Int. J. Plant Bas. Pharm. (2022) 2(1), 51-63

INTERNATIONAL JOURNAL OF PLANT BASED PHARMACEUTICALS

https://ijpbp.com

RESEARCH ARTICLE

OPEN ACCESS

Comparative toxicity of cinnamon oil, cinnamaldehyde and their nano-emulsions against *Culex pipiens* (L.) larvae with biochemical and docking studies

Nehad E.M. Taktak[a], Mohamed E.I. Badawy[b,*], Osama M. Awad[a], Nadia E. Abou El-Ela[a]

[a] *Alexandria University, High Institute of Public Health, Department of Environmental Health, 165 El-Horreya Avenue, El-Ibrahimia, Alexandria, Egypt*
[b] *Alexandria University, Faculty of Agriculture, Laboratory of Pesticide Residues Analysis, Department of Pesticide Chemistry and Technology, Aflatoun St., 21545 El-Shatby, Alexandria, Egypt*

ARTICLE INFO

Article History:

Received: 02 December 2021
Revised: 15 January 2022
Accepted: 15 January 2022
Available online: 19 January 2022

Edited by: B. Tepe

Keywords:
Culex pipiens
Cinnamon oil
Cinnamaldehyde
Nano-emulsions
Larvicidal activity
Biochemical studies
Molecular docking

ABSTRACT

The larvicidal activity of cinnamon oil and its main component, cinnamaldehyde, was compared with their nano-emulsions (NEs) against *Culex pipiens* mosquito larvae. Oil-in-water (O/W) NEs preparation was based on the coarse emulsion followed by high-energy ultra-sonication. The droplet size, polydispersity index (PDI), viscosity, zeta potential, and pH of NEs were investigated. The droplet sizes of the NEs were 95.67 nm for cinnamon oil and 174.59 nm for cinnamaldehyde. The NEs recorded high negative zeta potentials (-30.0 and -21.20 for cinnamon oil and cinnamaldehyde, respectively). The larvicidal activity results showed that the cinnamaldehyde (LC_{50} = 94.46 and 72.91 mg/l for T and NE, respectively) had higher activities than cinnamon oil (LC_{50} = 154.08 and 123.13 mg/l for T and NE, respectively) after 24 h of exposure against *C. pipiens* larvae. These results proved that NE formulation enhanced the activity of tested compounds against larvae. The *in vitro* effect on the acetylcholinesterase (AChE), adenosine triphosphatase (ATPase), and gamma-aminobutyric acid transaminase (GABA-T) were demonstrated, and the data proved that the NEs formulations were higher than their pure compounds. Non-formulated cinnamon oil and cinnamaldehyde caused 17.26% and 30.83% of AChE, respectively, while their NEs caused 46.40% and 60.59% inhibition. Furthermore, the molecular docking studies indicated that the affinity binding of cinnamaldehyde on AChE and GABA-T was higher than ATPase. This work describes bio-products with potential use against *C. pipiens* larvae as eco-friendly products.

1. Introduction

The mosquitoes are at the center of worldwide entomological research due to their importance as vectors of several viral and parasitic diseases affecting about 700 million people worldwide (Li et al., 2022; Wilder-Smith et al., 2017). According to the World Health Organization (WHO), more than 80 % of the world's population lives in areas at risk of at least one vector-borne disease that causes more than 700.000 deaths annually (WHO 2017). *Culex* is a predominant mosquito that mainly thrives in tropical and sub-tropical areas, breeds in dirty waters like congested drains and impaired septic tanks near human dwellings. In Egypt, *Culex* species can transmit a nematode worm (*Wuchereria bancrofti*), responsible for the filarial disease (Holder et al., 1999). Filariasis is the fastest spreading insect-borne disease to humans worldwide; 146 million cases are reported worldwide every year. About 3492 species of mosquito are recorded; 100 species of them are vectors and can transmit many diseases to humans and mammals (Ghosh et al., 2012). Once limited to tropical and subtropical zones, numerous vector-borne diseases have emerged in temperate areas because of climate change. Hence, temperate countries may be the most threatened by the emergence and re-emergence of vector-borne diseases (Karuppusamy et al., 2021; Rocklöv and Dubrow, 2020).

Vector control is the most critical step due to the absence of a drug or vaccine to prevent disease outbreaks. Most mosquito control

* Corresponding author:
E-mail address: mohamed.badawy@alexu.edu.eg (M.E.I. Badawy)
e-ISSN: 2791-7509
doi: https://doi.org/10.62313/ijpbp.2022.16

Please cite this article as: Taktak, N.E.M., Badawy, M.E.I., Awad, O.M., El-Ela, N.E.A., 2022. Comparative toxicity of cinnamon oil, cinnamaldehyde and their nanoemulsions against Culex pipiens (L.) larvae with biochemical and docking studies. International Journal of Plant Based Pharmaceuticals, 2(1), 51-63, https://doi.org/10.62313/ijpbp.2022.16.

programs target the larval stage in their breeding sites with larvicides because they are most concentrated, immobile, sensitive, and accessible at this stage. At the same time, the use of adulticides only reduces the adult population temporarily (Rehman et al., 2014). Moreover, the insecticide application is in a defined area, decreasing environmental contamination. Pyrethroids and organophosphates are the main groups used to control mosquitos in Egypt and worldwide. Organophosphate insecticides were used outdoor as larvicides, while pyrethroids were used indoors as adulticides (Zahran et al., 2017). However, synthetic insecticides to control mosquitoes have created resistance among target species, adverse effects on non-target organisms, and a secondary impact on the environment and public health.

These drawbacks have prompted researchers to explore new insect-selective products with low toxicological risks, environmentally safer, target-specific, and cost-effective insecticides as an alternative vector control tool (Alsaraf et al., 2021; Zeghib et al., 2020). Therefore, enormous efforts have been made to explore the efficiency of plant-based products against mosquitoes (Wangai et al., 2020; Zahran et al., 2017; Zeghib et al., 2020). Plant-based insecticides have bioactivity, biodegradability, and human and environmental safety compared to traditional insecticides. They also have various novel modes of action, which is crucial in combating insect resistance (Norris et al., 2018). Insecticidal properties of natural bioactive compounds derived from plants, especially essential oils, are suitable against mosquito larvae (El Gohary et al., 2021). These essential oils are complex natural mixtures of volatile, semi-volatile organic compounds, odorous, and secondary metabolites, principally mono- and sesquiterpenes, which are considered among the best alternatives for the control of disease vectors (Alsaraf et al., 2021; Taktak and Badawy, 2019). Monoterpenes are secondary plant metabolites with insecticidal, nematicidal, acaricidal, and larvicidal properties (El-Sabrout et al., 2020; Kweka et al., 2016). The physical and chemical properties of monoterpenes, such as chemical instability, volatility, and low water solubility, rendered them unsuitable for widespread use against Culex larvae (Moretti et al., 2002). As a result, incorporating essential oils (EOs) and monoterpenes into NEs could improve the system to be more effective than bulk substances. In addition, NEs provide several benefits, such as a substantial increase in water solubility, dissolution rate, dispersion uniformity, and significant bioavailability after application (El Gohary et al., 2021).

It is generally understood that insects exposed to these products may encounter toxic compounds that have relatively nonspecific effects on a wide range of molecular targets. Among the targets are proteins (enzymes, receptors, signaling molecules, ion-channels, structural proteins), nucleic acids, biomembranes, and other cell components (Rattan, 2010). As a result, insect physiology is impacted in several ways, including the nervous system malfunctioning, which is the most important one. Rattan (2010) reviewed several physiological effects of plant secondary metabolites on insects, including inhibition of acetylcholinesterase (AChE), blockage of the gamma-aminobutyric acid (GABA)-gated chloride channel, disruption of sodium and potassium ion exchange, and inhibition of cellular respiration (Rattan, 2010). In particular, inhibition of AChE activity is significant since it is responsible for terminating nerve impulse transmission through the synaptic pathway (Badawy et al., 2018; Rattan, 2010).

Therefore, the main objective of this study was to prepare NEs of cinnamon EO and cinnamaldehyde as larvicidal agents against *C. pipiens*. Droplet size distribution, polydispersity index (PDI), viscosity, pH, stability, and surface morphology of the produced NEs were examined. The larvicidal effect against *C. pipiens* of the technical grades (T) was compared to the NEs. The *in vitro* biochemical studies were also investigated on AChE, adenosine triphosphatase (ATPase), and GABA transaminase (GABA-T). In addition, molecular docking of cinnamaldehyde and a reference larvicide temephos was examined with some enzymes.

2. Materials and methods

2.1. Chemicals and reagents

Cinnamon (*Cinnamomum verum*) EO was purchased from LUNA Co. for the Perfumes and Cosmetics Industry (6 October City, Giza, Egypt). A trace gas chromatography Ultra/Mass Spectrophotometer ISQ (GC/MS Thermo Scientific) instrument was used to analyze the components of this oil. Cinnamaldehyde (≥ 95%) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). A reference larvicide temephos (90%) was purchased from Kalyani Industries Pvt. Ltd. Acetylthiocholine iodide (ATChI), adenosine triphosphate (ATP), bovine serum albumin (BSA), dimethyl sulfoxide (DMSO), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), Folin-Ciocalteu phenol reagent, gamma-aminobutyric acid (GABA), α-ketoglutarate, 2-mercaptoethanol, β-nicotinamide adenine dinucleotide (β-NAD), *p*-nitrophenyl phosphate, trichloroacetic acid (TCA), Tris-HCl [tris (hydroxymethyl) aminomethane hydrochloride], triton X-100 and Tween 80 were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other commercially available solvents and chemicals were purchased from El-Gomhouria for Trading Chemicals And Medical Appliances Co., (Adeb Ishak St, Manisha, Alexandria, Egypt) and used without further purification. Cinnamon oil was stored in dark vials at 4 °C until tested.

