Research Article

Cell Cytotoxicity, Antimicrobial, and Mosquitocidal Activity of Prepared Cinnamon Oil Formulations

Hesham A. Mahran[1,2*], Shawky M Aboelhadid[3], Saad S. Alqahtani[4,5], Mohamed Z. Sayed Ahmed[4,5]

[1]Health Informatics Department, College of Public Health and Tropical Medicine, Jazan University, Jazan, Saudi Arabia; [2]Hygiene, Zoonoses and Epidemiology Department, Faculty of Veterinary Medicine, Beni-Suef University, 52611, Beni-Suef, Egypt; [3]Parasitology Department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt; [4]Pharmacy Practice Research Unit, College of Pharmacy, Jazan University, Jazan, Saudi Arabia; [5]Clinical Pharmacy Department, College of Pharmacy, Jazan University, Jazan, Saudi Arabia.

Abstract | The goal of the current study was to create safe, antibacterial formulations based on cinnamon oil that were effective against *Culex pipiens* adults and pupae. Cinnamon nanoemulsion (CNE) was synthesized and characterized. The binary mixture of cinnamon with sesame oil (CS) was prepared at a rate of one part cinnamon to 3 parts sesame oil. Multiple concentrations from these formulations were prepared and tested against laboratory-reared adults and pupae of *C. pipiens*. The safety results showed that the ordinary form of cinnamon oil was safer for human skin cell fibroblast and human epidermoid Skin carcinoma than the other forms at high concentrations. Meanwhile, CNE was potent antibacterial activity even at the lowest concentrations. Moreover, CNE showed better pupicidal activity than the other forms with LC_{50} at 322 ug/mL lower than pure cinnamon oil, 465 ug/mL against pupae. Additionally, the CNE achieved the lowest concentration after 90 min (15.80ug/mL). The high concentration of 69.60ug/mL (LC_{50}) and 135.00ug/mL (LC_{99}) caused toxicity to adult *C. pipiens* in a short time (15 min). Meanwhile, sesame oil has no toxic effect on adult *C. pipens*. In conclusion, CNE has better antimicrobial, pupicidal and adulticidal activity against *C. pipiens* than ordinary and binary mixtures with sesame oil.

Keywords | *Culex pipiens*, Nanoemulsion, Cinnamon, Sesame, Binary mixture

Received | June 18, 2022; **Accepted** | February 11, 2023; **Published** | February 23, 2023
***Correspondence** | Hesham A. Mahran, Health Informatics Department, College of Public Health and Tropical Medicine, Jazan University, Jazan, Saudi Arabia;
Email: hamaran@jazanu.edu.sa
Citation | Mahran HA, Aboelhadid SM, Alqahtani SS, Ahmed MZS (2023). Cell cytotoxicity, antimicrobial, and mosquitocidal activity of prepared cinnamon oil formulations. Adv. Anim. Vet. Sci. 11(3):475-484.
DOI | https://dx.doi.org/10.17582/journal.aavs/2023/11.3.475.484
ISSN (Online) | 2307-8316

INTRODUCTION

Essential oils are abundant sources of bioactive materials and are naturally occurring secondary metabolites of plants. The latter biodegrade into safe substances that are used to treat infections (Govindarajan and Sivakumar, 2011). The family Lauraceae includes the tropical tree and herb known as cinnamon, which is grown in Sri Lanka, East Asia, and Middle Asia (Shu et al., 2008). Cinnamon EO repels mosquitoes and has larvicidal, ovicidal, adulticidal, and other anti-mosquito properties (Dai et al., 2020). Numerous active substances, including cinnamaldehyde, trans-cinnamaldehyde, cinnamyl acetate, and eugenol, are present in the cinnamon extract (Singh et al., 2007).

A seed oil crop of the Pedaliaceae family, sesame (*Sesamum indicum* L.) is primarily cultivated and produced in India and Africa (Baranitharan et al., 2015). In the fight against

Spodoptera littoralis (Biosd.), sesame oil and the insecticide demonstrated synergistic effects (Abdel-Hafez and Abdel-Aziz, 2010). Additionally, an 8:2 binary mixture of sesame oil and clove oil had synergistic effects against adult *Callosobruchus maculatus* (Soe et al., 2019). In a recent study conducted by Ibrahium et al. (2022), sesame oil and geranium oil possess synergistic effect in controlling cattle ticks.

The effectiveness of natural insecticides has recently improved thanks to nanoemulsions technology (Pant et al., 2014; Khoshraftar et al., 2019). This method demonstrated the dispersion of poorly water-soluble compounds, such as EOS, in an aqueous medium, which protects the active components against degradation and inactivation, increases effectiveness against the target vector and minimizes the hazardous effect on the environment (Volpato et al., 2016).

The common house mosquito, *Culex pipiens*, is a blood-sucking pest that infects people worldwide with diseases (Taubes, 1997). Synthetic chemical insecticides are the most powerful technique for controlling mosquitoes. Although it works well, there are several downsides, including pesticide resistance, environmental residues, toxicity that is dangerous to both humans and animals, and pollutants for the ecosystem (Khan et al., 2015; Xing et al., 2021).

Cinnamon essential oil (EO) showed significant antibacterial activity against foodborne pathogens; *Escherichia coli and Staphylococcus aureus* (Zhang et al., 2016), and *Pseudomonas aeruginosa* (El-Atki et al., 2019) *S. typhimurium* (Park et al., 2017) and (Vasconcelos et al., 2018). The hematologic malignancy cell line is significantly inhibited from proliferating when the cinnamon extract is used (Schoene et al., 2005). Additionally, it has been noted that cinnamon's active ingredients increase the cytotoxicity of various cancer cell types (Dutta and Chakraborty, 2018).

The current study aimed to evaluate the safety, antibacterial and mosquitocidal activities of the produced formulations of cinnamon (nanoemulsion form and as a binary mixture with sesame oils).

MATERIAL AND METHODS

Source of the used materials

Cinnamon zylinicum and sesame seed oils were purchased from the local market. The binary mixtures from cinnamon and sesame oils were prepared at a rate of (1:3) for all concentrations. The analysis of cinnamon and sesame oils were performed at Nawah Scientific Educational Research Center, Egypt (https://nawah-scientific.com/) using GC-MS, TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA).

Preparation of nanoemulsions from the EOs

The technique used by Nirmala et al. (2020) to create the nanoemulsions (NE). Essential oils (EO) combined with Tween 80 as a surfactant (one oil to three T80), then water was added to achieve a concentration of 2.50%, and they mixed using a magnetic stirrer (speed of 500 rpm for 10 min). Using an ultrasonicator, the prepared macroemulsion sonicated for five minutes (750 W, Branson Probe sonicator-Advanced model, 20 kHz). The produced nanoemulsions were evaluated. using a UV-visible spectrophotometer (UV-2600, Shimadz, Japan) at 345 nm.

