Valorization of deodorizing distillate palm oil residue for larvicidal activity against Aedes aegypti and synergistic effect of their free fatty acids

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Abstract

Increased consumption of palm oil results in more palm oil by-products. To meet environmental sustainability, deodorizing distillate palm oil (DDPO) also calls attention to diversifying its technological or commercial application. Because of this, the present work, to present new economic-scientific alternatives for this by-product, aimed to evaluate the larvicide effect of DDPO, well as to investigate the synergistic effect of the combination of these fatty acid present in the composition of DDPO, against larvae of 3rd instar of *Ae. aegypti*. The DDPO larvicide assay showed a high mortality rate, with an LC_{50} of 6.18 μg.mL^{-1} at 24 h and 8.73 μg.mL^{-1} at 48 h of treatment. In addition, the results of LC_{50}, among saturated fatty foods and between the combination of fatty acid (myristic, lauric, stearic and oleic) with palmitic acid, were met positively by second-degree polynomial regression analysis. Finally, the study of molecular docking, corroborated, as potential inhibitors of the binding of juvenile hormones. Together, the results suggest that DDPO can be a potential natural larvicide agent, making it an alternative to the excessive use of synthetic insecticides, thus minimizing its impacts on the environment and promoting new technologies for the use of this palm oil by-product.

Statement of Novelty

With the worldwide increase in the production by-product of palm oil, and number of cases of diseases promoted by the vectors. Is it possible for deodorizing distillate palm oil (DDPO) to be effective in controlling *A. aegypti* larvae?

Introduction

Palm oil has been used for centuries as a food and medicine source [1]. Currently, the great demand for refined vegetable oils has provided the palm oil production chain, generating a large number of by-products (gums, neutralizing sludge, and deodorizing distillate) of low commercial value [1, 2]. Thus, the challenge, therefore, is to convert deodorizing distillate palm oil (DDPO) as a resource for energy and other higher value-added productive uses to promote ecological solid waste management and environmental practices, mitigating the negative impacts resulting from the disposal of these wastes in the environment [2].

DDPO is one of the by-products generated by the palm oil refining process, without any technological application or commercial value, but it is interesting as a raw material for the production of high-added-value monoalkyl esters [3]. This residue contains high levels of free fatty acids (FFAs) around 84% [3–5], in addition to tocopherols, squalene, and sterols [2]. Although many studies on the use of DDPO have focused on the synthesis of biodiesel [3–11], and biolubricant [12], as they make it possible to reduce the costs of production of renewable products, due to the use of low-grade raw material, it adds value to the residue and makes it possible to produce biodiesel and lubricant through a "clean" and environmentally compatible technology.

Mosquitoes are the protagonists of several diseases that cause a strong impact on human health. This fact is evidenced mainly in tropical and subtropical regions of the world [13–15]. The *Aedes aegypti* mosquito is a global vector of human diseases, involving the transmission of pathogens responsible for causing diseases in humans, such as yellow fever, dengue, Zika, and Chikungunya fever [16, 17]. Its impact on human health is related to the environmental conditions during the larval growth phase and to the set of characteristics that allow the vector to be extremely efficient [18, 19].

The Pan American Health Organization (PAHO) notes that some 500 million people in America are at risk of dengue fever. Dengue has grown dramatically in recent decades, and according to the World Health Organization (WHO), an estimated 50 to 100 million cases of dengue occur annually worldwide, of which 500,000 progress to severe dengue and 25.000 die [20, 21].

Derivatives of alkyl fatty acids are molecules of simple composition and easy access. The oilseeds found in the Amazon have in their chemical composition fatty acids and alkyl derivatives such as fatty amides, triglycerides, and alkyl esters [17, 22, 23]. With the need to find environmentally safe biocides with high vector selectivity, fatty acids and their alkyl derivatives (synthetic or natural) have been used successfully against *Ae. aegypti* [16, 23–30].

The oil extracted from *Syagrus coronata* seeds, rich in octanoic, decanoic, and dodecanoic fatty acids, has shown the larvicidal effect on 3rd instar larvae of *Ae. aegypti*, in addition to exerting a deterrent effect on gravid females of the mosquito [31]. Oleic, linoleic, linolenic, palmitic, and stearic fatty acids and their respective esters showed larvicidal activity against *Culex quinquefasciatus* 4th instar larvae, affecting the metabolism and the morphology of the midgut of the larvae [32].

So, know that are no reports on the larvicidal effect of DDPO, this study aims to investigate the larvicidal bioactivity of DDPO and fatty-free acid (myristic acid, palmitic acid, lauric acid, stearic acid, and oleic acid) against *Ae. aegypti* mosquito larvae.

Experimental method

2.1 Chemicals and reagents

Ethanol (99.5%) was Synth (São Paulo, Brazil). DDPO originating from the palm oil refining process was supplied by *Companhia Refinadora da Amazônia* (Belém, Brazil). The fatty acids used in this study were as follows: acid myristic acid (95%), palmitic acid (98%), lauric acid (98%), stearic acid (98%), and oleic (95%), all purchased from Sigma-Aldrich (São Paulo - Brazil).

2.2 Identification of the fatty acid present in the DDPO by Gas chromatography/mass spectrometry analysis

To identify the compounds, present in the DDPO, which contained fatty acids, the sample was submitted to an esterification according to Lopes et al. [5].

