	770 (–)	1249 (–)	0.005 ± 0.001
CNE after 120 h	1122 (928–1627)	2031 (1560–3618)	0.003 ± 0.001
CNE after 144 h	–	–	–

C = cinnamon, CNE = cinnamon nanoemulsion

oil, eugenol (96.5%), exhibited a more potent larvicidal effect against *Anopheles Gambia* compared to clove oil [37].

The LC_{50} of the tested *C. zeylanicum* oil against *Culex pipiens* larvae was 85.3 ug/mL but this decreased to 33.8 ug/mL when the nanoemulsion form was used. Other studies have found that the LC_{50} and LC_{90} values for the activity of the essential oil of *C. zeylanicum* L. against the *Cx. Tritaeniorhynchus* and *Anopheles subpictus* mosquito species are 124, 225 ppm, and 71, 123 ppm, respectively [38]. Previous work has similarly found that the LC_{50} value (24 h) of cinnamon oil against *C. pipiens* larvae to be 71.87 mg/L [39], while the LC_{50} value for cinnamaldehyde against *A. aegypti* larvae is 36 ppm [40] and 13.45 ppm [41]. A dosage of 100 ug/ml cinnamaldehyde obtained from *Cinnamomum osmophloeum* leaf essential oil has been shown to induce 100% larval mortality of *Aedes albopictus* [28]**.** Another work has shown that cinnamon oil is toxic to *Culex pallens* larvae with LC_{50} 66 ug/ml and LC_{90} 99 ug/ml [42]. In contrast, another study found only weak larvicidal activity of cinnamon oil, with an LC_{50} of 429.75 ug/ml [3]. *Cinnamomum zeylanicum* also has larvicidal activity against *Aedes stephensi, Aede. Aegypti* and *Culex quinquefasciatus*, with LD_{95} of 228.2, 276.9 and 277.4 ug/ml, respectively [43]. Our results are therefore consistent with the most previous studies.

Regarding the mechanism of the lethal effect of cinnamon essential oils on mosquito larvae, [33] observed that *Culex quinquefasciatus* larvae develop a wrinkled body and loss of their inner respiratory tube, which is associated with a lack of oxygen, irregular breathing, and damage to the nervous system [44]. Another study has explained that the lethality is caused by damage to the digestive tract of larvae [45].

Nanoparticles have been shown to improve the insecticidal efficacy of various compounds [46]. In the present study, the nanoemulsion form of cinnamon oil was approximately three times more effective at killing mosquito larvae than the ordinary form (the LC_{50} was 85.3 ug/mL for the ordinary form but just 28.30 ug/mL for the cinnamon nanoemulsion form). Similarly, a nanoemulsion of *Cinnamomum zeylanicum* oil was up to 32% more effective killing against *Anopheles stephensi* larvae than the ordinary form [47]. Furthermore, a nanostructured form of *C. zeylanicum* essential oil can be used to control meal worm (*Alphitobius diaperinus*) with the least toxicity to the environment and its fundamental arthropods [27]. Work in related areas has also shown that a nanoemulsion of phytochemical oil was 1.4–1.6 times more effective against *Sitophilus oryzae* (the rice weevil) than essential oil [48]. Moreover, a nanoemulsion made with *Pterodon emarginatus Vogel* oil produced 100% mortality in *Aedes egypti* at a dose of 250 ppm, with effectiveness lasting for up to 24 h [49]. In addition, the nanoemulsion of *Vitex negundo* essential oil revealed significant larval toxicity against *Aedes aegypti*, with LC_{50} values of 43.29 ppm, again lasting for up to 24 h [50]. Finally, a nanoemulsion of mint achieved higher larvicidal activity against *C. pipiens* and *M. domestica* than the regular oil [51].

In this study, the larvicidal power of crude sesame oil was found to be quite weak, with an LC_{50} of only 1265 ug/mL. However When combined with cinnamon essential oil, at a ratio of one part cinnamon to three parts sesame oil, the LC_{50} decreased to 159.00 ug/mL. Overall, the addition of cinnamon to seasame oil at the rate of 1:3 increased the potency of seasame oil by a synergistic factor of 10, and increased the activity of cinnamon by a synergistic factor of 2. These findings align with those obtained by [52], showing that a range of essential oils extracted from the leaves of *S. indicum* had high lethal efficacy against *Aedes aegypti* (349.88 mg/L), *An. stephensi* (338.27 mg/L) and *Culex quinquefasciatus* (304.84 mg/L). Assitionslly, an 8:2 mixture of clove oil: sesame oil has been found to have a synergistic effect on lethal activity against adult *Callosobruchus maculatus* [18]. Moreover sesame oil has shown a synergetic effect with clove oil on the cotton leaf-worm, *Spodoptera littoralis*, and with acetamiprid against the larvae of *Trogoderma granarium* [53, 54].