Characterization of nanoemulsions (NE)

The droplet size distribution (d, nm) (analysis by volume) and polydispersity index (PDI) of nanoemulsions were measured by a zeta sizer apparatus (dynamic light scattering technique) (Nano-ZS90, Malvern, UK). Prior to the experiment, all the samples were diluted to 10% with deionized water in order to reduce the effects caused by multiple scattering effects.

Evaluation of the safety of cinnamon formulations (cytotoxic effect) on normal and cancer cells of human skin fibroblast

The used cells (Human Skin Fibroblast, HSF, and Human epidermoid Skin carcinoma) were sustained in DMEM media (complemented with streptomycin 100 mg/L and penicillin 100 units/mL and fetal bovine serum 10%). This media is prepared at 5% (v/v) CO2 atmosphere at a temperature of 37°C. Five concentrations of the two forms of dlimonene (DL and DLN) (0.0003, 0.003, 0.030, 0.30, and 3.00%) were prepared to test the viability of the cells by SRB assay (Routine Analysis IC50). The cell suspension ($5x10^3$ cells) aliquots of 100 µL were put in plates (96-well) and incubated in the same media for 24h. Another treatment of these cells was done with aliquot of 100 µL media containing different concentrations of DL/DLN. At 72 h post-exposure of cells to the DL/DLN, it was fixed by changing media with 150 µL of 10% TCA, then incubated for 1 h at 4°C. The solution of TCA was castoff, and the cells were washed five times with distilled water. SRB solution aliquots of 70 µL (0.4% w/v) were added and then incubated in a dark place for 10 min at room temperature. Each treated plate was washed three times with acetic acid (1%) and air-dried overnight. Then, 150 µL of TRIS (10 mM) was added to dissolve the protein-bound SRB stain; the absorbance measured at 540 nm (BMG LABTECH®- FLUOstar Omega microplate reader, Ortenberg, Germany) according to Allam et al. (2018) and Skehan et al. (1990). This procedure was conducted at Nawah Scientific Inc., (Mokatam, Cairo, Egypt).

Antimicrobial activity of the prepared forms of cinnamon essential oil

Preparing inoculum (Colony suspension method)

Salmonella typhimurium, Staphylococcus aureus, and Escherichia coli discs were separately added to 100 ml of Tryptic Soy Broth medium and cultured for 24.0 2.0 hours at 37.0-1.0°C. A loopful of broth was spread over Tryptic Soy Agar medium and incubated at 37.0°C for 21.0-3.0 hours in order to prepare a fresh (18-24 h) culture agar plate. By adding three to four colonies (from an organism plate) to a straight sterile saline solution, a turbidity corresponding to a 0.5 McFarland standard was achieved, and the inoculum density was standardized using a 0.5 McFarland standard and a Densi chek optical instrument. *Salmonella typhimurium, Staphylococcus aureus, and Escherichia coli* suspensions were individually adjusted to contain about 1.0 X 108 CFU/mL. The suspension was diluted to 1.0 X 107 CFU/mL by dilution with 9.0 mL of buffered peptone water from 1.0 mL (BPW).

Broth micro-dilution method

From the buffered Peptone Water, 100 µl from was inoculated into a raw of 8 wells, (escaping the first well). Then 200 µl from each sample was directly inoculated in the first well in 96 wells plate (without dilution), then 100 µl was aspirated and transferred to the next well, previously filled with 100 µl BPW, to make 1:2 dilution, mixed well, then 100 µl was aspirated from 1:2 dilution using the new tip and added to the next 100 µl broth (1:4 dilution), this step was repeated for preparing at least eight dilutions for the remaining wells of each antimicrobial sample. A 100 µl of the prepared inoculum was added to each well. That should result in final concentration of 5.0 X 10^5 CFU/mL (the recommended concentration is 2-8 X 10^5 CFU/mL). Another 100 µl from each organism suspension was diluted and cultured (externally) to confirm inoculum density. A growth control well containing inoculated broth, without sample, was added to each sample/plate. A negative control well containing only the broth without a sample nor bacteria was added to each sample plate. All plates were incubated at 35.0°C±1.0°C for 24.0±2.0 hr. After incubation, plates were removed from the incubator and placed on a dark surface to check growth. All growth control wells yielded turbid solution of growth, indicating the validity of the test. All negative control wells were found clear, indicating the validity of the test. Inoculum density culture results were confirmed to have a concentration of 4-6 X 10^5 CFU/mL for all tested organisms.

Mosquitocidal activity

The adult and pupae of *Culex pipiens* were supplied by a mosquito colony in Parasitology department, faculty of veterinary medicine, Beni-Suef University, Beni-Suef, Egypt.

Pupicidal bioassay

In this bioassay, the World Health Organization's recommended technique was utilized (WHO, 2005). 250 mL plastic mugs were used. The tested concentrations of the produced compounds were dissolved in 70% ethyl alcohol at 0.312 to 10%, and then the working solution was made by aliquoting one mL of these dilutions and adding it to 99mL distilled water. Twenty *Culex pipens* pupae were added to the concentrations that had been created in the plastic cups (five replicates for each concentration). Pupae were exposed to one mL of the solvent diluted in water as part of the negative control. A dosage of 6.5 ppm of deltamethrin was utilized as positive control. After 24 hours, the immobile pupae that were dead were noted, and the average percentage of mortality was calculated.

Adulticidal bioassay

According to the recommendations of the Centers for Disease Control and Prevention, the adulticidal bioassay was completed (CDC, Centers for Disease Control and Prevention, 2012). A five concentrations (50, 20, 15, 10, and 7 mg/mL) were prepared by solubilizing, the cinnamon and sesame oils in ethyl alcohol at a 70% concentration. A 295 mL glass bottle was filled with 1.0 mL of each concentration. Deltamethrin (1.7 ug/ml) served as positive control, while ethanol (70%) served as a negative one. Rotating the container to coat all the sides, gently stirred the contents. The bottle's cap was then taken off, and it was continually rolled on its side to allow all the liquid to escape. The bottle was left horizontally for 12 h. Fifteen female mosquitoes were introduced into each test bottle by using a mouth aspirator. After 90 minutes, with readings every 15 minutes, the number of dead and live mosquitoes was counted. In this test, a mosquito is deemed alive if it can fly, regardless of how many of its legs are still attached; otherwise, it is deemed dead or knocked over if it is motionless, unable to fly, or unable to stand steadily. All bioassays were conducted at a 28 °C temperature of and 80% relative humidity.