2.2. C. pipiens mosquito rearing

A colony of a susceptible strain of *C. pipiens* culture was reared in the High Institute of Public Health insectary, Alexandria University, Alexandria, Egypt. The larvae were feeding on biscuits until pupated in shallow trays with 2-3 liters of dechlorinated water. In adult cages (30 x 30 x 30 cm), male adults were fed on 30% sucrose solution, while females were fed on pigeon blood four times a week. The use of a live pigeon in our research was approved by the High Institute of Public Health's Ethics committee and confirmed by Alexandria University under reference number 481. The egg rafts were moved from adult cages to white trays containing dechlorinated water for egg hatching.

2.3. NEs preparation

The cinnamon oil NE was prepared from 10% active ingredient as an organic phase with a polar phase mixture of Tween 80 (10%) and water (80%). While NE of cinnamaldehyde was prepared as follows: active ingredient was dissolved in DMSO to prepare the organic phase, Tween 80 dissolved in distilled water at 50 °C to prepare the polar phase. Considering the concentrations of the active ingredient, DMSO and Tween 80 were 2.5%, 5%, and 2.5%, respectively, in the prepared total volume (Abdelrasoul et al., 2020). The organic phase was dropped into the polar phase by stirring at room temperature for 30 min at 4000 rpm to form the coarse emulsion. Using a high-energy ultrasonic procedure, the ultrasonic probe was used to transform the coarse emulsion into a NE (Figure 1). The ultrasonication process was carried out for 5 min at a rate of 9 cycles per second and a power of 15 kHz for cinnamon oil and 5 kHz for cinnamaldehyde (Munawiroh et al., 2017).

2.4. Characterizations of the NEs

2.4.1. Droplet size and polydispersity index (PDI)

At room temperature, droplet size and PDI of the NE were determined by Dynamic Light Scattering (DLS) technique with a Zetasizer NanoZSlaser diffractometer (Malvern Instruments Ltd, Worcestershire, UK) working at 633 nm and equipped with a backscatter detector (173˚). At a fixed 90° angle, the droplet size (nm) was characterized by distribution curves in intensity (%). The NEs were 500-fold diluted in ultrapure water and sonicated for 5 min at 9 cycles/sec and 5 kHz power before being measured to prevent multiple scattering effects. The droplet size was expressed as a mean diameter in nanometer (Mibielli et al., 2021; Silva et al., 2012).

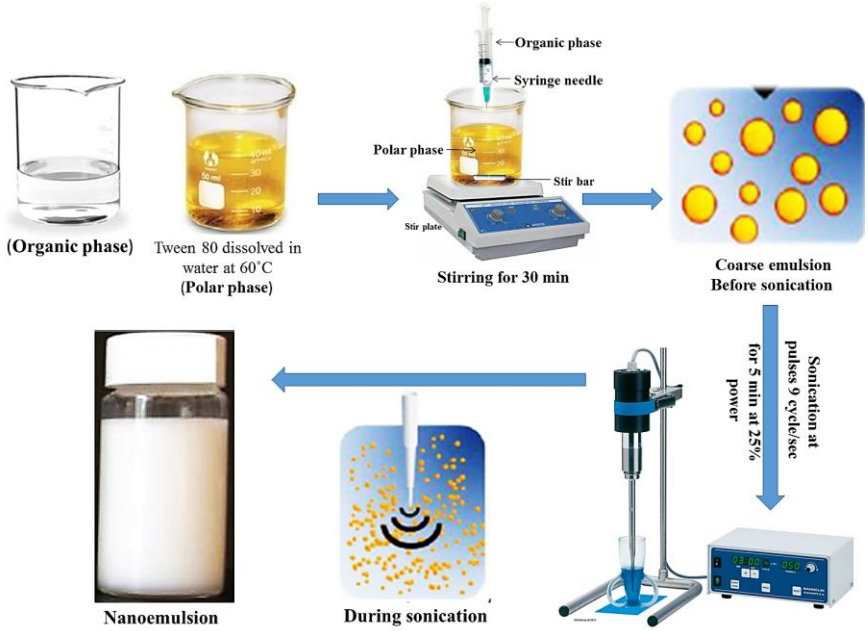

Figure 1. Schematic illustration of NEs preparation

2.4.2. Viscosity and pH measurements

The dynamic viscosity was determined without further dilution using a digital viscometer (a Rotary Myr VR 3000) with an L2 spindle spinning at 200 rpm at 25 °C. The viscosity was measured three times, and the results were expressed in mPa.s. The pH values of the prepared NE were determined using a digital pH meter (Crison pH Meter Basic 20, EU) (Badawy et al., 2017; Drais and Hussein, 2015).

2.4.3. Stability tests

Three samples from each formulation were centrifuged for 30 min at 5000 rpm, and phase separation, creaming, and cracking were observed. The NE should have enough stability without phase separation. Other thermodynamic stability tests were performed on stable formulations. Three samples of each NE were held at 25 and 40 °C then subjected to a heating-cooling test. The thermodynamic stability examinations were done by storing the NE at -21 °C for 24 h and then at 21 °C until melting for 24 h. A transparent tube was filled with 25 ml of each freshly prepared NE for three months at 25 °C as a storage period, the transition from steady-state to creaming and coalescence was investigated (Badawy et al., 2017; Drais and Hussein, 2015).

2.4.4. Zeta potential (ζ)

Using the Zetasizer NanoZSlaser (Malvern Instruments, Worcestershire, UK) at 25 °C, the zeta potential of NEs was investigated (Silva et al., 2012). Zeta potential was measured by transferring the samples to electrophoretic cells, then applying an electrical potential ± 150 mV. The formulations were 200-fold diluted in ultrapure water and sonicated for 5 min at 9 cycles/sec and 5kHz power before being analyzed to avoid multiple scattering effects (Smoluchowski, 1916).

2.4.5. TEM

TEM (JEOL JEM-1400 Plus transmission electron microscope, USA, Inc.) equipped with 20 mM aperture at 20 Kv was used to examine the surface morphology and topology of prepared NEs. Bright-field imaging with increasing magnification and diffraction modes was chosen. The sample from each NE was diluted with distilled water (1:100) and added to 200-mesh form war-coated copper TEM sample holders (EM Sciences, Hatfi eld, PA, Japan).

2.5. Toxicity assay against C. pipiens larvae

The larval bioassay was performed on *C. pipiens* larvae to compare the effect of cinnamon oil and cinnamaldehyde and their NEs by dipping method (WHO, 2005). Tested compounds and their NEs were tested against third instar larvae of *C. pipiens* to obtain the LC_{50} values. The technical cinnamaldehyde and pure cinnamon oil were dissolved in Tween 80/DMSO (0.05%) then dissolved in de-chlorinated water. In comparison, their NEs were dissolved directly in de-chlorinated water. After preliminary screening for all products, different stock solutions for each compound were prepared to determine the toxicity. The tested concentrations of cinnamaldehyde were between 50 to 600 mg/l. In contrast, the tested concentrations of cinnamon oil were between 20 to 300 mg/l. The acute toxicity of technical temephos (90%) was also evaluated by mixing different concentrations (2, 4, 6, 8, 10, 20, 30, and 40 µg/l) prepared in DMSO with dechlorinated tap water. In a control experiment, DMSO and Tween 80 were mixed with de-chlorinated water. Twenty *C. pipiens* larvae were put into plastic

cups containing 100 ml of de-chlorinated water. Mortality caused by the compounds was recorded as larval mortalities percentage of dead versus live larvae numbers after 24 and 48 h. The 50% lethal concentration (LC_{50}) values of the compounds were calculated using probit analysis (Finney, 1971).

2.6. Biochemical studies

2.6.1. Preparation of enzyme homogenates

After 24 h of exposure to LC_{50} values of tested compounds, surviving larvae were homogenized in 10 mM NaCl (1%, w/v) Triton X-100 and 40 mM sodium phosphate buffer (pH 7.4) at 4 °C to assess ATPase, AChE, and GABA enzyme activities. The homogenate was centrifuged at 4 °C for 20 min at 5000 rpm. The supernatant was either used right away for an enzymatic assay or processed at -20 °C for later use (Taktak and Badawy, 2019).

2.6.2. Total protein assay

According to Lowry et al. (1951) method, total protein in crude enzyme was determined using BSA for the standard curve. Protein extract (200 µl) was added to 1900 µl alkaline copper reagent [48 ml of sodium carbonate 2% (w/v) in 0.1 N sodium hydroxide + 1ml sodium-potassium tartrate 1% (w/v) + 1 ml copper sulfate 0.5% (w/v)] and immediately mixed with 200 µl Folin-Ciocalteu phenol reagent. After 30 min of incubation at 25 °C, the absorbance was recorded at 600 nm. By comparing the protein content of the measured samples to the BSA standard curve, the protein content of the samples was calculated.

2.6.3. Acetylcholinesterase (AChE) assay

AChE activity was determined by Ellman colorimetric method (Ellman et al., 1961). The reaction mixture contained 10 µl ATChI (0.075 M), 1340 µl phosphate buffer (pH 8), 100 µl of the crude enzyme, and 50 µl DTNB (0.01 M). Reaction mixture free of crude enzyme sample was checked as a blank, while a mixture containing non-treated larval extract was also studied as a control. Using a Unico 1200 Spectrophotometer (Laxco Inc, USA), the absorbance was measured at 412 nm after 10 minutes of incubation at 37 °C. The enzyme activity was expressed as OD_{412}.mg protein^{-1}.min^{-1}.

2.6.4. Adenosine triphosphatase (ATPase) assay

The Koch method was used to measure the activity of ATPase (Koch, 1969). The reaction mixture was prepared with 400 mM Na^+, 20 mM K^+, 5 mM Mg^+, and 5 mM ATP, and 200 l of the crude enzyme was transferred. After that, the volume was completed to 950 µl with tris-HCl buffer (pH 7.4). The reaction was stopped with 200 µl of TCA after 10 min of incubation at 37 °C. 10 ml of a fresh color reagent containing 5 g of ferrous sulfate dissolved in a solution of ammonium molybdate and sulfuric acid (10 N) was added to the reaction mixture. A reaction mixture free of crude enzyme sample was examined as a blank. As a control, a reaction mixture containing untreated larval extract was tested. The enzyme activity was measured according to the absorbance of the developed color at 740 nm and expressed as OD_{740}.mg protein^{-1}.min^{-1} (Kessler et al., 2014).

