











Research Article

Chemical characterization, insecticidal activity and *in silico* study of *Tephrosia vogelii* Hook.f. (Fabaceae) flower essential oil on *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae)

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Abstract. Pest control is one of the main challenges in sustainable agriculture. A promising pest-control method in these systems is the use of plants with insecticidal properties. This study aimed to identify the chemical components of *Tephrosia vogelii* Hook.f. (Fabaceae) and evaluate the potential of flower essential oil to control fire ant, *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae), a significant pest of strawberry crops. The essential oil analysis revealed the presence of farnesol, α -pinene, nerolidol, spathulenol, ocimene, and limonene as major constituents. The oil demonstrated strong insecticidal activity, with LC₅₀ and LC₉₀ values of 0.105 and 0.298 mL/L, respectively, in *S. saevissima*, suggesting a possible neurotoxic effect with rapid action. In bioassays, a neutral detergent was used to solubilize the essential oil, proving an effective and economical alternative to traditional solubilizers. In addition, to support a possible mechanism of action, docking against acetylcholinesterase (AChE; PDB 4EY6) was performed: farnesol exhibited the best affinity ($\Delta G = -7.1$ kcal/mol; $K_i \approx 6.20$ μ M), followed by nerolidol (-6.8) and, with lower affinities, α -pinene (-6.3) and limonene (-6.2). These results suggest a cholinergic contribution to the observed toxicity, to be confirmed with enzymatic assays and insect AChE models.

Keywords: Agroecology, Phytosanitation, Pest Management, Phytochemistry, Strawberry crops.

Introduction

Strawberry is one of the most socioeconomically important fruits worldwide (Alves et al. 2020). However, its cultivation faces significant phytosanitary challenges, particularly due to its susceptibility to various pests and pathogens, which severely impact both productivity and fruit quality (Morris 2009). The frequent use of chemical pesticides to manage these threats has contributed to the development of pest resistance, ecological imbalances, and raised concerns about contamination, making strawberries one of the most pesticide-contaminated fruits (Martins et al. 2021; Santos et al. 2023).

Among the various pests that threaten strawberry plants, the fire ant *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae) is particularly problematic. This species damages plants by attacking their roots and shoots and exacerbates infestations of other pests, such as aphids (Hemiptera: Aphididae). This interaction complicates pest management efforts in strawberry cultivation (Porter & Fowler 1992; Simões et al. 2007; Souto et al. 2012; Lopes et al. 2022; Santos et al. 2023).

Given the need for environmentally friendly pest control solutions, essential oils have emerged as a promising alternative. These natural products, extracted from plants, contain bioactive compounds with insecticidal, fungicidal, and bactericidal properties, offering a potential solution to reduce the reliance on synthetic pesticides (Isman 2000). Demonstrating the efficacy of these compounds is crucial to developing safer, more sustainable pest control options that minimize environmental degradation and preserve non-renewable natural resources (Peixoto et al. 2023; Santos et al. 2023).

The essential oil of *Tephrosia vogelii* Hook.f. (Fabaceae), a leguminous plant known for its insecticidal properties shows significant potential for controlling agricultural pests. Preliminary studies have indicated that its major compounds, such as α -pinene, germacrene D, and caryophyllene, possess potent insecticidal activity by disrupting insect nervous systems (Olayemi et al. 2015). However, the chemical composition and biological activity of the flower essential oil remain largely unexplored, representing a significant knowledge gap. In addition to chemical characterization and bioassays, *in silico* approaches, such as molecular docking, enable us to mechanistically predict how essential oil constituents interact with insect neurophysiological targets. Among these targets, acetylcholinesterase (AChE) plays a central role in cholinergic transmission, and there is evidence that mono- and sesquiterpenes can modulate it (Lopez & Pascual-Villalobos 2010; Abdelgaleil et al. 2016; Abou-Taleb et al. 2016; Georgiev et al. 2022). Although predictive, this tool, when integrated with mortality and potency data (LC₅₀/LC₉₀), helps prioritize bioactive constituents and guide subsequent enzymatic assays.

Thus, this study characterized by GC-MS/GC-FID the essential oil of *T. vogelii* flowers, evaluated its formicidal activity against *S. saevissima* workers at different concentrations and times with LC₅₀/LC₉₀ estimates via probit analysis, and investigated, by docking on AChE, the plausibility of a cholinergic component in the mode of action, focusing on the major constituents (farnesol, nerolidol, α -pinene, limonene).

Material and Methods

Plant material. Fresh flowers of *T. vogelii* plants were collected



at the Sítio Highland, located in the district of Victor Hugo, in the municipality of Marechal Floriano, ES. The collection area is located at an altitude of 900 m and the coordinates 40°51'35.4069" W; 20°25'36.5197" S. The collections were carried out in the months of June, July, August, and September of 2017, all during the morning period. The plant material was dried for 3 days in a Marconi circulation oven (model MA033/630) at 40°C until a constant mass was achieved. After drying, the flowers were weighed (approximately 60 g), placed in sealed plastic bags, and stored in a freezer at temperatures below 0°C. The identification was made by the IFES Herbarium, Campus de Alegre, and the VIES Herbarium, from UFES, located in the Municipality of Jerônimo Monteiro, where a voucher was deposited with registration number VIES 42096.

Essential oil extraction. The essential oil was extracted through hydrodistillation. Approximately 60 g of plant material (*T. vogelii* flowers) and 700 mL of distilled water were added to a 2000 mL round-bottom flask and distilled for 4 (four) hours. The hydrosol (water and essential oil) was separated and centrifuged at 6000 RPM for 10 min. After centrifugation, the essential oils were collected, weighed, and stored in amber glass bottles below 0°C.

