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Screening and Toxicity of Essential Oils From the Piperaceae Family on Spodoptera Frugiperda (Lepidoptera: Noctuidae) Under Laboratory Conditions

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Abstract

Spodoptera frugiperda (J. E. Smith, 1797), known as the fall armyworm, is an insect pest of economic importance that causes significant damage to maize crops, resulting in losses exceeding 50%, this species exhibits high polyphagy, with over 350 hosts plant species. Given the need for lower-impact alternatives, the insecticidal activity of essential oils from *P. marginatum, P. fuligineum, P. hispidum, P. tuberculatum, P. arboreum, P. aduncum* and *P. divaricatum* was evaluated against eggs (0–24 hours) and second-instar larvae of *S. frugiperda*. A completely randomized design was adopted with tem replicates, six concentrations (0.25 to 8.00%) and two controls. Chemical composition was determined by GC-MS and data were analyzed using generalized linear models with binomial distribution and Tukey correction (α = 0.05). Ovicidal activity, *P. marginatum* rendered 99% of eggs inviable at 4% and *P. fuligineum* at 8%. For larvicidal activity, only *P. marginatum* and *P. fuligineum* exceeded 50% mortality at 1%. The lowest LC50 values were *P. marginatum* (1.02%), *P. aduncum* (1.03%) and *P. fuligineum* (1.11%); the lowest LC90 for *P. fuligineum* (2.67%), *P. aduncum* (3.49%) and *P. marginatum* (3.59%). *P. arboreum* and *P. hispidum* showed the lowest percentages. GC-MS analysis identified 50 compounds, with germacrene D (24.67%) and germacrene B (19.80%) in *P. fuligineum* and *P. marginatum*; β-pinene (28.17%) and α-pinene (26.82%) in *P. tuberculatum*; and cis-ocimene (25.23%) associated with myristicin (18.88%) in *P. aduncum*. *P. fuligineum*, *P. marginatum* and *P. aduncum* showed the best ovicidal and larvicidal properties, with *P. fuligineum* the Greatest insecticidal potential.

INTRODUCTION

The Piperaceae family comprises more than 450 species cataloged within Brazilian territory; numerous species in this family are notable for the presence of bioactive compounds, particularly for their insecticidal activity (Lima 2015). Several studies have highlighted the potential of their essential oils, underscoring the importance of this genus for the development of alternatives in the management of insect pests (Guedes et al. 2020; Dutra et al. 2020).

The use of natural compounds emerges as an alternative to the synthetic products widely employed in pest management (Isman 2020). Essential oils are generally composed of various molecules with distinct mechanisms of action, which can hinder the development of resistance in insects a significant advantage over synthetic insecticides (Oliveira et al. 2018; Guedes et al. 2020). Although Brazil is a leader in research on botanical insecticides, few plant-based products are registered for agricultural use, with the notable exception of neem oil (*Azadirachta indica* A. Juss) (Isman 2020).

In this context, the management of pests such as the fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera, Noctuidae) a highly polyphagous pest that feeds on more than 353 plant species, including major economically important crops such as maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merrill), rice (*Oryza sativa* L.), and cotton (*Gossypium hirsutum* L.) has become an increasingly studied subject (Wang et al. 2025).

This broad diversity of hosts, combined with the pest's high adaptability, enables it to become established across a wide range of ecosystems (Demis and Jemal 2025). Currently, its distribution extends beyond the Americas, with a documented presence in West Africa, Ethiopia, and various regions across Asia, where infestations affect millions of hectares of crops (Tay et al. 2023).

The damage resulting from this widespread distribution includes severe harm to foliage and reproductive structures (Bakry and Abdel-Baky 2024), which impairs vegetative growth and directly impacts crop yields (Zhang et al. 2024). Losses range from 21% to 53%, potentially reaching as high as 80% in cases of severe infestation (Day et al. 2017). Managing this pest is challenging due to its high reproductive capacity; a single female can lay up to 1,500 eggs over the course of her life cycle (Kenis et al. 2023).

Compounding this situation, the use of synthetic insecticides has exerted strong selection pressure on pest populations, leading to increasing levels of resistance (Zenner de Polania 1996; Gutiérrez-Moreno et al. 2019; Kenis et al. 2023). In this context, secondary metabolites found in essential oils have shown the potential to target the pest’s early developmental stages, preventing larvae from reaching their most voracious phase and thereby averting more significant economic losses (Nonci et al. 2025).

Among the phytochemicals identified in these species, amides, monoterpenes, sesquiterpenes, and other alkaloids characteristic components of the essential oils of these plants stand out (Parmar et al. 1997). These compounds act on insect pests through various mechanisms, such as neurotoxicity and the inhibition of growth and feeding (Scott et al. 2008). In a study testing the essential oils of 18 Brazilian Piperaceae species against the eggs of *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera, Noctuidae), Krinski et al. (2018) observed a reduction in larval hatching for 16 of them, a result associated with the presence of dillapiole, myristicin, and spathulenol.