Statistical analysis

Five repetitions of each treatment were carried out, and mean, and SE values were calculated. ANOVA was used to analyze larval mortality, followed by Duncan's multiple range tests (p 0.05). Probit analysis was used to calculate the LC_{50} and LC_{90} values with their respective 95% confidence limits (Finney, 1952). All statistical analyses were performed using SPSS for Windows (version 22.0). The synergistic factor (SF) was calculated by dividing the LC_{50} value of the individual test pesticide by the equivalent LC_{50} value of the test insecticide + synergist mixture (Chou, 2006). If the synergistic component is = 1 - No effect, > 1 - Synergistic effect.

RESULTS AND DISCUSSION

GC-MS PHYTOCHEMICAL COMPOSITION OF CINNAMON ESSENTIAL OIL

Cinnamaldehyde represents the most common constituent of cinnamon essential oil (63.42%). Other constituents were cinnamaldehyde dimethyl acetal (13.16%), cinnamaldehyde à-hexyl- (11.75%), 2-propenoic acid, 3 phenyl-methyl ester (6.09%), and traces of benzene acetaldehyde, n-Butyl cinnamate, 1-Hexen-3-ol, 5-nitro-1-phenyl-, (R*,R*)- (Supplementary Table 1).

CHARACTERIZATION OF CINNAMON NANOEMULSION (CNE) FORMATION

The mean droplet size of nanoemulsion was around 391.7 d, nm with polydisperisty index (PDI) 0.979. The homogeneous size distribution of cinnamon indicated by the low value of PDI (Supplementary Figure 1).

CELL CYTOTOXICITY

At all dilutions, the cinnamon standard form (2.5% dissolved in 70% ethyl alcohol) proved safe for the normal cell line of human skin fibroblast (Table 1, Supplementary Figure 2). CNE form was entirely poisonous at a concentration of 2.50%, having been safe up to a concentration of 0.025% (Table 1, Supplementary Figure 2). At the high dose of 2.5%, the CS form was hazardous to 75% of normal cells (Table 1, Supplementary Figure 2). The common form of cinnamon was safe for the cancer cell line of Human epidermoid Skin carcinoma (Table 2, Supplementary Figure 3). At the highest dosage, 2.5% CNE form was fully toxic to cancer cells (Table 2, Supplementary Figure 3). Up to the highest concentrations, the CS form was safe for cancer cells, but after that point, the cells started to die, and just 42.07 percent of them were still alive (Table 2, Supplementary Figure 3).

ANTIMICROBIAL ACTIVITY

For all the investigated bacteria, *Staphylococcus aureus, Escherichia coli,* and *Salmonella typhimurium*, the lowest inhibitory concentration of regular cinnamon was 0.625% (Table 3). While cinnamon in the form of nanoemulsion significantly reduced microbial growth in which *S. aureus* was inhibited at 0.078%, while *S. typhimurium and E. coli* were inhibited at 0.039%. On the other hand, the cinnamon-sesame combination, demonstrated the least effective antibacterial action at a concentration of 1.25% (Table 3).

PUPICIDAL ACTIVITY OF CINNAMON PREPARED FORMULATIONS

With 322 ug/mL for CNE and 465 ug/mL for ordinary essential oil, the CNE form achieved LC_{50}, which lower than the cinnamon standard form. Additionally, the LC_{50} for CS was 928 ug/mL as opposed to 1170 ug/mL for sesame oil alone. At LC_{50} of 2375ug/mL (594C + 1792S),

Table 1: Cytoxicity of cinnamon formulations on human skin fibroblast cell line.

Concentrations (µg/ml)	Cinnamon		CNE		CS	
	Mean viable cells	STD	Mean viable cells	STD	Mean viable cells	STD
Control negative	100.00	00.00	100.00	0.00	100.00	0.00
0.00025%	96.73	0.409	99.32	0.18	98.60	0.22
0.0025%	92.52	0.466	97.50	0.16	94.51	0.41
0.025%	85.30	0.590	94.69	1.59	93.01	1.32
0.25%	83.93	0.260	34.64	0.95	86.38	1.37
2.50%	82.22	0.79	0.104	0.054	24.17	0.84
Blank	100.00	100.00	100.00	0.00	100.00	0.00

Table 2: Cytoxicity of cinnamon different formulations on human epidermoid skin carcinoma.

Concentrations (µg/ml)	Cinnamon		CNE		CS	
	Mean viable cells	STD	Mean viable cells	STD	Mean viable cells	STD
Control negative	100.00	00.00	100.00	0.00	100.00	0.00
0.00025%	94.18	1.08	94.07	1.39	97.52	1.04
0.0025%	92.27	1.07	90.44	0.97	95.01	0.07
0.025%	87.57	0.76	85.21	1.07	91.22	0.43
0.25%	83.08	1.68	69.13	0.59	86.77	0.16
2.50%	77.72	1.29	0.21	0.11	42.07	0.95
Blank	100.00	100.00	100.00	0.00	100.00	0.00

C= Cinnamon, CNE= cinnamon nanoemulsion, S = sesame oil, CS = cinnamon-sesame mixture at rate of 1:3.

Table 3: Antimicrobial effect by minimum inhibitory concentration of cinnamon formulations against *Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium.*

Dilution (%) organism	Cin form	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019	0.009
Staphylococcus aureus	C	S	S	S	R	R	R	R	R	R
Salmonella typhimurium		S	S	S	R	R	R	R	R	R
Escherichia coli		S	S	S	R	R	R	R	R	R
Staphylococcus aureus	CNE	S	S	S	S	S	S	R	R	R
Salmonella typhimurium		S	S	S	S	S	S	S	R	R
Escherichia coli		S	S	S	S	S	S	S	R	R
Staphylococcus aureus	CS	S	S	R	R	R	R	R	R	R
Salmonella typhimurium		S	S	R	R	R	R	R	R	R
Escherichia coli		S	S	R	R	R	R	R	R	R

C= Cinnamon, CNE= cinnamon nanoemulsion, S = sesame oil, CS = cinnamon-sesame mixture at rate of 1:3. S= susceptible, R= resistant.

Table 4: LC_{50} and LC_{90} of different formulations of cinnamon oil against *Culex pipiens* pupae.

Treatment	LC_{50} (95% CI) (ug/mL)	LC_{90} (95% CI) (ug/mL)	SLOPE ± SE	Synergistic factor
C	465 (382-571)	912 (740-1289)	0.005 ± 0.001	-
CNE	322 (258-408)	787 (629-1133)	0.005 ± 0.001	1.45
S	1170 (867-1506)	3202 (2542-4699)	0.001 ± 0.00	-
CS (1:3)	928 (232C+696S) (694-1193)	2375 (594C+1792S) (1860-3751)	0.002 ± 0.00	1.70 for S 1.50 for C

C= Cinnamon, CNE= cinnamon nanoemulsion, S = sesame oil, CS = cinnamon-sesame mixture at rate of 1:3.