2.3 Larvicidal bioassay

The larvae of *Ae. aegypti*, the used Rokeffeller colony, are from the Arthropod Laboratory of the Federal University of Amapá (ARTHROLAB), all larvae used were 3 stages, to avoid that during the experiments reached the pupal stage. They were kept in standard weather conditions with a temperature of 25 ± 2°C and relative humidity of 75 ± 5% and a photoperiod of 12 hours according to the World Health Organization [26].

The samples DDPO and free fatty acids stand [palmitic (PA), myristic (MA), stearic (SA), lauric (LA), and oleic (OA)] were prepared in different concentrations (15, 7.5, 5, 2.5 and 1 $\mu g.mL^{-1}$) solubilized in DMSO (0.5%). In each bioassay was used 10 larvae in controlled conditions (25 ± 2°C). The distilled water and DMSO (0.5%) were used with negative controls. All assays were performed in quintuplicate.

The content mortality count was carried out in periods of 24 and 48 hours after exposure to the larvae solutions. They were considered dead larvae that were not able to reach the water surface and using the readings was possible to establish the lethal concentrations (LC_{50} and LC_{90}) by probit analysis. All bioassay experiments were conducted according to standard [33].

2.4 Synergy between the free fatty acid

To determine whether free fatty acid mixtures increase larvicidal efficacy compared with the constituted DDPO or free fatty acid alone was preparing a combined solution in PA + MA, PA + SA, PA + LA, and PA + OA basis of LC_{50} value of each free fatty acid. In each bioassay was used 10 larvae in controlled conditions (25 ± 2°C). The distilled water and DMSO (0.5%) were used with negative controls. All assays were performed in quintuplicate [34].

2.4 Statistical analysis

For larvicidal activity, the lethal concentrations (LC_{50} and LC_{90}) were determined after 24 and 48 h of incubation and calculated using Probit analysis with StatGraphics Centurion.

1.5 Molecular Docking Simulations

2.5.1 Receiver selection

The 3D structure of the juvenile hormone binding crystal elucidated by X-ray diffraction was downloaded from the Protein Data Bank (PDB) with the code PDB ID 5V13 (*Ae. aegypti*), complexed with the ligand methyl (2*E*,6*E*)-9-[(2*R*)-3,3-dimethyloxiran-2-yl]-3,7-dimethylnona-2,6-dienoate (JHIII) with resolution 1.84 Å [35]. JHIII and Pyriproxyfen were used as control ligands in molecular docking studies based on an established standard protocol [36–39].

2.5.2 Docking study with AutoDock 4.2/Vina 1.1.2 via Graphical Interface PyRx (Version 0.8.30)

The assignment of protonation and tautomeric states of ligands was performed with the Discovery Studio® program 2.0 (2008), while the hydrogen atoms of the respective proteins were added from PROPKA using pH 6. In the docking study, specific complexed ligands were used in the AutoDock 4.2/Vina 1.1.2 and PyRx 0.8.30 (https://pyrx.sourceforge.io). The validation of the molecular docking protocols was performed by overlapping between the crystallographic ligand (experimental pose) and the best conformation obtained (docking pose) based on the value of the mean square deviation of the root (RMSD) [36–40].

Results and discussions

This result chemical composition of the DDPO showed that the fatty acid profile (FA) consists of 62.3% saturated and 33.4% monounsaturated fatty acid. Being predominantly of palmitic acid (54.3%), followed by oleic acid (33.4%), according to Lopes et al. [5]. Some other studies have shown relevant chemical compositions for saturated fatty acids from PFAD. Fernandes et al. [12] reported that the fatty-acid composition of palm fatty-acid distillates consisted mainly of palmitic acid (43.7%) and oleic acid (42.8%). Chang et al. [41] also showed that palmitic acid was found as the dominant saturated fatty acid 38.63–45.30%, whereas oleic acid was the major unsaturated fatty acid 33.54–44.05% from sample PFAD.

Knowing the lipid composition of the DDPO, the main fatty acids were also selected to evaluate the mortality potential among the selected and combined *Aedes* larvae. The toxicity of DDPO and all fatty acid (PA, MA, SA, LA, and OA) was evaluated by a direct contact mortality bioassay on third-instar larvae of *Ae. aegytpi*. Responses LC_{50} and LC_{90} varied according to the compound tested (Table 1) using the Probit analysis.

The larval mortality of the *Ae. aegytpi* after 24 or 48h treatment with DDPO showed highest activity (Fig. 1), with LC_{50} 6.18 $\mu g.mL^{-1}$ (5.06 ± 7.89 $\mu g.mL^{-1}$, confidential interval) and LC_{90} 8.73 $\mu g.mL^{-1}$ (7.29 ± 13.30 $\mu g.mL^{-1}$, confidential interval) at 24h, and at 48h showed LC_{50} 4.57 $\mu g.mL^{-1}$ (3.62 ± 5.62 $\mu g.mL^{-1}$, confidential interval) and LC_{90} 6.35 $\mu g.mL^{-1}$ (5.37 ± 8.76 $\mu g.mL^{-1}$, confidential interval).