Given this potential, the present study aimed to evaluate the bioactivity of Essential oils from various *Piper* species against the eggs and caterpillars of *S. frugiperda*, as well as to identify the specific compounds present in these Essential oils.

MATERIAL AND METHODS

The research was conducted at the Centro de Pesquisas, Estudos e Desenvolvimento Agroambientais (CPEDA) at UNEMAT (Tangará da Serra, MT), which included the extraction of essential oils and the performance of ovicidal and larvicidal bioassays; the chemical characterization of the *Piper* essential oils, was carried out at the Analytical Center the UFPEL. Seven native *Piper* species (*Piper aduncum, Piper arboreum, Piper divaricatum, Piper fuligineum, Piper hispidum, Piper marginatum*, and *Piper tuberculatum*) were collected in Tangará da Serra in September 2024, between 1:00 PM and 2:00 PM (Fig. 1). Morphological identification was confirmed by comparison with specimens from the TANG Herbarium (UNEMAT) and, in the case of *P. divaricatum*, from the UnB herbarium with the nomenclature validated using taxonomic databases.

Extraction of essential oil from *Piper*

To obtain the essential oils fresh leaves from six *Piper* species (*P. marginatum, P. hispidum, P. arboreum, P. aduncum, P. fuligineum*, and *P. divaricatum*) and the infructescences of *P. tuberculatum* were used. For each species, a 300 g sample was collected and processed in 100 g replicates (triplicates). Each replicate was ground with 1 liter of distilled water, transferred to a 2 L round-bottom flask, and subjected to hydrodistillation for 4 hours at approximately 100°C using a modified Clevenger-type apparatus (Tran et al. 2019). The resulting Essential oils were stored in amber glass vials and kept in a freezer at approximately − 12°C.

Source: Qgiz

Figure 1 Location of collection sites for species of the genus *Piper* in the municipality of Tangará da Serra, Mato Grosso. The geographic distribution of the seven collected species is indicated by colored markers: 1. *Piper fuligineum;* 2. *Piper divaricatum*; 3. *Piper marginatum*; 4. *Piper aduncum;* 5 *Piper tuberculatum;* 6. *Piper hispidum* e 7. *Piper arboreum.*

Conducting the Bioassays

The study of the insecticidal activity of the essential oils was extended through two different bioassays one ovicidal and one larvicidal against the target pest *S. frugiperda*. In both bioassays, a completely randomized design was employed, testing the same EO concentrations (0.25%, 0.5%, 1.0%, 2.0%, 4.0%, and 8.0%) for each of the seven *Piper* species, alongside two negative controls (distilled water and acetone). All samples were kept under controlled conditions in BOD (Biochemical Oxygen Demand) incubators at a temperature of 25 ± 1°C, a relative humidity of 70 ± 5%, and a 12-hour photoperiod, consistent with the rearing conditions.

Ovicidal activity bioassay

To evaluate ovicidal activity, *S. frugiperda* eggs no more than 24 hours old (post-oviposition) were used. The eggs were first moistened with distilled water to facilitate separation, then affixed to white cardstock strips (8 x 2 cm) using a fine-bristled brush and non-toxic white glue. The bioassay comprised 56 distinct treatments (seven species, each with eight treatments, including the various concentrations and the control), with ten replicates per treatment, each containing 30 eggs. This resulted in a total of 2,400 eggs per *Piper* species, amounting to 16,800 eggs evaluated in total.

The Essential oils were applied to the cardstock strips using a precision airbrush, calibrated to 15 psi and equipped with a 0.3 mm nozzle. For each treatment, 2 mL of the solution was applied across ten replicates each consisting of 30 eggs evenly distributed on pre-prepared cardstock. A fixed distance of 15 cm was maintained, and a constant pendulum-like motion was employed to ensure uniform deposition of the liquid.

Following application, the treatments were left to dry for 30 minutes. Subsequently, each replicate was placed into a flat-bottomed test tube, which was then sealed with cotton. Evaluations were conducted using a stereomicroscope (40x magnification) on the second, third, and fourth days post-treatment, with records made regarding egg inviability or the hatching of neonate larvae (Sombra et al. 2020).

Larvicidal activity bioassay

Toxicity bioassays to evaluate the larvicidal efficacy of *Piper* Essential oils against *S. frugiperda* larvae were conducted using second-instar individuals. Each bioassay consisted of eight treatments with 20 replicates per *Piper* species, totaling 1,120 individuals. The Essential oils were applied topically by depositing 2 µL of the diluted solution onto the dorsal surface of the larvae using a micropipette. Mortality was assessed daily for seven days; larvae that failed to respond to mechanical stimuli applied with a fine-bristled brush were considered dead.

Characterization of essential oils

The chemical composition of the essential oils was analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS-QP2010 Plus system equipped with an RTX-5 capillary column (30 m × 0.25 mm × 0.25 µm; Restek). The chromatographic conditions were as follows: injector temperature of 250°C, helium as the carrier gas at a flow rate of 0.81 mL/min, and a split ratio of 1:100. The oven temperature was initially set at 50°C and increased to 220°C at a rate of 4°C/min. Essential oils samples were diluted in ethyl acetate to a concentration of 1,500 ppm, and aliquots of 1.0 µL were injected into the GC-MS system.

