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Research Article

Keywords: acetylcholinesterase, botanical insecticide, Coleoptera, Curculionidae, emulsifier, GC-MS, hydrodistillation, terpenoids

Posted Date: November 23rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10907909/v1

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Additional Declarations: No competing interests reported.

Insecticidal activity of *Piper aduncum* essential oil against Cosmopolites sordidus: chemical composition and molecular docking of major constituents

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ABSTRACT

The chemical composition and laboratory toxicity of Piper aduncum L. (Piperaceae) leaf essential oil were evaluated against adult banana weevils, Cosmopolites sordidus (Germar) (Coleoptera: Curculionidae). Molecular docking was performed to investigate the possible interactions of its major constituents with insect acetylcholinesterase. The essential oil was obtained by hydrodistillation and characterized using GC-FID and GC-MS. Ten compounds were identified, with eucalyptol (1,8-cineole; 50.04%) as the main constituent, followed by α-pinene (11.31%), (E)-β-ocimene (9.19%), β-pinene (7.67%), and bicyclogermacrene (7.14%). Adult banana weevils were exposed to filter paper impregnated with essential oil solutions at concentrations of 0–20 mL/L, prepared separately using polysorbate Tween 80® and neutral detergent as emulsifiers. Mortality was assessed from 24 to 216 h and increased with the concentration and exposure time of both emulsifiers. At 72 h, the solution prepared with neutral detergent showed lower LC_{50} and LC_{90} point estimates (7.66 and 28.53 mL/L, respectively) than that prepared with Tween 80® (9.29 and 59.42 mL/L, respectively). Molecular docking was conducted using G119S mutant acetylcholinesterase-1 from Anopheles gambiae (PDB ID: 6ARY) as a surrogate target. The predicted binding scores ranged from −5.72 to −7.93 kcal/mol, with bicyclogermacrene showing the most favorable score among the compounds. Under laboratory conditions, the essential oil caused concentration- and time-dependent mortality in adult C. sordidus individuals. The docking results suggest possible interactions with acetylcholinesterase; however, experimental confirmation using the enzyme from the target species is required.

Keywords: acetylcholinesterase, botanical insecticide, Coleoptera, Curculionidae, emulsifier, GC-MS, hydrodistillation, terpenoids

1. INTRODUCTION

Banana is one of the most widely produced and consumed fruits worldwide, with a global production of approximately 139 million tons in 2023 (FAO 2024). Brazil is currently the sixth-largest banana producer, behind India, China, Indonesia, Nigeria, and Ecuador (FAO 2024). In the 2023/2024 harvest, Brazilian production reached approximately 6.9 million tons, most of which was consumed domestically. São Paulo, Bahia, and Minas Gerais were among the main producing states (IBGE 2024). Because banana production is intended primarily for the domestic market, factors that reduce crop productivity can have direct economic consequences for producers and the supply chain.

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae), is one of the major insect pests of banana and plantain crops and occurs in most banana-producing regions worldwide (Gold et al. 2001; Dantas et al. 2011; Al-Sarar et al. 2024). Larval feeding damages the corm and may compromise plant development and crop productivity. Susceptibility varies among banana cultivars. Under Brazilian conditions, the cultivars Maçã and Terra have been reported to be more susceptible than Prata, Nanica, and Nanicão (Batista Filho et al. 1990). However, none of these cultivars can be considered completely resistant, indicating that additional management measures are required.

Chemical insecticides have been employed against *C. sordidus*, but the development of resistance to organophosphorus compounds has been documented (Collins et al. 1991). Furthermore, no single management practice provides complete control of this pest, thus supporting integrated approaches that combine compatible control strategies (Gold et al. 2001). These limitations encourage the investigation of plant-derived products as potential alternatives or complementary tools for banana weevil management.

Essential oils contain mixtures of volatile compounds that exhibit insecticidal, repellent, and deterrent activities against agricultural pests (Isman 2006). Species of the genus *Piper* are recognized as sources of biologically active secondary metabolites (Da Silva et al. 2017). Essential oils obtained from *Piper aduncum* L. have demonstrated insecticidal activity against different insect pests, including *Sitophilus zeamais*, *Tenebrio molitor*, *Cerotoma tingomarianus*, *Solenopsis saevissima*, and *Helicoverpa armigera* (Estrela et al. 2006; Fazolin et al. 2007; Souto et al. 2011; Santos et al. 2017; Assunção et al. 2024). However, the chemical composition of *P. aduncum* essential oil is not uniform. Oils dominated by dillapiole, piperitone, camphor, viridiflorol, or other terpenoids have been reported, reflecting the influence of geographical origin, environmental conditions, plant development, and other biological factors (Lima et al. 2003; Morais 2009; Monzote et al. 2017). Therefore,

chemical characterization is necessary before the biological activity of an oil can be interpreted or compared with that of oils in previous studies.

The practical application of essential oils may be limited by volatility, low stability, poor water miscibility, and loss of active constituents. Formulation is therefore important for improving essential oil dispersion, handling, stability, and delivery (López et al. 2021; Singh and Pulikkal 2022). Because surfactants and other formulation conditions may influence emulsion properties and biological performance, the results obtained with different delivery vehicles should be interpreted separately. In the present study, polysorbate Tween 80® and neutral detergent were evaluated as separate delivery vehicles for *P. aduncum* essential oil.

The mechanisms underlying the insecticidal effects of essential oils may involve several molecular targets in insects. Acetylcholinesterase terminates cholinergic neurotransmission by hydrolyzing acetylcholine and is a well-established molecular target for organophosphate and carbamate insecticides. Some plant-derived monoterpenoids can modulate insect acetylcholinesterase, although their potency and inhibition mechanisms vary among compounds and insect species (Zarrad et al. 2017). *Piper aduncum* essential oil has also been reported to alter acetylcholinesterase activity in arthropods, with effects dependent on the exposure concentration (Pereira Filho et al. 2024). These findings support acetylcholinesterase as a plausible, but not exclusive, molecular target for some essential oil constituents. Thus, this study aimed to characterize the chemical composition of the essential oil obtained from *P. aduncum* leaves, evaluate its laboratory toxicity to adult *C. sordidus* when formulated with polysorbate Tween 80® or neutral detergent, estimate lethal concentrations, and explore the predicted interactions of the five major constituents with a surrogate insect acetylcholinesterase.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Essential Oil

The work was carried out at the Phytochemistry and Catalysis Laboratory of the Federal Institute of Espírito Santo (IFES) - Alegre Campus and at the Analytical Center of the Center for Exact, Natural and Health Sciences – CCENS Alegre Campus (UFES). The experiment involved collecting plants for botanical characterization and leaves for extraction and identification of the chemical constituents of the essential oil.

Branch samples with leaves of *P. aduncum* were collected at the roadside of ES 146 km 30.2 in Sítio Estrela do Norte, Barra do Batatal, municipality of Alfredo Chaves - ES. The collection area was located at an altitude of 80 meters (IBGE 1977) and at the coordinates 40º46’44.8508”W – 20º36’51.8207” S. The collection was carried out in May 2019, in the morning.