CS caused the most fatalities (Table 4). Regarding the LC_{90}, the CNE form had 100% pupicidal action at a concentration of 787ug/mL, whereas cinnamon oil only attained 100% at the highest dose of 912ug/mL (Table 4). The LC_{90} of sesame oil was 3202 ug/mL causing significant mortality of pupae reached 95% (Table 4). The binary mixture of cinnamon + sesame oil showed a synergistic effect of 1.70 for Sesame and 1.50 for cinnamon (Table 4). The toxicity of C and CNE against pupae was reported in Table 5. The low concentrations of ≤ 250 ug/mL caused non-significant mortality to the pupae. Deltamethrin demonstrated 100% mortality of pupae (Table 5). Moreover, the lowest doses of sesame oil and the CS mixture revealed negligible mortality (Tables 6 and 7).

Table 5: Pupicidal effect of cinnamon oil at different concentrations.

Treatment	Pupicidal / 20 (5 replicates) Mean mortality ±Std. Error	
	Nano emulsion	Ordinary form
DMSO	0.40±.2^{a}	0.40±.2^{a}
Delta 1.7	20.0±.0^{e}	20.0±.0^{e}
1000 ug/mL	20.0±.0^{e}	19.4±.2^{e}
500 ug/mL	15.8±.3^{d}	11.8±.3^{c}
250 ug/mL	11.2±.3^{c}	4.8±.3^{b}
125 ug/mL	4.4±.2^{b}	1.0±.3^{a}
62.50 ug/mL	0.6±.2^{a}	0.2±.2^{a}

Superscript of the same letter in cells of the same column is non-significant with the control. Superscript of different letters in cells of the same column is significant with the control ($P \leq 0.05$).

Table 6: Pupicidal activity of sesame oil.

Item	Sesame oil Mean mortality rate ± Std. error
Control negative DMSO	0.4±.2^{a} (2%)
Control deltamethrin 1.7	20.0±.0^{e} (100%)
3000 ug/mL	19.4±.2^{e} (97%)
1500 ug/mL	15.8±.3^{d} (79%)
750 ug/mL	7.8±.3^{c} (39%)
375 ug/mL	2.6±.2^{b} (13%)

Superscript of the same letter in cells of the same column is non-significant with the control. Superscript of different letters in cells of the same column is significant with the control ($P \leq 0.05$).

Table 7: Pupicidal activity of the binary mixture cinnamon-sesame oils at a rate of (1:3)

Item	Cinnamon-sesame (CS) Mean mortality rate (± Std. error)
Control negative DMSO	0.8±.2^{a} (4%)
Control Deltamethrin 3.5uL/L	20.0±.0^{e} (100%)
2000 ug/mL (1500ugS +500ug C/mL)	20.0±.0^{e} (100%)
1000 ug/mL (750ugS+ 250ug C /mL)	16.4±.2^{d} (82%)
500 ug/mL (375ug S+ 125ug C /mL)	9.8±.3^{c} (49%)
250 ug/mL (187ug S + 63ug C/mL)	2.6±.2^{b} (13%)

Superscript of the same letter in cells of the same column is non-significant with the control. Superscript of different letters in cells of the same column is significant with the control ($P \leq 0.05$).

Table 8: LC_{50} and LC_{90} of different formulations of cinnamon oil against *Culex pipiens* adult.

Treatment time (minutes)	LC_{50} (95% CI)	LC_{90} (95% CI)	SLOPE ± SE
Cinamon 15 min	69.6 (56.7-90.1)	135 (108-202)	0.03 ± 0.008
Cinamon Emulsion 15 min	55.5 (40-75)	146 (111-240)	0.026 ± 0.006
Cinamon 30 min	55.75 (40-76)	150 (114-249)	0.024 ± 0.006
Cinamon Emulsion 30 min	27 (17-37)	69 (52-131)	0.05 ± 0.01
Cinamon 60 min	39 (24-54)	113 (84-212)	0.03 ± 0.009
Cinamon Emulsion 60 min	21 (6-30)	62 (45-147)	0.05 ± 0.02
Cinamon 90 min	23 (11-31)	59 (44-134)	0.06 ± 0.02
Cinamon Emulsion 90 min	15.8 (-)	23.6 (-)	± 2.4

Adulticidal activity of cinnamon-prepared formulations

Cinnamon Eos's adulticidal action demonstrated a dose/time reverse relationship. Adult insect toxicity was caused by the high concentrations of 69.60 ug/mL (LC_{50}) and 135.00 ug/mL (LC_{90}) in a short amount of time (15 min) (Table 8). At 90 minutes, the concentration was at its lowest (23.00 ug/mL) as time passed, and the concentration fell. After 90 minutes, the CNE's concentration was at its lowest (15.80ug/mL) (Table 8). The mature *C. pipiens* is not harmful when exposed to such, though. The adulticidal action of cinnamon oil increased with exposure time, with 100% mortality being reached at the highest concentrations (250-125ug/mL) after only 15 minutes of exposure. Meanwhile, 100% mortality was reached at the lowest concentration (62.5ug/mL) during the greatest exposure time of 90 min. Additionally, CNE form increased the efficacy; after 90 minutes, 31.25ug/mL produced 100% mortality. After 15 minutes, a reference insecticide (1.7 ug/ml detramethrin) demonstrated 100% death (Table 8).

The massive use of chemical insecticides linked to a huge public health risks also environmental damage, and insect resistance (Govindarajan and Sivakumar, 2011). Cinnamon oil has powerful insecticidal properties (Volpato et al., 2016; Thomas et al., 2017). Our research sought to increase the effectiveness of cinnamon oil against various pupae and adult stages of *C. pipiens* by applying it in nanoemulsion form and combining it with sesame oil.

Results pertaining to the safety of prepared forms of cinnamon show that all prepared concentrations of the ordinary form are safe for both normal and malignant cells. For both types of cells, the nanoemulsion form is hazardous at the greatest concentration (2.5%). Additionally, the maximum dosage of the cinnamon-sesame oil mixture is harmful to both cell types. According to Larasati and Meiyanto (2018), cinnamon and its constituents exhibit anti-cancer and cancer prevention activities through various mechanisms, including suppression of tumor-promoted inflammation, immunomodulation, induction of cell death, anti-angiogenesis, and modulation of redox homeostasis. Additionally, Yufei et al. (2020) discovered that cinnamaldehyde, one of the primary components of cinnamon, may aid in the therapy of breast cancer by interacting with 59 significant potential targets. Sadeghi et al. (2019) showed that cinnamon, when used as a medicinal drug, inhibits various cancer cell apoptosis-related processes. Here, we demonstrate cinnamon nanoemulsion ability to fight cancer, which mostly happens via altering apoptosis-related processes. Moreover, CS at the concentration of 2.5% was toxic to 58% of cancer cells. This finding is supported by the results of Majdalawieh et al. (2020), who found that sesame oil increased the cytotoxic activity of natural killer cells against YAC-1 tumor cells.