Chemical characterization. The essential oil of the *T. vogelii* flower was analyzed by gas chromatography with a flame ionization detector (GC-FID) (Shimadzu GC-2010 Plus device) and by gas chromatography coupled with mass spectrometry (GC-MS) (Shimadzu device QPMS-2010). The following chromatographic conditions were used in both analyses: fused silica capillary column (30 m × 0.25 mm) with a DB5 stationary phase (0.25 μm film thickness); N₂ (GC-FID analysis) and Helium (GC-MS analysis) as the carrier gas with a flow rate of 3.0 mL/min; the oven temperature followed a program in which it remained at an initial temperature of 40°C for 3 min and then gradually increased at 3°C/min until reaching 240°C, remaining at this temperature for 5 min; injector temperature of 250°C; detector temperature 280°C; split ratio of 1:30.

GC-MS analyses were carried out using equipment operating via electronic impact with an impact energy of 70 eV, scan speed of 1,000, scan range of 0.5 fragments/second, and detected fragments of 29–400 (m/z). The chemical components of the oils were identified by comparing their mass spectra with those available in the Wiley7, NIST05, and NIST05s spectral databases, by co-injection with standards, and by LTPRI retention indices. A mixture of linear n-alkanes (C7–C40) was used to calculate linear temperature-programmed retention indices (LTPRI). The LTPRI Index is a retention index used with linear temperature programming during chromatographic runs. The LTPRI calculated for each compound was compared with values from the literature (Adams 2007) and estimated using the following equation:

$$LTPRI = 100Z + 100 \times \frac{[(t'_{RX}) - (t'_{RZ})]}{(t'_{RZ} + 1) - (t'_{RZ})} \quad (1)$$

Equation S1: Formula used to determine LTPRI index, where:

X: Compound of interest;

t'_{RX}: Compound retention time;

Z: Number of carbon atoms in the hydrocarbon with a retention time immediately preceding the retention time of X;

t'_{RZ}: retention time of the hydrocarbon immediately preceding the retention time of X;

t'_{RZ} + 1: retention time of the hydrocarbon immediately after the retention time of X.

The relative percentage of each essential oil compound was calculated by dividing the integral area of its respective peaks by the total area of all sample constituents.

Residual contact bioassay. We conducted a residual-contact bioassay to evaluate insecticidal activity. The experiments were conducted in the IFES – Campus de Alegre microbiology laboratory in climatic chambers, with a temperature of 25 ± 2°C, a relative air humidity of 75%, and a 12-hour photophase. To conduct the bioassays, worker ants of *S. saevissima* were collected from an organic strawberry crop on a family-run farm in Guaçuí, ES, Brazil. To prepare the oils, they were diluted using 20 μL of neutral Ype clear detergent (Linear Sodium

Alkyl Benzene Sulfonate) and distilled water, in all six concentrations tested: T1 (0.80 mL/L); T2 (0.60 mL/L); T3 (0.40 mL/L); T4 (0.10 mL/L); T5 (0.05 mL/L); T6 (0.025 mL/L) which are presented in Tab. 1. As a control (control), 10 mL of distilled water and 20 μL of the same detergent were used. The methodology employed was adapted from Souto et al. (2012), using glass Petri dishes (150 mm in diameter and 20 mm in height) with a lid and circular filter paper (150 mm in diameter) impregnated with different concentrations of *T. vogelii* essential oil (Tab. 1).

Three replications were carried out for each essential oil concentration and the control, each containing 20 worker ants. In each treatment, using an airbrush and a pressure of 15 lb/in², 1 mL of the solution was sprayed onto the plate lid and 1 mL onto the filter paper. After applying the oils, the ants were added to the plates using flexible rods soaked in a honey solution, and they were fed 10% honey. Then, the plates were sealed with PVC film, incubated at 25 ± 2°C under a 12-hour photoperiod, and evaluated 24 and 48 h after application of the solutions.

The assessments were made by observing the motionless ants, which were marked with a brush on the plate where they were dead. The experiment was performed in triplicate with different populations of *S. saevissima* workers to obtain more representative results. The ExpDes.pt package was used for data analysis in the R statistical program, and the results were corrected using the formula of Abbott (1915): $mortcor = [(mort-mean_control)/(100-mean_control) \times 100]$. Therefore, the ANOVA did not include a control (witness). The lethal concentrations of both essential oils, LC₅₀ and LC₉₀, were estimated by probit analysis (bioRssay package) using the R statistical software (version 3.4.0).

Molecular docking procedure. To explore the possible molecular basis of the observed toxic effects, AChE was selected as the target protein for molecular docking analysis. The crystal structure with PDB ID 4EY6 was chosen for this purpose, as it provides a high-resolution (2.35 Å) representation of human AChE complexed with a known inhibitor. This structure is widely used in computational studies due to its well-defined active site and suitability for ligand-binding assessments. Utilizing the 4EY6 structure allows for a detailed investigation of potential interactions between the essential oil constituents and a key neuroregulatory enzyme, thereby supporting the interpretation of insecticidal activity at the molecular level (Singh et al. 2017; Happi et al. 2024).

