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Aquatic Larvicidal Efficacy of *Periplaneta americana* Chitin and Essential Oils Against *Culex pipiens*: A Comparative Assessment

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ABSTRACT

With increasing resistance to chemical insecticides and mounting ecological concerns, the demand for eco-friendly mosquito control strategies has grown significantly. This study evaluated the physicochemical properties and larvicidal potential of chitin extracted from *Periplaneta americana* and compared its efficacy with several essential oils: orange, azadirachtin, jojoba, and an avocado–coconut oil blend against third-instar *Culex pipiens* larvae. FTIR and XRD analyses confirmed the extracted chitin as highly crystalline α-chitin, structurally comparable to commercial shrimp chitin. Elemental analysis and Degree of Acetylation (DA) values further verified high purity of extracted chitin. Despite its excellent structural integrity, chitin exhibited minimal larvicidal activity (LC_{50} = 2428.54ppm; TI = 10.55) at 48 hours post-treatment. In contrast, orange oil showed the highest larvicidal efficacy (LC_{50} = 256.30ppm; TI = 100), with larvae displaying pronounced neurotoxic symptoms, including uncoordinated movements and spasms. These observations prompted molecular docking analysis targeting the insecticide-resistant acetylcholinesterase (AChE) mutant of *Anopheles gambiae* (PDB ID: 6ARY). Key orange oil constituents, particularly sinensal and neryl acetate exhibited strong binding affinities and critical interactions with active-site residues, comparable to those of the co-crystallized inhibitor and chlorpyrifos. To validate these *in-silico* findings, biochemical assays were conducted and confirmed significant AChE inhibition, alongside notable reductions in larval carbohydrate, lipid, and protein levels. While chitin itself lacked direct larvicidal potency, its physicochemical stability, biocompatibility, and structural purity support its potential use as a delivery platform. We propose the development of chitin- or chitosan-based nano-formulations to encapsulate essential oils like orange oil, enhancing their stability, bioavailability, and sustained release for more effective and environmentally responsible mosquito control.

INTRODUCTION

Mosquitoes remain among the most significant vectors of infectious diseases worldwide, transmitting a broad array of pathogens including viruses, bacteria, and parasites that pose serious health risks to both humans and animals **(Gorris *et al*., 2021;**

Adly *et al*., 2022; Dhiman & Singh, 2024; Rady *et al*., 2024). Among the most widespread and epidemiologically important mosquito species is *Culex pipiens*, commonly known as the northern house mosquito **(Gorris *et al*., 2021)**. This species is a known vector of several severe diseases, including the West Nile virus, St. Louis encephalitis, and lymphatic filariasis, and thrives in polluted, stagnant water bodies often found in urban and peri-urban environments **(Vitek *et al*., 2008; Hamer *et al*., 2009; Gorris *et al*., 2021)**. The impact of climate change has further complicated vector control efforts by altering the distribution, abundance, and seasonal activity of many arthropod vectors, including mosquitoes potentially expanding the geographical range of vector-borne diseases and increasing transmission risks in previously unaffected areas **(Kamal *et al*., 2018; Abou Elhassan *et al*., 2024; Okely *et al*., 2025)**.

Conventional control strategies have historically relied on synthetic insecticides, such as pyrethroids, organophosphates, and carbamates, particularly as larvicides in aquatic environments **(Vinogradova, 2000; Meier *et al*., 2022)**. However, long-term and widespread use of these chemicals has led to the emergence of insecticide resistance **(Vereecken *et al*., 2022)** and raised significant environmental concerns due to their toxicity to non-target aquatic organisms, including fish, amphibians, crustaceans, and beneficial insects **(Ray & Ghosh, 2006; Antwi & Reddy, 2015)**. These ecological disruptions pose a serious threat to biodiversity and aquatic ecosystem stability. As a result, there is increasing interest in natural, eco-friendly alternatives for mosquito control.

One of the natural methods applied for controlling mosquito larvae involves the use of aquatic insect families such as Dytiscidae, Veliidae, Gerridae, and Notonectidae **(Lundkvist *et al*., 2003; Ahmed & Gadalla, 2005a, b; Blaustein *et al*., 2008)**. Furthermore, biopolymers such as chitin and its deacetylated derivative, chitosan, have gained attention due to their biodegradability, biocompatibility, and low toxicity **(Badawy & Rabea, 2011; Mohan *et al*., 2024)**. While extensively applied in pharmaceutical and cosmetic industries **(Baharlouei & Rahman, 2022; Kulka & Sionkowska, 2023; Szulc & Lewandowska, 2023)**, their role in pest management has been more limited. Chitosan has mainly been studied as a formulation aid or delivery matrix for other insecticidal agents, rather than as a standalone larvicide. Similarly, chitin especially when sourced from insects like *Periplaneta americana* remains underexplored as a direct bioactive agent in mosquito control, despite its structural robustness, environmental compatibility, and low production cost. Systematic investigations into the independent larvicidal efficacy of these biopolymers under standardized conditions are still scarce **(Abenaim & Conti, 2023; Mei *et al*., 2024; Mohan *et al*., 2024)**. This gap presents a promising opportunity to evaluate insect-derived chitin and chitosan in the context of sustainable vector control, particularly in ecologically sensitive aquatic environments.

In this study, we evaluated the larvicidal potential of raw chitin extracted from *Periplaneta americana*, comparing its efficacy to four naturally derived essential oils: Azadirachtin, orange oil, jojoba oil, and a blend of avocado and coconut oils each recognized for insecticidal properties **(Isman, 2006a; Baz *et al*., 2022)**. Initial bioassays were conducted against third-instar *Culex pipiens* larvae to determine relative effectiveness. Given the pronounced neurotoxic symptoms observed in orange oil treated larvae, molecular docking simulations were subsequently performed to explore potential mechanisms of action, focusing on interactions between key oil constituents and the active site of the insecticide-resistant acetylcholinesterase (AChE) G119S mutant (PDB: 6ary). To validate the *in-silico* predictions, biochemical assays were then carried out to assess AChE inhibition and changes in larval carbohydrate, lipid, and protein levels. This stepwise approach beginning with larval toxicity screening, followed by computational modeling and biochemical validation was designed to provide a comprehensive understanding of the effectiveness and underlying mechanisms of action of the tested natural compounds, particularly orange oil, while evaluating the direct bioactivity of insect-derived chitin under standardized laboratory conditions.

MATERIALS AND METHODS

1. Collection and processing of *Periplaneta americana* specimens for chitin extraction

Adult *Periplaneta americana* specimens were manually collected using hand-picking and sweeping nets from multiple sites in Egypt, including Cairo (Abbassia and Ain Shams, 2022; Hadyk El Koba, 2023), Giza (Abu Rawash, 2022), and Ismailia (2023). Collected specimens were euthanized using ethyl acetate and transported in labeled containers to the laboratory. Following taxonomic confirmation, they were rinsed with distilled water to remove debris and air-dried. Exoskeletons were then dehydrated in a drying oven (60–80°C) and ground into a fine powder using a mechanical grinder. This powder served as the starting material for chitin extraction.

2. Extraction and preparation of chitin for structural characterization

Chitin was extracted from finely ground *Periplaneta americana* exoskeleton powder using a modified protocol based on **Kaya and Baran (2015a)**, **Salama *et al*. (2017)** and **Kamal *et al*. (2020)**. The process included deproteinization and demineralization steps to obtain high-purity chitin for characterization and biological testing.

For deproteinization, 100g of exoskeleton powder were treated with 500mL of 1 M NaOH at 90°C for 48 hours with continuous stirring. The residue was filtered and washed with distilled water until neutral pH was achieved. Demineralization followed by soaking the material in 300mL of 1 M HCl at room temperature for 2 hours with

occasional stirring. The solid was filtered and rinsed with distilled water to neutrality. To enhance purity, the product was rinsed with ethanol, oven-dried at 60°C, and stored in dry conditions for further use.

3. Physicochemical characterization of extracted chitin

3.1. Fourier transform infrared (FTIR) characterization of chitin

The structural characteristics of chitin extracted from *Periplaneta americana* were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. The analysis was performed with a Euro EA FTIR spectrometer equipped with a PLATINUM-ATR (attenuated total reflectance) accessory. Spectral data were collected over the wavenumber range of 4000–500cm^{-1}. All measurements were carried out at the Central Laboratory, Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt.