The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, with the ion source and interface temperatures maintained at 250°C. Hydrocarbon standards were obtained commercially from Sigma-Aldrich and analyzed under the same chromatographic conditions. Compounds were identified by comparing their mass spectra with those available in the NIST05 and NIST08 mass spectral libraries and by comparison of linear retention indices (LRI) given in literature (Adams, 2017). The LRI of the constituents of oils were determined relative to (C8-20 and C10) n-alkanes and using a solution of EO dissolved in hexane.

$$KI = 100.n + 100.\left(\frac{t_{ri} - t_{rn}}{t_{rm} - t_{rn}}\right) \text{ (Eq. 1)}$$

Laboratory Rearing of *Spodoptera frugiperda*

S. frugiperda egg masses and larvae were collected from corn fields in Tangará da Serra, Mato Grosso (MT), and kept in quarantine until hatching. Newly hatched larvae were reared on an artificial diet (Greene et al. 1976) in a BOD (biochemical oxygen demand) incubator at 25 ± 1°C, 70 ± 10% relative humidity (RH), and a 12-hour photoperiod. At the second instar, the larvae were placed individually into tubes containing the diet. Upon reaching the pupal stage, they were disinfected with a 1% copper sulfate solution for three minutes, left to dry on paper towels, and then placed in containers until adult emergence. Adults were kept in PVC mating chambers (30 × 20 cm) lined with bond paper for oviposition and were provided with cotton wool soaked in a 10% honey solution.

Statistical Analysis

Generalized linear models (GLMs) with a binomial distribution for the response variable were applied to both datasets analyzed. For the analysis of larval mortality, data collected 168 hours after treatment application were used. For the analysis of egg non-viability, data collected 96 hours after treatment application were used. Regarding larvicidal activity, death was coded as a success (1) and a live larva as a failure (0). For ovicidal activity, non-viability was treated as a binary variable (success = non-viable egg; failure = viable egg), which allowed for the direct modeling of the probability of non-viability as a function of the treatments.

The models were fitted in the R environment using the glm function with a logit link function appropriate for binomial data which establishes a non-linear relationship between the mean of the response variable and the predictors. A completely randomized experimental design was employed, with the various treatments (essential oil concentrations, along with distilled water and acetone controls) serving as the explanatory factor; analyses were conducted separately for each *Piper* species.

Based on the fitted models, the mean probabilities of success for each treatment were estimated as "estimated marginal means" using the emmeans function from the emmeans package (Lenth et al. 2026). These estimates were obtained on the original response scale (probability) by applying the inverse transformation of the link function ($type = \text{response}$), which allows for a direct biological interpretation of the results.

Multiple comparisons between treatments were performed using pairwise contrasts of the estimated marginal means, with a Tukey adjustment to control for Type I error implemented via the $pairs$ function from the $emmeans$ package. Treatments were grouped based on statistical similarity using the cld function from the $\mu < comp$ package (Hothorn et al. 2008), with a significance level of 5% (α = 0.05).

95% confidence intervals were calculated for the estimated probabilities; these were first computed on the model scale (logit) and subsequently transformed back to the probability scale.

Due to the non-linear nature of the link function, these intervals may exhibit asymmetry particularly for values near the distribution boundaries (0 or 1) thereby accurately reflecting the uncertainty associated with the estimates. In situations where the data exhibited complete or quasi-complete separation (i.e., treatments with responses consisting exclusively of 0s or 1s), classical binomial models may yield biased estimates or unreliable confidence intervals. In such cases, Firth's penalized logistic regression was employed using the logistf function from the logistf package (Heinze and Schemper, 2002) which corrects maximum-likelihood bias in small samples or datasets with separation, thereby providing stable estimates and more robust confidence intervals.

To evaluate larvicidal activity, the lethal concentrations (LC50 and LC90) of the essential oils were estimated using dose-response regression models fitted with the drm function from the drc package in the R environment (Ritz et al., 2015). Mortality was modeled as a binary variable (dead = 1; alive = 0), assuming a binomial distribution. Log-logistic models with a probit link function widely used in toxicological data analysis to describe the relationship between mortality probability and the concentrations tested were applied (Finney, 1971).

Estimates of the lethal doses (LC50 and LC90) and their corresponding 95% confidence intervals were obtained using the ED function, based on the fitted model parameters (Ritz et al., 2015). This approach allows for the direct inference of toxicological parameters from the dose-response curve, accounting for the non-linear nature of the relationship between concentration and effect. All analyses were performed using the R software (R Core Team, 2024).