Among the three forms of cinnamon, CNE showed potent antimicrobial activity against the selected microorganisms. By employing minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against five common foodborne pathogenic bacteria, Shan et al. (2007) reported the antibacterial efficacy of cinnamon stick extract (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, and Salmonella anatum*). Fish products can benefit from cinnamon essential oil's natural antibacterial component, which helps maintain quality and increase shelf life (Chuesiang et al., 2020). Additionally, cinnamon essential oil interacts with bacterial cell membranes to impede enzymatic activity and cell wall production, which contributes to the antibacterial action of the compound (Chuesiang et al., 2019). Gram-negative bacteria were more susceptible to the antimicrobial effects of the cinnamon essential oil nanoemulsions than Gram-positive bacteria (Liu et al., 2021). They demonstrated that nanoemulsions with enhanced stability and smaller particle size were created.

According to the constituents of cinnamon essential oil, cinnamonaldehyde (E)- (63.42%), was the main component of *C. zylinicum*. These results corroborated those of Cheng et al. (2009), Aungtikun and Soonwera (2021), and Xing et al. (2021), who noted that cinnamaldehyde, though present in varying amounts, was the main element of cinnamon essential oil. According to Volpato et al. (2016),

41.27% of *C. zeylanicum* oil was cinnamaldehyde, which proved beneficial in controlling mealworms (*Alphitobius diaperinus*). According to Deletre et al. (2013), it has an irritating, repulsive, and poisonous effect on A. gambia at a rate of 80%. Cinnamaldehyde (98.3%), which Andrade-Ochoa (2018) discovered in C. verum, was very effective against *Culex quinquefasciatus* larvae and pupae. Benelli et al. (2018) measured cinnamaldehyde at a rate of 82.7% and achieved a highly promising pesticide action against the housefly *Musca domestica and Culex quinquefasciatus.* According to Nakasen et al. (2021), cinnamon repels *Culex quinquefasciatus* adults, eggs, and larvae stages quite effectively. According to studies by Batiha et al. (2020), Kallel et al. (2019), and Liang et al. (2019), the essential oil from the bark of *C. zeylanicum* had the highest concentration of cinnamaldehyde (77.34%), had a strong antioxidant effect, and was effective against mouse piroplasm. At the same time, Thomas et al. (2017) found that the constituent of cinnamon essential oils including eugenol (96.5%), achieved a high larvicidal effect against *Anophles Gambia* more than clove oil.

Regarding pupicidal action, cinnamon oil had a smaller impact on pupa than on *C. pipiens* larvae (465 ug/ml), while the impact was more in CNE form (322 ug/ml). These results support Andrade-Ochoa's (2018) observation that pupae are more resistant to cinnamon oil than larvae. The cinnamon oil's LC_{50} against pupae of *C. quinquefasciatus* after a 24-hour exposure was 216.7 ug/ml, compared to 24.5 ug/ml for larvae. According to Suresh et al. (2020), the LC_{50} value of sea fennel essential oil was 17.911 L/L, which was very harmful to *Ae. aegypti* pupae. In a 2019 study, Kaura et al. (2019) found that eucalyptus had a pupicidal impact on *Aedes* mosquitoes with LC_{50} values of 144.5 ppm and LC_{90} values of 741.3 ppm, while neem oil had LC_{50} values of 19,054 ppm and LC_{90} values of 19,952 ppm. *Zanthoxylum limonella* oil was found to be toxic to *A. aegypti* pupae after 24 hours of exposure, with mortality of 18% at 1% concentration and rising to 100% at a concentration of 10%. Mortality of *C. quinquefasciatus* pupae at 1%, 5%, and 10% concentrations was noted as 7%, 59%, and 100% for pupal stage after 24 hours of exposure to the oils, respectively. The pupicidal activity increased with the nanoemulsion form, which was in agreement with Mishra et al. (2019), who reported that permethrin nanoemulsion had significantly higher pupicidal activity than the ordinary form against *Culex tritaeniorhynchus* and *Aedes aegypti*, with LC_{50} values at 24 h for PNE being 0.021 and 0.048 mg L-1, respectively. Additionally, *Citrus hystrix* nanoemulsion essential oil demonstrated larvicidal and pupicidal effect on larva and pupa of *Aedes aegypti*, according to Subekti et al. (2020), and it is more environmentally friendly.

The LC_{50} and LC_{90} values in a short time (15 min) were 69.60 ug/mL and 135.00 ug/mL, respectively, for the adulticidal impact of cinnamon Eos. The LC_{90} was 23.00ug/mL after 90 minutes. After 90 minutes, the CNE's LC_{90} value was the lowest (15.80 ug/mL). This conclusion is corroborated by a study by Prajapati (2005) who exposed that adults of *A. stephensi, A. aegypti, and C. quinquefasciatus* were vapor toxic to the essential oil *C. zeylanicum* after 1h with LD95 values of 286.2, 285.9, and 275.9 mg/mat, respectively.

After a 24-hour exposure, Chansang et al. (2018) and Aungtikun and Soonwera (2021) found that cinnamon oil's LC_{50} against *Ae Aegypti* was less than 3.5 ug/mg female. Using of cinnamon oil at 10%/cm2 by impregnated paper method by Ramar et al. (2017) recorded 96% mortality against adult *Culex quinquefasciatus* mosquitoes after 24 hours. *Zanthoxylum limonella* oil caused adult mortality after 24 hours, according to Soonwera and Phasomkusolsil's (2017), with LC_{50} values of 6.0% for *Ae. aegypti* and 5.7% for *C. quinquefasciatus*. Olivera et al. (2021) discovered that *Thymus vulgaris* L. and *Origanum vulgare* L. have addulticidal effects against *Aedes aegypti* L, with LC_{50} values of 14.3 and 11.7 mg/mL, respectively. The results showed that cinnamon oil (10%) and nanoemulsion (5%) were 100% effective against *M. domestica* after 90 minutes of exposure. Boito et al. (2018) found that the addulticidal effect of cinnamon oil against *C. pipiens* was significantly improved by the nanoemulsion form. Mossa et al. (2017) investigated the higher insecticidal activity of camphor nanoemulsion against wheat weevil, *Sitophilus granaries*. Additionally, Heydari et al. (2020) exposed that the pest cotton aphid showed comparatively high contact toxicity to peppermint oil nanoemulsion, with LC_{50} of 3879.5 l/L.