To enable structural comparison, a commercial shrimp-derived chitin sample was analyzed under identical conditions and used as a reference standard. Infrared spectra of both insect- and shrimp-sourced chitin were interpreted based on established peak assignments reported in the literature **(Kaya & Baran, 2015a)**.

The degree of acetylation (DA)—a key indicator of chitin purity and structural preservation—was calculated by measuring the absorbance ratio of the amide I peak at 1655cm^{-1} (A_{1655}) to the hydroxyl group peak at 3450cm^{-1} (A_{3450}), using the following equation **(Liu *et al*., 2012)**:

$$\mathbf{DA} = (\mathbf{A_{1655}}/\mathbf{A_{3450}}) * \mathbf{115}$$

Peak identification and absorbance quantification were conducted using OriginLab® Origin 8.5.1 software.

3.2. X-ray diffraction (XRD) analysis of chitin structure

The crystallinity of chitin extracted from *Periplaneta americana* was assessed using X-ray diffraction (XRD), with a commercial shrimp-derived chitin sample analyzed in parallel for comparison. Measurements were conducted on a Bruker D2 Phaser (2nd generation) diffractometer using CuKα radiation (λ = 1.5418 Å) at an operating voltage of 30 kV and current of 10 mA. Diffraction patterns were recorded over a 2θ range of 5° to 80°, with a step size of 0.02° and a counting time of 0.2 seconds per step. All analyses were carried out at the Desert Research Center, Matariya, Egypt.

To quantify crystallinity, the Crystallinity Index (CrI) was calculated using the following formula, widely cited in chitin research **(Kaya & Baran, 2015a; Kamal *et al*., 2020)**:

$$\boldsymbol{CrI} = \left[\frac{(\boldsymbol{I_{110}} - \boldsymbol{I_{am}})}{\boldsymbol{I_{110}}}\right] * \mathbf{100}$$

Where, I_{110} represents the intensity of the principal crystalline peak (typically around $2\theta \approx 19°$), and I_{am} denotes the intensity of the amorphous background near $2\theta \approx 14.5°$.

Diffraction data were analyzed using OriginLab® Origin 8.5.1 software, which facilitated peak identification, baseline correction, and precise measurement of the intensities required for CrI determination.

3.3. Elemental composition analysis of extracted chitin

Elemental composition analysis was conducted to determine the carbon (C), hydrogen (H), and nitrogen (N) content of chitin extracted from *Periplaneta americana*, with a commercial shrimp-derived chitin sample used as a reference. Measurements were performed using a Flash EA 1112 Series elemental analyzer (Thermo Finnigan, Italy), equipped with a MAS 200 autosampler and operated via Eager 300 software on a Windows-based platform. All procedures adhered to the manufacturer's standard operating protocols and were carried out at the Micro Analytical Center, Faculty of Science, Cairo University.

The degree of acetylation (DA), a key indicator of chitin purity and structural integrity, was calculated from the nitrogen content using a widely accepted equation **(Kaya *et al*., 2014; Kaya & Baran, 2015a; Kamal *et al*., 2020)**:

$$DA = \left[\frac{([C/N] - 5.14)}{1.72}\right] * 100$$

Where, C/N represents the carbon-to-nitrogen ratio derived from the elemental analysis. This method provides a reliable estimate of the acetylation level, which is critical for assessing the suitability of chitin for various applications.

4. Assessment of larvicidal activity of extracted chitin and selected natural oils

To evaluate the potential of chitin as a natural and environmentally sustainable larvicide, a comparative bioassay was conducted to assess its insecticidal activity against *Culex pipiens* larvae. Chitin extracted from *Periplaneta americana* was tested alongside several naturally derived essential oils: Azadirachtin, orange oil, jojoba oil, and a blended mixture of avocado and coconut oils. These substances were selected based on their botanical origin and established insecticidal properties, providing a relevant benchmark for assessing chitin's efficacy as a biological control agent. The primary aim of this study was to determine whether raw chitin could serve as a safe and effective alternative to conventional chemical larvicides, supporting the broader shift toward environmentally responsible pest management strategies.

4.1. Rearing of Culex pipiens mosquitoes

Fifth-generation *Culex pipiens* egg rafts were obtained from the insectary of the Department of Entomology, Faculty of Science, Ain Shams University, Egypt. Egg rafts were placed in white enamel trays (35–40cm diameter, 10cm depth) containing 1500mL of distilled water. Freshly hatched larvae were fed daily with finely powdered Tetra-Min® fish food (Germany), evenly distributed across the water surface. Water was stirred daily and replaced every two days to prevent microbial buildup. Aeration was applied for 5 minutes daily using a small air pump to ensure oxygenation. Pupae were collected and transferred to plastic containers with distilled water for adult emergence. Adults were housed in screened wooden cages and provided with a 10% sucrose solution. Female mosquitoes received a blood meal from a live pigeon to stimulate oviposition. Rearing procedures including feeding, water management, pupal transfer, and adult maintenance—were conducted under controlled laboratory conditions following established protocols **(Adly *et al*., 2022; El-Helw *et al*., 2024, 2025; El-Sayed *et al*., 2024; Haikal *et al*., 2025; Khalil *et al*., 2025; Ramadan *et al*., 2025)**. Environmental parameters were maintained at $27 \pm 2°C$, $70 \pm 10\%$ RH, and a 14:10h light-dark photoperiod.

4.2. Comparative larvicidal bioassay of Periplaneta americana chitin and natural essential oils

This study evaluated the larvicidal potential of chitin extracted from *Periplaneta americana*, in comparison with four natural essential oils: Orange oil, azadirachtin oil, jojoba oil, and Avo-coco oil Mix.

Larval bioassays were performed on early third-instar *Culex pipiens* larvae using the WHO-recommended dipping method **(WHO, 2005)**. Each treatment was tested at 50, 100, and 150ppm in triplicate. For each replicate, 25 larvae were placed in 100mL of the corresponding solution under controlled laboratory conditions ($27 \pm 2°C$; $70 \pm 10\%$ RH; 14:10h light–dark cycle). Distilled water served as a negative control, while an ethanol–water mixture matching the ethanol content used in oil formulations was used as a solvent control.

Larval mortality was recorded at 24-, 48-, and 72-hours post-treatment; however, LC_{50} values were calculated based exclusively on 48-hour mortality data. Larvae were considered dead if they failed to reach the water surface or did not respond to gentle prodding. LC_{50} values were estimated using Finney's probit analysis method **(Finney, 1971)**. This step was supported with mortality corrections applied via Abbott's formula **(Abbott, 1925)**:

$$\textit{Corrected Mortality}\ (\%) = \left[\frac{\textit{Observed Mortality}\ \% - \textit{Control Mortality}\ \%}{100 - \textit{Control Mortality}\ \%}\right] * 100$$

Essential oils were supplied by Shoura Chemicals Company (Cairo–Alexandria Desert Road, Egypt). Stock solutions (10,000 ppm) were prepared by dissolving 0.5mL of oil in 49.5mL of ethanol, and working concentrations were diluted in distilled water.

5. Molecular docking analysis for mode of action prediction

Molecular docking simulations were performed using Molecular Operating Environment (MOE) software (version 2024.06; https://www.chemcomp.com/en/index.htm) to investigate the potential mechanism underlying the larvicidal activity of orange oil, which demonstrated lowest LC_{50} during bioassay test.

Major constituents of orange oil were selected based on their consistent identification in the literature **(Lawrence, 2001; Njoroge *et al*., 2009; González-Mas *et al*., 2019)**. Their 2D structures were drawn in Chem Draw 20.0, converted to 3D using MOE, and optimized through protonation, partial charge assignment, and energy minimization.

Docking simulations targeted the insecticide-resistant acetylcholinesterase (AChE) G119S mutant of *Anopheles gambiae* (PDB ID: 6ARY), co-crystallized with the inhibitor BT7 [(1S)-2,2-difluoro-1-[1-(pentan-3-yl)-1H-pyrazol-4-yl] ethan-1-ol]. The BT7 ligand was retained during receptor preparation to accurately define the active site.

Protein preparation involved the removal of water molecules and non-essential ligands (excluding BT7), addition of hydrogens, and energy minimization. The receptor was treated as rigid, while ligand flexibility was fully enabled.

Each ligand underwent 100 docking iterations. The top 20 poses were ranked by docking score (London ΔG), and the best pose was selected based on the lowest binding energy (S-score), RMSD < 2 Å, and interaction with key catalytic residues. Binding interactions such as hydrogen bonds, π–π stacking, and hydrophobic contacts were examined particularly with TRP245(B), SER280(B), GLU359(B), HIS600(B), and TYR282(B) to elucidate potential AChE inhibition mechanisms.