Table 1. The chemical constituents of the cinnamon EO isolated from *C. verum* by GC/MS

Rt (min)	Compound	Molecular formula	Molecular weight	Area (%)	RI
6.60	α-Pinene	$C_{10}H_{16}$	136	1.03	919
8.49	β-Pinene	$C_{10}H_{16}$	136	0.73	980
10.65	β-Cymene	$C_{10}H_{14}$	144	1.01	1050
13.10	1,8-Cineole	$C_{10}H_{18}O$	154	6.12	1129
18.99	Linalool	$C_{11}H_{18}O_2$	182	2.02	1319
24.40	*(-)*-Camphor	$C_{10}H_{16}O$	152	0.54	1494
25.43	*(E)*-Cinnamaldehyde	C_9H_8O	132	80.09	1527
26.01	Eugenol	$C_{10}H_{12}O_2$	164	1.06	1545
26.89	Methyl linoleate	$C_{19}H_{36}O_2$	296	3.18	1574
29.55	*(E)*-Cinnamyl acetate	$C_{11}H_{12}O_2$	176	2.56	1660
32.09	β-Terpinyl acetate	$C_{12}H_{20}O_2$	196	0.43	1742
36.89	Caryophyllene	$C_{15}H_{24}$	204	0.63	1896
	Total			99.40	

Rt: Retention time. RI: Retention index.

2.6.5. Gamma aminobutyric acid transaminase (GABA-T) assay

The activity of GABA-T was assayed according to Pandey and Singh (1985) with minor modifications. Enzyme extract (100 µl) was added to 1450 µl of 50 mM Tris-HCl (pH 8.5), 100 µl of 2 mM α-ketoglutarate, 100 µl of 2-mercaptoethanol (20 mM), and 20 µl of β-NAD (1.1 mM). The reaction was started by adding 200 µl of GABA (3 mM) to the mixture. Finally, 30 µl of 1% triton-x 100 were added to the previous mixture. Samples including the blank and control samples were incubated at 25 to 30 °C for 30 min. The absorbance was measured at 340 nm, and the enzyme activity was presented as OD_{340}.mg protein^{-1}.min^{-1}.

2.7. Molecular docking

Using molecular docking studies, digital values were used to determine the effectiveness of cinnamaldehyde and temephos against various enzyme targets. The crystal structures of AChE (PDB: 5X61), ATPase (PDB: 4BYG), and GABA-T (PDB: 1SF2) were obtained from protein data bank (PDB) (http://www.rcsb.org/pdb/home/home.do). The selection of AChE PDB was based on the model for *C. pipiens* AChE1 that was built using the template protein of malaria mosquito vector *Anopheles gambiae* (PDB: 5X61, Chain B), with 99% query coverage and 92.36% similarity (Rao et al., 2021). 3D structure of the tested enzymes was visualized using Molecular Operating Environment (MOE) 2014.13 software (Chemical Computing Group Inc, Montreal, Quebec, Canada) and examined for missing atoms, bonds, and contacts (Chemical Computing Group, 2008). The tested compounds were converted to 3D, and the energy was minimized by the MMFF94 function (Halgren, 1999). A triangle-matching algorithm was chosen to dock the compounds into the active sites of the enzymes. The contributions of hydrophobic, ionic, hydrogenated, and Van der Waals interactions were used to quantify the free energy of binding. A ligand was considered adequate for a minimum docking score value (or interaction energy calculation) of an enzyme-ligand complex.

2.8. Statistical analysis

Statistical analysis was performed using the IBM SPSS software version 25.0 (SPSS, Chicago, IL, USA) (IBM, 2017). Mortality percentages were calculated for each treatment and corrected using Abbott's equation (Abbott, 1925). Means and standard error (SE) were obtained from three independent replications for each treatment. According to the probit analysis, the log dose-response lines (LdP line) were used to determine the LC_{50} values (Finney, 1971). The least-square regression analysis was used to determine the 95% confidence limits. Analysis of variance (ANOVA) of the biochemical data was conducted and means property values were separated ($p \leq 0.05$) with Student-Newman-Keuls (SNK).

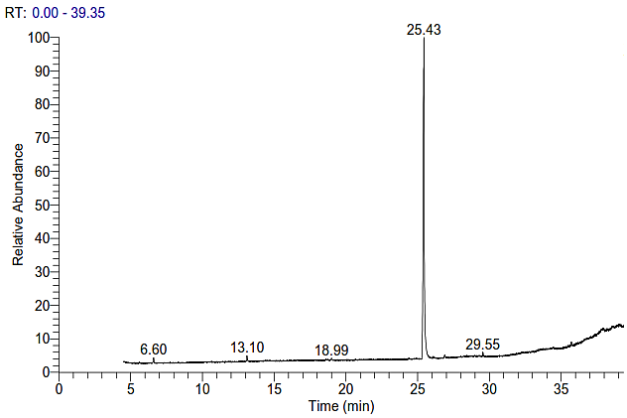

Figure 2. GC/MS chromatogram of cinnamon essential oil

3. Results and discussion

3.1. GC/MS analysis of cinnamon oil

The GC/MS chromatogram of the oil is shown in Figure 2. The chemical composition of cinnamon oil was done using GC/MS analysis. A chromatogram of *C. verum* EO constituents showed that the oil is a mixture of 12 different components, representing 99.40% of the total oil (Table 1). The identified chemical compounds belong to olefinic hydrocarbons, monoterpenes, sesquiterpenes, and other essential phytochemicals. The major components detected in the oil, according to retention time and their relative abundances, were α-pinene (1.03%), β-cymene (1.01%), 1,8-cineole (6.12%), linalool (2.02%), *(E)*-cinnamaldehyde (80.09%), eugenol (3.18%), methyl linoleate (1.06%) and *(E)*-cinnamyl acetate (2.56%). However, other constituents, including β-pinene, *(-)*-camphor, β-terpinyl acetate, and caryophyllene, were less abundant with a percentage of lower than 1%. The GC/MS analysis results of the cinnamon EO showed that cinnamaldehyde was the major component of this essential oil. According to studies carried out by several investigators, the major component of cinnamon EO was cinnamaldehyde in the range of 44-97% (Adinew, 2014; Wang et al., 2009; Yu et al., 2020). These findings agree with the current study since the concentration of cinnamaldehyde was 80.09%. On the contrary, the results obtained by Ghosh et al. (2013) proved that eugenol was found to be the main component of cinnamon oil (*C. zeylanicum*) with 59.918% of the total peak area. In conclusion, most *C. verum* oil components were similar to those reported in this oil chemistry.

Table 2. The visual appearance, droplet size, PDI, zeta potential, dynamic viscosity, and pH of the prepared NEs

Nanoemulsion	Visual appearance	Droplet size (nm) ± SE	PDI ± SE	Zeta potential (mV)	Viscosity (mPa.s) ± SE	pH	Stability after centrifugation at 5000 rpm
Cinnamon oil NE	Milky	95.67±0.04	0.33±0.05	-30.3	7.03±0.02	6.11	√
Cinnamaldehyde NE	Milky	174.59±0.08	0.58±0.13	-21.20	6.93±0.07	6.08	√

(√) refer to the stable state, (×) refer to the non-stable state. PDI: Polydispersity index.

3.2. Characterizations of the prepared NEs

3.2.1. Droplet size and polydispersity index (PDI)

Table 2 presented the droplet size and PDI data of produced NEs. There is a significant difference between the droplet size values for cinnamon oil and cinnamaldehyde, which were 95.67 and 174.59 nm, respectively. In general, the scale of 10-500 nm corresponding to the average droplet size of O/W NEs (Izquierdo et al., 2002; Kabri et al., 2011). This finding showed that all compounds were successfully prepared at the nanometric scale. In agreement with our results, the findings obtained by Abdelrasoul et al. (2018) reported that the mean particle size of nano-sized cinnamaldehyde was 128.07 nm. While the particle size detected by Mibielli et al. (2021) in the carvacrol, NE was 354.20 nm. Under room temperature and accelerated stability assessment, no phase separation, creaming, or sedimentation were observed (Balaji et al., 2017; Qin et al., 2017). The tiny droplets in a NE contribute to its long-term physical stability, which is why this sort of formulation is often known as "approaching thermodynamic stability". (Izquierdo et al., 2002; Solans et al., 2005). NEs have several advantages over traditional emulsions due to the small size of the droplets: higher optical visibility, more excellent stability against droplet aggregation and gravitational separation, and increased bioactivity of encapsulated components (McClements, 2012). For these reasons, NEs have emerged as an alternative drug, pesticide carriers, and other vital applications (Feng et al., 2018; Tang et al., 2013).

The PDI of the prepared NEs were 0.33 and 0.58 for cinnamon and cinnamaldehyde, respectively (Table 2). The PDI reflects the distribution, homogeny, and stability of particle size in the emulsion. These findings indicated that the size distribution of all NEs was

relatively narrow. In agreement with our results, the PDI value of cinnamaldehyde NE prepared by Abdelrasoul et al. (2018) was 0.322. While the PDI value of carvacrol NE formulated by Mibielli et al. (2021) was 0.104. The PDI, which ranges from zero to one, is a dimensionless estimate of the width of the size distribution measured from the cumulate analysis (Kentish et al., 2008). A lower PDI value indicates the presence of a uniform droplet size distribution (homogeneous system), while a PDI value closer to one indicates the presence of a large variety of droplet sizes (heterogeneity of the system) (Tyagi et al., 2012).