Molecular docking simulations were performed to assess the binding interactions between the identified essential oil constituents and the AChE enzyme. The three-dimensional crystal structure of human AChE (PDB ID: 4EY6; resolution: 2.35 Å) was retrieved from the RCSB Protein Data Bank. Protein preparation was carried out using AutoDock Tools 1.5.7 by removing water molecules and non-standard ligands, adding polar hydrogens, and assigning Gasteiger charges. The processed protein structure was saved in PDBQT format. Ligand structures were drawn in ChemDraw and converted into 3D structures using the same software tools. The resulting molecular files were then prepared for docking by converting them to PDBQT format using AutoDock Tools, during which torsional flexibility and atomic charges were assigned.

Docking simulations were conducted using AutoDock Vina 1.2.0. The grid box was centered on the active site of AChE with coordinates: X = 11, Y = -53, Z = -25, encompassing the catalytic pocket. The exhaustiveness parameter was set to 8 to ensure reliable sampling. The best binding poses and binding affinity values (kcal/mol) were recorded. The resulting docking conformations and molecular interactions were visualized and analyzed using Discovery Studio Visualizer 2021 (Trott & Olson 2010)

Results

Chemical characterization of *T. vogelii* flowers essential oil. The yield of essential oil from *T. vogelii* flowers was between 0.75% and 0.99% (w/w). GC-MS analysis identified six primary compounds, representing 78.96% of the oil's composition, with notable components



being α -pinene and limonene, both recognized for insecticidal and antimicrobial effects (Gundidza 2008; Sumitomo et al. 2015) (Fig. 1; Tab. 2). The farnesol was the most abundant constituent, representing 32.26% of the total composition. The oil was predominantly composed of mono- and sesquiterpenes, including the monoterpenes α -pinene (15.26%), limonene (7.70%), and (E)- β -ocimene (6.10%), as well as the sesquiterpenes nerolidol (13.16%) and spathulenol (4.48%). Together, these terpenoid classes accounted for the majority of the chemical profile, indicating a composition rich in volatile compounds typically associated with insecticidal, antimicrobial, and neuroactive properties. Specifically, α -pinene has demonstrated antimicrobial activity, particularly against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, and functions as an AChE inhibitor at low concentrations (Gundidza 2008; Sumitomo et al. 2015). Limonene, another significant compound, has been shown to induce rapid paralysis and death in insects by causing motor coordination loss (Viegas Junior 2003). All major identified constituents have demonstrated insecticidal properties, disrupting insect physiological functions by obstructing respiratory tracts or dissolving exoskeleton lipids, leading to dehydration and death (Menezes 2005; Corrêa & Salgado 2011). This study is pioneering in characterizing the essential oil of *T. vogelii* flowers, whereas prior research focused predominantly on leaf extracts.

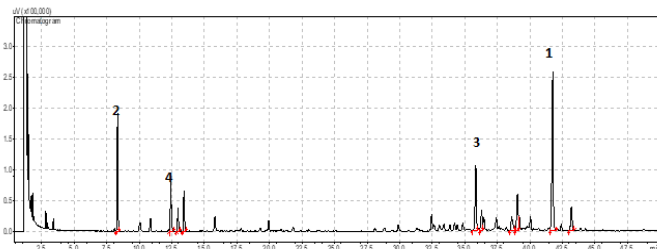


Figure 1. Chromatogram (GC-MS) of the essential oil from *Tephrosia vogelii* Hook.f. (Fabaceae) flower showing the main compounds. (1: farnesol; 2: α -pinene; 3: nerolidol; 4: limonene).

Insecticide potential. In bioassays, essential oil concentrations of 0.80 mL/L, 0.60 mL/L, and 0.40 mL/L achieved 100% ant mortality, with 0.40 mL/L being the most effective due to its lower oil usage. Lower concentrations, however, were less effective, with mortality rates below 80%. Using probit analysis, lethal concentration values were estimated as LC_{50} at 0.105 mL/L (CI_{95} : 0.0839 – 0.133) and LC_{90} at 0.298 mL/L (CI_{95} : 0.222 – 0.454). The control treatment with only distilled water and detergent proved that detergent alone had no insecticidal effect, confirming its suitability as an alternative solubilizer for essential oils in bioassays (Fig. 2; Tab. 3).

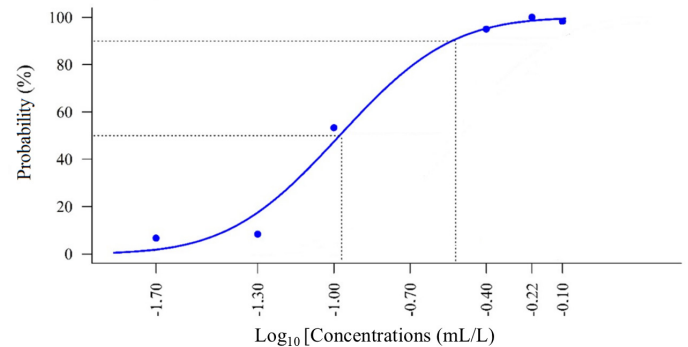


Figure 2. Graph of the concentration-mortality curve. Concentrations were transformed by $\log_{10} X$.

Binding insights from docking simulations. Molecular docking analysis was conducted to evaluate the potential interactions of selected essential oil constituents with the AChE enzyme (Tab. 1). Among the compounds tested, limonene and α -pinene exhibited relatively weaker binding affinities, with binding energies of -6.2 and -6.3 kcal/mol and estimated inhibition constants (K_i) of $28.354 \mu M$ and $23.948 \mu M$, respectively. Although α -pinene displayed the highest ligand efficiency ($LE = 0.630$), its overall binding profile remained modest in terms of fit quality and binding efficiency. Nerolidol demonstrated a slightly stronger interaction (-6.8 kcal/mol, $K_i = 10.293 \mu M$), accompanied by

Table 1. Treatments and their respective concentrations and methods of preparing the solutions.