RESULTS

Ovicidal action

The viability of *S. frugiperda* eggs varied as a function of the concentration of essential oils in the seven *Piper* species evaluated ($P < 0.001$; Table 2). The fitted models explained between 40% and 73% of the variation, with the best fits obtained for *P. marginatum* (McFadden's $R^2 = 0.73$; $\chi^2 = 2400$), *P. divaricatum* ($R^2 = 0.70$; $\chi^2 = 1385$), and *P. fuligineum* ($R^2 = 0.68$; $\chi^2 = 2222$), and the lowest for *P. tuberculatum* ($R^2 = 0.41$; $\chi^2 = 1367$). The water and acetone controls did not differ from each other in any species, nor did they differ among species ($P > 0.05$ and $P = 1.00$, respectively), which rules out any effect of the solvent on embryonic viability.

The response followed the concentration across all species, with two distinct patterns. In six of them, inviability increased progressively across the tested range. In *P. divaricatum*, the effect remained at the control level up to 4% and appeared only at 8% ($P < 0.001$), representing an abrupt response. The comparison between species was significant at all EO concentrations ($P < 0.01$; Table 2) and not significant in the two controls (water, $P > 0.05$; acetone, $P = 1.00$). The greatest separation among species occurred at 4% ($R^2 = 0.53$; $\chi^2 = 1064$) and the least at 8% ($R^2 = 0.17$; $\chi^2 = 136.75$), a concentration at which most of the oils had already reached their maximum response.

At 8%, *P. arboreum*, *P. fuligineum*, and *P. marginatum* reached 99% inactivity, while *P. aduncum* recorded the lowest value (83%), differing from all others ($P < 0.01$). At 4%, *P. marginatum* (99%) and *P. arboreum* (97%) formed the top group, while *P. divaricatum* stood alone with the lowest value (9%). At 2%, *P. fuligineum* and *P. marginatum* (80%) and *P. arboreum* (73%) had the highest values, while *P. divaricatum* remained at 8%. At the two lowest concentrations (0.50% and 0.25%), only *P. aduncum, P. arboreum, P. tuberculatum*, and *P. hispidum* differed from the controls, with embryo viability ranging from 10% to 40%; the others remained at the control level (Table 1).

Overall, the species with the highest embryonic nonviability at concentrations of 2% or higher were *P. fuligineum, P. marginatum*, and *P. arboreum*, all above 70%, and the species with the lowest response across nearly the entire range was *P. divaricatum*, which only matched the others at the highest concentration tested.

Table 1

Effect of different concentrations of essential oils from species of the genus *Piper* on the non-viability of *Spodoptera frugiperda* eggs 96 h after application. Statistics (mean and confidence intervals) are based on a binomial distribution, followed by pairwise analysis with a Tukey correction at a 5% probability level.

Species	**N**	**Water**	**Acetone**	**0,25%**	**0,50%**	**1%**	**2%**	**4%**	**8%**	**$R^2_{McFadden}$**	**χ^2**	**P valor**
Piper aduncum	20	0,01± (0,01–0,04)	0,01± (0,00–0,03)	0,10± (0,07–0,14)	0,40± (0,35–0,46)	0,60± (0,55–0,66)	0,60± (0,55–0,66)	0,88± (0,84–0,92)	0,83± (0,79–0,87)	0,4	1321	< 0.001
		eA	eA	dC	c A	bA	b B	a B	a C			
Piper arboreum	20	0,01± (0,01–0,04)	0,01± (0,00–0,03)	0,36± (0,31–0,42)	0,36± (0,31–0,42)	0,57± (0,52–0,63)	0,73± (0,69–0,79)	0,97± (0,97–1,00)	0,99± (0,98–1,00)	0,51	1699	< 0,001
		eA	eA	dA	dA	cA	b A	a A	a A			
Piper divaricatum	20	0,01± (0,01–0,04)	0,01± (0,00–0,03)	0,00± (0,00–0,03)	0,00± (0,00–0,03)	0,01± (0,01–0,04)	0,08± (0,06–0,12)	0,09± (0,07–0,13)	0,94± (0,91–0,97)	0,7	1385	< 0,001
		cA	cA	cD	cC	cB	bC	b C	a B			
Piper fuligineum	20	0,00± (0,00–0,02)	0,01± (0,00–0,03)	0,01± (0,01–0,04)	0,01± (0,01–0,04)	0,57± (0,52–0,63)	0,80± (0,76–0,85)	0,89± (0,86–0,93)	0,99± (0,98–1,00)	0,68	2222	< 0,001
		dA	dA	dD	dC	cA	bA	b B	a A			
Piper hispidum	20	0,00± (0,00–0,03)	0,01± (0,00–0,03)	0,23± (0,19–0,28)	0,22± (0,18–0,27)	0,57± (0,52–0,63)	0,50± (0,45–0,56)	0,90± (0,87–0,94)	0,94± (0,91–0,97)	0,44	1441	< 0,001
		dA	dA	cB	cB	bA	bB	a B	a B			
Piper marginatum	20	0,01± (0,01–0,04)	0,01± (0,01–0,04)	0,01± (0,01–0,04)	0,01± (0,01–0,04)	0,57± (0,52–0,63)	0,80± (0,76–0,85)	0,99± (0,97–1,00)	0,99± (0,98–1,00)	0,73	2400	< 0,001
		dA	dA	dD	dC	cA	bA	a A	a A			
Piper tuberculatum	20	0,01± (0,01–0,04)	0,00± (0,00–0,03)	0,36± (0,31–0,42)	0,32± (0,27–0,38)	0,57± (0,52–0,63)	0,60± (0,55–0,66)	0,91± (0,88–0,94)	0,94± (0,91–0,97)	0,41	1367	< 0,001
		dA	dA	cA	cAB	bA	bB	a B	a B			
$R^2_{McFadden}$		0,01	0	0,22	0,23	0,14	0,18	0,53	0,17			
χ^2		3,54	0,26	405	470	418,64	516,37	1064	136,75			
P valor		> 0,05	1	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01	< 001			