To validate the docking protocol, chlorpyrifos, a known AChE inhibitor, was docked using the same parameters. Its binding profile was compared with those of the orange oil constituents and the co-crystallized BT7 ligand, ensuring consistency and accuracy in the predicted interaction models **(El-Helw *et al*., 2024, 2025; El-Sayed *et al*., 2024; Haikal *et al*., 2025; Khalil *et al*., 2025; Ramadan *et al*., 2025)**.

6. Biochemical analysis of larval metabolism and acetylcholinesterase activity

To validate the molecular docking predictions specifically the proposed inhibition of acetylcholinesterase (AChE) by orange oil constituents, biochemical assays were performed to evaluate AChE activity in *Culex pipiens* larvae following treatment. Additionally, total carbohydrate, lipid, and protein contents were assessed to understand broader physiological impacts.

All assays were conducted using early third instar *Culex pipiens* larvae. Larvae were homogenized in chilled distilled water (50mg tissue per 1mL) using a glass–Teflon tissue homogenizer (ST–2 Mechanic-Preczyina, Poland). Homogenates were centrifuged at 8000 rpm for 15 minutes at 2°C, and the resulting supernatants were stored at –20°C for analysis.

AChE activity was measured using the colorimetric method of **Simpson *et al*. (1964)**. Each reaction contained 200µL of enzyme extract, 0.5mL of 0.067 M phosphate buffer (pH 7.0), and 0.5mL of 3 mM acetylcholine bromide. After 30 minutes of incubation at 37°C, 1mL of alkaline hydroxylamine was added to stop the reaction, followed by 0.5 mL HCl and 0.5 mL ferric chloride. Absorbance was measured at 515nm, and AChE activity was expressed as µg acetylcholine bromide hydrolyzed/min/100 larvae.

Total carbohydrate content was determined using the phenol–sulfuric acid method **(Aly *et al*., 2023)**. Homogenized tissue (1g) was extracted with 5mL of 0.3 N perchloric acid at 0°C, centrifuged, and reacted with 0.5mL of 20% phenol and 5mL of concentrated sulfuric acid. After 10 minutes of rest and 10–20 minutes at 25–30°C, absorbance was measured at 490nm. Carbohydrate levels were expressed as µg glucose equivalents per 100 larvae.

Total lipids were quantified by the phospho-vanillin method **(Knight *et al*., 1972)**. A 250µL aliquot of homogenate was mixed with 5mL concentrated sulfuric acid, heated for 10 minutes in a boiling water bath, cooled, then reacted with 6mL phospho-vanillin reagent for 45 minutes. Absorbance was measured at 525nm, and lipid content was calculated from a standard curve and reported as µg per 100 larvae.

Protein content was assessed using the Bradford method **(Bradford, 1976)**. A 50µL homogenate sample was diluted in 1mL of 0.1 M phosphate buffer (pH 6.6) and mixed with 5mL of Bradford reagent. After 2 minutes at room temperature, the absorbance was measured at 595nm. Protein concentrations were determined using a bovine serum albumin standard curve and reported as µg per 100 larvae.

All biochemical parameters were measured using three biological replicates per treatment. Statistical analyses were conducted in R **(R Core Team, 2015)** using one-way ANOVA where significant differences were detected ($P < 0.05$). Tukey's Honestly

Significant Difference (HSD) test was applied for pairwise comparisons. Results are reported as mean ± standard deviation (SD).

RESULTS

1. Physicochemical characterization of extracted chitin

1.1. FTIR spectral analysis of extracted chitin

Chitin is a natural polysaccharide composed of repeating units of N-acetyl-D-glucosamine linked through β-(1→4) glycosidic bonds. Its backbone is rich in functional groups such as hydroxyl (–OH), acetamido (–$NHCOCH_3$), and ether (C–O–C), which can be readily identified and characterized using Fourier Transform Infrared (FTIR) spectroscopy to confirm chitin's presence and structural features.

The FTIR spectrum of chitin extracted from *Periplaneta americana* (Fig. 1) displayed several peak characteristics of the α-chitin polymorph. Strong transmittance bands were observed at 3420 and 3255cm^{-1}, corresponding to O–H and N–H stretching vibrations, respectively, along with bands at 2957, 2919, and 2873cm^{-1} attributed to C–H stretching. A key structural indicator was the splitting of the Amide I band into two distinct peaks at 1650 and 1620cm^{-1}, confirming the presence of α-chitin and suggesting intramolecular hydrogen bonding (CO···H–OCH_2). Additional spectral features included peaks at 1549cm^{-1} (Amide II, N–H bending), 1416 and 1373cm^{-1} (C–H bending), 1305cm^{-1} (Amide III, C–N stretching), and 1153cm^{-1} (C–O–C asymmetric stretching). The fingerprint region, ranging from 1258 to 894cm^{-1}, exhibited bands corresponding to C–N and C–O stretching, as well as ring and skeletal vibrations, further affirming the chitin structure.

Similarly, the FTIR spectrum of commercial shrimp-derived chitin (Fig. 1) revealed comparable features. O–H and N–H stretching vibrations were noted at 3444 and 3263cm^{-1}, while C–H stretching appeared at 2930 and 2853cm^{-1}. The Amide I region again showed peak splitting at 1659 and 1629cm^{-1}, consistent with α-chitin. The Amide II and III bands were located at 1559 and 1318cm^{-1}, respectively, with additional peaks at 1419, 1381, 1160, 1069, 1030, 952, and 895cm^{-1}, collectively confirming a well-defined chitin signature. The degree of acetylation (DA), calculated using Formula (1) based on the absorbance ratio of the Amide I (~1655cm^{-1}) and hydroxyl (~3450cm^{-1}) bands, was found to be 111.80% for *Periplaneta americana* chitin and 107.2% for shrimp chitin. While these values exceed the theoretical maximum of 100%, such results are not uncommon in FTIR-based estimations of natural biopolymers and may be attributed to spectral variability, moisture interference, or baseline shifts. Nevertheless, the close similarity in DA values and spectral features strongly supports the conclusion that chitin extracted from *Periplaneta americana* is structurally equivalent to high-quality commercial α-chitin.

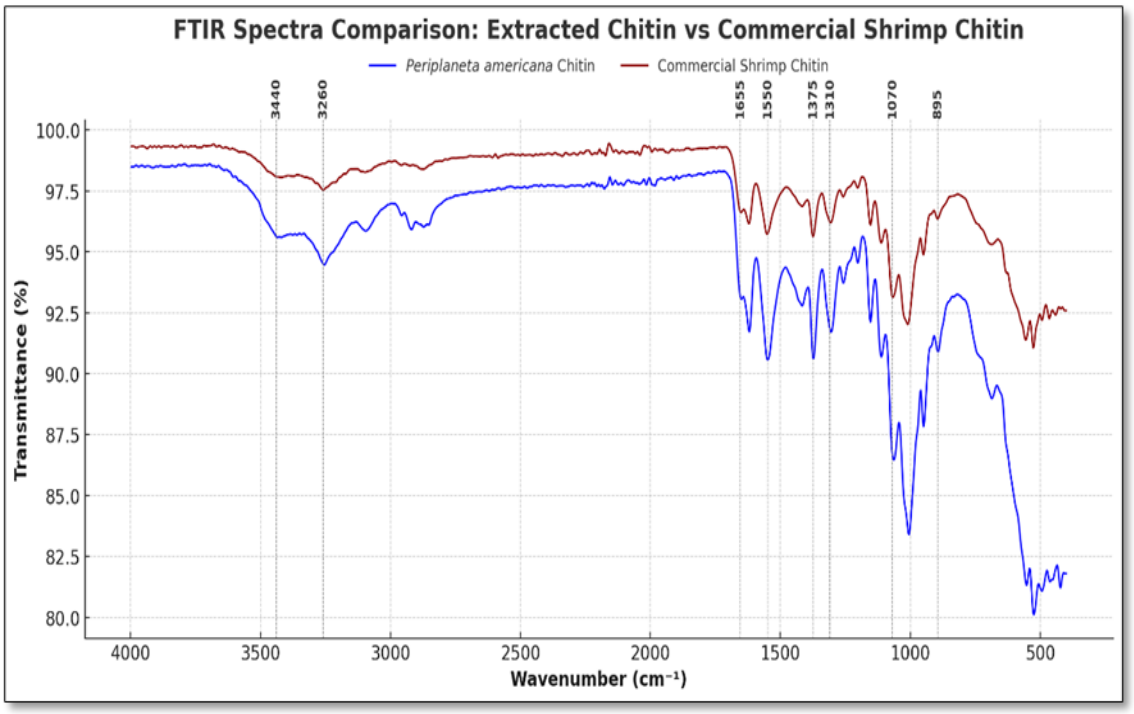

Fig. 1. The FTIR spectra of chitin extracted from *Periplaneta americana* and commercial shrimp chitin demonstrate a high degree of similarity, with characteristic transmittance bands appearing around 3440cm^{-1} (O–H/N–H stretching), 1655cm^{-1} (amide I), 1550cm^{-1} (amide II), and 1375cm^{-1} (C–H bending). The alignment of these distinctive peaks validates the the successful extraction of chitin from *Periplaneta americana*. successful extraction of chitin from *Periplaneta americana*