3.2.2. Zeta potential

Table 2 and Figure 3 show that the NEs revealed negative zeta potential (-30.30 and -21.20 for cinnamon and cinnamaldehyde, respectively). This finding implies a sufficiently high negative surface charge for droplet-droplet repulsion and, as a result, improved NE stability (Bruxel et al., 2012). The zeta potential is a better way to optimize sample stability and save time consumed in shelf-life tests. It is considered a potent indicator of NE stability, resist flocculation and aggregation for more extended periods, and is associated with to surface potential of the droplets (Benita and Levy, 1993). The charge acquired by a particle or molecule in a given medium is its zeta potential. It arises from the surface charge, concentration, and types of ions in the solution. Since particles of similar charge will repel each other, those with high charges will resist flocculation and aggregation for more prolonged periods making such samples more stable. This finding means that the stability can be modified by altering the pH, the ionic concentration, the type of ions, and additives such as surfactants and polyelectrolytes (Mahdi et al., 2011).

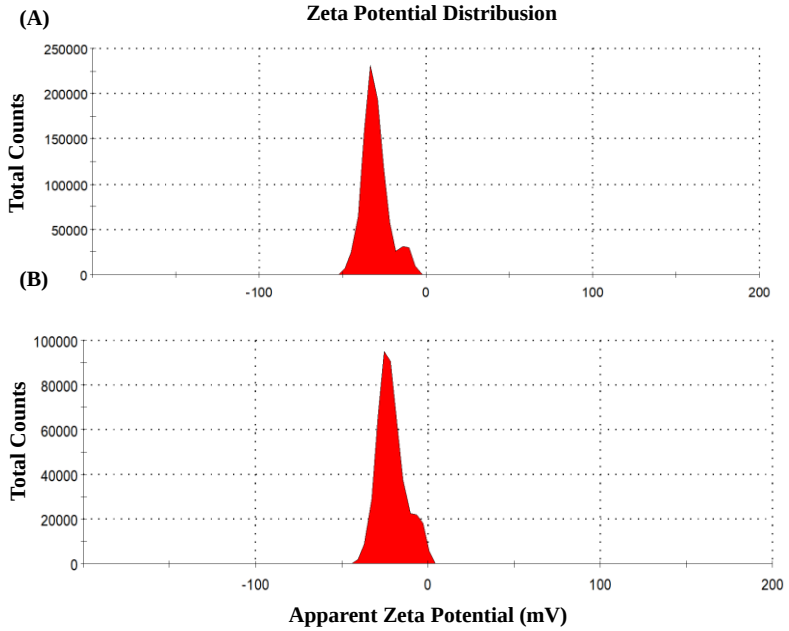

Figure 3. Zeta potential distribution graph of NEs of cinnamon oil (A) and cinnamaldehyde (B)

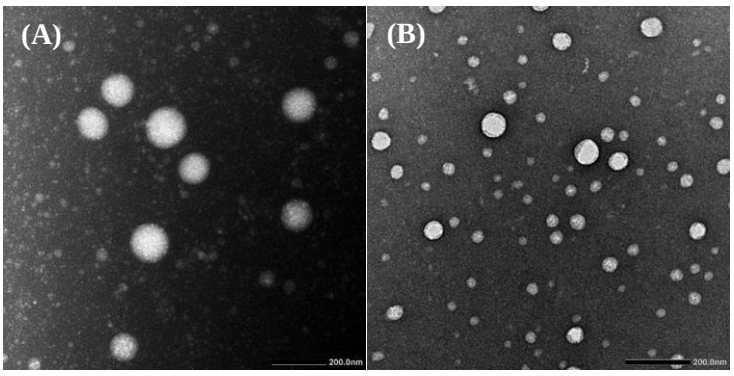

Figure 4. Transmission electron micrograph of cinnamon NE (A) and cinnamaldehyde NE (B). The TEM was performed on a JEOL JEM-1400 Plus transmission electron microscope operating at an acceleration voltage of 80.0 kV with a 20 mm aperture. Print magnification 30000x.

3.2.3. Viscosity and pH measurements

The viscosity values of the NEs were 7.03 and 6.93 for cinnamon and cinnamaldehyde, respectively (Table 2). The pH values were 6.11 and 6.08 for cinnamon and cinnamaldehyde, respectively (Table 2). As mentioned previously, the pH range of a NE has a significant impact on its stability. The surface charge of the globule changes as the pH value changes, affecting their stability during storage. A rise in globule surface charge facilitates electrostatic repulsion and decreases NE flocculation and dissolution (Pengon et al., 2018).

3.2.4. Thermodynamic stability studies

An accelerated storage testing was carried out to predict the long-term physical stability of the NEs (Badawy et al., 2017). The recorded data of visual stability, centrifugation, and heating-cooling

stability of NEs showed that all prepared NEs were stable at 5000 rpm of centrifugation, at 25 °C for up to 3 months, and under heating-cooling cycle test.

3.2.5. TEM

The morphological study of the structure of NE droplets is carried out using TEM. TEM micrographs of NEs were presented in Figure 4, showing their spherical form and typical NE appearance at magnification 30.000x.

3.3. Larvicidal efficacy of tested compounds and their NEs

After 24 and 48 h of exposure, the larvicidal activity of cinnamon oil and technical (T) cinnamaldehyde was compared with their NEs and temephos against *C. pipiens*. Table 3 presented the LC_{50} values with 95% confidence limits and other statistical parameters. Cinnamaldehyde T and NE were more effective (LC_{50} = 70.44-94.46 mg/l) than the EO products (LC_{50} = 112.34-154.08 mg/l). Cinnamaldehyde NE gave the LC_{50} value of 72.90 mg/l more than the T form (94.46 mg/l) after 24 h of the experiment. At the same time, this compound gave LC_{50} values 70.44 and 84.91 mg/l after 48 h of exposure for NE and T, respectively. NE form of EO gave an LC_{50} value of 123.13 mg/l, which was more effective than non-formulated EO (LC_{50} = 154.08 mg/l) after 24 h of exposure. It can be observed that the larvicidal behavior of the NE was the best. Temephos as a reference insecticide against mosquito larvae exhibited the LC_{50} of 0.012 and 0.009 mg/l after 24 and 48 h, respectively.

Table 3. Larvicidal activity of cinnamon EO, its main component cinnamaldehyde, and their NEs against *C. pipiens*

EOs	Time of exposure (h)	Formulation	LC_{50}[a] (mg/L)	95% confidence limits (mg/L)		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
				Lower	Upper			
Cinnamon oil	24	EO	154.08	144.56	163.65	7.28±0.46	-15.94±1.02	12.81
		NE	123.13	117.53	128.15	6.96±0.52	-14.55±1.12	4.18
	48	EO	145.75	136.52	154.65	7.48±0.48	-16.18±1.06	12.73
		NE	112.34	105.47	118.18	6.19±0.52	-12.69±1.12	9.13
Cinnamaldehyde	24	T	94.46	87.52	103.56	10.23±0.61	-20.25±1.21	56.15
		NE	72.90	69.05	76.23	7.01±0.60	-13.05±1.16	5.88
	48	T	84.91	77.09	90.17	9.95±0.92	-19.20±1.82	10.79
		NE	70.44	60.61	77.33	7.02±0.62	-12.97±1.19	9.18
Temephos	24	T	0.012	0.008	0.017	3.33±0.19	-3.54±0.20	90.08
	48	T	0.009	0.007	0.011	4.26±0.28	-3.99±0.26	57.96

EO: Essential oil, T: Technical, NE: Nanoemulsion, [a] The concentration causing 50% mortality, [b] Slope of the concentration-mortality regression line, [c] Intercept of the regression line, [d] Chi-squared value, SE: Standard error

Table 4. The activity of cinnamon EO, its main component cinnamaldehyde, and their NEs on AChE, ATPase, and GABA-T activity in *C. pipiens* larvae after 24 h of the treatment with LC_{50} of each treatment

Treatment	Type of formulation	Enzyme activity (OD.mg protein^{-1}.min) ± SE			Change in activity (%) ± SE		
		AChE	ATPase	GABA-T	Inhibition of AChE	Activation of ATPase	Activation of GABA-T
Untreated larvae	-	0.93^{a}±0.00	1.07^{e}±0.01	0.110^{e}±0.00	0.00^{e}±0.00	0.00^{e}±0.00	0.00^{e}±0.00
Cinnamon oil	EO	0.77^{b}±0.00	1.38^{d}±0.02	0.161^{c}±0.00	17.26^{d}±0.08	28.56^{d}±2.80	46.13^{c}±5.58
	NE	0.50^{d}±0.00	1.88^{c}±0.02	0.213^{b}±0.00	46.40^{b}±0.57	75.42^{c}±2.51	93.14^{b}±6.62
Cinnamaldehyde	T	0.64^{c}±0.00	2.37^{b}±0.01	0.146^{d}±0.01	30.83^{c}±0.49	120.53^{b}±2.06	32.21^{d}±4.36
	NE	0.37^{e}±0.01	2.93^{a}±0.05	0.409^{a}±0.00	60.59^{a}±1.59	172.59^{a}±3.07	271.23^{a}±14.05
Temephos	T	0.18^{f}±0.01	-	-	80.65^{a}±1.01	-	-

EO: Essential oil, T: Technical, NE: Nanoemulsion, OD: Optical density, SE: Standard error, AChE: Acetylcholinesterase, ATPase: Adenosine triphosphatase, GABA-T: Gamma-aminobutyric acid transaminase. Values in the column with different letters are significantly different at $p \leq 0.05$ using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) test.

Based on the literature review, a natural product with LC_{50} < 100 mg/l could be a promising candidate for mosquito control (Dias and Moraes, 2014; Kharoubi et al., 2021; Ríos et al., 2017). In our earlier study, twenty-one monoterpenes from different chemical groups were evaluated against *C. pipiens* larvae, and the results indicated that (±)-β-citronellol and cinnamyl acetate had the highest mortality (LC_{50} = 5.520 and 5.603 mg/l, respectively) (Taktak and Badawy, 2019). In addition, our results are approximately similar to the data obtained by Pavela (2015), who proved that the LC_{50} values of *p*-cymene and linalool against *C. quinquefasciatus* larvae were 21 and 247 mg/l, respectively (Pavela, 2015). Moreover, Michaelakis et al. (2014) proved that the LC_{50} value of linalool was more than 200 mg/l against *C. pipiens* larvae (Michaelakis et al., 2014). In addition, the data observed for cinnamon and eucalyptus oils showed a 100% larvicidal effect at 1000 mg/l and a 100% knockdown effect at 10% against *C. quinquefasciatus* (Manimaran et al., 2012).