When the larvae of *Ae. aegypti* were exposed to the solution containing free fatty acid, individually, lauric acid (C12:0) show high-rate mortality for larvae of *Ae. aegypti*, with LC_{50} 2.46 $\mu g.mL^{-1}$ and LC_{90} 3.64 $\mu g.mL^{-1}$ at 24h. The free fatty acid more present in DDPO, palmitic acid (C:16), showed a moderate rate of larvicidal toxicity, in general, less that the DDPO mixture, with LC_{50} 12.75 $\mu g.mL^{-1}$ and LC_{90} 23.14 $\mu g.mL^{-1}$ at 24h and LC_{50} 4.74 $\mu g.mL^{-1}$ and LC_{90} 12.42 $\mu g.mL^{-1}$ at 48 h. In following the myristic acid (C14:0) with LC_{50} 4.20 $\mu g.mL^{-1}$ and LC_{90} 8.41 $\mu g.mL^{-1}$ at 24 h too with interest activity. However, low toxicity was observed for stearic acid (C18:0) with LC_{50} 65.03 $\mu g.mL^{-1}$ and LC_{90} 44.41 $\mu g.mL^{-1}$ at 24h (Table 1). The effect of unsaturated carbon chain bonds was evaluated using oleic acid (C18:1) as a model, which demonstrated increased activity compared to C18:0, with LC_{50} 8.35 $\mu g.mL^{-1}$ and LC_{90} 6.85

$\mu g.mL^{-1}$ at 24h, and at 48h with LC_{50} 6.85 $\mu g.mL^{-1}$ and LC_{90} 11.91 $\mu g.mL^{-1}$ at 48h (Table 1). Previous studies reported that the insecticidal activity of fatty acids against mosquitoes is mainly due to presence of long-chain unsaturated fatty acids [42–43].

The disturbances induced by this free fatty acid in the lipid structure of the membrane involve changes in membrane fluidity, phase behavior, permeability, membrane fusion, lateral pressure, and flip-flop dynamics, in certain instances, these disturbances can lead to cellular lysis [42], being one of the possible mechanisms of action in *Ae. aegypti* larvae.

Table 1
Determination of lethal concentrations (LC_{50} and LC_{90}) of DDPO and free fatty acid, against *Ae. aegypti* larvae during 24 h and 48 h of exposure.

Sample	LC_{50} ($\mu g.mL^{-1}$)		LC_{90} ($\mu g.mL^{-1}$)	
	24 h	48 h	24 h	48 h
DDPO	6.18	4.57	8.73	6.35
Lauric acid (C12:0)	2.46	2.23	3.64	3.45
Myristic acid (C14:0)	4.20	2.94	8.41	6.74
Palmitic acid (C16:0)	12.75	4.74	23.14	12.42
Stearic acid (C18:0)	65.03	44.41	65.03	44.41
Oleic acid (C18:1)	8.35	6.85	14.6	11.91

Free fatty acids are also important molecules for the mosquito reproduction process, as a source of energy for the developing embryo, factors of resistance or susceptibility to viruses, in the female mosquito, lipid metabolism is connected with the gonadotrophic cycle, and the levels of free fatty acids as C16:0 and C18:1 act as two of the regulatory factors of lipid metabolism [42].

Through the polynomial fit of second order was found a strong relationship between larvicidal effect of saturated fatty acids, identified in the sample of DDPO. Polynomial regression describes the fitting of a nonlinear relationship between the value of x (fatty acid) and the conditional mean of y (LC_{50}). The relationship found was of positive effect. The correlation best fitted to the model was determined for both LC_{50} ($\mu g.mL^{-1}$) and LC_{90} ($\mu g.mL^{-1}$) in 24, with $R^2 = 0.9806$ and $R^2 = 0.9936$, while for the effect of larval mortality in 48h, the correlation decreases slightly to $R^2 = 0.9344$ and $R^2 = 0.9711$, in LC50 ($\mu g.mL^{-1}$) and LC_{90} (μ g.mL^{-1}), respectively (Fig. 2).

To investigate the synergistic or antagonistic effect among the fatty acids present in the DDPO sample on larval mortality of *Ae. aegypti*, palmitic acid was chosen as the baseline for all combinations, as GC-MS analysis revealed it to be the most abundant fatty acid. The palmitic acid with oleic acid (PA + OA)

combination showed a mortality rate of 68% (LC_{50} = 10.82 µg.mL^{-1} and LC_{90} = 15.57 µg.mL^{-1}), followed by the combination of palmitic acid and lauric acid (PA + LA) with a mortality rate of 58% within 24 hours (LC_{50} = 11.56 µg.mL^{-1} and LC_{90} = 16.01 µg.mL^{-1}) (Fig. 3).

After analyzing the mortality readings, it was possible to establish the LC_{50} and LC_{90} values for the combinations of free fatty acids. It can be observed that when combined with palmitic acid, lauric acid, and myristic acid showed higher LC_{50} and LC_{90} values both at 24 hours and 48 hours compared to the results of the isolated substances (Fig. 3).

When larvae were exposed to a combination of palmitic acid and stearic acid (PA + SA), it was observed that there was a decrease in the LC_{50} and LC_{90} values to 13.7 µg.mL^{-1} and 19.2 µg.mL^{-1}, respectively, at 24 hours, indicating a synergistic effect in the acid combination. A synergistic effect occurs when two or more combined compounds have a greater effect than the sum of their individual effects. In this case, the combination of the compounds produces an enhanced effect, as observed in the test.