The nanoemulsion form was more soluble, dispersible in water, and, therefore more efficient than the conventional form in achieving high pupicidal and adulticidal action (Duarte et al., 2015). In comparison to bulk/original EO, nanoemulsions' high surface area and low surface tension that's disseminate and permeate active ingredients in the target region, which may enhance the pharmacodynamics capabilities of nanoemulsions (Pavoni et al., 2019). Additionally, nanoemulsion forms are efficient, secure, and environmentally beneficial because they degrade naturally and have few adverse impacts on both the environment and non-target organisms (Sundararajan et al., 2018; Mohafrash et al., 2020).

CONCLUSIONS AND RECOMMENDATIONS

Better pupicidal and adulticidal effects than the standard cinnamon Eos form were produced using nanoemulsion technology and binary mixing of sesame oil with

cinnamon Eos. This formulation for controlling *C. pipiens* is economical and environmentally friendly.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deputyship for Research and Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number ISP20-5.

NOVELTY STATEMENT

The novelty of the work Preparation of cinnamon formulations effective against mosquitoes.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the conception, design, data collection, analysis, interpretation of results, and writing of the manuscript. They all provided critical feedback and approved the final version for submission.

SUPPLEMENTARY MATERIAL

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.aavs/2023/11.3.475.484

Abbreviations

C. pipiens= *Culex pipiens;* EO= essential oil; C= cinnamon; CNE= cinnamon nanoemulsion; S= sesame oil; CS= 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.

Data availability statement

All data are available in the manuscript and its supplementary materials

Conflicts of interest

The authors have declared no conflict of interest.

REFERENCES

Abdel-Hafez HF, Abdel-Aziz MA (2010). Synergistic effects of some plant extracts to biorational product, spinosad against the cotton leaf worm, *Spodoptera littoralis* (Biosd.) (Lepidoptera: Noctuidae). Egypt. J. Biol. Pest Contr., 20: 27-23.

Allam RM, Al-Abd AM, Khedr A, Sharaf OA, Nofal SM, Khalifa AE, Mosli HA, Abdel-Naim AB (2018). Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. Toxicol. Lett., 291: 77-85. https://doi.org/10.1016/j.toxlet.2018.04.008

Andrade-Ochoa S, Sánchez-Aldana D, Chacón-Vargas KF (2018). Oviposition deterrent and larvicidal and pupaecidal activity of seven essential oils and their major components against *Culex quinquefasciatus* Say (Diptera: Culicidae): Synergism-antagonism effects. Insects, 9(1): 25. https://doi.org/10.3390/insects9010025

Aungtikun J, Soonwera M (2021). Improved adulticidal activity against *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) from synergy between *Cinnamomum* spp. essential oils. Sci. Rep., 11(1): 4685. https://doi.org/10.1038/s41598-021-84159-z

Baranitharana M, Dhanasekaran S, Gokulakrishnanbb J, Krishnappac K, Deepab J (2015). Mosquito larvicidal properties of *Sesamum indicum* L. against *Aedes aegypti* (Linn.), *Anopheles stephensi* (Liston), *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Life Sci. Arch., 1(2): 72–77.

Batiha GE, Beshbishy AM, Guswanto A, Nugraha A, Munkhjargal T, Abdel-Daim M, Mosqueda J, Igarashi I (2020). Phytochemical characterization and chemotherapeutic potential of cinnamomum verum extracts on the multiplication of protozoan parasites *in vitro* and *in vivo*. Molecules, 24, 25(4): 996. https://doi.org/10.3390/molecules25040996

Benelli G, Pavela R, Giordani C, Casettari L, Curzi G, Cappellacci L, Petrelli R, Maggi F (2018). Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly Musca domestica. Ind. Crop Prod., 112: 668-680. https://doi.org/10.1016/j.indcrop.2017.12.062

Boito JP, Da Silva AS, dos Reis JH, Santos DS, Gebert RR, Biazus AH, Machado G (2018). Insecticidal and repellent effect of cinnamon oil on flies associated with livestock. Rev. MVZ Córdoba, 23(2): 6628-6636. https://doi.org/10.21897/rmvz.1337

CDC, Centers for Disease Control and Prevention (2012). Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. CDC Methods, pp. 1–28.

Chansang A, Champakaew D, Junkum A, Amornlerdpison D, Chaithong U, Jitpakdi A, Pitasawat B (2018). Potential of natural essential oils and cinnamaldehyde as insecticides against the dengue vector Aedes aegypti (Diptera: Culicidae). Southeast Asian J. Trop. Med. Publ. Health, 49(1): 6-22.

Cheng SS, Liu JY, Huang CG, Hsui YR, Chen WJ, Chang ST (2009). Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species. Bioresour. Technol., 100: 457-464. https://doi.org/10.1016/j.biortech.2008.02.030

Choi WS, Park BS, Lee YH, Jang DY, Yoon HY, Lee SE (2006). Fumigant toxicities of essential oils and monoterpenes against *Lycoriella mali* adults. Crop Prot., 25: 398–401. https://doi.org/10.1016/j.cropro.2005.05.009

Chuesiang P, Sanguandeekul R, Siripatrawan U (2020). Phase inversion temperature-fabricated cinnamon oil nanoemulsion as a natural preservative for prolonging shelf-life of chilled Asian seabass (*Lates calcarifer*) filets. LWT Food Sci. Technol., 125: 109–122. https://doi.org/10.1016/j.lwt.2020.109122

Chuesiang P, Siripatrawan U, Sanguandeekul R, McClements DJ, McLandsborough L (2019). Antimicrobial activity of PIT-fabricated cinnamon oil nanoemulsions: Effect of surfactant concentration on morphology of foodborne pathogens. Food Contr., 98: 405–411. https://doi.org/10.1016/j.

foodcont.2018.11.024

Dai DN, Chung NT, Huong LT, Hung NH, Chau DTM, Yen NT, Setzer WN (2020). Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils from five species of cinnamomum growing wild in north central Vietnam. Molecules, 25(6): 1303. https://doi.org/10.3390/molecules25061303

de Oliveira AA, Franaca LP, Ramos AS, Ferreira JLP, Maria ACB, Oliveira KM, Jr ESA, da Silva JN, Branches ADS, Barros GA, da Silva NG, Tadei WP, Amaral ACF, de Andrade Silva JR (2021). Larvicidal, adulticidal and repellent activities against *Aedes aegypti* L. of two commonly used spices, *Origanum vulgare* L. and *Thymus vulgaris* L. S. Afr. J. Bot., 14: 17-24. https://doi.org/10.1016/j.sajb.2021.03.005