A voucher specimen of *P. aduncum* was deposited at the herbarium Capixaba da Universidade Federal do Espírito Santo under accession number 6718. The collected leaves were dried for essential oil extraction. The leaves underwent hydrodistillation, which involved soaking the plant material in water under heat, and the resulting volatile product was condensed and collected using a Clevenger apparatus, following the methodology described by Souza et al. (2017). Approximately 100 g of *P. aduncum* leaves and 700 mL of distilled water were added to a 2000 mL round-bottom flask. The heating mantle was set to 140 ºC; once boiling was reached, the temperature was lowered to 100 °C, and the mixture was distilled for 4 hours. The essential oil was separated and subjected to centrifugation at 6000 rpm for 10 minutes. After centrifugation, the essential oil was collected, weighed, subjected to yield calculation (ranging from 0.9% to 1.19%), and stored in an amber glass bottle and cooled to a temperature below 0 °C.

2.2 Analysis of Essential Oil by Gas Chromatography

The essential oil sample from the leaves of *P. aduncum* was subjected to analysis by gas chromatography with flame ionization detection (GC-FID) (Shimadzu GC-2010 Plus apparatus) and by gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu GCMS-QP2010 apparatus) following the methodology of Souza et al. (2017). The following chromatographic conditions were used in the analysis: fused silica capillary column (30m × 0.25 mm) with DB5 stationary phase (0.25 µm film thickness); N2 (in GC/FID analysis) and helium (in GC/MS analyses) as carrier gas with a flow rate of 3.0 mL/min^{-1}; oven temperature followed a program where it remained for 3 minutes at an initial temperature of 40 °C and then gradually increased by 3 °C min^{-1} until reaching 240 ºC, maintaining this temperature for 5 minutes; injector temperature of 250 °C; detector temperature of 280 °C; and split ratio of 1:30.

Diluted essential oil samples were analyzed using GC–FID to quantify the chemical constituents and determine the retention indices (RI). Each essential oil component was quantified based on its relative peak area in the chromatogram. The substances present in the essential oil were identified by comparing their mass spectra with those in the database (WILEY7n). The RI was calculated using GC data from a homologous series of saturated aliphatic hydrocarbons ranging from C7 to C40 (Sigma-Aldrich) performed on the same column and under the GC conditions used for the essential oils, using the equation proposed by Van Den Dool and Kratz (1963), as indicated in Equation 1:

$$LTPRI = 100n + 100\frac{t_{R(i)} - t_{R(n)}}{t_{R(n+1)} - t_{R(n)}} \qquad (1)$$

For the calculation of the linear temperature-programmed retention index (LTPRI), A mixture of linear n-alkanes (C7–C40) was used to calculate the linear temperature-programmed retention index (LTPRI). The LTPRI index is a retention index used for linear temperature programming during chromatographic analyses (Von Mühlen 2009).

2.3 Insecticidal Assay

The experiments were conducted in the physical chemistry and microbiology laboratory of the Agricultural Family School of Alfredo Chaves - MEPES in Alfredo Chaves, ES, in a climate chamber at 25 ± 2°C, 70% relative humidity, and a 12-hour photoperiod. To perform the biotests, 880 adults of the rhizome weevil (*C. sordidus*) were selected, collected in a banana plantation of the silver group, cultivar Vitória, at the Active Germplasm Bank (BAG), in the experimental farm of Incaper, located in the municipality of Alfredo Chaves – ES, at coordinates 40º 44' 31.8969" W and 20º 38' 11.1928" S. For capturing the weevils, tile-type baits were used, made from the pseudostem of the banana plant, measuring 50 cm in length and distributed near the banana clumps (Mesquita 2003).

For seven days, the borers were captured, placed in a 1.0 L container with a perforated lid to allow gas exchange, and fed pieces of banana rhizome. For the bioassays, borers measuring 10 mm in length were selected using a caliper and subsequently placed in another container with a perforated 1.0 L lid for testing. The two stock solutions used in the bioassays were prepared at the Applied Chemistry Laboratory of the Federal Institute of Espírito Santo (IFES) - Alegre Campus, each containing 200 mL/L of *P. aduncum* essential oil. One was composed of the emulsifier polysorbate TWEEN 80 and the other of a neutral detergent.

For the preparation of each solution, 3.0 mL (20% v/v) of *P. aduncum* essential oil was added to the bottom of two test tubes, followed by 1.5 mL (10% v/v) of polysorbate in one of the containers, and in the other container, 1.5 mL (10% v/v) of detergent, which were subsequently vortexed® for 10 minutes. After reaching a cream-like appearance, 10.5 mL of distilled water was slowly added while the device was agitated for another 10 minutes. The final volume of each stock solution was 15.0 mL. Two negative control solutions were also prepared: the first contained 0.3 mL of polysorbate TWEEN 80 (1% v/v) and 30 mL of distilled water. For the second negative solution, only the composition of the 0.3 mL was changed, which was filled with neutral detergent (1% v/v). The components in their respective solutions were homogenized in a vortex® shaker for 10 minutes. Biotests were conducted using the impregnated filter-paper protocol (FAO, Method No. 15, 1974) according to the methodology adapted from Souto et al. (2011), using Petri dishes (90 mm × 15 mm) with lids and circular filter

paper of 90 mm diameter impregnated with different concentrations of *P. aduncum* oil: 0 mL/L; control, 2 mL/L; 4 mL/L; 6 mL/L; 8 mL/L; 10 mL/L; 12 mL/L; 14 mL/L; 16 mL/L; 18 mL/L, and 20 mL/L.

Table 1 here

For each essential oil concentration and control with its respective emulsifier, two tests were conducted. Each test comprised four replicates of five rhizome borers, corresponding to 220 insects per test for each emulsifier. Thus, 440 insects were used for each emulsifier and 880 insects in total. The treatments were applied by spraying 1.0 mL onto the Petri dish lid and 4.0 mL of each concentration onto the filter paper using an airbrush. After the oils were applied, the rhizome borers were placed on the plates with tweezers and fed pieces of banana rhizome weighing approximately 50 g. Subsequently, the plates were sealed with crepe tape® and placed in a BOD incubator at a temperature of 25 ± 2°C, under a 12-hour photoperiod, relative humidity of 70%, and evaluated at 24, 48, 72, 96, 120, 144, and 216 hours after the application of the concentrations. The evaluations were made by observing the borers that remained immobile after 30 seconds. They were considered dead when, upon being touched on the thorax with a brush, they showed no signs of life.