Treatment	Concentration of essential oil (mL/L)	Method of preparation
Control	0.00	20 μL of neutral detergent + 10 mL of distilled water
T1	0.800	8 μL of essential oil + 20 μL of neutral detergent + 10 mL of distilled water
T2	0.600	6 μL of essential oil + 20 μL of neutral detergent + 10 mL of distilled water
T3	0.400	20 μL of essential oil + 20 μL of neutral detergent + 50 mL of distilled water
T4	0.100	5 μL of essential oil + 20 μL of neutral detergent + 50 mL of distilled water
T5	0.050	5 μL of essential oil + 20 μL of neutral detergent + 100 mL of distilled water.
T6	0.025	5 μL of essential oil + 20 μL of neutral detergent + 200 mL of distilled water.

Table 2. Identification of the composition of the essential oil of *Tephrosia vogelii* Hook.f. (Fabaceae) flower*.

Compounds	Area (%)	RT	IKCal	IKTab	m/z (Relative Intensity)
α -pinene	15.26	8.331	931	932	41(14); 67(8); 77(25); 79(20); 91(38); 92(38); 93(100); 105(10); 121(11); 136(8).
limonene	7.7	12.411	1027	1024	41(22); 67(73); 68(100); 79(31); 91(22); 92(24) 93(78); 94(31);107(20); 136(22).
NI	3.35	12.969	1039	-	39(17); 41(24); 44(17); 77(27); 79(32); 91(43); 92(40); 93(100); 105(14).
ocimene <(E)- β ->	6.1	13.422	1049	1044	39(21); 41(29); 77(31); 79(39); 80(37); 91(45); 92(25); 93(100); 105(17); 136(6).
nerolidol	13.16	35.848	1565	1561	41(77); 43(49); 55(33); 67(27); 69(100); 71(33); 81(29); 93(71); 107(37); 204(2).
spathulenol	4.48	36.29	1577	1577	41(58); 43(100); 69(38); 91(46); 93(47); 105(39); 119(39); 121(75); 155(51); 220(0.2).
NI	4.17	38.605	1638	-	41(50); 43(100); 79(42); 91(65); 93(43); 105(48); 109(41); 119(63); 159(56); 202(23); 220(2).
NI	8.31	39.047	1650	-	41(54); 43(67); 79(41); 91(66); 93(40); 105(52); 117(44); 119(35); 131(59); 159(100); 220(0.63).
farnesol	32.26	41.769	1726	1722	41(52); 55(11); 67(14); 68(11); 69(100); 81(27); 93(24); 95(10); 107(11); 222(0.13).
NI	5.21	43.191	1766	-	

*RT (Retention Time – minutes); IKCal (Calculated LTPRI Index); IKTab (Tabled Kovats Index) and m/z (mass/charge ratio and relative intensity). NI (not indicated). Source: Adams (2007).

**Table 3.** Concentration-response of the essential oil of *Tephrosia vogelii* Hook.f. (Fabaceae) flower on *Solenopsis saevissima* (Smith, 1855) (Hymenoptera: Formicidae), worker ants.

N	Slope (\pm EP)	LC ₅₀ (mL/L)	LC ₉₀ (mL/L)	X ²	DF	p-value
360	2.898 \pm 0.355	0.105 (0.0839 – 0.133) ¹	0.298 (0.222 – 0.454) ¹	8.37	4	0.921283

N: Number of insects used; X²: Chi-square; DF: Degrees of freedom. ¹ Confidence interval CI₉₅ (α = 0.05)**Table 4.** Docking results of volatile compounds with AChE*.

Compound	Binding Energy (kcal/mol)	LE	FQ	BEI	Ki (μ M)
Farnesol	-7.1	0.444	0.607	0.032	6.202
α -pinene	-6.3	0.630	0.436	0.046	23.948
Nerolidol	-6.8	0.425	0.581	0.031	10.293
Limonene	-6.2	0.620	0.430	0.046	28.354

* BEI: Binding Efficiency Index; FQ: Fit Quality; Ki: Estimated Inhibition Constant; LE: Ligand Efficiency.

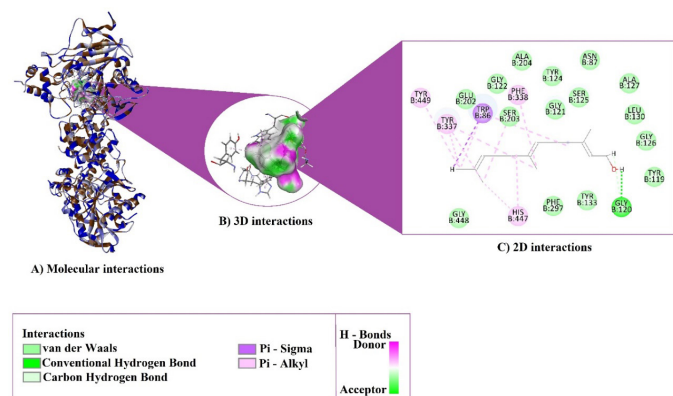
Table 5. Hydrogen bonding, π -interactions, and hydrophobic contacts formed by farnesol within the AChE binding site.