The second-order polynomial regression analysis indicates that there is a relationship between the combinations of palmitic acid and the other fatty acids present in the DOD and their respective larvicidal effects. It is noteworthy that the synergistic effect observed in the PA + AS mixture was more accentuated than the other mixtures, which favored the adjustment of the model.

It is possible to observe that the concentration values presented by the mixture of all the acids in DDPO are lower compared to palmitic, stearic, and oleic acids. We can assume that the relationship between the acids in the mixture causes a synergistic effect aiming to improve the efficacy of the residue.

Mosquito-borne diseases are budding as a global dispute as they are hastily increasing and grueling to control due to the resistance development, which stemmed from the failure of chemical insecticides [43]. Several studies are being conducted to evaluate the synergistic effect of substances for the control of vectors of public health importance [43–46].

The combination of synergistic substances offers an alternative approach for developing new methods to control resistant strains of these vectors. This approach also enables the use of environmentally friendly materials, thereby reducing the impact on the environment [43]. It is important to conduct appropriate studies and tests to assess the interaction between compounds and determine whether a synergistic or antagonistic effect occurs, aiming for improved efficacy in controlling larvae of the *Ae. aegypti* vector.

The structure of compounds directly affects their site and mode of action, and understanding the mechanisms by which the compound interacts with its target can impact characteristics such as efficacy. There are several mechanisms associated with larval mortality, such as substances that directly affect the central nervous system [47], compounds that act on acetylcholine receptors and GABA ion channels [48], and alterations in juvenile hormone [49].

In the study, it was observed that larvae treated with DDPO at a concentration of 15 µg.mL^{-1} showed alterations in their external structure after the exposure period (Fig. 4). It is known that changes in the cuticle can compromise the larvae's ability to feed, respire, and protect themselves from predators, as well as interfere with their molting and metamorphosis process. Therefore, we can assume that the synergy of the oils may have affected the integrity of the larvae's cuticle, leading to a rupture of the protective layer and resulting in dehydration and death.

Alterations in the external structure can also be a mode of action for larvicides against *Ae aegypti.* Changes in the external structure, such as the cuticle can affect the integrity and function of the cuticle, resulting in impacts on larval survival and development, dehydration is a common example that can occur due to the action of chemical larvicides or natural products.

Altered characteristics observed in *Ae. aegypti* larvae, as documented in the literature, include modifications in the external structure such as anal papillae, distorted body shape, and variations in body coloration (darker or paler), with changes in the cuticle being more frequently observed. The effect of intoxication leading to larval death can also result in aberrations in the structure of the larvae [50].

In addition to external changes, authors also report that these toxic substances can cause alterations in the internal portion of larvae, as it is immersed in a liquid environment. These alterations can affect the intestine, as well as the expulsion of the peritrophic matrix as a defense mechanism during intoxication [51].

As a complementary study to better elucidate the larvicidal potential of DDPO and its fatty acids presents, the molecular docking study was performed. The validation of the molecular docking protocols was performed with overlapping of the crystal structure of the reference ligand and the best docking pose for the biological target, in which a similar bioactive conformation of the JHIII was sought (Fig. 5). The RMSD value obtained was 1.34Å.

The validation result was considered satisfactory since the crystallographic binder pose and the docking pose were considered similar (Fig. 4). By recovering the pose of the juvenile hormone binding inhibitor (JHIII) it was possible to perform the validation of the molecular docking protocols, calculating the value of the mean square root of the deviation (RMSD) of 1.34 Å. According to reports in the literature, a RMSD value of up to 2 Å is considered adequate, so the docking protocol was considered satisfactory because it presents a similarity to the experimental model [37–39].

Considering the larvicidal activity assay (Table 1) of lauric acid and myristic acid fatty acids with better LC50 values [(24h 2.46 and 4.20 µg.mL^{-1}) and (48h 3.63 and 2.94 µg.mL^{-1}), respectively] about the other isolated fatty acids and binary mixtures, were chosen and evaluated in an *in silico* study for elucidation of the chemical mechanism of action, via molecular docking study. In the juvenile hormone binding receptor, the binding affinity of the ligands tested was observed compared to the JHIII and pyriproxyfen controls used in the molecular docking study (Table 2).

Table 2

Binding affinity values (kcal.mol^{-1}) of controls and potential ligands at the juvenile hormone binding receptor.

Moléculas	Afinidade de Ligação (kcal.mol^{-1})
JHIII	−8.7
Pyriproxyfen	−10.3
Lauric acid	−6.8
Myristic acid	−7.1

When compared to the controls (*in silico*), lauric acid and myristic acid, present in the DDPO, close values were shown for the binding affinity to the controls used in the docking study with − 6.8 and − 7.1 kcal.mol^{-1}, respectively, so the inhibitors are considered potential insecticide/larvicidal agents because they act on mortality or in the retardation of the morphological growth process of *Ae. aegypti*.

The site of interaction with JHIII (PDB ID 5V13) showed α-helix interactions between amino acid residues Leu33-Leu37, Ile44Val51, Tyr59-Glu71, and Cys122-His136 and in β-leaf between waste Leu72-Arg73 [35]. The interactions classify as hydrophobic for all residues (Fig. 6).