2.4 Data Analysis

Mortality at 72 h was subjected to probit analysis to estimate the LC_{50} and LC_{90} values and their 95% confidence intervals for the EO formulated with Tween 80® or neutral detergent. The experiments were conducted in a completely randomized design with a split-plot arrangement over time, in which the plots comprised 11 concentrations and the subplots comprised seven exposure times. Analysis of variance was performed for the concentration × exposure time interaction. Regression models were assessed using the F-test, and their parameters were assessed using the Student's t-test.

2.5 Molecular Docking Analysis

Molecular docking was performed to investigate the potential interactions between the five major constituents identified by GC-MS, namely, eucalyptol (1,8-cineole), α-pinene, (E)-β-ocimene, β-pinene, and bicyclogermacrene and insect acetylcholinesterase. Because no experimentally resolved three-dimensional protein structure of C. sordidus is available in the Protein Data Bank, the G119S mutant acetylcholinesterase-1 from Anopheles gambiae (PDB ID: 6ARY) was selected as a surrogate target. This mutation is associated with resistance to anticholinesterase insecticides, and the structure contains a co-crystallized difluoromethyl ketone inhibitor that

enabled the definition of the binding site and the validation of pose reproduction (Cheung et al. 2018). Therefore, docking was interpreted as a predictive complement to bioassays and not as direct evidence of enzyme inhibition in *C. sordidus*.

The protein structure was obtained from the Protein Data Bank (PDB). The co-crystallized ligand and crystallographic water molecules were removed, polar hydrogen atoms were added, and Gasteiger charges were assigned using AutoDockTools version 1.5.7 before conversion to the PDBQT format. Three-dimensional ligand structures were obtained from PubChem in SDF format, converted to PDB format using BIOVIA Discovery Studio Visualizer 2021, and prepared in AutoDockTools 1.5.7 by assigning Gasteiger charges, defining rotatable bonds, and converting the structures to the PDBQT format.

Docking simulations were performed using AutoDock Vina (version 1.2.5) (Trott and Olson 2010; Eberhardt et al. 2021). The grid was centered on the binding pocket defined by the co-crystallized ligand at x = −59.554, y = 58.668, and z = 17.089, with dimensions of 18 × 18 × 18 Å. An exhaustiveness value of 16 was used, and nine poses were generated for each ligand in each docking run. The pose with the lowest predicted binding energy was selected for the interaction analysis. Redocking of the native ligand yielded an RMSD of 0.85 Å, which was below the 2.0 Å criterion used to assess the reproduction of the experimental binding pose. Three-dimensional poses and two-dimensional interaction diagrams were examined using BIOVIA Discovery Studio Visualizer 2021.

3. RESULTS

3.1 Composition of the Essential Oil

GC-FID and GC-MS analysis identified 10 constituents in *P. aduncum* EO (Figure 1; Table 2). Eucalyptol was the main constituent (50.04%), followed by α-pinene (11.31%), (E)-β-ocimene (9.19%), β-pinene (7.67%), and bicyclogermacrene (7.14%). Together, these five constituents accounted for 85.35% of the relative chromatographic area. Myrcene, (Z)-β-ocimene, α-terpineol, (E)-caryophyllene, and germacrene D accounted for the remaining identified fraction.

Figure 1 here

Table 2 here

3.2 Insecticidal Assay

The percentage of insects killed as a function of the concentration of the essential oil from *P. aduncum* leaves diluted in polysorbate TWEEN 80® and the exposure time to the product is shown in Figure 2. Significant

relationships between the percentage of insects killed and the concentration of *P. aduncum* essential oil were noted for all exposure times; that is, the percentage of insects killed increased significantly with increasing concentrations, as shown in Figure 2 and the respective regression models, significance, and R2 values in Table 3. Significant functional relationships were also observed between the percentage of insects killed and exposure time to the product, as indicated by regression models, significance, and R2 values (Figure 3 and Table 4).

Figures 2 and 3 here

Table 3 and 4 here

With neutral detergent, mortality exceeded 50% at 10 mL/L after 72 h. Mortality increased with concentration at all exposure times and with exposure time across the evaluated concentrations (Figures 4 and 5; Tables 5 and 6). No mortality was observed in the neutral detergent control.

Figures 4 and 5 here

Tables 5 and 6 here

3.3 Determination of LC_{50} and LC_{90}

At 72 h, the LC^{50} point estimates were 9.29 mL/L (95% CI: 7.52–11.21) for polysorbate and 7.66 mL/L (95% CI: 5.17–9.97) for neutral detergent. The corresponding LC_{90} estimates were 59.42 mL/L (95% CI: 39.67–117.09) and 28.53 mL/L (95% CI: 19.71–62.32), respectively (Table 7).

Table 7 here

3.4 Molecular Docking

Docking of the five major EO constituents against the G119S mutant A. gambiae acetylcholinesterase produced predicted scores ranging from −5.72 to −7.93 kcal/mol (Figure 6). Bicyclogermacrene showed the most favorable score (−7.93 kcal/mol), followed by eucalyptol (−6.40 kcal/mol), β-pinene (−6.28 kcal/mol), α-pinene (−6.14 kcal/mol), and (E)-β-ocimene (−5.72 kcal/mol). Bicyclogermacrene was predicted to form hydrophobic π-alkyl and π-σ contacts with Trp441, with no hydrogen bond identified (Figures 7 and 8).

Figures 6, 7 and 8 here

4. DISCUSSION

The present study demonstrated that *P. aduncum* essential oil caused concentration- and time-dependent mortality in adult *C. sordidus* under laboratory conditions. This response was observed with both polysorbate Tween 80® and neutral detergent as delivery vehicles, although the neutral detergent formulation yielded lower LC_{50} and LC_{90} point estimates at 72 h. These findings extend the known insecticidal spectrum of *P. aduncum* essential oil to the banana weevil and are consistent with its previously reported activity against insects of different orders, including *Helicoverpa armigera*, *Solenopsis saevissima*, *Cerotoma tingomarianus*, *Tenebrio molitor*, and *Sitophilus zeamais* (Estrela et al. 2006; Fazolin et al. 2007; Souto et al. 2011; Santos et al. 2017). Because these studies differed in target species, exposure method, oil composition, and concentration units, their toxicity estimates are not directly comparable with those obtained here; however, together, they support the insecticidal potential of chemically distinct *P. aduncum* oils.

The oil evaluated in the present study was characterized by an eucalyptol-rich profile, with eucalyptol (50.04%), α-pinene (11.31%), (E)-β-ocimene (9.19%), β-pinene (7.67%), and bicyclogermacrene (7.14%) accounting for 85.35% of the relative chromatographic area. This profile differs from that of *P. aduncum* oils, which are dominated by dillapiole, piperitone, camphor, or viridiflorol (Monzote et al. 2017), but is similar to that of the eucalyptol-rich material reported from the Cerrado biome of northern Minas Gerais, Brazil (Oliveira et al. 2013). Variability in the chemical composition of *P. aduncum* has been associated with geographical origin and environmental and biological factors that influence terpene production (Lima et al. 2003; Morais 2009). This variability is relevant when comparing biological responses because oils from the same species may contain different main constituents and proportions.