In recent years, nanotechnology in pest management has been increasing, and one of the most important goals is to prepare nano pesticides (Jesser et al., 2020). Essential oils or monoterpenes in nanometric emulsions enhanced mosquitocidal activity (Nenaah et al., 2021). For example, the NE of neem oil with the hydrodynamic size of 30.12 nm and PDI of 0.262 exhibited noteworthy larvicidal activity against *C. quinquefasciatus* (LC_{50} = 11.75 mg/l after 24 h) (Mishra et al., 2018). Currently, extensive efforts are being made to study the potential toxic behavior of prevalent nano pesticides in mosquitoes and other pest ecosystems (Kumar et al., 2020; Mishra et al., 2018). However, limited experimental studies have been carried out to shed light on the toxicity profile of these nanometric pesticides towards the non-target species prevalent in the target ecosystem (Côa et al., 2020; Taktak et al., 2021).

3.4. Biochemical studies

To explain some biochemical actions of the tested compounds on *C. pipiens* larvae, the effect of the LC_{50} values of these compounds on the activity of AChE, ATPase, and GABA-T extracted from larvae after 24 h of the treatment was studied. Generally, the activities of AChE and GABA-T were inhibited after treatment, while ATPase activity was increased (Table 4). The activity of enzymes in untreated larvae was 0.93, 1.07, and 0.11 for AChE, ATPase, and GABA-T, respectively. Cinnamaldehyde NE inhibited the AChE enzyme by 60.59%, while the unformulated form decreased the activity by 30.83% compared to the untreated larvae. At the same time, the

effectiveness of cinnamon NE (46.40% inhibition) was more than the pure oil (17.26% inhibition) on AChE activity. NE of cinnamon oil caused 75.42% activation of ATPase enzyme compared to its pure oil (28.56% activation). In contrast, cinnamaldehyde NE caused more effect (172.56% activation) than the T form (120.53%). All tested compounds, at the level of the LC_{50}, proved an activation of GABA-T activity. Cinnamaldehyde NE was the most active product, which caused 271.23% activation for GABA-T compared to 32.21, 93.14, and 46.16% activation for technical cinnamaldehyde, cinnamon NE, and cinnamon EO, respectively.

Table 5. Molecular docking, binding scores, and binding interactions of cinnamaldehyde and temephos within the active sites of AChE (PDB ID: 5X61), ATPase (PDB ID: 4BYG), and GABA-T (PDB ID: 1SF2)

Enzyme	Compound	Docking score (ΔG, kcal/mol)	Van der Waals	H-Bond			Hydrophobic Interactions (π-interactions)			RMSD
				(Amino acid-ligand atom)	Interaction	Distance (Å)	(Amino acid-ligand atom)	Interaction	Distance (Å)	
AChE	Cinnamaldehyde	-4.32	Gly A445, Gly B445, Ile A231, Ile A446, Ile B231, Ile B446, Phe A490, Phe B490, Trp A441, Trp B441, Tyr A282, Tyr A493, Tyr A494, Tyr B282, Tyr B493, Tyr B494	Cys A447-O1 Cys B447-O1	HBA HBA	3.04 3.04	-	-	-	1.274
	Temephos	-7.41	Asn B438, Asp A233, Asp B233, Gly A445, Gly B445, Ile A231, Ile B231, Leu B444, Phe B449, Trp A441, Trp B441, Tyr A282, Tyr A493, Tyr A494, Tyr B282, Tyr B493, Tyr B494, Val B232, Val B235	Cys A447-S1 Cys A447-S1 Ile A446-S1 Ile A446-S1	HBA HBA HBA HBA	3.78 3.75 4.45 4.45	-	-	-	1.301
ATPase	Cinnamaldehyde	-2.62	Ala B486, Glu A488, Glu B488, Gly A497, Gly B497, His B503, His B515, Val B496	-	-	-	-	-	-	2.353
	Temephos	-4.53	Ala B489, Arg B268, Glu A488, Glu B488, Thr B299, Pro B490	Gly B300-S1 Ser B301-S1	HBA HBA	4.14 4.05	-	-	-	1.835
GABA-T	Cinnamaldehyde	-4.06	Arg A192, Arg C192, Glu A270, Glu B270, His A206, His C206, Phe A189, Phe C189, Phe B351, Phe D351, Lys A329, Tyr B348, Tyr D348, Thr B353, Thr D353, Ile A72, Ile C72	Lys C329-O1	HBA	3.19	-	-	-	0.982
	Temephos	-3.06	Glu A229, Glu B229, Gln A395, Gln B395, Ile A402, Ile B402, Phe A230, Phe B230, Pro A226, Pro B226, Ser A403, Ser B403, Tyr A225, Tyr B225	Arg A222-S1 Arg B222-S1 Pro A399-S12 Pro B399-S12	HBD HBD HBA HBA	3.39 3.46 3.47 3.47	-	-	-	2.007

RMSD: The root means square deviation of the pose, in Å, from the original ligand. This field is present if the site definition is identical to the ligand definition.

According to some authors, the inhibition of acetylcholinesterase by terpenes has no relationship with their larvicidal properties (Cai et al., 2018; Seo et al., 2015). Miyazawa and Yamafuji (2005) proved that bicyclic hydrocarbons with allylic methyl group uncompetitive inhibitors for AChE. In contrast, bicyclic alcohols and ketones exhibited weak inhibition. Inhibition of AChE by α-pinene, terpineol, linalool, β-myrcene, nerol, and geraniol was found at high concentrations (Zarrad et al., 2017). In another study, fenchone, S-carvone, and linalool, followed by estragole, produced a higher inhibition on AChE, but *(E)*-anethole was not active (López and Pascual-Villalobos, 2010). The effects of twenty-one monoterpenes on AChE, acid and alkaline phosphatases, ATPase, and GABA-T were investigated (Taktak and Badawy, 2019). The authors proved that all compounds proved a significant inhibition of all tested enzymes except ATPase. Monoterpenes such as thymol, linalool, menthol, camphor, carvone, and borneol have been discovered to bind to ionotropic GABA receptors in insects (Tong, 2010). Glycolysis produces ATP in the insect body, both in the cell wall and cytoplasm. Consequently, essential oils and their constituents affect the intracellular and extracellular ATP balance (Faleiro, 2011). *In vivo* activation of ATPase at these points may be due to changes in ATP balance as a result of the influence of essential oils and their constituents on cell membranes.

3.5. Molecular docking

Table 5 and Figure 5 show the docking results of cinnamaldehyde with AChE (PDB: 5X61), ATPase (PDB: 4BYG), and GABA-T (PDB: 1SF2), respectively. The data presented as docking scores and different interactions include H-bonds, Van der Waals, and hydrophobic. The binding affinity values of cinnamaldehyde were -4.32, -2.62, and -4.06 kcal/mol on the active sites of AChE, ATPase, and GABA-T, respectively. It can be noted that the highest binding affinity was found with AChE and GABA-T. However, temephos as a reference insecticide exhibited binding affinity values -7.41, -4.53, and -3.06 kcal/mol on the active sites of AChE, ATPase, and GABA-T, respectively (Table 5).

Figure 5A shows the recognized binding modes and molecular orientations (2D and 3D) of cinnamaldehyde with AChE. Seventeen amino acids surround the compound through Van der Waals interactions. The oxygen atom formed two HBA interactions with the amino acids Cys A447 and Cys B447. No hydrophobic interactions were observed. Cinnamaldehyde exhibited low binding affinity with high docking energy toward the active sites of the ATPase (ΔG = -2.62 kcal/mol). It was interacted with ATPase by Van der Waals through ten amino acids (Ala B486, Glu A488, Glu B488,

Gly A497, Gly B497, His B503, His B515, and Val B496). Cinnamaldehyde did not show hydrogen bonds to amino acids or hydrophobic interactions with ATPase in the active pockets. The binding confirmation of the cinnamaldehyde with ATPase is shown in Figure 5B. This compound is surrounded by fourteen amino acids through Van der Waals interactions in GABA-T (Figure 5C). The O1 atom formed one HBA with the amino acid Lys C329 via hydrogen bonding interaction (3.19 Å).