1.2. XRD analysis of chitin crystallinity

The X-ray diffraction (XRD) pattern of chitin extracted from *Periplaneta americana* (Fig. 2) exhibited a prominent diffraction peak at 2θ ≈ 19.68°, corresponding to the (110) crystalline plane of α-chitin. Additional minor peaks were observed at 6.43°, 22.90°, and 24.24°, further supporting the identification of the α-polymorph. The crystallinity index (CrI), calculated using Formula (2) described in the methodology section, was determined to be 78.66%, indicating a well-ordered crystalline structure. In comparison, the commercial shrimp-derived chitin sample presented a similar diffraction profile. A strong primary peak appeared at 2θ ≈ 19.54°, accompanied by minor peaks at 6.52°, 22.31°, and 23.45°. The calculated CrI for shrimp chitin was slightly higher, at 82.23%, yet remained closely aligned with the value obtained for *Periplaneta americana* chitin.

The strong similarity in diffraction peak positions and CrI values between the two samples confirms that chitin extracted from *Periplaneta americana* possesses a

crystalline structure comparable to that of commercial α-chitin, reinforcing its suitability as a high-quality, natural biopolymer.

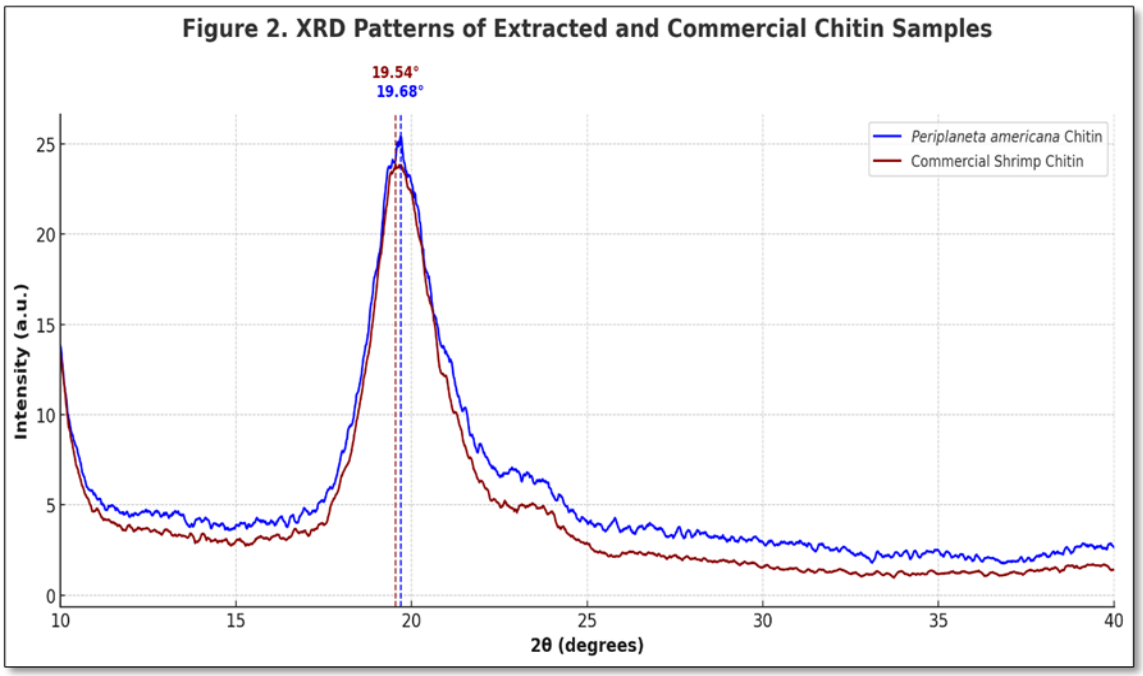

Fig. 2. XRD patterns of chitin extracted from *Periplaneta americana* and commercial shrimp chitin. Both samples show characteristic crystalline peaks of α-chitin. The main diffraction peaks are located at 2θ ≈ 19.68° for *Periplaneta americana* chitin and 2θ ≈ 19.54° for shrimp chitin. The close similarity in peak positions and intensities indicates comparable crystallinity between the two samples

1.3. Elemental composition of extracted chitin

Elemental analysis was performed to determine the carbon (C), hydrogen (H), and nitrogen (N) content of chitin extracted from *P. americana*, with a commercial shrimp-derived chitin sample used as a reference. The degree of acetylation (DA) was subsequently calculated using Formula (3), as outlined in the methodology. The results are summarized in Table (1).

The chitin sample from *Periplaneta americana* exhibited carbon, hydrogen, and nitrogen contents of 43.48, 6.90, and 6.13%, respectively, resulting in a calculated DA of 113.54%. In comparison, the shrimp chitin sample contained 43.32% carbon, 6.85% hydrogen, and 6.41% nitrogen, corresponding to a DA of 94.00%.

These DA values indicate a high proportion of acetylated glucosamine units, characteristic of well-preserved α-chitin. The slightly elevated DA observed in the *Periplaneta americana* sample may be attributed to minor experimental variability or the

presence of residual organic matter, which could influence nitrogen quantification. Nevertheless, the high DA values in both samples align with the expected chemical profile of natural α-chitin and further confirm the successful extraction of structurally intact chitin from *Periplaneta americana*.

Table 1. Elemental analysis of chitin extracted from *Periplaneta americana* and commercial shrimp chitin includes measurements of carbon, hydrogen, and nitrogen percentages, along with the calculated Degree of Acetylation (DA)

Chitin Sample	C %	H %	N %	DA %
P. americana	43.48	6.90	6.13	113.54
Shrimp	43.32	6.85	6.41	94

The elevated DA values indicate a substantial presence of acetylated glucosamine units, which is a defining feature of well-preserved α-chitin.

2. Comparative larvicidal activity of extracted chitin and natural oils

The larvicidal efficacy of chitin extracted from *Periplaneta americana* was assessed in comparison with four natural biocidal agents: Orange oil, azadirachtin oil, jojoba oil, and a blended mixture of avocado and coconut oils against third-instar *Culex pipiens* larvae. Mortality was evaluated 48 hours post-treatment, and the results are summarized in Table (2) and Fig. (3).

Among all tested compounds, chitin exhibited the lowest larvicidal activity. Its LC_{50} value was calculated at 2428.54ppm, the highest of the group, indicating that significantly higher concentrations were required to achieve 50% mortality. The corresponding Toxicity Index (TI) was 10.55, making it only 10.55% as potent as orange oil, which served as the reference standard. Despite its limited efficacy, chitin's regression model demonstrated strong reliability, with a high coefficient of determination ($r^2 = 0.98$) and a good fit to the probit model ($\chi^2 = 0.11$, $P = 0.75$).

In contrast, orange oil showed the highest larvicidal potency, with an LC_{50} of 256.30 ppm and a TI of 100. The regression model fit was strong ($r^2 = 0.90$), and the goodness-of-fit test ($\chi^2 = 1.924$, $P = 0.17$) confirmed the reliability of the dose–response prediction. Larvae treated with orange oil also displayed notable neurotoxic symptoms, including uncoordinated and erratic movements, suggesting disruption of acetylcholinesterase (AChE) activity or interference with octopaminergic or GABAergic pathways. Physical deformities, such as siphon loss and, in some cases, head detachment, were also observed, likely due to membrane destabilization or cuticular degradation by the oil's constituents. Conversely, larvae exposed to chitin showed neither neurotoxic symptoms nor morphological abnormalities, further supporting its distinct and likely non-neuroactive mode of action.