The residual effect of both forms of cinnamon oil increased with increasing concentration. The ordinary form of cinnamon essential oil maintained a residual effect in water for up to 72 h when applied at a concentration of 1000 ug/ml, while the nanoemulsion form continued to have an effect for up to 120 h at the same dose. Similarly, a nanoemulsion of *Cinnamomum zeylanicum*

Table 7 Residual effect of cinnamon essential oil formulations of on survival of *Culex pipiens* larvae

	Cinnamon ordinary						Cinnamon nanoemulsion					
	Post 24 h Mean ± Std. Error	Post 48 Mean ± Std. Error	Post 72 h Mean ± Std. Error	Post 96 h Mean ± Std. Error	Post 120 h Mean ± Std. Error	Post 144 h Mean ± Std. Error	Post 24 h Mean ± Std. Error	Post 48 Mean ± Std. Error	Post 72 h Mean ± Std. Error	Post 96 h Mean ± Std. Error	Post 120 h Mean ± Std. Error	Post 144 h Mean ± Std. Error
Ethyl alcohol 70%	$2 \pm .2^{a}$	$.4 \pm .2^{a}$	$.8 \pm .2^{a}$	$.8 \pm .2^{a}$	$.8 \pm .2^{a}$	$.8 \pm .2^{a}$	$.2 \pm .2^{a}$	$.4 \pm .2^{a}$	$.8 \pm .2^{a}$	$.8 \pm .2^{a}$	$.8 \pm .2^{a}$	$.8 \pm .2^{a}$
Delta 3.5ug/L	$20.0 \pm .0^{c}$	$20.0 \pm .0^{e}$	$20.0 \pm .0^{e}$	$20.0 \pm .0^{c}$	$20.0 \pm .0^{b}$	$16.2 \pm .4^{b}$	$20.0 \pm .0^{b}$	$20.0 \pm .0^{d}1$	$20.0 \pm .0^{d}$	$20.0 \pm .0^{b}$	$20.0 \pm .0^{c}$	$16.2 \pm .4^{c}$
1000 ug/	$20.0 \pm .0^{c}$	$20.0 \pm .0^{e}$	$15.8 \pm .6^{d}$	$2.6 \pm .5^{b}$	$1.0 \pm .3^{a}$	$1.0 \pm .3^{a}$	$20.0 \pm .0b$	$20.0 \pm .0^{d}$	$20.0 \pm .0^{d}$	$19.4 \pm .4^{b}$	$9.6 \pm .5^{b}$	$1.0 \pm .3^{a}$
500 ug/ ml	$20.0 \pm .0^{c}$	$17.8 \pm .4^{d}$	$5.0 \pm .3^{c}$	$1.0 \pm .3^{b}$	$1.0 \pm .3^{a}$	$.6 \pm .2^{a}$	$20.0 \pm .0^{b}$	$20.0 \pm .0^{d}$	$15.4 \pm .5^{c}$	$1.0 \pm .3^{a}$	$1.0 \pm .3^{a}$	$1.0 \pm .3^{a}$
250 ug/ml	$20.0 \pm .0^{c}$	$9.2 \pm .4^{c}$	$2.0 \pm .4^{b}$	$1.4 \pm .2^{b}$	$1.0 \pm .3^{a}$	$1.0 \pm .3^{a}$	$20.0 \pm .0^{b}$	$16.4 \pm .2^{c}$	$7.2 \pm .6^{b}$	$1.4 \pm .2^{a}$	$1.2 \pm .2^{a}$	$1.0 \pm .3^{a}$
125 ug/ml	$16.8 \pm .4^{b}$	$1.4 \pm .2^{b}$	$1.2 \pm .2^{a}$	$.8 \pm .4^{b}$	$.6 \pm .2^{a}$	$.6 \pm .2^{a}$	$20.0 \pm .0^{b}$	$2.8 \pm .4^{b}$	$1.4 \pm .2^{a}$	$1.0 \pm .3^{a}$	$1.0 \pm .3^{a}$	$.0 \pm .0^{a}$

Superscript of the same letter in cells of the same column indicates a non-significant effect compared to the control. Superscript of different letters in cells of the same column indicates a significant effect compared to the control ($P \leq 0.05$)

Table 8 Residual effect of SO on survival of *Culex pipiens* larvae

	24 H Mean ± Std. Error	48 H Mean ± Std. Error	72 H Mean ± Std. Error	96 H Mean ± Std. Error	120 H Mean ± Std. Error	144 H Mean ± Std. Error
Cont. DEMSO	.8 ± .4^{a}	.8 ± .4^{a}	.8 ± .4^{a}	.8 ± .4^{a}	.8 ± .4^{a}	.8 ± .4^{a}
Delta 3.5	20.0 ± .0^{d}	20.0 ± .0^{d}	20.0 ± .0^{c}	20.0 ± .0^{b}	20.0 ± .0^{b}	16.2 ± .4^{b}
SO 750	10.4 ± .5^{c}	3.8 ± .4^{b}	1.0 ± .3^{a}	1.0 ± .3^{a}	1.0 ± .3^{a}	1.0 ± .3^{a}
SO 1500	10.4 ± .5^{c}	3.6 ± .4^{b}	1.0 ± .3^{a}	1.0 ± .3^{a}	1.0 ± .3^{a}	1.0 ± .3^{a}
SO 3000	20.0 ± .0^{d}	14.8 ± .4^{c}	5.4 ± .5^{b}	2.0 ± .4^{a}	1.0 ± .3^{a}	1.0 ± .3^{a}