Compounds	H-Bond	π -Stacking / π -Electrostatic	Alkyl interactions
Farnesol	H26 – GLY120:O H25 – GLY120:O (Carbon H-Bond)	H2 – TRP86 (Pi-Sigma)	TRP86 – [001] TRP86 – C12 TYR337 – C12 TYR337 – C13 TYR337 – C14 PHE338 – C13 PHE338 – C14 HIS447 – [001] HIS447 – C13 TYR449 – C12

moderate LE (0.425) and BEI (0.031) values, indicating a balanced but not exceptional inhibitory potential.

Among all the compounds analyzed, farnesol emerged as the most promising AChE binder, exhibiting the lowest binding energy (-7.1 kcal/mol) and a favorable inhibition constant ($K_i = 6.202$ μ M). The combination of its LE (0.444), fit quality ($FQ = 0.607$), and binding efficiency index ($BEI = 0.032$) suggests that farnesol possesses a highly favorable interaction profile with the enzyme, supporting its potential as a strong candidate for AChE inhibition.

The molecular docking analysis revealed that farnesol establishes multiple stabilizing interactions within the active site of AChE (Fig. 3; Tab. 2). Two hydrogen bonds were identified between the ligand and GLY120, involving H26 and H25, the latter corresponding to a carbon-hydrogen bond. A pi-sigma interaction was observed between H2 of farnesol and the aromatic ring of TRP86, indicating pi-based stabilization within the binding pocket. In addition, extensive hydrophobic interactions were detected between farnesol and key aromatic and nonpolar residues, including TRP86, TYR337, PHE338, HIS447, and TYR449. Notably, the C12, C13, and C14 atoms of farnesol were frequently involved in these interactions, reinforcing its close fit within the enzyme's hydrophobic gorge. Collectively, these binding features suggest a strong and favorable interaction profile for farnesol with AChE at the structural level.

**Figure 3.** Binding interactions of farnesol with the target protein: (A) Docked complex view, (B) 2D interaction diagram, and (C) 3D interaction map showing key non-covalent contacts.

Discussion

The essential oil of *T. vogelii* flowers demonstrated strong insecticidal efficacy against *S. saevissima*, achieving total mortality at low concentrations (0.40 mL/L). Beyond the quantitative outcomes, this biological potency reflects a likely neurophysiological disruption mediated by the terpenoid constituents identified, particularly farnesol, nerolidol, α -pinene, and limonene, which are known to interfere with the insect nervous system (Viegas Junior 2003; Lopez & Pascual-Villalobos 2010; Abdelgaleil et al. 2016; Georgiev et al. 2022). These compounds act primarily by altering acetylcholinesterase (AChE) activity, leading to the accumulation of acetylcholine at synaptic clefts and resulting in continuous neuronal excitation, paralysis, and death.

The docking analyses reinforce this hypothesis by revealing that farnesol and nerolidol exhibit high binding affinities to the AChE active site ($\Delta G = -7.1$ and -6.8 kcal/mol, respectively), forming hydrogen bonds and hydrophobic interactions with catalytic residues such as TRP86, TYR337, and HIS447. Such molecular features suggest that these sesquiterpenes may act as competitive inhibitors, blocking substrate access to the catalytic triad, as reported for other essential oil constituents (Abou-Taleb et al. 2016; Saad et al. 2018). This mechanistic inference provides a rational explanation for the rapid toxicity observed in bioassays and aligns with previous studies demonstrating that mono- and sesquiterpenes can impair neural signal transmission in insects (Lopez & Pascual-Villalobos 2010; Georgiev et al. 2022).

Furthermore, the lipophilic nature of the oil components facilitates their penetration into the insect cuticle and tracheal system, thereby enhancing contact toxicity (Corrêa & Salgado 2011). Once internalized, these volatile molecules can destabilize neuronal membranes or interfere with ion channels, compounding the neurotoxic effects initiated by AChE inhibition. The synergistic interplay among terpenes- α -pinene's membrane-disruptive potential and limonene's motor-coordination impairment-likely amplifies overall toxicity (Viegas Junior 2003; Gundidza et al. 2008).

From an ecological and applied perspective, the high activity of *T. vogelii* oil at low doses underscores its potential as a sustainable botanical insecticide suitable for integrated pest management (IPM). Its selectivity toward insects, low environmental persistence, and renewable botanical origin align with agroecological principles aimed



at reducing dependence on synthetic pesticides (Isman 2000; Peixoto et al. 2023).

Overall, the present findings not only demonstrate the practical insecticidal performance of *T. vogelii* flower oil but also elucidate plausible biochemical and neurophysiological mechanisms underlying its toxicity. By integrating chemical, biological, and computational evidence, this study supports the hypothesis that terpenoid-induced disruption of AChE and neuronal signaling constitutes the principal mode of action, thereby offering a mechanistic foundation for future enzymatic and behavioral validation studies.

The probit analysis provides significant insight into the lethal concentrations, with LC₅₀ at 0.105 mL/L and LC₉₀ at 0.298 mL/L, indicating that even relatively low concentrations are effective against *S. saevissima*. The confidence intervals indicate reliable efficacy at these concentrations, supporting the potential of *T. vogelii* essential oil as a consistent and powerful insecticide. Given these LC values, the oil could be integrated into pest management systems, especially for organic agriculture, where synthetic insecticides may not be viable.

The successful use of detergent as a solubilizer in this study aligns with other research, affirming its compatibility with essential oils for insecticidal applications. The lack of toxic effects from the detergent itself supports its use as an economical and effective alternative to more costly solubilizers like Tween 80, acetone, or ethanol (Obrzut & Carvalho 2017; Moreira et al. 2015). This finding could encourage the adoption of more accessible materials in essential oil bioassays and commercial formulations, enhancing the practicality and affordability of essential oil-based insecticides.