The avocado–coconut oil mixture demonstrated moderate larvicidal activity, with an LC_{50} of 395.10ppm and a TI of 64.87. The model fit was excellent (r^2= 0.99), and the probit curve demonstrated a strong goodness-of-fit (χ^2= 0.005, *P*= 0.95). Azadirachtin oil showed a similar trend, with an LC_{50} of 689.21ppm and a TI of 37.19, supported by high model consistency (r^2= 0.99; χ^2 = 0.008, *P*= 0.93). Jojoba oil was the least effective among the essential oils tested, with an LC_{50} of 933.37ppm and a TI of 27.46, though it still demonstrated a satisfactory model fit (r^2= 0.93; χ^2 = 0.914, *P*= 0.34). The slopes of the probit regression curves provide insight into the uniformity of larval responses across concentrations. A steeper slope reflects a more homogeneous population response to increasing dosages, while a lower slope indicates broader variability. The avocado–coconut oil blend had the steepest slope (1.82 ± 0.53), suggesting consistent toxic effects. Orange oil exhibited a more gradual slope (1.13 ± 0.40), implying a wider range of larval sensitivity, despite its higher potency. Intermediate slopes were recorded for azadirachtin (1.45 ± 0.54), jojoba (1.44 ± 0.61), and chitin (1.34 ± 0.84). The relatively high standard error associated with chitin's slope suggests greater inconsistency in larval susceptibility to this compound.

In summary, orange oil emerged as the most potent larvicide among all tested agents, supported by strong statistical performance and observable physiological impacts on the larvae. In contrast, *Periplaneta americana* chitin showed minimal direct toxicity. However, its high structural quality and consistent regression fit indicate potential as a biologically compatible matrix for formulating or delivering active botanical agents in future mosquito control strategies, rather than serving as a primary larvicide on its own.

Table 2. Median lethal concentrations (LC_{50}), slope ± standard error (SE), regression statistics, and Toxicity Index (TI) of various biocidal agents against third-instar *Culex pipiens* larvae at 48 hours post-treatment. The table includes calculated chi-square (χ^2) values (compared to tabulated value = 3.8), corresponding *P*-values, and coefficients of determination (r^2) indicating the goodness of fit for the probit regression models.

Tested compound	LC50/ppm	Slope ± SE	χ2cal. (tab.=3.8)	*P*-value	r^2 (tab.=0.99)	Toxicity index
Orange Oil	256.30	1.13 ± 0.40	1.924	0.17	0.90	100.00
Avo-Coco Oil Mix	395.10	1.82 ± 0.53	0.005	0.95	0.99	64.87
Azadirachtin Oil	689.21	1.45 ± 0.54	0.008	0.93	0.99	37.19
Jojoba Oil	933.37	1.44 ± 0.61	0.914	0.34	0.93	27.46
Chitin	2428.54	1.34 ± 0.84	0.11	0.75	0.98	10.55

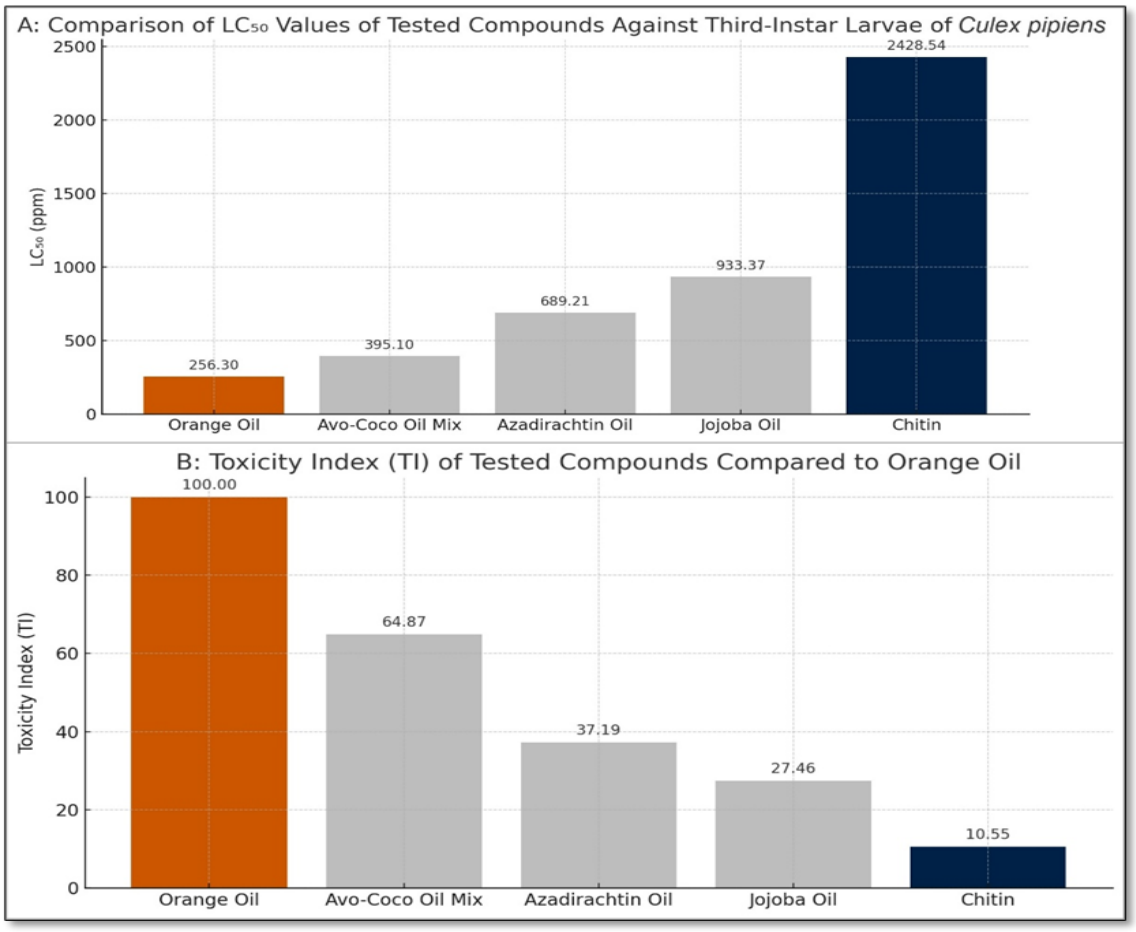

Fig. 2. Comparative larvicidal efficacy of *Periplaneta americana*-derived chitin and selected essential oils against third-instar *Culex pipiens* larvae after 48 hours of exposure. (A) Median lethal concentrations (LC_{50}, ppm) indicating the concentration required to achieve 50% larval mortality. Orange oil demonstrated the highest potency (LC_{50} = 256.30ppm), while chitin exhibited the lowest (LC_{50} = 2428.54ppm). (B) Toxicity Index (TI) values calculated relative to orange oil (TI = 100), highlighting the significantly reduced larvicidal activity of the other agents particularly chitin (TI = 10.55)

3. Molecular docking analysis for mode of action prediction

Molecular docking simulations were conducted to investigate the potential mechanism underlying the larvicidal activity of orange oil, with a particular focus on its interaction with insect acetylcholinesterase (AChE). Orange oil was selected for this analysis based on its superior larvicidal efficacy (LC_{50} = 256.30ppm) and the distinct neurotoxic symptoms observed in treated *Culex pipiens* larvae, including uncoordinated and erratic movements. These symptoms indicated potential interference with neural

signaling pathways, providing strong justification for targeting AChE—a critical enzyme involved in insect neuromuscular function.

Docking was carried out using Molecular Operating Environment (MOE) software against the insecticide-resistant G119S AChE mutant from *Anopheles gambiae* (PDB ID: 6ARY). The enzyme's active site was defined based on the co-crystallized difluoromethyl ketone inhibitor (BT7), which was retained to guide docking precision and ensure relevance to real binding interactions.

Among the tested constituents, Sinensal exhibited the highest binding affinity (docking score = –6.60 kcal/mol; RMSD = 1.95 Å), forming a hydrogen bond with TYR282 and a π–hydrogen (H–π) interaction with TRP245. Neryl acetate followed closely (–6.45 kcal/mol; RMSD = 1.34 Å), interacting with SER280 and TRP245. Geranyl acetate (–6.08 kcal/mol) showed similar behavior, forming a hydrogen bond with SER280. Undecanal (–5.98 kcal/mol) established interactions with TYR282 and TRP245, while Citral and Nerol also showed favorable binding, engaging SER280 and HIS600 respectively.