Eucalyptol deserves particular consideration because it is the main constituent of the oil and has previously been identified as an electrophysiologically active volatile and laboratory attractant for *C. sordidus* (Ndiege et al. 1996). Attraction and mortality are distinct biological responses, and the forced exposure assay used in this study did not evaluate orientation, repellency, or attraction. Therefore, the reported behavioral activity of eucalyptol neither explains nor contradicts the mortality observed in this study. This suggests that future studies should investigate whether the eucalyptol-rich profile affects banana weevil behavior, in addition to causing toxicity under contact exposure conditions.

Mortality increased with both concentration and exposure time, indicating that the biological response depended on the intensity and duration of exposure. The impregnated-filter-paper method maintained the insects

in contact with the treated surface throughout the evaluation period; consequently, the experiment established laboratory toxicity under continuous exposure but did not distinguish the relative contributions of contact and volatile exposure. The absence of mortality in the neutral-detergent control indicates that mortality in that formulation was associated with the essential-oil treatment under the evaluated conditions. The concentration- and time-dependent pattern is consistent with previous laboratory studies in which *P. aduncum* oils exhibited insecticidal activity after exposure by contact or on treated surfaces (Estrela et al. 2006; Fazolin et al. 2007; Souto et al. 2011; Santos et al. 2017).

The two delivery vehicles produced different LC point estimates, with lower values obtained for the neutral-detergent formulation. Essential oils have limited miscibility in water, and formulation conditions can influence their dispersion, handling, and biological performance (López et al. 2021; Singh and Pulikkal 2022). Nevertheless, the physicochemical properties of the formulations were not measured in this study. Thus, the observed point estimates cannot be attributed to differences in droplet size, stability, surface tension, or cuticular penetration, and the data do not identify the physicochemical basis of the response. The results support reporting the two formulations separately rather than assigning a specific mechanistic explanation to the emulsifier effect.

The docking results provide a complementary hypothesis concerning the possible molecular targets of the major constituents. The ranking did not follow the compound abundance; eucalyptol represented 50.04% of the oil, whereas bicyclogermacrene represented 7.14% but produced the most favorable predicted score against the surrogate acetylcholinesterase. Bicyclogermacrene was predicted to establish hydrophobic contacts with Trp441, but no hydrogen bonds were identified. Experimental studies have shown that some monoterpenoids can modulate insect acetylcholinesterase, and that *P. aduncum* essential oil can alter acetylcholinesterase activity in arthropods (Zarrad et al. 2017; Pereira Filho et al. 2024). These reports make acetylcholinesterase a plausible target for investigation; however, the present scores alone do not demonstrate binding affinity, enzyme inhibition, or the mechanism responsible for mortality.

The mortality bioassay used the complete essential oil rather than isolated compounds; therefore, the observed activity cannot be assigned to bicyclogermacrene, eucalyptol, or any other individual components. Additive, synergistic, or antagonistic interactions among the constituents are also possible. In addition, the docking target was the resistance-associated G119S mutant acetylcholinesterase-1 from *Anopheles gambiae,* rather than the enzyme from *C. sordidus* (Cheung et al. 2018). Redocking with an RMSD of 0.85 Å supports the reproduction of the crystallographic pose within the adopted protocol; however, it does not convert the predicted scores into experimental affinities or inhibitory potencies. Enzyme assays, tests with isolated constituents and defined

mixtures, behavioral experiments, and field evaluations are required to determine the relationship between the chemical profile, formulation, and predicted acetylcholinesterase interactions and the insecticidal activity observed in the laboratory.

5. CONCLUSIONS

The essential oil from *P. aduncum* leaves, characterized by eucalyptol (50.04%) as the main constituent, caused concentration- and time-dependent mortality in adult *C. sordidus* under laboratory conditions. The neutral detergent formulation yielded lower LC_{50} and LC_{90} point estimates than the polysorbate Tween 80® formulation at 72 h. Molecular docking ranked bicyclogermacrene as the constituent with the most favorable predicted score against a surrogate G119S acetylcholinesterase; however, this prediction did not identify the compound responsible for mortality or demonstrate an acetylcholinesterase-mediated mechanism in *C. sordidus*. These results support the further evaluation of this eucalyptol-rich oil as a botanical option for banana weevil management, including field efficacy, residual activity, phytotoxicity, formulation performance, constituent interactions, and experimental assessment of acetylcholinesterase inhibition.

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Conflict of interest

The authors declare no conflicts of interest.

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Table 1. Treatments, concentrations of the *P. aduncum* stock solution based on polysorbate TWEEN 80® or neutral detergent, and method of solution preparation.

Treatment	Concentration Essential oil (mL/L)	Preparation method
T0	0	5 (mL) of the negative stock solution;
T1	2	50 (µL) of the *Piper* stock + 4950 (µL) of distilled water
T2	4	100 (µL) of the *Piper* stock + 4900 (µL) of distilled water
T3	6	150 (µL) of the *Piper* stock + 4850 (µL) of distilled water
T4	8	200 (µL) of the *Piper* stock + 4800 (µL) of distilled water
T5	10	250 (µL) of *Piper* stock + 4750 (µL) of distilled water
T6	12	300 (µL) of the *Piper* stock + 4700 (µL) of distilled water
T7	14	350 (µL) of the *Piper* stock + 4650 (µL) of distilled water
T8	16	400 (µL) of the *Piper* stock + 4600 (µL) of distilled water
T9	18	450 (µL) of the *Piper* stock + 4550 (µL) of distilled water
T10	20	500 (µL) of *Piper* stock + 4500 (µL) of distilled water

Table 2. Characterization of the *P. aduncum* leaf essential oil by the LTPRI index and GC-MS [a].

Peak	Retention time	Calculated retention index[b]	Tabulated retention index[c]	Name	Area%[d]
1	8.343	931	932	*α*-Pinene	11.31
2	10.085	973	974	*β*-Pinene	7.67
3	10.856	992	988	Myrcene	2.35
4	12.568	1030	1026	Eucalyptol	50.04
5	12.989	1040	1032	(Z)- β-Ocimene	3.48
6	13.447	1050	1044	(E)-β-Ocimene	9.19
7	19.959	1191	1186	*α*-Terpineol	3.22
8	29.892	1416	1417	(E)-Caryophyllene	1.24
9	32.450	1479	1484	Germacrene D	4.37
10	33.100	1494	1500	Bicyclogermacrene	7.14

[a] Compounds identified by the LTPRI index and by GC-MS using a DB5 column. [b] Calculated using a mixture of saturated n-alkanes (C7–C40). [c] Tabulated indices based on Adams, 2007. [d] Relative area based on the chromatogram in Figure 1, identifying only compounds with a relative area > 1%.