This research offers novel insights by focusing on *T. vogelii* flowers, rather than on leaf extracts, as in previous studies. The detected terpenoids, such as α -pinene and limonene, have shown insecticidal effects comparable to or even greater than those observed in leaf extracts, suggesting a shift toward using flower oils for pest control. Studies on terpenoids and sesquiterpenes have corroborated their effectiveness in insecticide applications, as demonstrated by Simas et al. (2004) and Menezes (2005), reinforcing their value in integrated pest management (IPM) systems.

The inhibitory effects of compounds present in *T. vogelii* essential oil, evaluated against the AChE enzyme using *in silico* methods, on *S. saevissima* individuals have not been previously reported in the literature. AChE inhibitors are widely used in Alzheimer's treatment and play a critical role in organophosphorus insecticides, which exert toxicity by inhibiting AChE in insects, leading to their death (Georgiev et al. 2022). Molecular docking analyses with AChE revealed that farnesol exhibited the highest binding affinity, with a binding energy of -7.1 kcal/mol, and formed strong, stable interactions with the enzyme. This compound was followed by nerolidol (-6.8 kcal/mol), while α -pinene (-6.3 kcal/mol) and limonene (-6.2 kcal/mol) showed weaker interaction profiles with lower binding energies. These findings suggest that farnesol is a strong candidate with high inhibitory potential against AChE, whereas nerolidol shows moderate inhibitory activity, and α -pinene and limonene exhibit limited inhibitory activity. The *in silico* results from our study are consistent with those reported by Saad et al. (2018) for AChE inhibitors, supporting the notion that compounds with high binding affinity may exhibit inhibitory activity. Indeed, α -pinene and α -terpinene have been previously reported by Lopez & Pascual-Villalobos (2010) to inhibit AChE activity. Additionally, several essential oils have been reported to inhibit AChE activity (Abdelgaleil et al. 2016; Abou-Taleb et al. 2016). Our *in silico* analyses indicate that farnesol and nerolidol compounds have potential as AChE inhibitors; these findings are supported by *in vivo* studies, demonstrating that molecular-level data correlate with biological effects.

This study's findings suggest that *T. vogelii* essential oil is a viable candidate for sustainable pest control, especially in agricultural settings seeking environmentally friendly alternatives. Future research could investigate the specific modes of action of each compound within the essential oil, such as the precise AChE inhibition pathways, to further refine its applications. Additionally, examining its effects on other pest species and in various environmental conditions would enhance the understanding of its broader applicability. This research marks a critical

step in advancing botanical insecticides and promotes *T. vogelii* flower essential oil as a promising, sustainable pest control resource.

Compared with other botanical insecticides, the efficacy of *T. vogelii* flower essential oil is notably high, even at low concentrations, placing it among the most potent plant-derived products tested against ants and related pests. For instance, essential oils from *Piper* (Piperaceae) species, such as *P. aduncum* and *P. macedoi*, have shown LC₅₀ values ranging from 0.12 to 0.45 mL/L against *S. saevissima* and *Cerosipha forbesi* (Weed, 1889) (Hemiptera: Aphididae), depending on their terpenoid composition (Souto et al. 2012; Santos et al. 2023). Similarly, *Cymbopogon* (Poaceae) and *Eucalyptus* (Myrtaceae) oils, rich in monoterpenes like citral, 1,8-cineole, and α -pinene, display insecticidal activity through mechanisms involving AChE inhibition and cuticular disruption, with LC₅₀ values generally between 0.20 and 0.60 mL/L (Abdelgaleil et al. 2016; Abou-Taleb et al. 2016; Georgiev et al. 2022). The LC₅₀ of *T. vogelii* (0.105 mL/L), therefore, indicates a superior potency, likely due to synergistic effects among sesquiterpenes such as farnesol and nerolidol that enhance AChE inhibition and neurotoxicity. In this context, *T. vogelii* not only performs comparably to other essential oils traditionally used in pest management but also broadens the chemical and ecological spectrum of bioinsecticidal agents available for integrated pest management programs.

When interpreting the molecular docking results presented in this study, it is also important to acknowledge the inherent limitations of the structural model employed. Although the human AChE crystal structure (PDB: 4EY6) is widely used in computational toxicology due to its well-resolved active site, structural divergence between human and insect AChE enzymes may influence ligand-binding profiles. Therefore, the interactions reported here should not be regarded as definitive mechanistic evidence but rather as predictive hypotheses suggesting a potential cholinergic contribution. Verification of this hypothesis will require future biochemical inhibition assays using insect-derived AChE or advanced *in silico* analyses based on insect-specific homology models.

Farnesol and nerolidol, identified as major constituents of the essential oil and exhibiting high predicted affinity toward AChE, also emerge as priority candidates for future mechanistic investigations. Assessing these compounds individually and in combination through targeted bioassays would help clarify their specific contributions to the insecticidal activity observed in the whole essential oil. Such studies would not only provide experimental validation of the predictive *in silico* findings reported here but also enable a more refined understanding of the mechanistic roles and structure–activity relationships underlying their biological effects.