Deletre E, Martin T, Campagne P, Bourguet D, Cadin A, Menut C, Bonafos R, Chandre, F (2013). Repellent, irritant and toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* mosquito. *PLoS One*, 8(12): e82103. https://doi.org/10.1371/journal.pone.0082103

Duarte JL, Amado JRR, Oliveria AEMFM, Cruz RAS, Ferreira AM, Souto RNP, Falcão DQ, Carvalho JCT, Fernandes CP (2015). Evaluation of larvicidal activity of a nanoemulsion of *Rosmarinus officinalis* essential oil. Rev. Bras. Farmacogn., 25(2): 189–192. https://doi.org/10.1016/j.bjp.2015.02.010

Dutta A, Chakraborty A (2018). Cinnamon in anticancer armamentarium: A molecular approach. J. Toxicol. Article ID 8978731. https://doi.org/10.1155/2018/8978731

El-Atki Y, Aouam I, El-Kamari F, Taroq A, Nayme K, Timinouni M, Lyoussi B, Abdellaoui A (2019). Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. J. Adv. Pharm. Technol. Res., 10(2): 63–67. https://doi.org/10.4103/japtr.JAPTR_366_18

Finney DJ (1952). Probit analysis: A statistical treatment of the sigmoid response curve. Cambridge University Press, Cambridge.

Govindarajan M, Sivakumar R (2011). Larvicidal and repellent effect of some *Tribulus terrestris* L., (*Zygophyllaceae*) extracts against the dengue fever mosquito, Aedes aegypti (Diptera: Culicidae). Asian Pac. J. Trop. Med., (3): 941-947.

Heydari M, Amirjani A, Bagheri M, Sharifian I, Sabahi Q (2020). Eco-friendly pesticide based on peppermint oil nanoemulsion: Preparation, physicochemical properties, and its aphicidal activity against cotton aphid. Environ. Sci. Pollut. Res. Int., 27(6): 6667-6679. https://doi.org/10.1007/s11356-019-07332-y

Ibrahium SM, Aboelhadid SM, Wahba AA, Farghali AA, Miller RJ, Abdel-Baki AAS, Al-Quraishy S (2022). Preparation of geranium oil formulations effective for control of phenotypic resistant cattle tick *Rhipicephalus annulatus*. Sci. Rep., 12(1): 1-13. https://doi.org/10.1038/s41598-022-14661-5

Kallel I, Hadrich B, Gargouri B, Chaabane A, Lassoued S, Gdoura R, Ben Messaoud E (2019). Optimization of cinnamon (*Cinnamomum zeylanicum* Blume) essential oil extraction: Evaluation of antioxidant and antiproliferative effects. Evid. Based Complement. Altern. Med., https://doi.org/10.1155/2019/6498347

Kaura T, Mewara A, Zaman K, Sharma A, Agrawal SK, Thakur V (2019). Utilizing larvicidal and pupicidal efficacy of Eucalyptus and neem oil against Aedes mosquito: An approach for mosquito control. Trop. Parasitol., 9(1): 12-17.

Khan I, Badshah T, Saeed M, Khan GZ (2015). Testing efficacy of botanical and mineral kerosene oils on Culex quinquefasciatus mortality and their repellency in field ovitraps. Sci. Postprint, 1(2): e00050. https://doi.org/10.14340/spp.2015.05A0001

Khoshraftar Z, Safekordi AA,Shamel A, Zaefizadeh M (2019). Synthesis of natural nano pesticides with the origin of Eucalyptusglobulus extract for pest control. Green Chem. Lett. Rev., 12: 286-298. https://doi.org/10.1080/17518253.2019.1643930

Larasati YA, Meiyanto E (2018). Revealing the potency of cinnamon as an anti-cancer and chemopreventive agent. Indones. J. Cancer Chemoprev., 9(1): 47-62. https://doi.org/10.14499/indonesianjcanchemoprev9iss1pp47-62

Liang Y, Li Y, Sun A, Liu X (2019). Chemical compound identification and antibacterial activity evaluation of cinnamon extracts obtained by subcritical n-butane and ethanol extraction. Food Sci. Nutr., 7(6): 2186-2193. https://doi.org/10.1002/fsn3.1065

Liu X, Chen L, Kang Y, He D, Yang B, Wu K (2021). Cinnamon essential oil nanoemulsions by high-pressure homogenization: Formulation, stability, and antimicrobial activity. 147: 111660. https://doi.org/10.1016/j.lwt.2021.111660

Majdalawieh AF, Farraj JF, Carr RI (2020). *Sesamum indicum* (sesame) enhances NK anti-cancer activity, modulates Th1/Th2 balance, and suppresses macrophage inflammatory response. Asian Pac. J. Trop. Biomed., 10: 316-324. https://doi.org/10.4103/2221-1691.284946

Mishra P, Dutta S, Haldar M, Dey P, Kumar D, Mukherjee A, Chandrasekaran N (2019). Enhanced mosquitocidal efficacy of colloidal dispersion of pyrethroid nanometric emulsion with benignity towards non-target species. Ecotoxicol. Environ. Saf., 176: 258-269. https://doi.org/10.1016/j.ecoenv.2019.03.096

Mohafrash SM, Fallatah SA, Farag SM, Mossa ATH (2020). Mentha spicata essential oil nanoformulation and its larvicidal application against Culex pipiens and Musca domestica. Ind. Crops Prod., 157: 112944. https://doi.org/10.1016/j.indcrop.2020.112944

Mossa ATH, Abdelfattah NAH, Mohafrash SMM (2017). Nanoemulsion of camphor (*Eucalyptus globulus*) essential oil, formulation, characterization and insecticidal activity against wheat weevil, sitophilus granarius. Asian J. Crop Sci., 9: 50-62. https://doi.org/10.3923/ajcs.2017.50.62

Nakasen K, Wongsrila A, Prathumtet J, Sriraj P, Boonmars T, Promsrisuk T, Laikaew N, Aukkanimart R (2021). Bio efficacy of cinnamaldehyde from *Cinnamomum verum essential* oil against Culex quinquefasciatus; (Diptera: Culicidae). J. Entomol. Acarol. Res., 53(1). https://doi.org/10.4081/jear.2021.9400

Nirmala MJ, Durai L, Gopakumar V, Nagarajan R (2020). Preparation of celery essential oil-548 based nanoemulsion by ultrasonication and evaluation of its potential anticancer and 549 antibacterial activity. Int. J. Nanomed., 15: 7651-7666. https://doi.org/10.2147/IJN.S252640