In the juvenile hormone binding protein, the JHIII ligand is present in the binding pocket of the *N*-terminal domain, and the crystal conformation is similar to the three protein chains. In JHIII it is observed the presence of an epoxy group located in the center of the domain and a methyl ester oriented towards the surface. The epoxy group forms hydrogen bonds with the phenolic hydroxyl of Tyr129 and the remainder of the isoprenoid chain is surrounded by hydrophobic side chains including Phe144, Tyr64, Trp53, Val65, Val68, Leu72, Leu74, Val51 and Tyr33 [52].

The lauric acid molecule with the free energy of -6.8 Kcal/mol presents hydrophobic category interactions (pi-Alkyl type) with Val68, Leu72, and Phe144; and a hydrogen bond with Trp50. Myristic acid (-7.1 Kcal/mol) presents a hydrogen bond with the amino acid residue Ser69 and with hydrophobic (alkyl) interactions with Val34, Leu37, Val51, and Phe269.

According to the docking study, myristic acid with the highest binding affinity potential, presents a hydrogen bond in Ser69, with a location close to the amino acid residue chain present in pyriproxyfen with Tyr64, such interactions are directly related to the type of orientation, pose and torsion that the molecule will present at the binding site.

The study of molecular docking allowed the evaluation of potential inhibitors of juvenile hormone binding through the similarity of interactions with amino acid residues at the binding site, fatty acids (myristic acid and lauric acid) present in DDPO, present significant stability of insecticide/larvicide activity. In addition, they present an excellent profile for the inhibition of juvenile hormone binding, since the

interactions established in the receptors used in the docking study were similar to the controls (JHIII and Piriproxifen) used, reinforcing the potential application of DDPO residue in the larval control of the *Ae. aegypti.*

Conclusion

The results of the present study (experimental and docking molecular) suggest that the DDPO residue is a potential natural larvicide for the control of the *Ae. aegypti* vector with good LC_{50} (6.18 $\mu g.mL^{-1}$ up to 24h). The larval mortality test carried out with the fatty acids, individually, showed that the efficiency in the larval mortality rate of the PADD is affected by the presence of lauric acid (C12:0) had the highest larval mortality (LC_{50} = 2.46 $\mu g.mL^{-1}$ at 24h), followed by myristic acid (C14:0) with LC_{50} 4.20 $\mu g.mL^{-1}$ up to 24h. Furthermore, the docking study clarified the interactions established in the receptors of myristic acid and lauric acid with the controls JHIII and Piriproxifen. Also was observed that the increase in the carbonic chain and amount of unsaturation (C16:0; C18:0 and C18:1) among the fatty acids, present in the DDPO, reduced the larvicidal effect when compared to the toxicity of DDPO only. Palmitic acid, the majority among the acids present in the residue of DDPO, showed a synergistic effect with all the evaluated fatty acids. In conclusion, the DDPO residue demonstrated promising larvicidal toxicity against *Ae. aegypti*, presenting a potential alternative to address environmental concerns associated with the disposal of DDPO residue and the excessive use of synthetic insecticides. Moreover, it offers a solution to the public health issue of controlling disease-transmitting vectors.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the present study.

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Figures

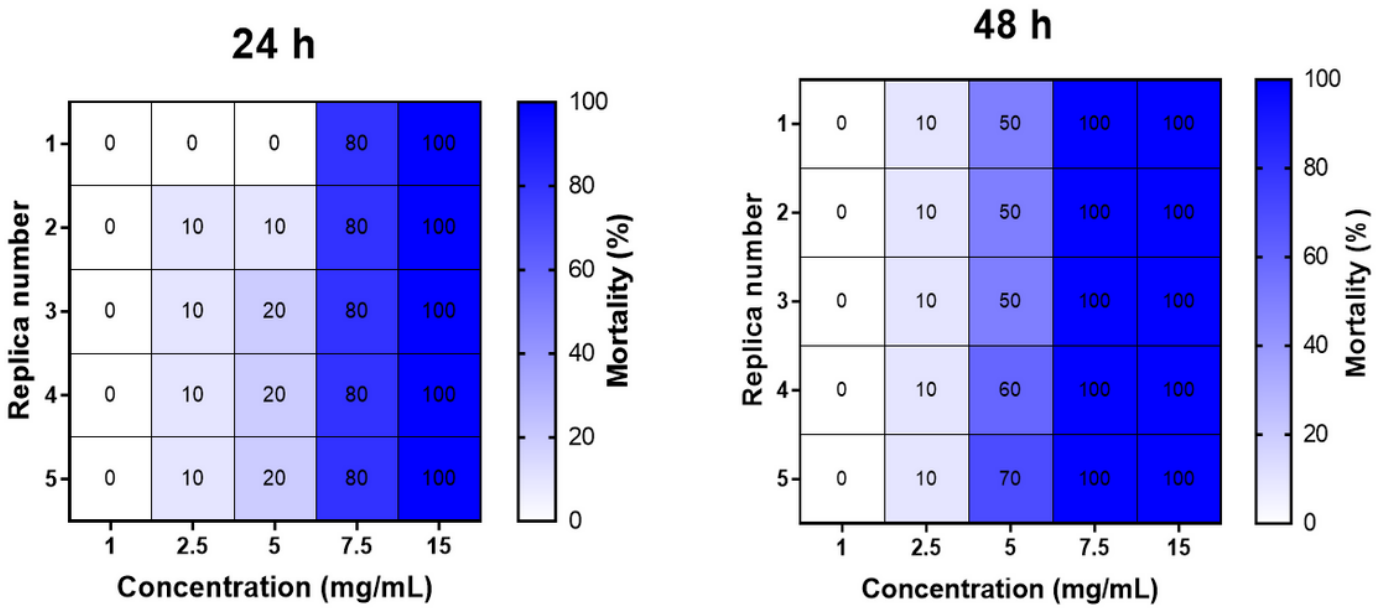

Figure 1

Frequency mortality larvae of *Ae. aegypti* after 24 h and 48 h of exposure to the DDPO.