Limonene, a major component of orange oil, demonstrated moderate binding (–5.38 kcal/mol) and formed a stable H–π interaction with TRP245. While not the most potent binder, its high abundance may enhance overall larvicidal activity through synergistic or additive effects.

As a point of reference, the insecticide chlorpyrifos (–6.66 kcal/mol; RMSD = 1.38 Å) interacted with key catalytic residues HIS600 and TRP245. The co-crystallized ligand (BT7) reinforced the validity of the docking site, showing multiple hydrogen bonds with GLU359, SER280, GLY279, and ALA361, along with π-interactions involving TRP245.

Across the docked ligands, TRP245 emerged as a central anchoring residue, particularly for π-mediated interactions, while SER280, TYR282, and HIS600 were also frequently involved. RMSD values ranged from 0.54 to 1.95 Å, confirming the stability of the predicted ligand conformations.

The docking results, summarized in Table (3) and illustrated in Fig. (4), provide molecular-level evidence that several orange oil constituents can effectively interact with the AChE active site, mimicking key binding patterns observed with chlorpyrifos and the co-crystallized ligand. These computational findings were subsequently supported by biochemical assays designed to validate AChE inhibition, thereby strengthening the proposed mechanism of action underlying orange oil's larvicidal activity.

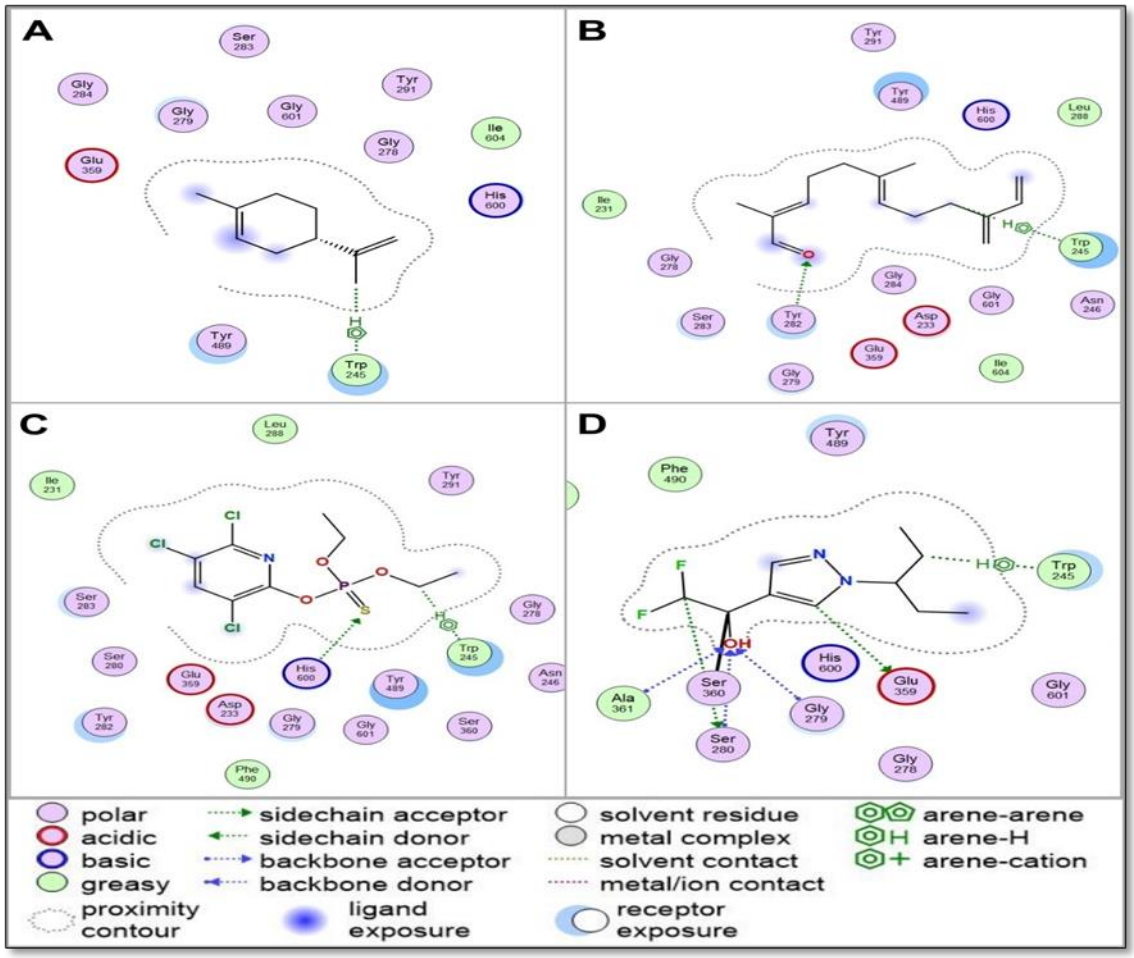

Fig. 3. Two-dimensional interaction diagrams showing the binding of orange oil constituents and reference ligands to acetylcholinesterase (AChE, PDB ID: 6ARY). (A) Limonene, the major orange oil component, displays hydrophobic interactions with TRP245 and TYR489. (B) Sinensal, the strongest binder, forms hydrogen bonds and H–π interactions with TYR282 and TRP245. (C) Chlorpyrifos, a reference AChE inhibitor, interacts with HIS600 and TRP245. (D) Co-crystallized ligand (BT7) defines the active site via hydrogen bonds with GLU359, SER280, and TRP245. The similarity in binding modes supports AChE inhibition as a likely mechanism of orange oil's larvicidal action

Table 3. Molecular docking results of orange oil constituents targeting the insecticide-resistant acetylcholinesterase (AChE) G119S mutant of *Anopheles gambiae* (PDB ID: 6ARY). Docking was conducted within the active site defined by the co-crystallized difluoromethyl ketone inhibitor. The table presents each compound's docking score (S, kcal/mol), RMSD (Å), interacting residues, interaction types, bond distances (Å), and bond energy (E, kcal/mol). Chlorpyrifos, a well-known organophosphate AChE inhibitor, is included as a reference. These results identify key constituents likely responsible for AChE inhibition and contribute to understanding the larvicidal efficacy of orange oil

Chemical Class	Constituent	S	Rmsd Å	Residues	Interaction type	Distance Å	E (kcal/mol)
Terpenes	**Limonene**	-5.38	0.90	TRP245	H-pi	4.1	-0.7
	Myrcene	-5.30	0.99	TRP245	H-pi	4.03	-0.5
	Sabinene	-5.32	1.92	**No detected Bonding**			
	Valencene	-5.46	1.04	TYR489	H-pi	4.7	-0.5
Terpenoids	**Citronellal**	-5.71	0.85	GLU359	H-donor	3.63	-0.5
				TRP245	H-pi	4.23	-0.7
	Geraniol	-5.52	1.61	GLY278	H-donor	3.31	-0.5
				TRP245	H-pi	3.79	-0.6
	Citral	-5.85	1.09	SER280	H-acceptor	3.07	-1.3
	Geranyl Acetate	-6.08	1.21	SER280	H-acceptor	3.06	-1.7
	Linalool	-5.66	0.54	HIS600	H-acceptor	3.32	-0.6
				TRP245	H-pi	4.2	-0.5
	Nerol	-5.92	1.44	HIS600	H-donor	3.04	-0.9
	Neryl acetate	-6.45	1.34	SER280	H-acceptor	2.93	-1.4
				TRP245	H-pi	3.74	-0.6
Aldehydes	**Nonanal**	-5.71	1.19	TYR282	H-acceptor	2.96	-0.9
				TRP245	H-pi	3.84	-0.6
	Perillaldehyde	-5.37	0.68	TRP245	H-pi	3.86	-0.7
	Sinensal	-6.60	1.95	TYR282	H-acceptor	2.93	-1.2
				TRP245	H-pi	4.04	-0.5
	Undecanal	-5.98	1.46	TYR282	H-acceptor	3.18	-1.4
				TRP245	H-pi	3.72	-0.6
	Decanal	-5.50	1.82	SER280	H-acceptor	3.2	-0.9
	Octanal	-5.07	1.26	HIS600	H-acceptor	3.46	-0.5
				TRP245	H-pi	3.64	-0.7
Organophosphate	**Chlorpyrifos**	-6.66	1.38	HIS600	H-acceptor	3.57	-0.9
				TRP245	H-pi	3.88	-0.6
Binding interactions of the co-crystallized ligand at the active site of acetylcholinesterase (AChE), as defined in the crystal structure PDB: 6ARY.				GLU359	H-donor	3.39	-0.9
				SER280	H-donor	3.11	-0.6
				GLY279	H-acceptor	3.19	-1.6
				SER280	H-acceptor	2.85	-2.9
				ALA361	H-acceptor	2.88	-1.8
				TRP245	H-pi	3.62	-0.8

4. Biochemical effects of chitin and natural oils on *Culex pipiens* larvae

Biochemical assessments of *Culex pipiens* third-instar larvae revealed significant differences in carbohydrate, lipid, and protein content, as well as acetylcholinesterase (AChE) activity, following treatment with various natural compounds compared to untreated controls, as presented in Table (4) and visualized in Fig. (5).