Table 3. Average percentage of insects killed as a function of essential oil concentration and the coefficients of determination R^2

Time (hour)	Concentration (ml/L)	
	Equation	R^2
24	y = 2.575**x - 9.833	0.9019**
48	y = 3.348**x - 3.833	0.9648**
72	y = 3.424**x + 5.833	0.9374**
96	y = 3.318**x + 13	0.9233**
120	y = 3.386**x + 14.5	0.9371**
144	y = -0.097*x2 + 5.855**x + 7.291	0.9856**
216	y = 3.166*x + 32.66	0.8247**

* = significant at 5%; ** = significant at 1%, by F and t tests; ns = not significant.

Table 4. Average percentage of insects killed as a function of exposure time and the coefficients of determination R^2

Concentration (ml/L)	Time (h)	
	Equation	R^2
2	y = -0.0007*x2 + 0.290**x - 6.057	0.9508**
4	y = 0.271**x - 2.878	0.9423**
6	y = -0.0007**x2 + 0.414**x - 3.9273	0.9962**
8	y = -0.0016*x2 + 0.6506**x - 3.8089	0.9493**
10	y = -0.0015*x2 + 0.5706**x + 5.3375	0.9451**
12	y = -0.0013*x2 + 0.5579**x + 3.621	0.9633**
14	y = 0.2961**x + 22.763	0.9600**
16	y = -0.0008**x2 + 0.4446**x + 32.889	0.9975**
18	y = -0.0023*x2 + 0.7583**x + 25.573	0.8828*
20	y = -0.0017*x2 + 0.631**x + 31.193	0.9354**

* = significant at 5%; ** = significant at 1%, by F and t tests; ns = not significant.

Table 5. Average percentage of insects killed as a function of the concentration of *P. aduncum* essential oil diluted in neutral detergent and coefficients of determination R^2.

Time (hour)	Concentration (ml/L)	
	Equation	R^2
24	y = 4.7273**x - 18	0.9452**
48	y = 5.1894**x - 13.333	0.9065**
72	y = 4.803**x - 0.8333	0.9460**
96	y = 4.5**x + 7	0.9246**
120	y = 4.5833**x + 9.8333	0.9377**
144	y = 4.8106x + 9.3333	0.9250**
216	y = 4.7045**x + 13.5	0.9268**

* = significant at 5%; ** = significant at 1%, by F and t tests; ns = not significant.

Table 6. Average percentage of insects killed as a function of exposure time and the coefficients of determination R^2

Concentration (ml/L)	Time (h)	
	Equation	R2
2	y = -0.001**x2 + 0.339**x - 3.0176	0.9424**
4	y = -0.0005*x2 + 0.2382**x - 3.8938	0.9598**
6	y = -0.0005**x2 + 0.2382**x - 3.8938	0.9598**
8	y = -0.0013*x2 + 0.4724**x + 2.5234	0.9342**
10	y = -0.0022*x2 + 0.7982**x + 1.5037	0.9242**
12	y = -0.0012**x2 + 0.4538**x + 32.596	0.9865**
14	y = -0.0012*x2 + 0.4377**x + 51.37	0.9423**
16	y = -0.0007*x2 + 0.3193**x + 55.573	0.9289**
18	y = -0.001*x2 + 0.4033**x + 56.052	0.9532**
20	y = -0.0007*x2 + 0.2596*x + 75.283	0.8417

* = significant at 5%; ** = significant at 1%, by F and t tests; ns = not significant.

Table 7. Probit estimates for mortality of *C. sordidus* adults exposed for 72 h to *P. aduncum* essential oil formulated with two emulsifiers.

Emulsifier	GL1	N2	Inclination±SEM3	LC_{50} (IC_{95}) (mL/L)	LC_{90} (IC_{95}) (mL/L)	$\chi 2$
Polysorbate	8	400	1.82±0.25	9.29 (7.52-11.21)	59.42 (39.67-117.09)	9.29
Detergent	6	320	2.24±0.38	7.66 (5.17-9.97)	28.53 (19.71-62.32)	11.02

CI, confidence interval; LC_{50} and LC_{90}, concentrations estimated to cause 50% and 90% mortality, respectively.

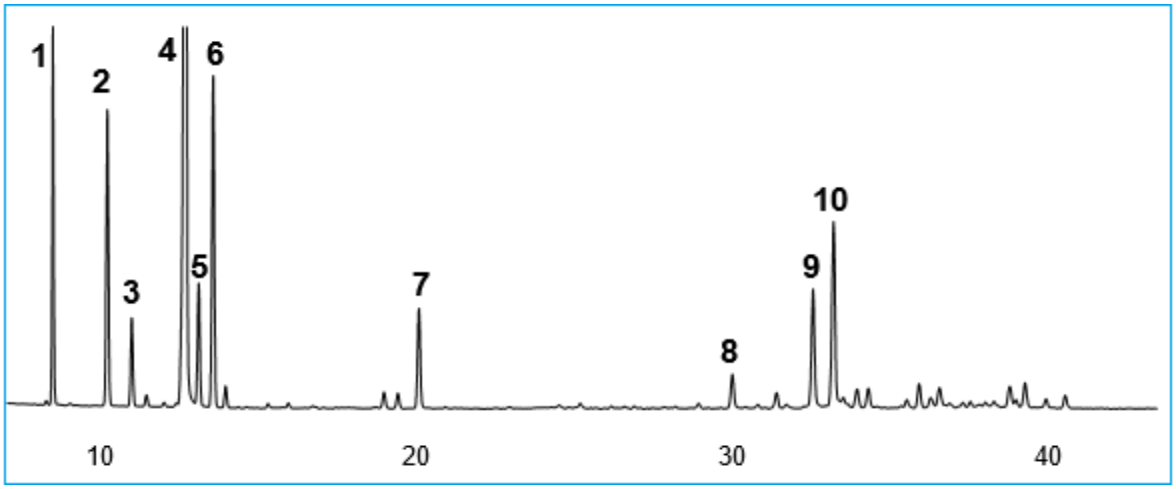

Figure 1. Chromatogram of the essential oil from *P. aduncum* leaves obtained using a GC-MS apparatus (Shimadzu GCMS-QP2010).