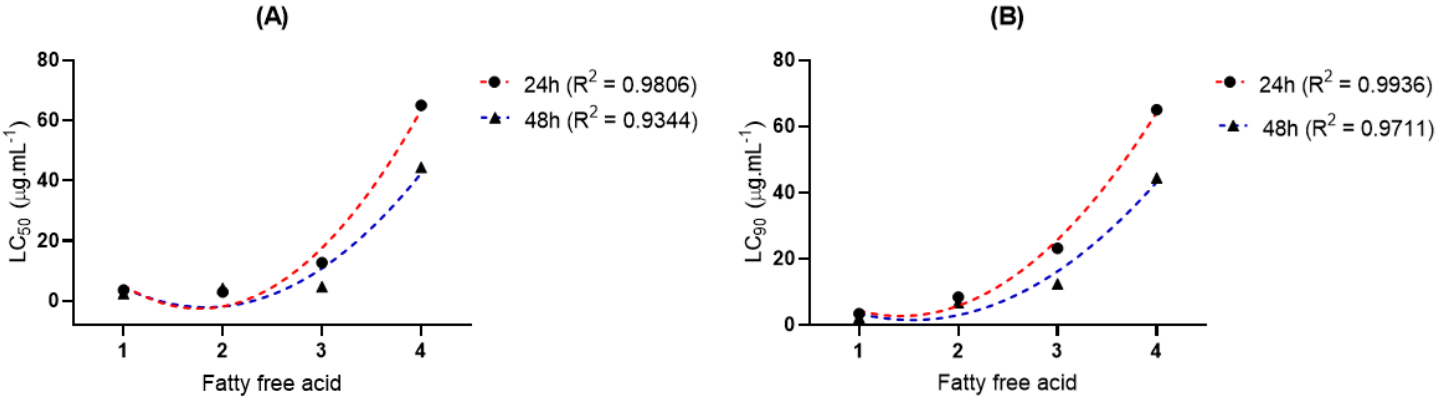

Figure 2

The second-degree polynomial regression analysis showing the effect between saturated fatty acids, identifiable in DDPO, about LC_{50} and LC_{90} ($\mu g.mL^{-1}$). X Legend = 1 (LA); 2 (MA); 3 (PA); and 4 (SA).

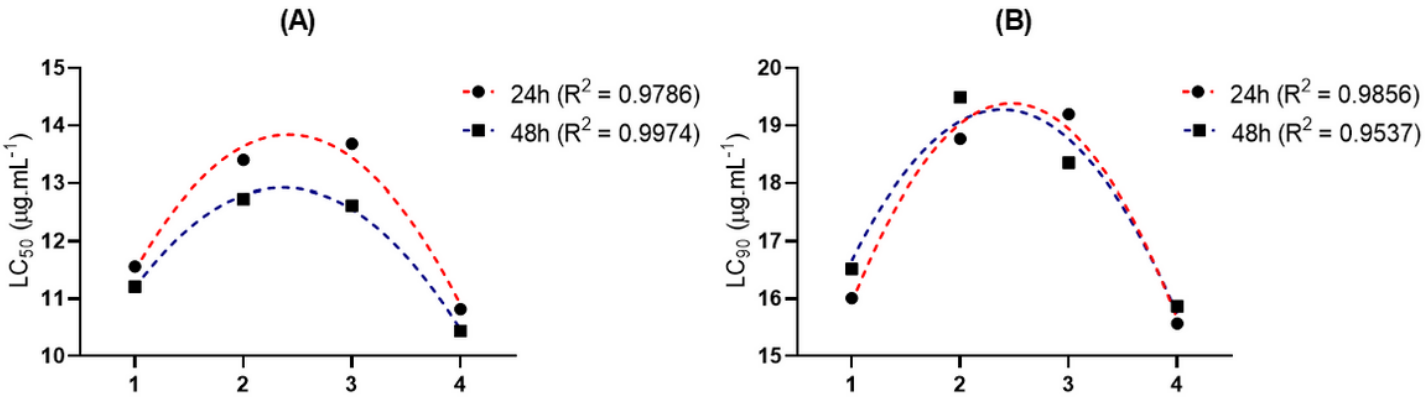

Figure 3

The second-degree polynomial regression analysis showing the effect between palmitic acid combination to other fatty acids, identifiable in DDPO, about LC_{50} and LC_{90} (µg.mL^{-1}). X Legend = 1 (PA+LA); 2 (PA+MA); 3 (PA+SA); and 4 (PA+OA).