Superscript of the same letter in cells of the same column indicates a non-significant effect compared to the control. Superscript of different letters in cells of the same column indicates a significant effect compared to the control ($P \leq 0.05$)

Table 9 Residual effect of a cinnamon-sesame oil combination at a rate of one part cinnamon to three parts sesame oil on the survival of *Culex pipiens* larvae

	24 H Mean ± Std. Error	48 H Mean ± Std. Error	72 H Mean ± Std. Error	96 H Mean ± Std. Error	120 H Mean ± Std. Error	144 H Mean ± Std. Error
Cont. Ethyl alcohol 70%	0.80 ± .4^{a}	0.80 ± .4^{a}	0.80 ± .4^{a}	0.80 ± .4^{a}	0.80 ± .4^{a}	0.80 ± .4^{a}
Delta 3.5	20.0 ± .0^{b}	20.0 ± .0^{d}	20.0 ± .0^{c}	20.0 ± .0^{b}	20.0 ± .0^{b}	16.2 ± .4^{b}
500 C + SO 1500	20.0 ± .0^{b}	3.0 ± .3^{b}	1.4 ± .2^{a}	1.2 ± .4^{a}	0.80 ± .4^{a}	0.6 ± .2^{a}
1000 C + SO 3000	20.0 ± .0^{b}	10.8 ± .4^{c}	3.0 ± .3^{b}	1.2 ± .4^{a}	0.80 ± .4^{a}	0.6 ± .2^{a}

Superscript of the same letter in cells of the same column indicates a non-significant effect compared to the control. Superscript of different letters in cells of the same column indicates a significant effect compared to the control ($P \leq 0.05$)

oil was able to kill 100% of *Anopheles stephensi* larvae for up to three days [48], a nanoemulsion of *Protium heptaphyllum* resin essential oil had a residual larvicidal effect for 72 h after application against *Aedes aegypti* larvae at a dose of 40 ug/ml [51], and *Eucalyptus* oil retained a residual effect against *Aedes* mosquitoes for up to eight days at a dose of 1000 ppm [53].

It has previously been recognized that nanoemulsion forms can be applied more easily than the ordinary forms of essential oils since they have better solubility, supporting dispersion in aqueous media, and also better stability, thus increasing the residual time [54]. Nanoemulsions also benefit from reduced oil particle size, low surface tension, and a greater surface area, all of which help them to disperse the active components of the essential oils across the target site, and permeate the target itself. This explains why nanoemulsion technology offers better biological activity compared to bulk/original essential oils [55]. Additionally, the nanoemulsion forms retain essential oils basic safety and environmentally friendly characteristics since they are biodegradable and have few side effects on non-target organisms, and the environment [56].

5 Conclusions

Cinnamon essential oil has significant larvicidal activity which improved when delivered either in a nanoemulsion form or in a mixture of one part cinnamon oil to three parts sesame oil. This is an important finding since sesame oil is cheaper than cinnamon oil. Moreover, the nanoemulsion form of the cinnamon essential oil was found to maintain a residual effect against *C. pipiens* in water for longer than the ordinary form or a sesame oil formulation which may have a role in controlling house mosquito in Jazan district, KSA. Further investigation of the safety and environmental impact of these formulations to ensure they meet regulatory standards and can be safely integrated into mosquito control programs.

Abbreviations

C. pipiens	*Culex pipiens*
EO	Essential oil
C	Cinnamon
CNE	Cinnamon nanoemulsion
SO	Sesame oil
CSO	Cinnamon + sesame oil
PDI	Polydispersity index
LC_{50}	Concentration killed 50% of treated larvae
LC_{90}	Concentration killed 90% of treated larvae
SF	Synergistic factor

Acknowledgements
The authors extend their appreciation to Dr. Ahlam Gamal (PhD) for helping in mosquito colony stablishment.

Author contributions
HAM contributed to Conceptualization, investigation, funding, writing and review**;** SMA contributed to Conceptualization, investigation, methods, analysis, writing and review; KMH contributed to Investigation, data curation, data analysis, drafting writing.

Funding
We have not received any funding.

Availability of data and materials
It will be made available on request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Professor Shawky M. Aboelhadid is a co-author of this study and a Managing Editor of the journal. He was not involved in handling this manuscript during the submission and review processes. The rest of the authors have no conflict of interest to declare.

Received: 23 July 2023 Accepted: 6 December 2023
Published online: 19 December 2023

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