Lowercase letters = comparisons across rows, analyzing the effect of the essential oils concentrations for each species, along with the controls. Uppercase letters = comparisons across columns, analyzing the effect among the *Piper* species at the same concentration. R^2 = McFadden's R^2.

Larvicidal Activity

The mortality of *S. frugiperda* caterpillars showed a dose-dependent pattern as a function of the concentrations of essential oils from different *Piper* species (P < 0.001) (Table 2). At the highest concentrations (8% and 4%), mortality ranged from 75% to 97% for *P. aduncum*, *P. arboreum, P. divaricatum, P. fuligineum, P. marginatum*, and *P. tuberculatum*, with no statistically significant differences among them. In contrast, *P. hispidum* exhibited the lowest mortality rates at high concentrations, with 64% and 83% mortality at 4% and 8% concentrations, respectively.

At a 2% concentration, *P. aduncum* and *P. tuberculatum* exhibited mortality rates of 48% and 45%, respectively. Conversely, concentrations of 1%, 0.5%, and 0.25% for *P. aduncum* showed a drastic reduction in mortality, ranging from 25% to 8%, while for *P.*

tuberculatum, mortality did not differ from the controls. The 1% and 2% concentrations for the species *P. divaricatum, P. fuligineum, P. hispidum*, and *P. marginatum* did not differ statistically from one another, with mortality ranging from 7% to 69%. At concentrations of 0.5% and 0.25%, *P. divaricatum* maintained its bioactivity against *S. frugiperda* caterpillars, with lethality rates of 7% and 16%, in that order.

On the other hand, *P. fuligineum* and *P. marginatum* were bioactive only at a concentration of 0.5%, with mortality rates ranging from 30% to 16%. As for *P. hispidum*, no bioactivity was observed at lower concentrations. The species *P. arboreum* exhibited bioactivity at concentrations ranging from 2% to 0.25%, although with low lethality, ranging from 7% to 21%. Regarding the *Piper* species, there was no significant difference at the 8% concentration, where larval mortality ranged from 83% to 97%. It is worth noting that *P. fuligineum* and *P. marginatum* were already biologically active at a concentration of 1%, with mortality rates exceeding 50%.

The LC50 values showed significant differences in larvicidal activity among the tested species (Table 3). The essential oils of *P. marginatum* (LC50 = 1.02%), *P. aduncum* (LC50 = 1.03%), *P. fuligineum* (LC50 = 1.11%), *P. divaricatum* (LC50 = 1.44%), and *P. tuberculatum* (LC50 = 1.89%) exhibited the lowest lethal concentrations. In contrast, the essential oils of *P. arboreum* (LC50 = 2.43%) and *P. hispidum* (LC50 = 3.35%) differed from those of the other tested species. As for the LC90, the essential oil of *P. fuligineum* stood out the most, requiring only 2.67% to kill 90% of the caterpillars, followed by *P. aduncum* (3.49%) and *P. marginatum* (3.59)

The EC_{50} values for *P. tuberculatum* (3.98%) and *P. divaricatum* (4.57%) required higher concentrations to control the same percentage observed in the other species. However, the OEs of *P. arboreum* and *P. hispidum* required even higher concentrations to achieve the same level of control; it was not possible to determine the LC90 for these species within the range of concentrations evaluated.

Chemical Composition of Essential Oils from *Piper* spp.

The chromatographic results for the chemical composition of the essential oils (Table 5) revealed a total of 50 compounds; a total of 21 compounds were selected as major components, each accounting for more than 5% of the area. The essential oil of *P. marginatum* consisted mainly of Germacrene B, with 19.8% area percentage, and Cis-ocimene (15.88%). Other significant constituents were β-caryophyllene (10.19%), α-phellandrene (9.22%), γ-terpinene (6.89%), trans-ocimene (6.52%), and germacrene D (5.08%).

The essential oil of *P. tuberculatum* exhibited a high concentration of monoterpenes, with β-pinene being the major compound at 28.17%, followed by α-pinene (26.86%) and limonene (15.39%). β-Bisabolene (8.66%) was also a major constituent. Similarly, the essential oil of *P. divaricatum* also exhibited a high concentration of α-pinene (25.92%), followed by β-pinene (10.45%), pentadecane (14.08%), germacrene D (12.51%), α-phellandrene (12.14%), and silvestrene (7.95%).