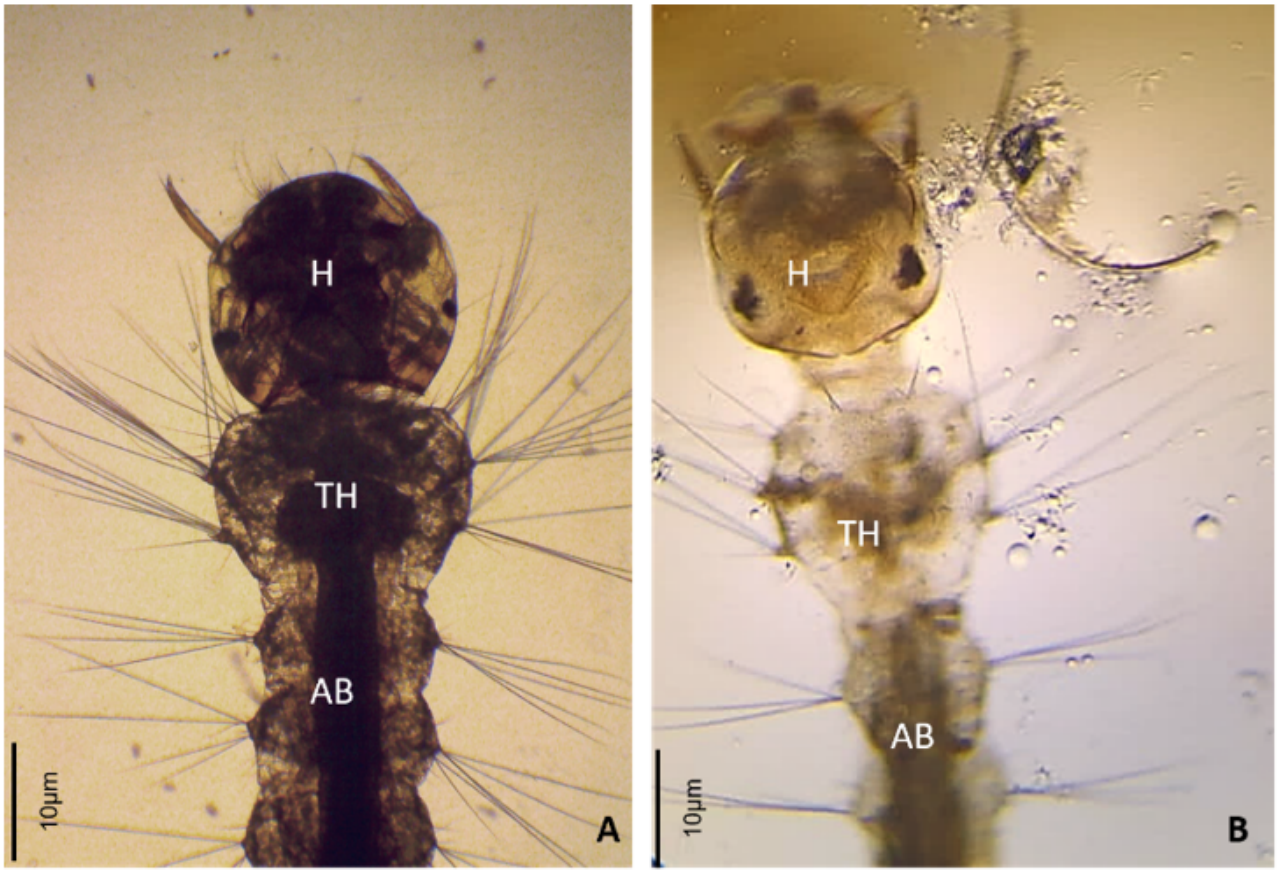

Figure 4

Ae. aegypti larvae treated with DDPO 15 µg.mL^{-1} (B) and DMSO control (A) with the following structures: head (H), thorax (TH), and abdomen (AB).