Conclusion

The essential oil from *T. vogelii* flowers demonstrates significant formicidal potential against *S. saevissima*. Its chemical composition, biological efficacy, and *in silico* interactions provide a consistent basis for its rational application in the management of agriculturally important ants. However, the precise mode of action of its key constituents and potential phytotoxicity remain to be clarified. The docking results highlight a plausible cholinergic contribution, but given their predictive nature, enzymatic validation using insect AChE models is required. This study reports, for the first time, the chemical characterization of *T. vogelii* flower essential oil, identifying terpenoids with recognized insecticidal, bactericidal, and fungicidal activities, and supports its promise as a sustainable tool for IPM.

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Authors' Contributions

ATBS: Formal analysis, Investigation, Methodology, Writing – review & editing; ANV: Formal analysis, Investigation, Methodology, Writing – review & editing; MABS: Formal analysis, Investigation, Methodology, Writing – review & editing; KAC: Formal analysis, Investigation, Methodology, Writing – review & editing; LAP: Formal analysis, Investigation, Methodology, Writing – review & editing; JSZ-J: Formal analysis, Investigation, Methodology, Writing – review & editing; HOGC: Formal analysis, Investigation, Methodology, Writing – review & editing; ECA: Formal analysis, Investigation, Methodology, Writing – review & editing; MFCS: Writing – original draft; LM: Conceptualization, Funding acquisition, Resources, Supervision.

Conflict of Interest Statement

Authors declare no conflict of interest.