Unlike the other species, the essential oil of *P. fuligineum* was characterized by a predominance of sesquiterpenes, especially germacrene D (24.67%) and germacrene B (10.93%). Other notable components were α-pinene (9.85%), α-phellandrene (7.23%), cis-ocimene (6.85%), β-caryophyllene (6.48%), and silvestrene (6.39%).

Table 2

Effect of different concentrations of essential oils from species of the genus *Piper* on the mortality of *Spodoptera frugiperda* caterpillars 168 hours after application. Statistics (mean and confidence intervals) based on a binomial distribution followed by a paired analysis with Tukey's correction at a 5% significance level.

Espécie	**Água**	**Acetona**	**0,25%**	**0,50%**	**1%**	**2%**	**4%**	**8%**	**R^2**	**Pv**
Piper aduncum	0,02± (0,00–0,05)	0,02± (0,00–0,05)	0,08± (0,05–0,14)	0,13± (0,08–0,20)	0,25± (0,19–0,33)	0,48± (0.40–0,57)	0,89± (0,84–0,94)	0,96± (0,92–0,99)	0,43	< 0,001
	e A	e A	d A	cd A	c A	bA	a A	a A		
Piper arboreum	0.02± (0.00–0.05)	0,02± (0,00–0,05)	0,11± (0,03–0,34)	0,11± (0,03–0,34)	0,07± (0,01–0,29)	0,21± (0,09–0,44)	0,75± (0,56–0,91)	0,92± (0,71–0,99)	0,48	< 0,001
	c A	c A	b A	b A	b A	b B	a AB	a A		
Piper divaricatum	0,02± (0,00–0,05)	0,02± (0,00–0,05)	0,16± (0,05–0,39)	0,07± (0,01–0,29)	0,21± (0,09–0,44)	0,50± (0,30–0,70)	0,97± (0,71–1,00)	0,97± (0,71–1,00)	0,56	< 0001
	d A	d A	c A	c A	bc A	b AB	a AB	a A		
Piper fuligineum	0,02± (0,00–0,05)	0,02± (0,00–0,05)	0,00± (0,02–0,05)	0,16± (0,06–0,39)	0,50± (0,30–070)	0,69± (0,47–0,85)	0,97± (0,71–1,00)	0,97± (0.71–1,00)	0,58	< 0,001
	d A	d A	d A	c A	bc A	ab A	a AB	a A		
Piper hispidum	0,02± (0,00–0,05)	0,02± (0,00–0,05)	0,11± (0,03–0,34)	0,07± (0,01–0,29)	0,07± (0,01–0,29)	0,16± (0,06–0,39)	0,64± (0,42–0,81)	0,83± (0,61–0,94)	0,41	< 0,001
	c A	c A	bc A	bc A	b A	b B	a B	a A		
Piper marginatum	0,02± (0.00–0.05)	0,02± (0,00–0,05)	0,07± (0,01–0,29)	0,30± (0,15–0,53)	0,54± (0,34–0,74)	0,59± (0,38–0,78)	0,92± (0,79–0,99)	0,96± (0,92–0,99)	0,42	< 0,001
	d A	d A	cd A	bc A	b A	b AB	a A	a A		
Piper tuberculatum	0.02± (0,00–0,05)	0,02± (0,00–0,05)	0,00± (0,00–0,05)	0,07± (0,01–0,29)	0,11± (0,03–0,34)	0,45± (0,26–0,66)	0,92± (0,71–0,99)	0,96± (0,92–0,99)	0,59	< 0,001
	c A	c A	c A	c A	c A	b AB	a A	a A		
$R^2_{McFadden}$	0	0	0,12	0,09	0,18	0,16	0,23	0,32		
P valor	1	1	0,16	0,16	< 0,01	< 0,01	< 0,01	0,07		

Lowercase letters = comparisons within rows, analyzing the effect of essential oil concentrations for each species, along with the controls. Uppercase letters = comparisons within columns, analyzing the effect among *Piper* species at the same concentration. R^2 = McFadden's R^2. $p < 0.001$ (highly significant). CV: Coefficient of Variation

Table 3
Lethal concentrations (LC50 and LC90) of various essential oils from species of the genus *Piper* on *Spodoptera frugiperda* 168 hours after application.

Species	CL_{50}	IC95% CL_{50}	CL_{90}	IC95% CL_{90}
P. tuberculatum	1,89	1,53–2,32	3,98	2,89–5,47
P. aduncum	1,03	0,79–1,36	3,49	2,26–5,37
P. arboreum	2,43	1,80–3,29	9,52	5,41–16,76
P. divaricatum	1,44	1,11–1,87	4,57	2,99–7,00
P. fuligineum	1,11	0,88–1,39	2,67	1,88–3,77
P. hispidum	3,35	2,35–4,78	15,55	7,46–32,42
P. marginatum	1,02	0,77–1,35	3,59	2,30–5,61

95% CI stands for 95% Confidence Interval.