Carbohydrate levels varied notably across treatments ($P< 0.05$, one-way ANOVA, Tukey's HSD). Larvae treated with the Avo-Coco oil mix (278.67 ± 12.06 µg/100 larvae) and chitin (335.67 ± 21.83 µg/100 larvae) maintained levels close to the control group (349.67 ± 14.74 µg/100 larvae). In contrast, orange oil treatment led to a pronounced depletion (58.00 ± 3.61 µg/100 larvae), representing the greatest reduction observed. Azadirachtin (107.67 ± 6.66 µg/100 larvae) and jojoba oil (106.00 ± 7.21 µg/100 larvae) also significantly reduced carbohydrate content.

Lipid content followed a similar trend. The highest values were recorded in control larvae (158.67 ± 12.06 µg/100 larvae), followed by chitin (152.33 ± 2.52 µg/100 larvae) and jojoba oil (146.67 ± 5.86 µg/100 larvae). Larvae exposed to orange oil again showed the most substantial reduction (58.67 ± 1.53 µg/100 larvae), while moderate decreases were observed with Avo-Coco (89.67 ± 3.51 µg/100 larvae) and azadirachtin (94.00 ± 2.00 µg/100 larvae) treatments.

Protein content was the highest in control (792.67 ± 39.51 µg/100 larvae) and chitin-treated larvae (775.67 ± 28.75 µg/100 larvae), with no statistically significant difference between them. All oil treatments caused significant reductions, with orange oil again showing the lowest value (483.00 ± 11.27 µg/100 larvae). Moderate declines were observed for jojoba (698.67 ± 14.05 µg/100 larvae), Avo-Coco (655.00 ± 14.18 µg/100 larvae), and azadirachtin (649.67 ± 25.50 µg/100 larvae).

AChE activity was significantly affected by treatment ($P< 0.05$). Control larvae exhibited the highest activity (16.67 ± 0.42 µg AchBr/min/100 larvae), followed by chitin-treated larvae (16.50 ± 0.89). Orange oil resulted in the greatest suppression (11.37 ± 0.31), supporting its known neurotoxic effect. Avo-Coco (11.47 ± 0.45), azadirachtin (12.23 ± 0.78), and jojoba oil (13.47 ± 0.55) also significantly inhibited AChE activity.

In summary, orange oil consistently induced the most severe biochemical disturbances across all measured parameters, reflecting its potent larvicidal and neurotoxic effects. In contrast, chitin treatment had a minimal physiological impact and maintained near-control values across most metrics. All observed differences were statistically significant as determined by one-way ANOVA followed by Tukey's HSD test ($P< 0.05$).

Table 4. Levels of total carbohydrates, lipids, proteins, and acetylcholinesterase (AChE) activity in *Culex pipiens* larvae subjected to various oil treatments and *Periplaneta americana* chitin. Values represent mean ± SD (n= 3). Distinct superscript letters indicate statistically significant differences between treatments (Tukey's HSD, α = 0.05).

Sample	AChE activity (µg AChBr/min/100 larvae)	Proteins (µg/100 larvae)	Carbohydrates (µg/100 larvae)	Lipids (µg/100 larvae)
Chitin	16.50 ± 0.89^{c}	775.67 ± 28.75^{c}	335.67 ± 21.83^{d}	152.33 ± 2.52^{c}
Jojoba Oil	13.47 ± 0.55^{b}	698.67 ± 14.05^{b}	106.00 ± 7.21^{c}	146.67 ± 5.86^{c}
Orange Oil	11.37 ± 0.31^{a}	483.00 ± 11.27^{a}	58.00 ± 3.61^{a}	58.67 ± 1.53^{a}
Avo-Coco Oil Mix	11.47 ± 0.45^{a}	655.00 ± 14.18^{b}	278.67 ± 12.06^{b}	89.67 ± 3.51^{b}
Azidirachtin Oil	12.23 ± 0.78^{a}	649.67 ± 25.50^{b}	107.67 ± 6.66^{c}	94.00 ± 2.00^{b}
Control	16.67 ± 0.42^{c}	792.67 ± 39.51^{c}	349.67 ± 14.74^{d}	158.67 ± 12.06^{c}

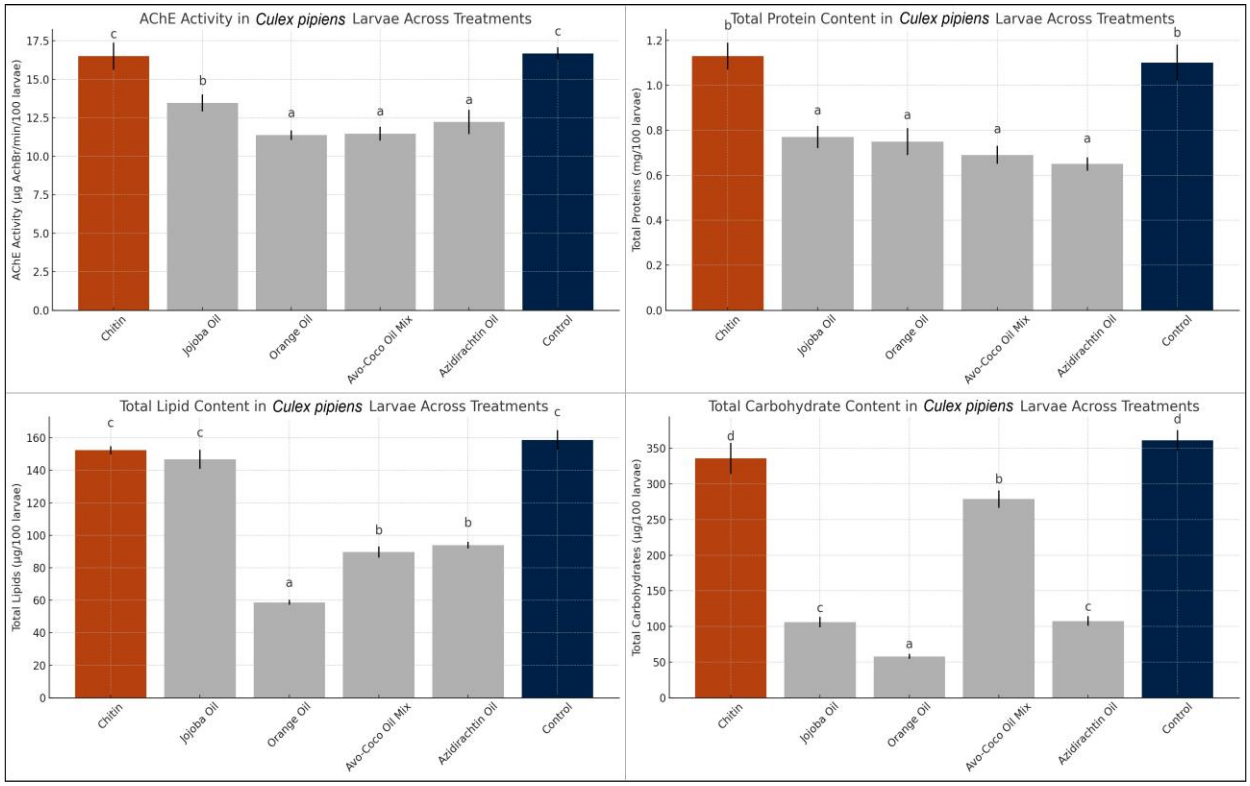

Fig. 5. Biochemical responses in *Culex pipiens* third-instar larvae following treatment with chitin, Azadirachtin oil, orange oil, jojoba oil, and an Avo-Coco oil mix, compared to untreated controls. The graphs show acetylcholinesterase (AChE) activity (µg AchBr/min/100 larvae), and total protein, lipid, and carbohydrate contents (µg/100 larvae). Bars represent mean ± SD (n = 3). Different letters above bars indicate statistically significant differences among treatments ($P < 0.05$), determined by one-way ANOVA followed by Tukey's HSD test. Chitin and control groups are highlighted using distinct colors for clarity

DISCUSSION

This study comprehensively evaluated the larvicidal potential of chitin extracted from *Periplaneta americana*, beginning with its structural characterization to confirm identity and purity. Following extraction, the toxicity of chitin was assessed against third-instar *Culex pipiens* larvae and compared to four botanical insecticides: Orange oil, azadirachtin, jojoba oil, and a blended avocado–coconut oil mix. To gain insight into possible mechanisms of action, molecular docking analyses were conducted targeting acetylcholinesterase (AChE). Finally, biochemical assays were performed to evaluate the physiological effects of each treatment on larval metabolism and enzyme activity.