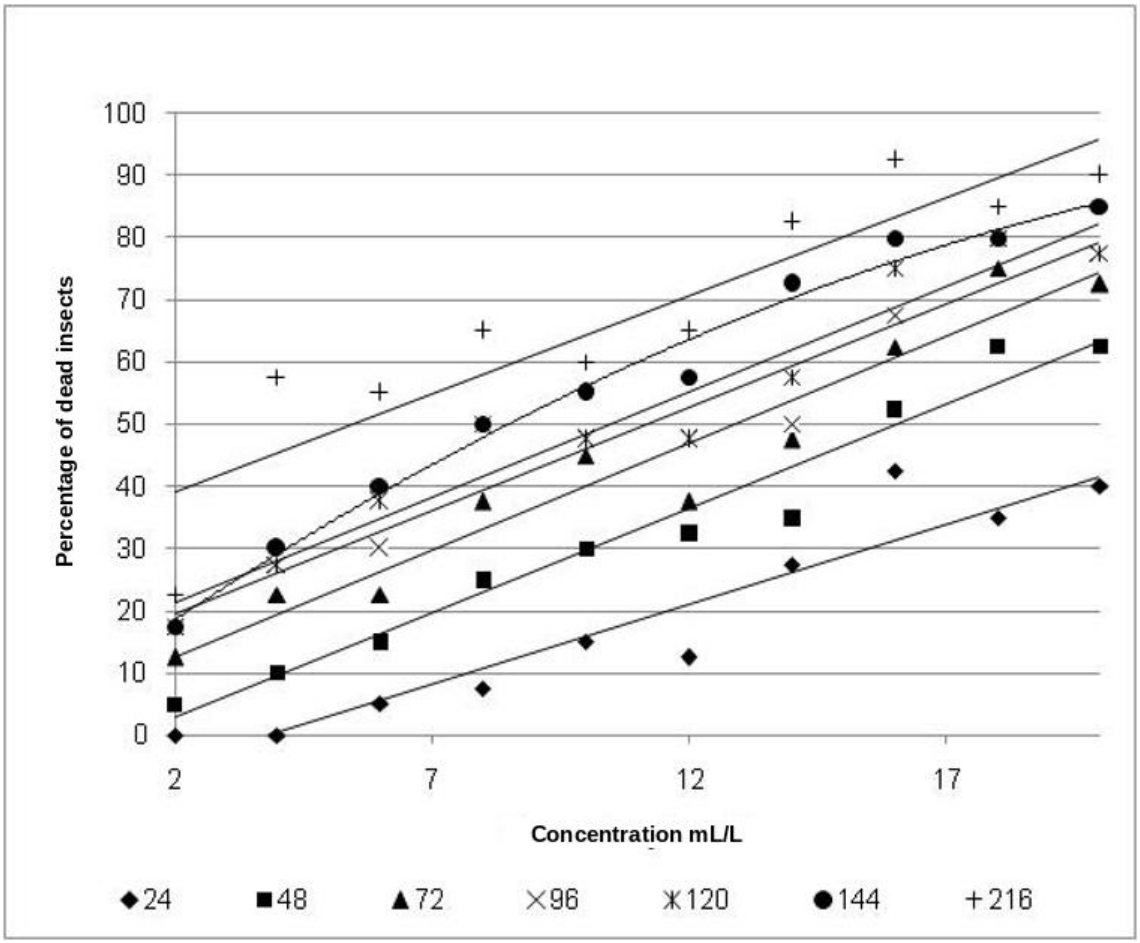

Figure 2. Mortality of *C. sordidus* adults as a function of *P. aduncum* essential oil concentration in the polysorbate Tween 80® formulation at different exposure times

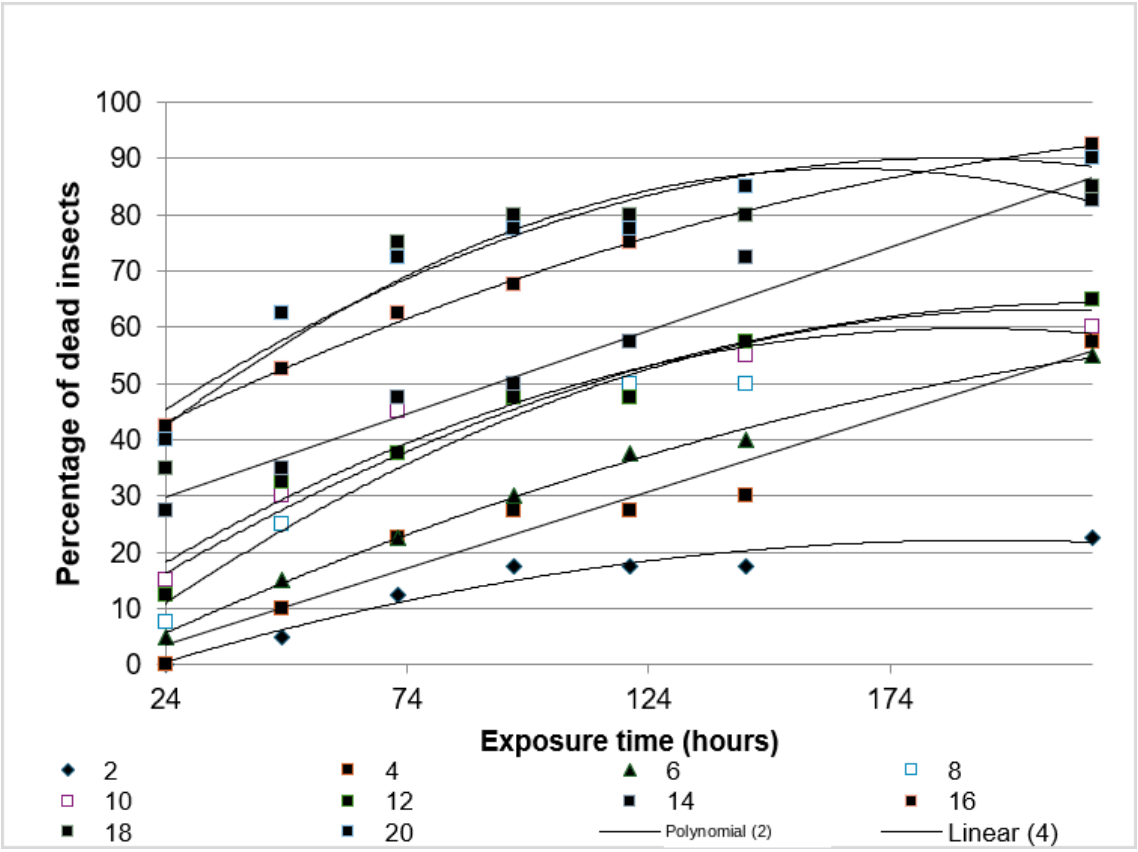

Figure 3. Mortality of *C. sordidus* adults as a function of exposure time to *P. aduncum* essential oil formulated with polysorbate Tween 80® at different concentrations