In line with this sesquiterpene profile, the essential oil of *P. hispidum* was characterized by a predominance of germacrene B (18.3%) and 3-carene (14.07%). The other major constituents were δ-cadinene (8.44%), β-caryophyllene (6.87%), α-bulnesene (5.89%), and β-pinene (5.53%). Similarly, the essential oil of *P. arboreum* had Germacrene B (18.52%), γ-muurolene (16.54%), β-caryophyllene (14.29%), and α-phellandrene (10.64%) as its main constituents. Also significant were α-pinene (6.7%), silvestrene (5.65%), and 3-carene (5.54%). Finally, the essential oil of *P. aduncum* stood out for its distinct composition, being rich in cis-ocimene (25.23%) and myristicin (18.88%). The remaining major constituents were 5,6-dimethoxy-1-indanone (11.98%), trans-ocimene (11.33%), germacrene B (9.81%), and germacrene D (6.67%).

Table 5
Chemical composition of essential oils from different *Piper* species.

		Area (%)							
Nº	**Compounds**	**Retention (KI)**	**Pm**	**Pt**	**Pf**	**Pd**	**Ph**	**Par**	**Pad**
1	α-pineno	939	-	26,82	9,85	25,92	-	6,70	-
2	β-pineno	979	-	28,17	-	10,45	5,23	-	-
3	α-felandreno	1002	9,22	-	7,23	12,14	-	10,64	-
4	3-careno	1011	-	-	-	-	14,07	5,54	-
5	Limoneno	1029	-	15,39	-	-	-	-	-
6	Silvestreno	1030	-	-	6,39	7,95	-	5,65	-
7	Cis-ocimeno	1037	15,88	-	6,85	-	-	-	25,23
8	Trans-ocimeno	1050	6,52	-	-	-	-	-	11,33
9	γ-terpineno	1059	6,89	-	-	-	-	-	-
10	β-cariofileno	1419	10,19	12,89	6,48	-	6,87	14,29	-
12	Germacreno D	1481	5,08	-	24,67	12,51	-	-	6,67
13	Valenceno	1496	-	-	-	-	-	-	-
14	Germacreno B	1561	19,80	-	10,93	-	18,30	18,52	9,81
15	γ-muuroleno	1479	-	-	-	-	-	16,54	-
16	β-bisaboleno	1505	-	8,66	-	-	-	-	-
17	α-bulneseno	1509	-	-	-	-	5,89	-	-
18	Miristicina	1518	-	-	-	-	-	-	18,88
19	δ-cadineno	1523	-	-	-	-	8,44	-	-
20	Pentadecano	-	-	-	-	14,08	-	-	-
21	5,6-dimetóxi-1-indanona	-	-	-	-	-	-	-	11,98

Pm = *Piper marginatum*; Pt = *Piper tuberculatum*; Pf = *Piper fuligineum*; Pd = *Piper divaricatum*; Ph = *Piper hispidum*; Par = *Piper arboreum*; and Pad = *Piper aduncum*.

DISCUSSION

Essential oils represent a potential alternative for managing insect pests, as they can act as substitutes for or adjuvants to chemical insecticides (Isman 2020). In this study, the Essential oils from the seven Piper species exhibited activity against the eggs and larvae of *S. frugiperda*, with responses that were predominantly concentration-dependent and markedly distinct among the different species. This variation indicates that the insecticidal activity of essential oils is not related solely to the amount applied, but also to the composition and relative proportions of their constituents.

Ovicidal Action

The high egg mortality observed at the highest concentrations demonstrates that the embryonic stage of *S. frugiperda* is susceptible to *Piper* Essential oils. The absence of differences among the controls confirms that the response was not due to water or acetone, reinforcing the oils' effect on embryonic viability. The dose-dependent pattern observed for most species is consistent with the need to reach a certain concentration of biologically active compounds for changes capable of compromising embryonic development to occur.

The greater activity of *P. marginatum, P. arboreum*, and *P. fuligineum* especially at concentrations of 2% and above suggests that their chemical profiles are more compatible with the physiological processes involved in maintaining egg viability. In contrast, *P. divaricatum*

exhibited a much weaker response at intermediate concentrations, with a significant effect observed only at 8%. This behavior demonstrates that egg susceptibility is not uniform among the OEs and that certain chemical profiles may require substantially higher concentrations to produce an effect.

The reduction in hatching may be associated with blockage of the chorion, preventing the exchange of CO_2 and O_2, with the interruption of embryonic development, or with the inability of the neonatal larva to break through the chorion (Kala et al. 2019; de Oliveira et al. 2022). Although these processes have not been directly evaluated, the lipophilic nature of constituents such as germacrene B, germacrene D, β-caryophyllene, α-pinene, and β-pinene may favor their interaction with insect structures and interfere with metabolic processes (Oussalah et al. 2007; de Souza et al. 2020).

According to Gupta et al. (2023), studies have shown that compounds present in Essential oils can promote membrane destabilization, enzyme inhibition, and metabolic changes, while Al-Nagar et al. (2020) observed effects on egg viability and larval survival in lepidoptera exposed to Essential oils rich in bioactive compounds.