Physicochemical characterization confirmed that *Periplaneta americana*-derived chitin exhibits the hallmark features of α-chitin. FTIR analysis revealed characteristic Amide I band splitting (1650 and 1620cm^{-1}), supported by distinct Amide II and III peaks, in close agreement with commercial shrimp chitin. XRD analysis showed strong diffraction peaks around $2\theta \approx 19.6°$, indicative of high crystallinity. The calculated Crystallinity Index (CrI) for *Periplaneta americana* chitin (78.66%) was comparable to shrimp chitin (82.23%). Additionally, the high degree of acetylation (DA = 113.54%) and elemental composition confirmed its structural integrity and purity **(Liu *et al*., 2012; Kaya *et al*., 2014; Kaya & Baran, 2015a; Kamal *et al*., 2020)**.

Despite its favorable physicochemical properties, chitin was the least effective larvicidal agent among all treatments, exhibiting the highest LC_{50} value (2428.54ppm) and the lowest toxicity index (TI = 10.55). No neurotoxic symptoms—such as tremors, spasms, or uncoordinated movement—were observed in larvae treated with chitin. Additionally, biochemical assays revealed no significant reductions in acetylcholinesterase (AChE) activity or in the levels of carbohydrates, lipids, or proteins, which remained comparable to those of the untreated control group.

This lack of both acute toxicity and neurophysiological disruption suggests that chitin operates through a mechanism distinct from that of neuroactive compounds like essential oils. Chitin is a high-molecular-weight polysaccharide composed of repeating N-acetylglucosamine units arranged in a semi-crystalline structure. Rather than interacting with biochemical targets such as enzymes, its biological activity is more plausibly attributed to physical or physiological interferences such as disrupting molting, impairing cuticular integrity, or hindering normal development **(Abenaim & Conti, 2023)**. These structural and functional characteristics also explain why chitin is not a suitable candidate for receptor-based computational approaches like molecular docking, which are designed to model discrete interactions between small ligands and protein active sites.

Orange oil demonstrated the highest larvicidal potency among all tested treatments, with the lowest LC_{50} value (256.30ppm) and the highest toxicity index (TI = 100). Larvae exposed to orange oil showed pronounced neurotoxic symptoms, including tremors, spasms, and uncoordinated movement, suggesting a potential disruption of the nervous system. These behavioral effects prompted further investigation into the mode of action through molecular docking simulations targeting acetylcholinesterase (AChE), a key enzyme in neural signal transmission **(El-Sayed *et al*., 2024; El-Helw *et al*., 2024, 2025; Haikal *et al*., 2025; Khalil *et al*., 2025; Ramadan *et al*., 2025)**.

Docking analysis revealed that several orange oil constituents, notably sinensal and neryl acetate, exhibited strong binding affinities to the active site of AChE (PDB ID: 6ARY). These compounds formed stabilizing interactions, including hydrogen bonds and H–π stacking, with critical catalytic residues such as TRP245, TYR282, and HIS600. The binding poses and interactions closely mirrored those observed with known AChE inhibitors like chlorpyrifos and the co-crystallized ligand BT7, reinforcing AChE inhibition as a likely mechanism of action.

Interestingly, limonene, the major component of orange oil, showed only modest binding affinity and lacked strong direct interactions with AChE's active site. This suggests that although limonene contributes to the overall volatility and penetration of the oil, the primary inhibitory effect may be driven by minor yet more bioactive constituents such as sinensal and neryl acetate.

These *in silico* findings were further supported by biochemical assays, which confirmed a significant reduction in AChE activity in orange oil-treated larvae, along with marked depletion of carbohydrate, lipid, and protein levels. The convergence of larvicidal bioassay results, observed neurotoxic symptoms, docking predictions, and biochemical validations strongly supports AChE inhibition as the principal toxic mechanism of orange oil in *Culex pipiens* larvae.

The avocado–coconut oil mix, azadirachtin, and jojoba oil demonstrated moderate larvicidal activities, with LC_{50} values of 395.10, 689.21, and 933.37ppm, respectively. Biochemical profiling showed partial AChE inhibition and moderate depletion in metabolic reserves, indicating slower or cumulative toxic effects characteristic of many plant-derived insecticides **(Su & Mulla, 1998; Nathan *et al*., 2004, 2006; Pavela, 2015)**. These agents, though less potent than orange oil, may offer value in integrated mosquito management strategies, especially when sustained exposure or synergistic formulations are feasible.

Although raw chitin lacks standalone larvicidal potency, its structural and biochemical neutrality, high purity, and similarity to pharmaceutical-grade shrimp chitin suggest utility in other vector control applications. Specifically, its biocompatibility and

physicochemical stability make it a promising carrier material for essential oils. We propose the development of chitin- or chitosan-based nano-formulations encapsulating orange oil. Such systems could enhance oil stability, protect active compounds from environmental degradation, and enable sustained release in aquatic habitats thereby improving larvicidal efficiency while minimizing ecological impact **(Kamaraj *et al*., 2010; Huston *et al*., 2021)**.

CONCLUSION

This study demonstrates that chitin extracted from *Periplaneta americana* possesses high structural integrity comparable to commercial shrimp chitin but lacks direct larvicidal activity. Its high LC_{50} (2428.54ppm) and low toxicity index (10.55) highlight its ineffectiveness as a standalone biocidal agent. In contrast, orange oil proved to be the most effective treatment, significantly reducing carbohydrate, lipid, and protein levels and inhibiting AChE activity in treated *Culex pipiens* larvae. Docking simulations confirmed strong binding interactions between key orange oil constituents and the AChE active site, supporting neurotoxic disruption as the principal mechanism of action.

The performance contrast between orange oil and chitin underscores the latter's potential role not as an insecticide but as a sustainable, biodegradable carrier. Given its high purity, compatibility, and ability to maintain the stability of volatile agents, *Periplaneta americana* chitin or its derivative, chitosan may serve as an ideal delivery platform in larvicide nano-formulations.

Future research should focus on developing and testing chitin- or chitosan-based nanoparticles loaded with orange oil. These formulations could enhance bioavailability, provide sustained release, and ultimately contribute to safer, more targeted mosquito control strategies with minimal environmental impact.

Author contributions

This study is part of Amina A. Rashad’s PhD research. She was primarily responsible for the practical execution of the work, including the synthesis and characterization of the chemical derivatives, the design and implementation of the larvicidal bioassays, the biochemical and insecticidal activity analyses, as well as drafting the manuscript. Mahmoud Kamal, a supervising author, closely assisted Amina A. Rashad in the experimental design, conceptualization of the research, and manuscript writing. He was responsible for designing and executing the *in-silico* studies, which encompassed molecular docking. Sohair M. Gad Allah, Rawda M. Badawy, Imam I. Ahmed, and Magdi G. Shehata served as academic supervisors. They offered scientific guidance throughout the study, played a key role in shaping the chemical aspects of the research, and conducted a thorough revision of the manuscript. All authors revised and approved the final version of the manuscript.

Statement on Data Availability

All data generated or analyzed during this study are included in this published article.

Ethics Declaration

This study was approved by the Research Ethics Committee at Ain Shams University (Approval code: ASU-SCI/ENTO/2025/5/3) and was performed in adherence to the National Institute of Health (NIH) guidelines, all methods are documented following the ARRIVE guidelines.

Conflict of Interest Statement

The author(s) reported no potential conflicts of interest.

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