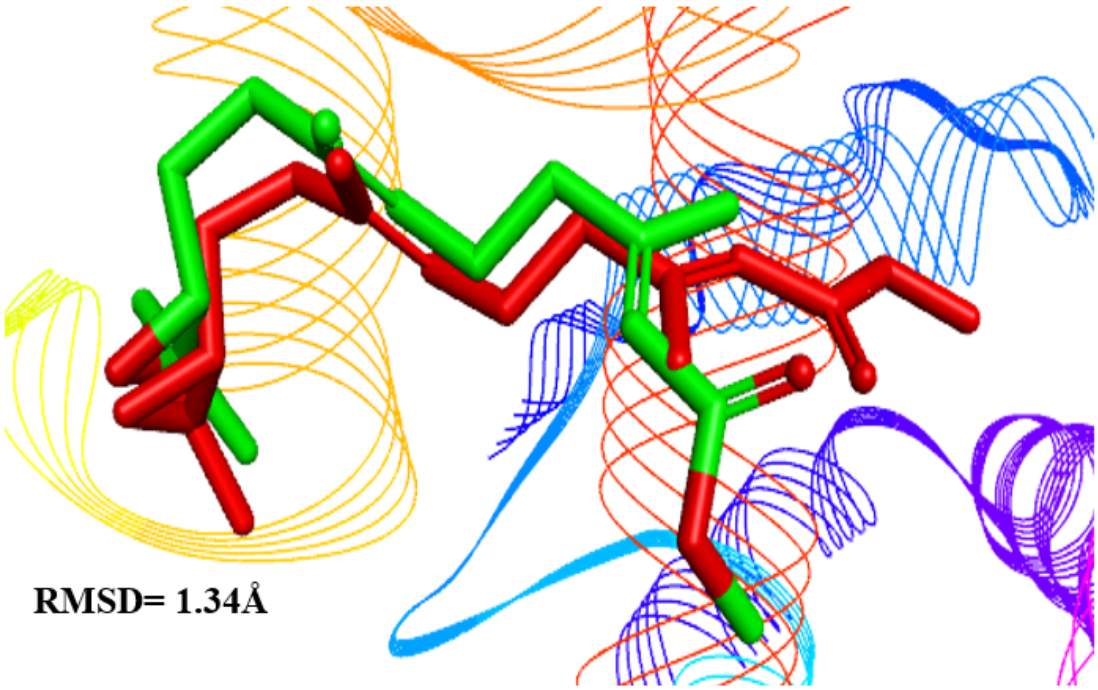

Figure 5

Overlays of the crystallographic binder pose (red JHIII) with the docking pose (green JHIII).

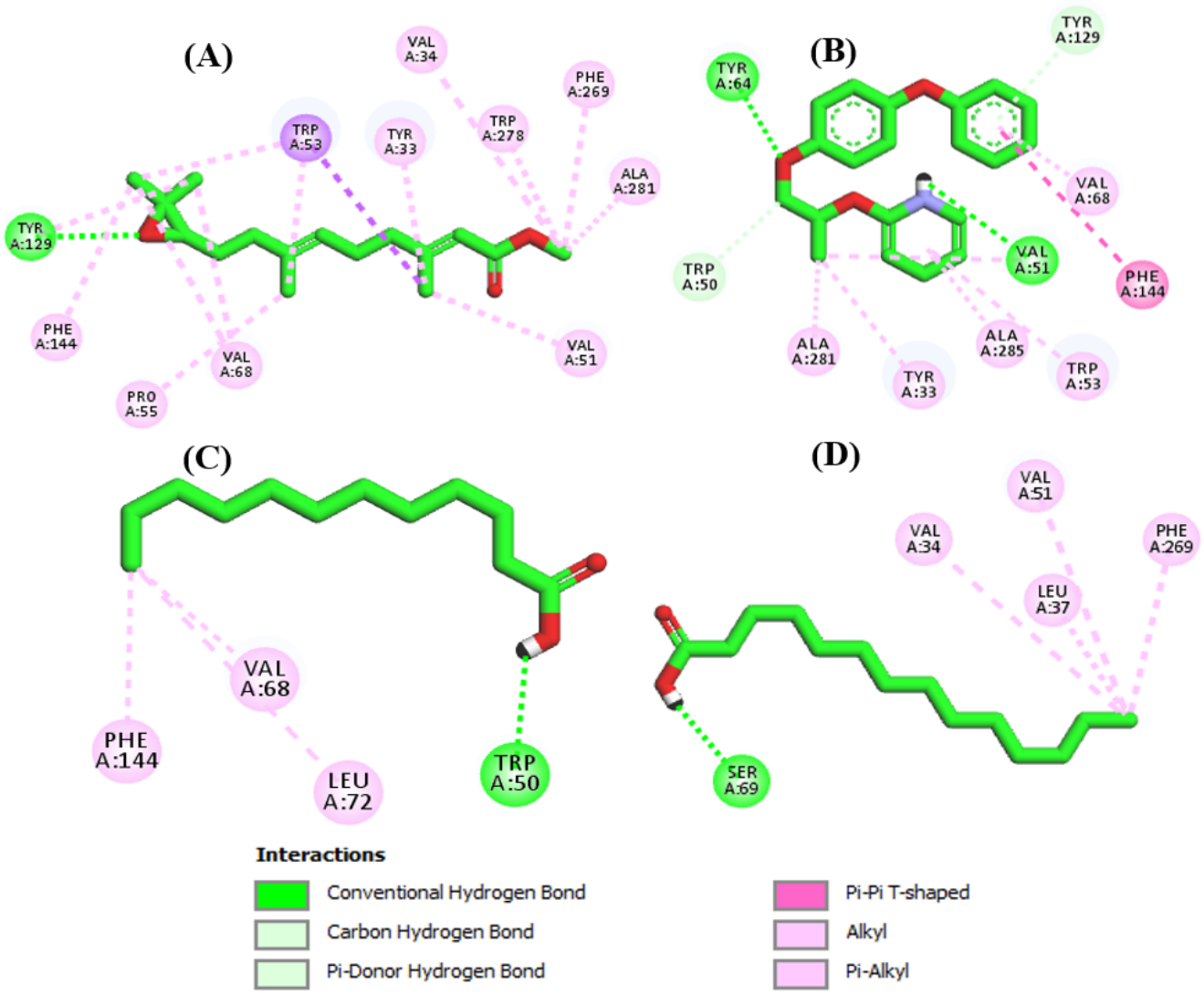

Figure 6

Interactions of controls JHIII (**A**), Pyriproxyfen (**B**), and potential inhibitors lauric acid (**C**) and myristic acid (**D**) with the active site of the receptor binding of the juvenile hormone (PDB ID 5V13).