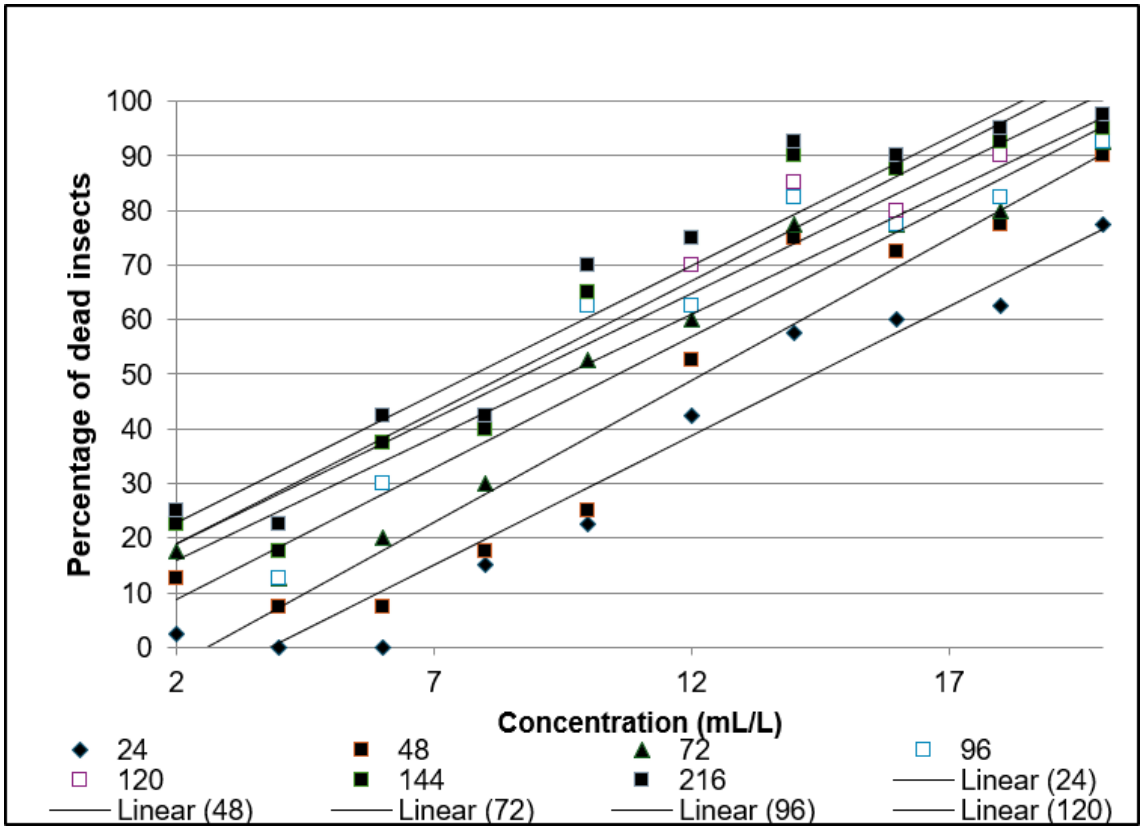

Figure 4. Mortality of *C. sordidus* adults as a function of *P. aduncum* essential oil concentration in the neutral-detergent formulation at different exposure times

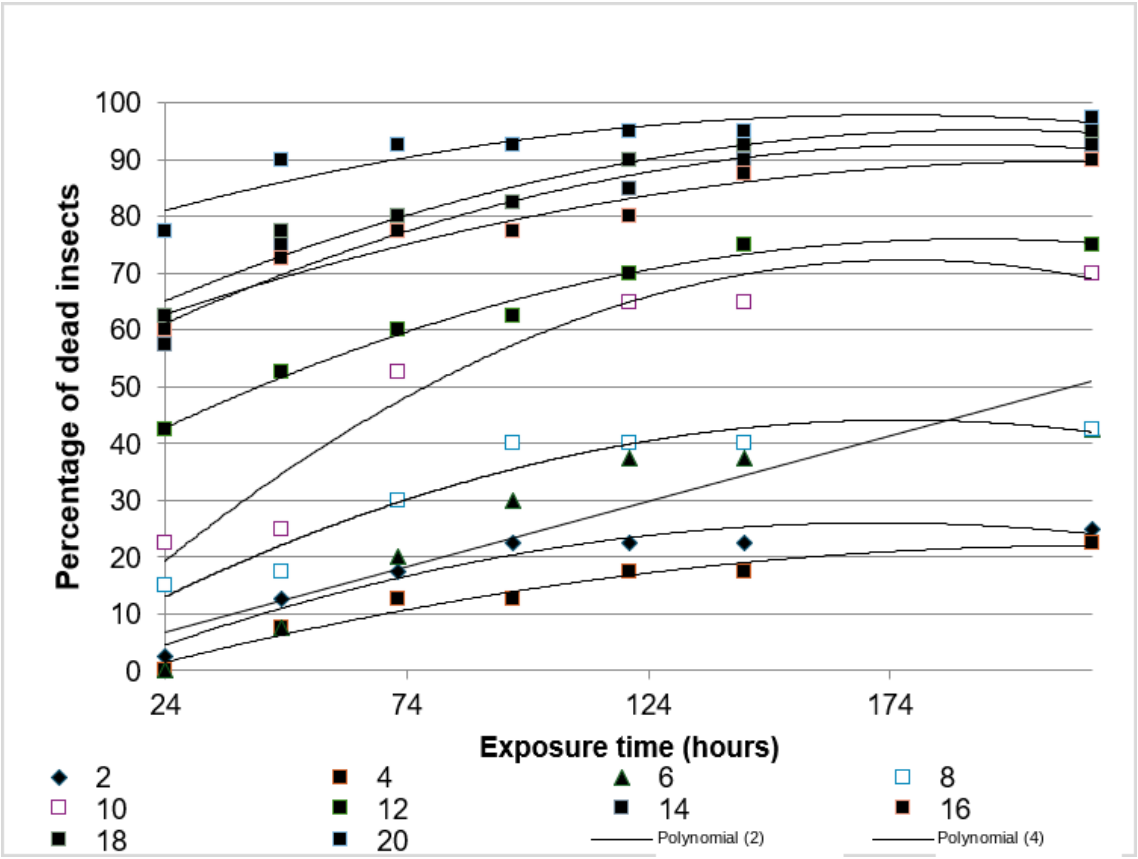

Figure 5. Mortality of *C. sordidus* adults as a function of exposure time to *P. aduncum* essential oil formulated with neutral detergent at different concentrations