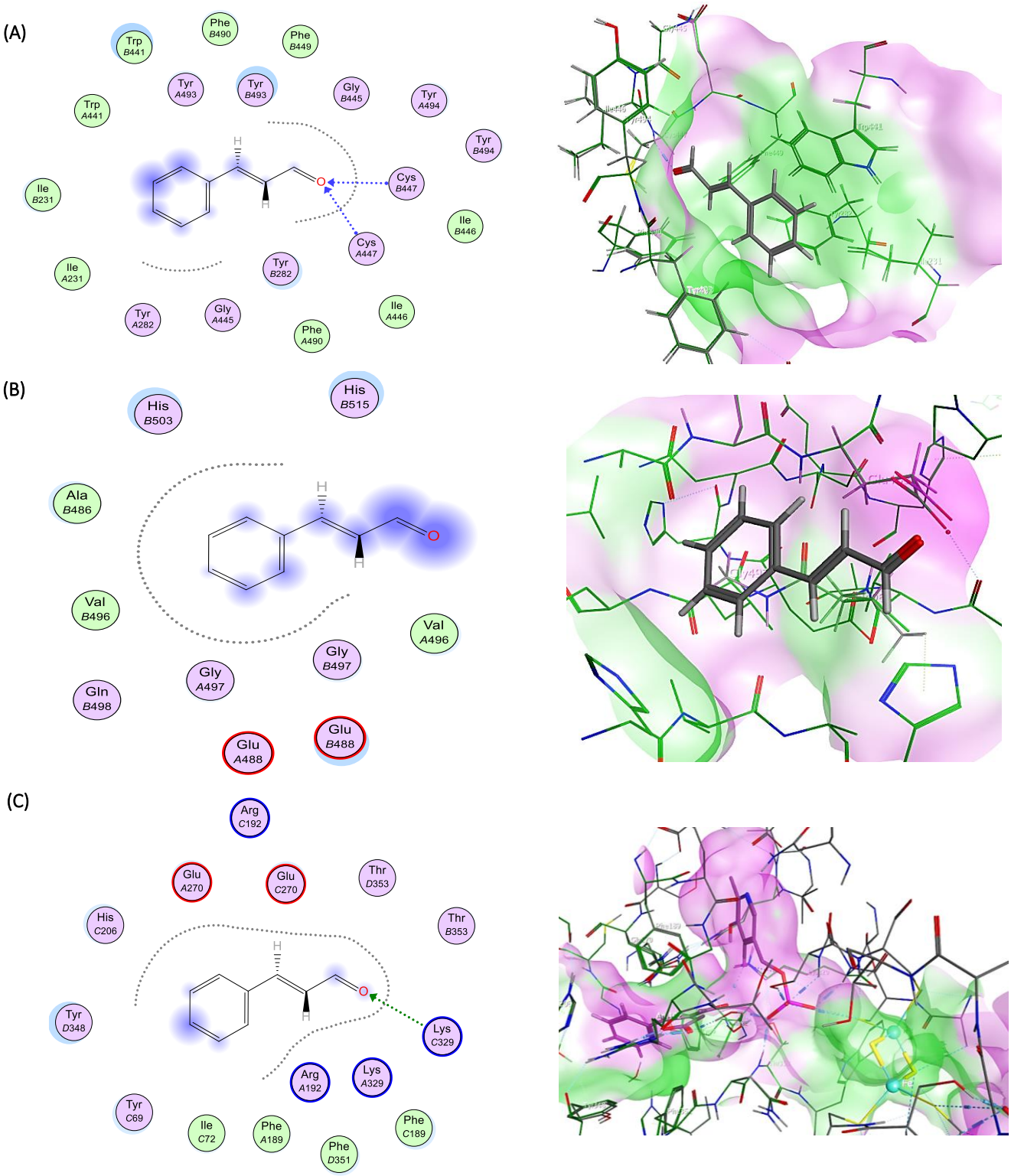

Figure 5. Docking view of cinnamaldehyde on the binding sites of AChE (PDB: 5X61) (A), ATPase (PDB: 4BYG) (B), and GABA-T (PDB: 1SF2) (C). Left are the 2D interaction diagrams, and right are the complex structures in 3D.

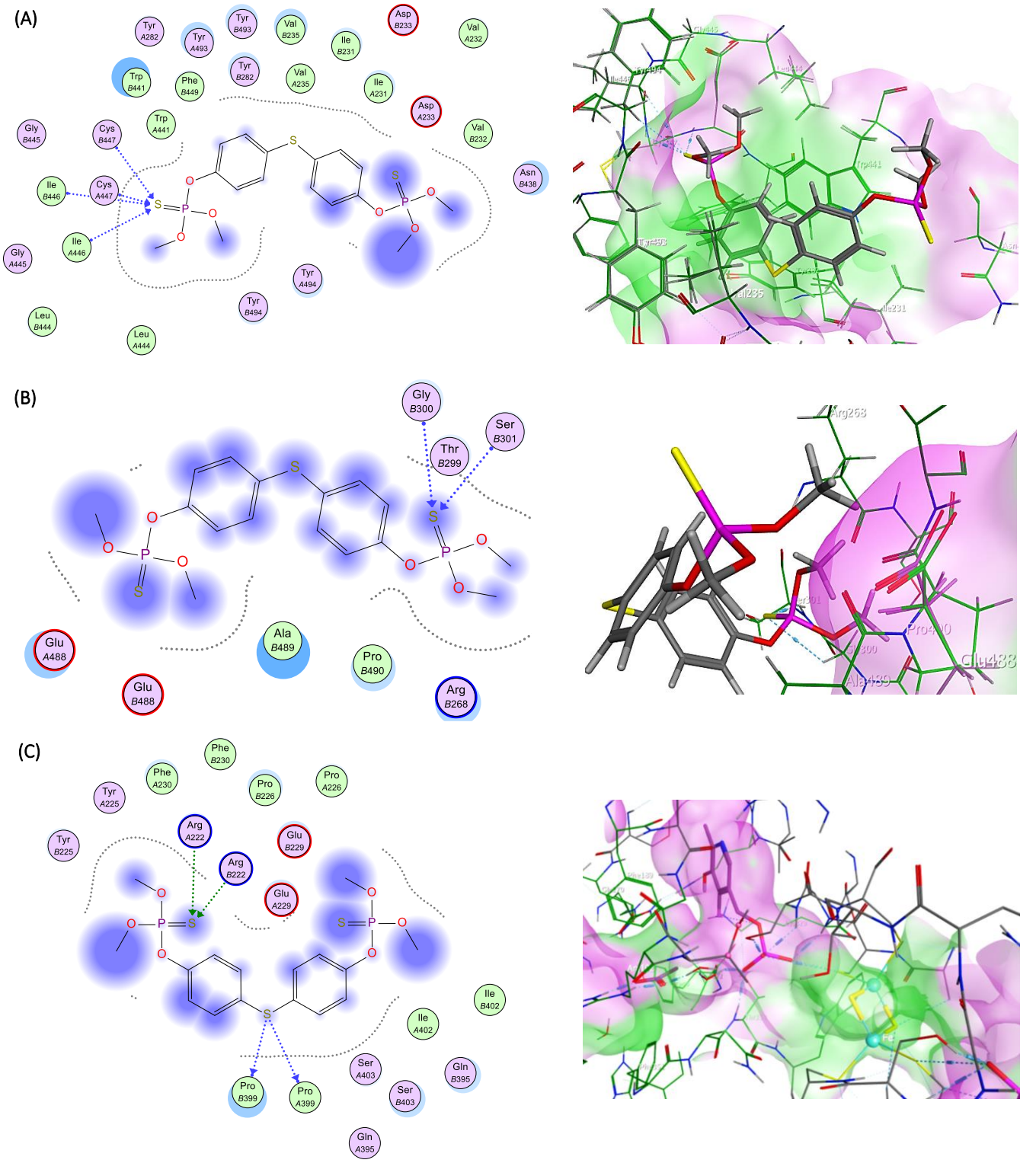

Figure 6. Docking view of temephos on the binding sites of AChE (PDB: 5X61) (A), ATPase (PDB: 4BYG) (B), and GABA-T (PDB: 1SF2) (C). Left are the 2D interaction diagrams, and right are the complex structures in 3D.

Figure 6 shows the recognized binding modes and molecular orientations (2D and 3D) of temephos with the target enzymes. The insecticide bonded with AChE Van der Waals, hydrogen bonding, and hydrophobic interactions. Sulfur atom in the thiophosphate group interacted with four amino acids, Cys A447-S1, Cys A447-S1, Ile A446-S1, and Ile A446-S1 through HBA (Figure 6A). No hydrophobic interactions were found. The binding confirmation with ATPase is shown in Figure 6B. The sulfur atom in the thiophosphate group formed two HBAs with the amino acids Gly B300 and Ser B301 with 3.39 and 3.46 Å, respectively. The insecticide is surrounded by fourteen amino acids through Van der Waals interaction in GABA-T (Figure 6C). The sulfur atom in the thiophosphate group formed two HBDs with the amino acids Arg A222 and Arg B222 with 3.39 and 3.46 Å, respectively. Moreover, the sulfur atom of sulfanylphenoxy moiety formed two HBAs with the amino acids Pro A399 and Pro B399 with 3.39 and 3.46 Å, respectively. No hydrophobic interactions were found.

Because the mechanism of larvicidal activity of monoterpenes is not wholly understood, molecular docking is a method to predict and understand molecular recognition. It detects the predominant binding mode and binding affinity between the protein and ligand and gives a 3D structural explanation of the interaction in several biological processes (Gumede et al., 2012; Lie et al., 2011). This tool was used to elucidate the biological activities of some larvicides with their pharmacokinetic properties (Kumar et al., 2018; Zeng et al., 2016). The docking study revealed that cinnamaldehyde has low binding energy and a high affinity for the functional pocket of the enzyme targets and can bind to the active sites of those enzymes.

4. Conclusions

Natural pesticides derived from natural resources are urgently needed to control mosquitoes without causing harm to beneficial organisms or the environment. Therefore, NEs of cinnamon oil and cinnamaldehyde were prepared and characterized as alternatives to synthetic mosquitocides. The NEs enhanced the mosquitocidal activity against *C. pipiens* larvae and displayed a remarkable effect. As a result, cinnamaldehyde had more larvicidal activity than cinnamon oil 24 h after exposure. Furthermore, the action of the NEs was higher than the unformulated products on the target enzymes (AChE, ATPase, and GABA-T). Additionally, the molecular docking study demonstrated that cinnamaldehyde had a greater affinity for AChE and GABA-T than ATPase. These results indicate that cinnamon oil and cinnamaldehyde NEs have an overall significant effect on *C. pipiens* larvae and might prove helpful as mosquitocide agents.

Acknowledgments

None.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

CRediT authorship contribution statement

All authors contributed to the study's conception and design. They performed material preparation, data collection, and analysis. Nehad E.M. Taktak performed the preparation, characterization of the NEs, the bioassay techniques, and molecular docking studies. In addition, she also wrote the draft of the manuscript. Osama M. Awad, Nadia E. Abou El-Ela, and Mohamed E.I. Badawy designed the experiments, assisted in the characterization of the NEs, analyzed and interpreted the data, and wrote the manuscript. All authors participated in article proofreading, sentence correction and approved the final manuscript.