Oliveira AE, Duarte JL, Amado JR, Cruz RA, Rocha CF, Souto RN, Ferreira RM, Santos K, da Conceiç˜ao EC, de Oliveira LA, Kelecom A (2016). Development of a larvicidal nanoemulsion with *Pterodon emarginatus* vogel oil. PLoS One, 11: e0145835. https://doi.org/10.1371/journal.pone.0145835

Pant M, Dubey S, Patanjali PK, Naik SN, Sharma S (2014). Insecticidal activity of eucalyptus oil nanoemulsion with Karanja and jatropha aqueous filtrates. Int. Biodeterior. Biodegrad., 91: 119-127. https://doi.org/10.1016/j.ibiod.2013.11.019

Park JB, Kang JH, Song KB (2017). Antibacterial activities of a cinnamon essential oil with cetylpyridinium chloride emulsion against Escherichia coli O157:H7 and *Salmonella typhimurium* in basil leaves. Food Sci. Biotechnol., 27(1): 47–55. https://doi.org/10.1007/s10068-017-0241-9

Pavoni L, Pavela R, Cespi M, Bonacucina G, Maggi F, Zeni V, Canale A, Lucchi A, Bruschi F, Benelli G, (2019). Green micro and nanoemulsions for managing parasites, vectors and pests. Nanomaterials, 9: 1285. https://doi.org/10.3390/nano9091285

Prajapati V, Tripathi A, Aggarwal, KK, Khanuja, SPS (2005). Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Bioresour. Technol., 96: 1749–1757. https://doi.org/10.1016/j.biortech.2005.01.007

Ramar M, Ignacimuthu S, Manonmani P, Murugan K (2017). Adulticidal activity of botanical oils by impregnated paper assay against culex quinquefasclatus say. Int. J. Pharm. Pharm. Sci., 9(5). https://doi.org/10.22159/ijpps.2017v9i5.15764

Sadeghi S, Amirhossein D, Mohammad HP, Nasrin S, Reza AN, Roxana S, Shirin AM, Amirhossein S, Hamed M (2019). Anti-cancer effects of cinnamon: Insights into its apoptosis effects. Eur. J. Med. Chem., 178: 131-140. https://doi.org/10.1016/j.ejmech.2019.05.067

Schoene NW, Kelly MA, Polansky MM, Anderson RA (2005). Water-soluble polymeric polyphenols from cinnamon inhibit proliferation and alter cell cycle distribution patterns of hematologic tumor cell lines. Cancer Lett., 230(1): 134–140. https://doi.org/10.1016/j.canlet.2004.12.039

Shan B, Cai YZ, Brooks JD, Corke H (2007). Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): Activity against foodborne pathogenic bacteria. J. Agric. Food Chem., 55(14): 5484-5490. https://doi.org/10.1021/jf070424d

Shu ZXL, Jie L, Van Der HW (2008). Cinnamomum Flora. China, 7: 166-187.

Singh G, Maurya S, Delampasona MP, Catalanca A (2007). A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem. Toxicol., 45: 1650-1661. https://doi.org/10.1016/j.fct.2007.02.031

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst., 82(13): 1107-1112. https://doi.org/10.1093/jnci/82.13.1107

Soe TN, Ngampongsai A, Sittichaya W (2019). Synergistic effect of sesame oil and clove oil on toxicity against the pulse beetle, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chysomelidae). Khon Kaen Agric. J., 47(suppl).

Soonwera M, Phasomkusolsil S (2017). Adulticidal, larvicidal, pupicidal and oviposition deterrent activities of essential oil from *Zanthoxylum limonella* Alston (Rutaceae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Asian Pac. J. Trop. Biomed., 7: 967–978. https://doi.org/10.1016/j.apjtb.2017.09.019

Subekti N, Indriyanti DR, Soegianto A (2020). Larvicidal and pupicidal activities from *Citrus hystrix* against Aedes aegypti mosquitoes. Ecol. Environ. Conserv., 26(3): 1313-1318.

Sundararajan B, Moola AK, Vivek K, Kumari BDR (2018). Formulation of nanoemulsion from leaves essential oil of *Ocimum basilicum* L. and its antibacterial, antioxidant and larvicidal activities (*Culex quinquefasciatus*). Microb. Pathog., 125: 475-485. https://doi.org/10.1016/j.micpath.2018.10.017

Suresh U, Murugan K, Panneerselvam C, Aziz AT (2020). Encapsulation of sea fennel (*Crithmum maritimum*) essential oil in nanoemulsion and SiO2 nanoparticles for treatment of the crop pest *Spodoptera litura* and the dengue vector *Aedes aegypti*. Ind. Crops Prod., 158: 113033. https://doi.org/10.1016/j.indcrop.2020.113033

Taubes G (1997). A mosquito bites back. N. Y. Times Mag., 40: 4624.

Thomas MHD, Manjurano A, Morona D, Kweka EJ (2017). Evaluation of active ingredients and larvicidal activity of clove and cinnamonessential oils against *Anopheles gambiae* (sensu lato). Adelina Parasit. Vectors, 10: 411. https://doi.org/10.1186/s13071-017-2355-6

Vasconcelos NG, Croda J, Simionatto S (2018). Antibacterial mechanisms of cinnamon and its constituents: A review. Microb. Pathog., 120: 198-203. https://doi.org/10.1016/j.micpath.2018.04.036

Volpato A, Baretta D, Zort´ea T, Campigotto G, Galli GM, Glombowsky P, Santos RCV, Quatrin PM, Ourique AF, Baldissera MD, Stefani LM, Da Silva AS (2016). Larvicidal and insecticidal effect of *Cinnamomum zeylanicum* oil (pure andnanostructured) against mealworm (*Alphitobius diaperinus*) and its possible environmental effects J. Asia Pac. Entomol., 19(4): 1159-1165. https://doi.org/10.1016/j.aspen.2016.10.008

World Health Organization (2005). Guidelines for laboratory and field testing of mosquito larvicid WHO/CDS/WHOPES/GCDPP/2005, 13: 7–20.

Xing H, Hu Y, Yang L, Lin J, Bai H (2021). Fumigation activity of essential oil of cinnamonum loureirii against red imported fire ant (Solenopsis invicta) Workers. https://doi.org/10.21203/rs.3.rs-450369/v1

Yufei L, An T, Wan D, Yu B, Fan Y, Pei X (2020). Targets and mechanism used by cinnamaldehyde, the main active ingredient in cinnamon, in the treatment of breast cancer. Front. Pharmacol., 11: (2020). www.frontiersin.org/article/10.3389/fphar.2020.582719. https://doi.org/10.3389/fphar.2020.582719

Zhang Y, Xiaoyu L, Yifei W, Pingping J, Siew YQ (2016). Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. Food Contr., 59: 282-289. https://doi.org/10.1016/j.foodcont.2015.05.032