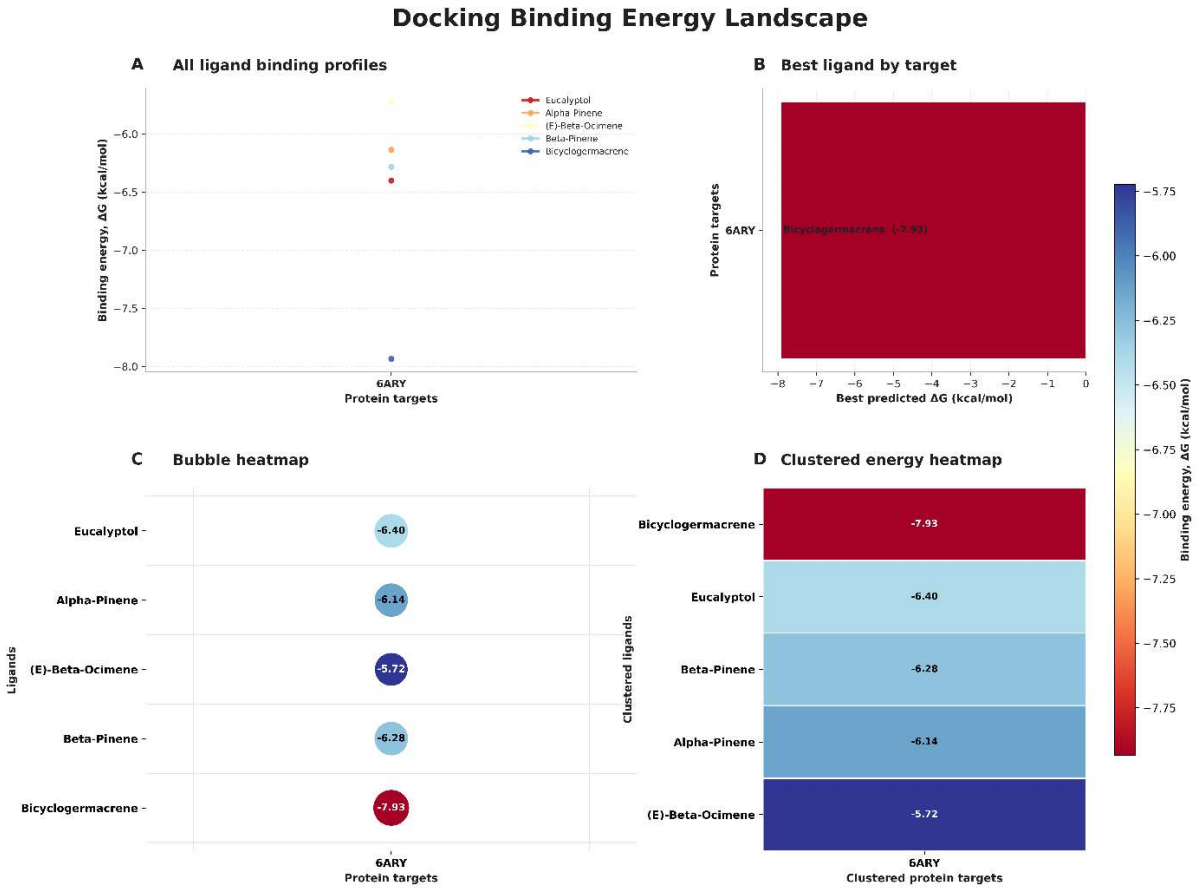

Figure 6. Comparative molecular docking results for the major constituents of *P. aduncum* essential oil against acetylcholinesterase (PDB ID: 6ARY). (A) Docking scores of compounds. (B) Ligand with the most favorable docking score. (C) Bubble heatmap of docking scores. (D) Clustered heatmap of docking scores.

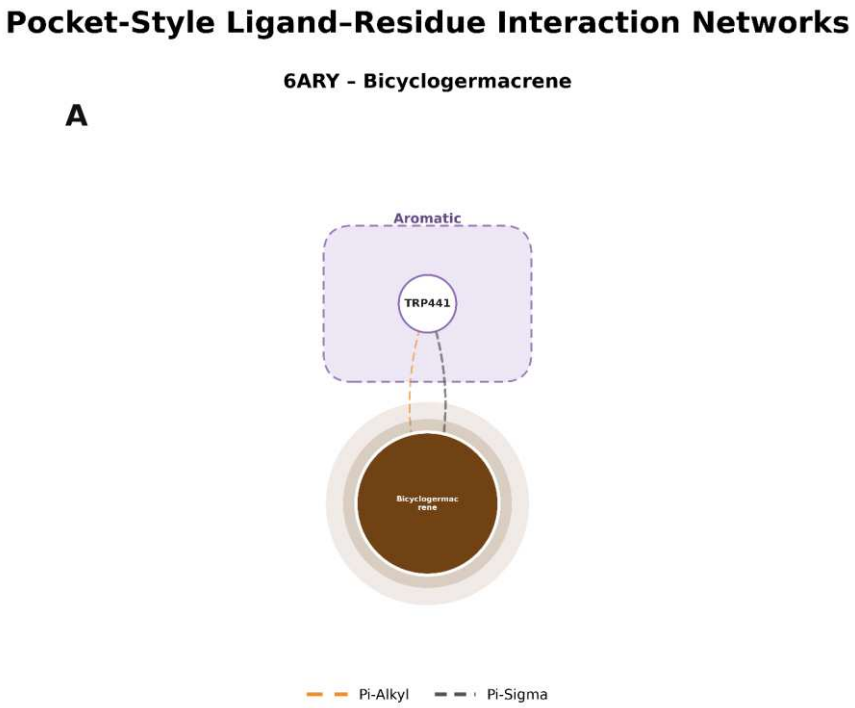

Figure 7. Predicted interaction network between bicyclogermacrene and acetylcholinesterase (PDB ID: 6ARY).

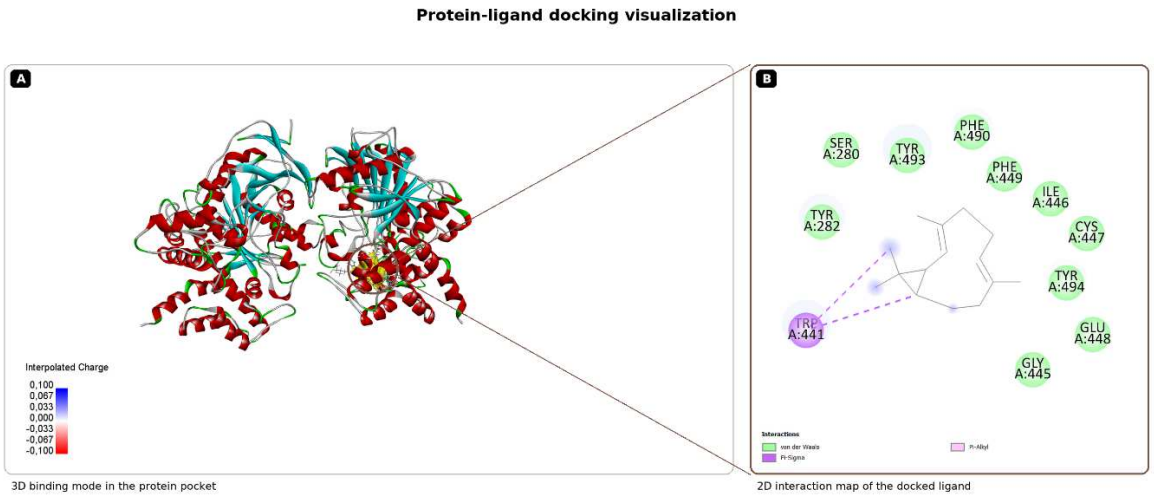

Figure 8. Predicted binding conformation and protein-ligand interactions of bicyclogermacrene with acetylcholinesterase (PDB ID: 6ARY). (A) Three-dimensional docking pose in the binding pocket. (B) Two-dimensional interaction map showing predicted residues and intermolecular contacts.