Ethics approval and consent to participate

Approval of the Ethics Committee of the High Institute of Public Health, Alexandria University, Egypt, was obtained on 10th July 2018 with a reference number of 481. The study was carried out in compliance with the International Guidelines for Research Ethics. Consent to Participate is not applicable.

ORCID Numbers of the Authors

N.E.M. Taktak: 0000-0002-5542-8851
M.E.I. Badawy: 0000-0002-6923-5452
O.M. Awad: 0000-0002-7372-5462
N.E.A. El-Ela: 0000-0000-0000-0000

Supplementary File

None.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18(2), 265-267.

Abdelrasoul, M.A., Ahmed, A.R., Badawy, M.E.I., 2018. Formulation, Characterizations and Antibacterial Activity of some Nanoemulsions Incorporating Monoterpenes. *Journal of Plant Protection and Pathology*, 9(10), 697-705.

Abdelrasoul, M.A., Eid, A.R., Badawy, M.E., 2020. Preparation, characterizations and antibacterial activity of different nano-emulsions incorporating monoterpenes: *in vitro* and *in vivo* studies. *Archives of Phytopathology and Plant Protection*, 53(7-8), 310-334.

Adinew, B., 2014. GC-MS and FT-IR analysis of constituents of essential oil from Cinnamon bark growing in South-west of Ethiopia. *International Journal of Herbal Medicine*, 1(6), 22-31.

Alsaraf, S., Hadi, Z., Akhtar, M.J., Khan, S.A., 2021. Chemical profiling, cytotoxic and antioxidant activity of volatile oil isolated from the mint (*Mentha spicata* L.,) grown in Oman. *Biocatalysis and Agricultural Biotechnology*, 43, 102034.

Badawy, M.E.I., Abdelgaleil, S.A., Mahmoud, N.F., Marei, A.E.S.M., 2018. Preparation and characterizations of essential oil and monoterpene nano-emulsions and acaricidal activity against two-spotted spider mite (*Tetranychus urticae* Koch). *International Journal of Acarology*, 44(7), 330-340.

Badawy, M.E.I., Saad, A.F.S., Tayeb, E.S.H., Mohammed, S.A., Abd-Elnabi, A.D., 2017. Optimization and characterization of the formation of oil-in-water diazinon nano-emulsions: Modeling and influence of the oil phase, surfactant and sonication. *Journal of Environmental Science and Health, Part B*, 52(12), 896-911.

Balaji, A.P.B., Sastry, T.P., Manigandan, S., Mukherjee, A., Chandrasekaran, N., 2017. Environmental benignity of a pesticide in soft colloidal hydrodispersive nanometric form with improved toxic precision towards the target organisms than non-target organisms. *Science of The Total Environment*, 579, 190-201.

Benita, S., Levy, M.Y., 1993. Submicron emulsions as colloidal drug carriers for intravenous administration: comprehensive physicochemical characterization. *Journal of Pharmaceutical Sciences*, 82(11), 1069-1079.

Bruxel, F., Laux, M., Wild, L.B., Fraga, M., Koester, L.S., Teixeira, H.F., 2012. Nanoemulsões como sistemas de liberação parenteral de fármacos. *Química Nova*, 35(9), 1827-1840.

Cai, X., Li, Q., Xiao, L., Lu, H., Tang, J., Huang, J., Yuan, J., 2018. Insecticidal and Acetylcholinesterase Inhibition Activity of *Veratrum nigrum* Alkaloidal Extract against the German Cockroach (*Blattella germanica*). Journal of Arthropod-Borne Diseases, 12(4), 414.

Chemical Computing Group M., 2008. Molecular Operating Environment. Montreal https://wwwchemcompcom/.

Côa, F., Bortolozzo, L.S., Petry, R., Da Silva, G.H., Martins, C.H.Z., de Medeiros, A.M.Z., Sabino, C.M.S., Costa, R.S., Khan, L.U., Delite, F.S., 2020. Environmental toxicity of nanopesticides against non-target organisms: the state of the art. *Nanopesticides*, 227-279.

Dias, C.N., Moraes, D.F.C., 2014. Essential oils and their compounds as *Aedes aegypti* L.(Diptera: Culicidae) larvicides. *Parasitology Research*, 113(2), 565-592.

Drais, H.K., Hussein, A.A., 2015. Formulation and characterization of carvedilol nano-emulsion oral liquid dosage form. *International Journal of Pharmaceutical Science*, 7(12), 209-216.

El Gohary, E.G., Farag, S., El-Sayed, A., Khattab, R., Mahmoud, D., 2021. Insecticidal Activity and Biochemical Study of the Clove Oil (*Syzygium aromaticum*) Nano-Formulation on *Culex pipiens* L.(Diptera: Culicidae). *Egyptian Journal of Aquatic Biology and Fisheries*, 25(1), 227-239.

Ellman, G.L., Courtney, K.D., Andres Jr, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7(2), 88-95.

El-Sabrout, A.M., Zoghroban, A.A., Abdelgaleil, S.A., 2020. Chemical composition and effects of four essential oils on mortality, development and physiology of the West Nile virus vector, *Culex pipiens*. *International Journal of Tropical Insect Science*, 40, 789-799.

Faleiro, M.L., 2011. The mode of antibacterial action of essential oils. *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances*, 2, 1143-1156.

Feng, J., Zhang, Q., Liu, Q., Zhu, Z., McClements, D.J., Jafari, S.M., 2018. Application of nano-emulsions in formulation of pesticides. In Nanoemulsions (pp. 379-413). Academic Press.

Finney, D.J., 1971. Probit Analysis: 3d Ed. Cambridge University Press.

Ghosh, A., Chowdhury, N., Chandra, G., 2012. Plant extracts as potential mosquito larvicides. *The Indian Journal of Medical Research*, 135(5), 581.

Ghosh, V., Saranya, S., Mukherjee, A., Chandrasekaran, N., 2013. Cinnamon oil nano-emulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. *Journal of Nanoscience and Nanotechnology*, 13(1), 114-122.