References

- Abbott, W. S. (1925) A method of computing the effectiveness of insecticide. *Journal of Economic Entomology*, 18: 265-267. doi: [10.1093/jee/18.2.265a](https://doi.org/10.1093/jee/18.2.265a)
- Abdelgaleil, S. A. M.; Mohamed, M. I. E.; Shawir, M. S.; Abou-Taleb, H. K. (2016) Chemical composition, insecticidal and biochemical effects of essential oils of different plant species from Northern Egypt on the rice weevil, *Sitophilus oryzae* L. *Journal of Pest Science*, 89(1): 219-229. doi: [10.1007/s10340-015-0665-z](https://doi.org/10.1007/s10340-015-0665-z)
- Abou-Taleb, H. K.; Mohamed, M. I. E.; Shawir, M. S.; Abdelgaleil, S. A. M. (2016) Insecticidal properties of essential oils against *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase and adenosine triphosphatases. *Natural Product Research*, 30(6): 710-714. doi: [10.1080/14786419.2015.1038999](https://doi.org/10.1080/14786419.2015.1038999)
- Adams, R. P. (2007) *Identification of Essential Oils Components by Gas Chromatography/Mass Spectroscopy*. Carol Stream, Illinois: Allured Publishing Corporation.
- Alves, M. C.; Matoso, E. S.; Peil, R. M. (2020) What is the profile of strawberry producers in the south Brazilian region and what do they think about substrate cultivation? *Horticultura Brasileira*, 38(4): 428-433. doi: [10.1590/s0102-0536202004014](https://doi.org/10.1590/s0102-0536202004014)
- Corrêa, J. C. R.; Salgado, H. R. N. (2011) Atividade inseticida das plantas e aplicações: revisão. *Revista Brasileira de Plantas Medicinais*, 13(4): 500-506. doi: [10.1590/s1516-05722011000400016](https://doi.org/10.1590/s1516-05722011000400016)
- Georgiev, B.; Nikolova, M.; Aneva, I.; Dzhurmanski, A.; Sidjimova, B.; Berkov, S. (2022) Plant products with acetylcholinesterase inhibitory activity for insect control. *BioRisk*, 17: 309-315. doi: [10.3897/biorisk.17.77052](https://doi.org/10.3897/biorisk.17.77052)
- Gundidza, M.; Gweru, N.; Mmbengwa, V.; Ramalivhana, N. J.; Magwa, Z.; Samie, A. (2008) Phytoconstituents and biological activities of essential oil from *Rhus lancea* L. f. *African Journal of Biotechnology*, 7(16): 2787-2789.
- Happi, G. M.; Haider, S.; Ahmed, S. A.; Ul-Haq, Z. (2024) Molecular docking assessment of limonoids from Cameroonian *Entandrophragma* species as potential inhibitors of *Anopheles gambiae* acetylcholinesterase (AChE). *AppliedChem*, 4(4): 320-332. doi: [10.3390/appliedchem4040020](https://doi.org/10.3390/appliedchem4040020)
- Isman, M. B. (2000) Plant essential oils for pest and disease management. *Crop Protection*, 19(8-10): 603-608. doi: [10.1016/s0261-2194\(00\)00079-x](https://doi.org/10.1016/s0261-2194(00)00079-x)
- Lopes, R. P.; Parreira, L. A.; Venancio, A. N.; Santos, M. F. C.; Menini, L. (2022) Chemical characterization and evaluation of acaricidal potential of rosemary essential oil and its main compound α -pinene on the two-spotted spider mite, *Tetranychus urticae*. *Natural Product Research*, 37(17): 2940-2944. doi: [10.1080/14786419.2022.2137799](https://doi.org/10.1080/14786419.2022.2137799)
- Lopez, M. D.; Pascual-Villalobos, M. J. (2010) Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. *Industrial Crops and Products*, 31(2): 284-288. doi: [10.1016/j.indcrop.2009.11.005](https://doi.org/10.1016/j.indcrop.2009.11.005)
- Martins, L. J.; Welter, P. D.; Soares, S. M. F.; Kavcic, W.; Miranda, C. B.; Fagherazzi, A. F.; Regianini, N. F.; Kretzschmar, A.; Rufato, L.; Baruzzi, G. (2021) Planting density interferes with strawberry production efficiency in Southern Brazil. *Agronomy*, 11(3): 408. doi: [10.3390/agronomy11030408](https://doi.org/10.3390/agronomy11030408)
- Menezes, E. A. (2005) *Inseticidas botânicos: seus princípios ativos, modo de ação e uso agrícola*. Seropédica: Embrapa Agrobiologia.
- Moreira, F. J. C.; Santos, C. D. G.; Innecco, R.; Silva, G. S. (2015) Controle alternativo de nematoide das galhas (*Meloidogyne incognita* raça 2) com óleos essenciais em solo. *Summa Phytopathologica*, 41(3): 207-213. doi: [10.1590/0100-5405/1967](https://doi.org/10.1590/0100-5405/1967)
- Morris, C. E. (2009) Strawberry: Global cultivation challenges. *Journal of Fruit Sciences*, 22(3): 245-260.
- Obrzut, V. V.; Carvalho, R. I. N. (2017) Utilização do óleo essencial de sassafrás para o manejo da mariposa oriental em pessegueiro. *Revista Acadêmica: Ciências Agrárias e Ambientais*, 9(1): 65-71. doi: [10.7213/cienciaanimal.v9i1.11090](https://doi.org/10.7213/cienciaanimal.v9i1.11090)
- Olayemi, O. M.; Adediran, I. A.; Fagbohun, O. A. (2015) Insecticidal properties of *Tephrosia vogelii* and its potential for agricultural pest management. *African Journal of Agricultural Science*, 10(5): 299-308.
- Peixoto, P. M. C.; Júlio, A. A.; Jesus, E. G.; Venancio, A. N.; Parreira, L. A.; Santos, M. F. C.; Menini, L. (2023) Fungicide potential of citronella and tea tree essential oils against tomato cultivation's phytopathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* and analysis of their chemical composition by GC/MS. *Natural Product Research*, 38(4): 667-672. doi: [10.1080/14786419.2023.2184358](https://doi.org/10.1080/14786419.2023.2184358)
- Porter, S. D.; Fowler, H. G. (1992) Fire ants (*Solenopsis saevissima*) and their impact on agricultural crops. *Entomological Research*, 41(2): 183-194.
- Saad, M. M. G.; Abou-Taleb, H. K.; Abdelgaleil, S. A. M. (2018) Insecticidal activities of monoterpenes and phenylpropenes against *Sitophilus oryzae* and their inhibitory effects on acetylcholinesterase and adenosine triphosphatases. *Applied Entomology and Zoology*, 53(2): 173-181. doi: [10.1007/s13355-017-0532-x](https://doi.org/10.1007/s13355-017-0532-x)
- Santos, A. T. B.; Dian, V. S.; Parreira, L. A.; Lourenço, M. P.; Zanuncio, J. J. S.; Jesus, E. G.; Santos, M. F. C.; Menini, L. (2023) Aphid *Cerosipha forbesi* control using *Piper macedoi* Yuncck essential oil in strawberry crop. *Natural Product Research*, 38(10):1753-1758. doi: [10.1080/14786419.2023.2214832](https://doi.org/10.1080/14786419.2023.2214832)
- Simas, N. K.; Lima, E. C.; Conceição, S. R.; Kuster, R. M.; Oliveira, F. A. M.; Lage, C. L. S. (2004) Produtos naturais para o controle da transmissão da dengue: atividade larvicida de *Myroxylon balsamum* (óleo vermelho) e de terpenoides e fenilpropanóides. *Química Nova*, 27(1). doi: [10.1590/s0100-40422004000100009](https://doi.org/10.1590/s0100-40422004000100009)
- Simões, J. C.; Fadini, M. A. M.; Venzon, M. (2007) Manejo integrado de pragas na cultura do morango. *Informação Agropecuária*, 28(236): 56-63.
- Singh, K. D.; Labala, R. K.; Devi, T. B.; Singh, N. I.; Chanu, H. D.; Sougrakpam, S.; Rajashekar, Y.; Nameirakpam, B. S.; Sahoo, D.; Rajashekar, Y. (2017) Biochemical efficacy, molecular docking and inhibitory effect of 2, 3-dimethylmaleic anhydride on insect acetylcholinesterase. *Scientific Reports*, 7(1): 12483. doi: [10.1038/s41598-017-12932-0](https://doi.org/10.1038/s41598-017-12932-0)
- Souto, R. N. P.; Harada, A. Y.; Andrade, E. H. A.; Maia, J. G. S. (2012) Insecticidal activity of *Piper* essential oils from the Amazon against the fire ant *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae). *Neotropical Entomology*, 41(6): 510-517. doi: [10.1016/j.indcrop.2009.11.005](https://doi.org/10.1016/j.indcrop.2009.11.005)



[10.1007/s13744-012-0080-6](https://doi.org/10.1007/s13744-012-0080-6)

- Sumitomo, K.; Akutsu, H.; Fukuyama, S.; Minoshima, A.; Kukita, S.; Yamamura, Y.; Sato, Y.; Hayasaka, T.; Osanai, S.; Funakoshi, H.; et al. (2015) Conifer-derived monoterpenes and forest walking. *Mass Spectrometry*, 4(1): A0042. doi: [10.5702/massspectrometry.a0042](https://doi.org/10.5702/massspectrometry.a0042)
- Trott, O.; Olson, A. J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2): 455-461. doi: [10.1002/jcc.21334](https://doi.org/10.1002/jcc.21334)
- Viegas Junior, C. (2003) Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Química Nova*, 26(3): 390-400. doi: [10.1590/s0100-40422003000300017](https://doi.org/10.1590/s0100-40422003000300017)