Gumede, N.J., Singh, P., Sabela, M.I., Bisetty, K., Escuder-Gilabert, L., Medina-Hernández, M.J., Sagrado, S., 2012. Experimental-like affinity constants and enantioselectivity estimates from flexible docking. *Journal of Chemical Information and Modeling*, 52(10), 2754-2759.
Halgren, T.A., 1999. MMFF VI. MMFF94s option for energy minimization studies. *Journal of Computational Chemistry*, 20(7), 720-729.
Holder, P., Browne, G., Bullians, M., 1999. The mosquitoes of New Zealand and their animal disease significance. *Surveillance*, 26(4), 12-15.
IBM 2017. Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.
Izquierdo, P., Esquena, J., Tadros, T.F., Dederen, C., Garcia, M.J., Azemar, N., Solans, C., 2002. Formation and stability of nano-emulsions prepared using the phase inversion temperature method. *Langmuir*, 18(1), 26-30.
Jesser, E., Yeguerman, C., Gili, V., Santillan, G., Murray, A.P., Domini, C., Werdin-González, J.O., 2020. Optimization and characterization of essential oil nano-emulsions using ultrasound for new ecofriendly insecticides. *ACS Sustainable Chemistry & Engineering*, 8(21), 7981-7992.
Kabri, T.H., Arab-Tehrany, E., Belhaj, N., Linder, M., 2011. Physico-chemical characterization of nano-emulsions in cosmetic matrix enriched on omega-3. *Journal of Nanobiotechnology*, 9(1), 1-8.
Karuppusamy, B., Sarma, D.K., Lalmalsawma, P., Pautu, L., Karmodiya, K., Nina, P.B., 2021. Effect of climate change and deforestation on vector borne diseases in the North-Eastern Indian state of Mizoram bordering Myanmar. *The Journal of Climate Change and Health*, 2, 100015.
Kentish, S., Wooster, T.J., Ashokkumar, M., Balachandran, S., Mawson, R., Simons, L., 2008. The use of ultrasonics for nano-emulsion preparation. *Innovative Food Science & Emerging Technologies*, 9(2), 170-175.
Kessler, A., Sahin-Nadeem, H., Lummis, S.C., Weigel, I., Pischetsrieder, M., Buettner, A., Villmann, C., 2014. GABAA receptor modulation by terpenoids from *Sideritis* extracts. *Molecular Nutrition & Food Research*, 58(4), 851-862.
Kharoubi, R., Rehimi, N., Khaldi, R., Haouari-Abderrahim, J., Soltani, N., 2021. Phytochemical Screening and Insecticidal Activities of Essential oil of *Mentha* x *piperita* L.(Lamiales: Lamiaceae) and their Enzymatic Properties against Mosquito *Culex pipiens* L.(Diptera: Culicidae). *Journal of Essential Oil Bearing Plants*, 24(1), 134-146.
Koch, R.B., 1969. Chlorinated hydrocarbon insecticides: inhibition of rabbit brain ATPase activities. *Journal of Neurochemistry*, 16(2), 269-271.
Kumar, A., Behera, P.C., Rangra, N.K., Dey, S., Kant, K., 2018. Computational tool for immunotoxic assessment of pyrethroids toward adaptive immune cell receptors. *Pharmacognosy Magazine*, 14(53), 124.
Kumar, D., Kumar, P., Singh, H., Agrawal, V., 2020. Biocontrol of mosquito vectors through herbal-derived silver nanoparticles: Prospects and challenges. *Environmental Science and Pollution Research*, 27(21), 25987-26024.
Kweka, E.J., Lima, T.C., Marciale, C.M., de Sousa, D.P., 2016. Larvicidal efficacy of monoterpenes against the larvae of *Anopheles gambiae*. *Asian Pacific Journal of Tropical Biomedicine*, 6(4), 290-294.
Li, S.L., Acosta, A.L., Hill, S.C., Brady, O.J., de Almeida, M.A., Cardoso, J.D.C., Messina, J.P., 2022. Mapping environmental suitability of *Haemagogus* and *Sabethes* spp. mosquitoes to understand sylvatic transmission risk of yellow fever virus in Brazil. *PLOS Neglected Tropical Diseases*, 16(1), e0010019.
Lie, M.A., Thomsen, R., Pedersen, C.N., Schiøtt, B., Christensen, M.H., 2011. Molecular docking with ligand attached water molecules. *Journal of Chemical Information and Modeling*, 51(4), 909-917.
López, M.D., Pascual-Villalobos, M.J., 2010. Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Industrial Crops and Products*, 31(2), 284-288.
Mahdi, E.S., Noor, A.M., Sakeena, M.H., Abdullah, G.Z., Abdulkarim, M.F., Sattar, M.A., 2011. Formulation and *in vitro* release evaluation of newly synthesized palm kernel oil esters-based nano-emulsion delivery system for 30% ethanolic dried extract derived from local *Phyllanthus urinaria* for skin antiaging. *International Journal of Nanomedicine*, 6, 2499.
Manimaran, A., Cruz, M.M.J.J., Muthu, C., Vincent, S., Ignacimuthu, S., 2012. Larvicidal and knockdown effects of some essential oils against *Culex quinquefasciatus* Say, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston). *Advances in Bioscience and Biotechnology*, 13, 855-862.
McClements, D.J., 2012. Nano-emulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter*, 8(6), 1719-1729.
Mibielli, R.B., Gerber, T., Mazzarino, L., Veleirinho, M.B., Yunes, R.A., Maraschin, M., 2021. Development of a Spectrophotometric Method for Quantification of Carvacrol in Nanoemulsions. *Revista Brasileira de Farmacognosia*, 31(1), 116-120.
Michaelakis, A., Vidali, V.P., Papachristos, D.P., Pitsinos, E.N., Koliopoulos, G., Couladouros, E.A., Kimbaris, A.C., 2014. Bioefficacy of acyclic monoterpenes and their saturated derivatives against the West Nile vector *Culex pipiens*. *Chemosphere*, 96, 74-80.
Mishra, P., Tyagi, B.K., Chandrasekaran, N., Mukherjee, A., 2018. Biological nanopesticides: a greener approach towards the mosquito vector control. *Environmental Science and Pollution Research*, 25(11), 10151-10163.
Miyazawa, M., Yamafuji, C., 2005. Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. *Journal of Agricultural and Food Chemistry*, 53(5), 1765-1768.
Moretti, M.D., Sanna-Passino, G., Demontis, S., Bazzoni, E., 2002. Essential oil formulations useful as a new tool for insect pest control. *An Official Journal of the American Association of Pharmaceutical Scientists*, 3(2), 64-74.
Munawiroh, S.Z., Nabila, A.N., Chabib, L., 2017. Development of water in olive oil (W/O) Nanoemulsions as lipstick base formulation. *International Journal of Pharma Medicine and Biological Sciences*, 6(2), 37-42.
Nenaah, G.E., Almadiy, A.A., Al-Assiuty, B.A., Mahnashi, M.H., 2021. The essential oil of *Schinus terebinthifolius* and its nano-emulsion and isolated monoterpenes: investigation of their activity against *Culex pipiens* with insights into the adverse effects on non-target organisms. *Pest Management Science*, In press.
Norris E.J., Bartholomay L., Coats J., 2018. Present and future outlook: the potential of green chemistry in vector control. In: Strickman D. (ed) Advances in the Biorational Control of Medical and Veterinary Pests. ACS Publications, pp 43-62.
Pandey, A., Singh, R., 1985. Response of the cockroach brain gamma-aminobutyric acid system to isonicotinic acid hydrazide and mercaptopropionic acid. *Biochemistry International*, 10(2), 213-220.
Pavela, R., 2015. Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. *Parasitology Research*, 114(10), 3835-3853.
Pengon, S., Chinatangkul, N., Limmatvapirat, C., Limmatvapirat, S., 2018. The effect of surfactant on the physical properties of coconut oil nano-emulsions. *Asian Journal of Pharmaceutical Sciences*, 13(5), 409-414.
Qin, H., Zhang, H., Li, L., Zhou, X., Li, J., Kan, C., 2017. Preparation and properties of lambda-cyhalothrin/polyurethane drug-loaded nano-emulsions. *RSC Advances*, 7(83), 52684-52693.
Rao, P., Goswami, D., Rawal, R.M., 2021. Revealing the molecular interplay of curcumin as *Culex pipiens* Acetylcholine esterase 1 (AChE1) inhibitor. *Scientific Reports*, 11(1), 1-18.
Rattan, R.S., 2010. Mechanism of action of insecticidal secondary metabolites of plant origin. *Crop Protection*, 29(9), 913-920.
Rehman, J.U., Ali, A., Khan, I.A., 2014. Plant based products: Use and development as repellents against mosquitoes: A review. *Fitoterapia*, 95, 65-74.
Ríos, N., Stashenko, E.E., Duque, J.E., 2017. Evaluation of the insecticidal activity of essential oils and their mixtures against *Aedes aegypti* (Diptera: Culicidae). *Revista Brasileira de Entomologia*, 61, 307-311.
Rocklöv, J., Dubrow, R., 2020. Climate change: an enduring challenge for vector-borne disease prevention and control. *Nature Immunology*, 21(5), 479-483.
Seo, S.M., Jung, C.S., Kang, J., Lee, H.R., Kim, S.W., Hyun, J., Park, I.K., 2015. Larvicidal and acetylcholinesterase inhibitory activities of Apiaceae plant essential oils and their constituents against *Aedes albopictus* and formulation development. *Journal of Agricultural and Food Chemistry*, 63(45), 9977-9986.
Silva, A.L., Alexandrino, F., Verissimo, L.M., Agnez-Lima, L.F., Egito, L.C.M., De Oliveira, A.G., Do Egito, E.S.T., 2012. Physical factors affecting plasmid DNA compaction in stearylamine-containing nano-emulsions intended for gene delivery. *Pharmaceuticals*, 5(6), 643-654.
Smoluchowski, M.V., 1916. Drei vortrage uber diffusion, brownsche bewegung und koagulation von kolloidteilchen. *Zeitschrift fur Physik*, 17, 557-585.
Solans, C., Izquierdo, P., Nolla, J., Azemar, N., Garcia-Celma, M.J., 2005. Nano-emulsions. *Current Opinion in Colloid & Interface Science*, 10(3-4), 102-110.
Taktak, N.E., Badawy, M.E.I., 2019. Potential of hydrocarbon and oxygenated monoterpenes against *Culex pipiens* larvae: Toxicity, biochemical, pharmacophore modeling and molecular docking studies. *Pesticide Biochemistry and Physiology*, 158, 156-165.
Taktak, N.E., Badawy, M.E.I., Awad, O.M., Abou El-Ela, N.E., Abdallah, S.M., 2021. Enhanced mosquitocidal efficacy of pyrethroid insecticides by nanometric emulsion preparation towards *Culex pipiens* larvae with biochemical and molecular docking studies. *Journal of the Egyptian Public Health Association*, 96(1), 1-19.
Tang, S.Y., Shridharan, P., Sivakumar, M., 2013. Impact of process parameters in the generation of novel aspirin nano-emulsions–comparative studies between ultrasound cavitation and microfluidizer. *Ultrasonics Sonochemistry*, 20(1), 485-497.
Tong, F., 2010. Investigation of mechanisms of action of monoterpenoid insecticides on insect gamma-aminobutyric acid receptors and nicotinic acetylcholine receptors. Iowa State University.
Tyagi S., Panda A., Khan S., 2012. Formulation and evaluation of diclofenac diethyl amine microemulsion incorporated in hydrogel. *World Journal of Pharmaceutical Research*, 1, 1298-1319.
Wang, R., Wang, R., Yang, B., 2009. Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. *Innovative Food Science & Emerging Technologies*, 10(2), 289-292.
Wangai, L.N., Kamau, K.K., Munyekenye, G., Nderu, D., Maina, E., Gitau, W., Otieno, F., 2020. Efficacy of Plant-based Repellents Against Anopheles Mosquitoes: A Systematic Review. *Biomedical Sciences*, 6, 44.
WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization.
WHO, 2017. Global vector control response 2017-2030. World Health Organization, 1-16.
Wilder-Smith, A., Gubler, D.J., Weaver, S.C., Monath, T.P., Heymann, D.L., Scott, T.W., 2017. Epidemic arboviral diseases: priorities for research and public health. *The Lancet Infectious Diseases*, 17(3), e101-e106.
Yu, T., Yao, H., Qi, S., Wang, J., 2020. GC-MS analysis of volatiles in cinnamon essential oil extracted by different methods. *Grasas y Aceites*, 71(3), 372.
Zahran, H.E.D.M., Abou-Taleb, H.K., Abdelgaleil, S.A., 2017. Adulticidal, larvicidal and biochemical properties of essential oils against *Culex pipiens* L. *Journal of Asia-Pacific Entomology*, 20(1), 133-139.

Zarrad K., Laarif A., Ben H.A., Chaieb I., Mediouni B.J.J., 2017. Anticholinesterase potential of monoterpenoids on the whitefly *Bemisia tabaci* and their kinetic studies. *Journal of Agricultural Science and Technology*, 19, 643-652.

Zeghib, F., Tine-Djebbar, F., Zeghib, A., Bachari, K., Sifi, K., Soltani, N., 2020. Chemical Composition and Larvicidal Activity of *Rosmarinus officinalis* Essential Oil Against West Nile Vector Mosquito *Culex pipiens* (L.). *Journal of Essential Oil Bearing Plants*, 23(6), 1463-1474.

Zeng, H.J., Yang, D., Hu, G.Z., Yang, R., Qu, L.B., 2016. Studies on the binding of pepsin with three pyrethroid insecticides by multi-spectroscopic approaches and molecular docking. *Journal of Molecular Recognition*, 29(10), 476-484.

Reviewed by:

Yahyea Baktiar Laskar: Assam University, Silchar, INDIA
Kemal Buyukguzel: Zonguldak Bulent Ecevit University, Zonguldak, TURKEY