The chemical composition of the Essential oils evaluated provides insights into some of these differences. *P. marginatum* and *P. arboreum*, which exhibited high ovicidal activity, contain α-pinene and β-caryophyllene among their main constituents compounds associated with cellular and neurophysiological changes in insects (Chaubey 2022). *P. aduncum*, in turn, exhibited a composition characterized by the presence of myristicin, a compound associated with insecticidal activity and interference with metabolic processes (Fazolin et al. 2007; Pavela et al. 2020). In *P. tuberculatum*, the high proportion of α- and β-pinene coincided with high ovicidal activity, consistent with the biological activity attributed to these compounds.

However, the chemical composition does not allow for establishing a direct relationship between a single compound and the intensity of the response. α-Pinene, for example, is present in *P. tuberculatum* and *P. divaricatum*, which exhibited distinct ovicidal responses, while germacrene B and β-caryophyllene occur in both highly active oils and species with lower activity. This lack of a direct correlation reinforces the hypothesis that the activity results from the combined action of the constituents and their relative proportions.

Furthermore, due to the complexity of the chemical composition of essential oils, the mechanisms of action are attributed to the various compounds and their ability to penetrate the target organism, potentially acting on specific enzymes of the central nervous system (Jankowska et al. 2018). Thus, the observed ovicidal activity likely results from multiple physiological interactions, although the specific mechanisms responsible for egg inviability were not measured in this study.

The high activity against eggs is particularly relevant given the scarcity of studies focused on pest management during embryonic development (Krinski and Foerster 2016; Guedes et al. 2020). Considering that effective ovicidal activity should prevent the hatching of at least 75% of the eggs (Krinski and Foerster 2016), the results obtained at the highest concentrations indicate the potential of *Piper* essential oils to act against *S. frugiperda* even at this stage of the development cycle.

Larvicidal Activity

Larvicidal activity was also concentration-dependent, but showed significant differences in potency among the essential oils. The absence or reduction of activity at lower concentrations for some species, combined with increased mortality at higher concentrations, suggests that the amount of compound available to reach the organism is a determining factor in the manifestation of the effect. This pattern is consistent with the need for the constituents to penetrate the cuticle and reach susceptible tissues or physiological systems (Fazolin et al. 2007; de Oliveira et al. 2022).

The LC50 analysis shows that *P. marginatum, P. aduncum*, and *P. fuligineum* were the OEs with the highest larvicidal potency, while *P. arboreum* and *P. hispidum* exhibited lower potency. The LC90 particularly highlights the performance of *P. fuligineum*, which required a lower concentration to achieve high mortality. Therefore, although several species produced high mortality at the highest concentrations, the amount required to achieve a given level of control differed, indicating real differences in potency among the chemical profiles.

The high activity of *P. fuligineum* may be related to the predominance of germacrene D and germacrene B in its essential oil. These sesquiterpenes are associated with neurotoxic action and interference with juvenile hormone production, potentially causing paralysis and developmental arrest (Huang et al. 2021) *P. marginatum*, which exhibited the lowest LC_{50}, contains germacrene B, β-caryophyllene, α-phellandrene, and germacrene D compounds associated with neurodisruptive effects, tissue necrosis, and larval morphophysiological

alterations (Guedes et al. 2020; de Oliveira et al. 2022; Lopes et al. 2024). In *P. aduncum*, the presence of myristicin, which is associated with insecticidal and metabolic effects, may also contribute to the high potency observed (Fazolin et al. 2007; Pavela and Benelli 2016).

On the other hand, the lower potency of *P. hispidum* and *P. arboreum* demonstrates once again that the presence of certain compounds is not sufficient to explain the activity. Both contain germacrene B and β-caryophyllene, which are also found in more toxic species. Thus, the ratio between the constituents and their interactions appears to be more important than the mere presence of specific molecules.

The volatility of monoterpenes may contribute to the difference in response among the oils, especially in contact bioassays, in which some of the compounds may be lost through evaporation before penetrating the seed coat (Monteiro et al. 2021). This characteristic may help explain differences in activity, but it does not constitute a standalone explanation, since species with a composition rich in monoterpenes exhibited distinct responses. Therefore, the observed toxicity should be understood as the result of the interaction between chemical composition, concentration, and the availability of the constituents during exposure.

Integration of Chemical Composition and Insecticidal Activity

A joint analysis of the ovicidal and larvicidal effects shows that *Piper* essential oils have the potential to act on different life stages of *S. frugiperda*, but that their efficacy varies depending on the target life stage and the chemical profile of the oil. *P. marginatum* and *P. fuligineum* stand out particularly for combining high activity against eggs with high larvicidal potency, while *P. arboreum* showed more significant performance against eggs and *P. aduncum* demonstrated greater efficacy against larvae.

The lack of a direct correlation between major compounds and activity underscores the complexity of essential oils. Monoterpenes and sesquiterpenes may act simultaneously on different physiological systems, including neurotransmission, hormonal regulation, cuticular integrity, metabolic processes, and cytotoxicity (Al-Nagar et al. 2020; Almadiy and Nenaah 2022). Thus, the results are best interpreted by considering the essential oil as a biologically active mixture, rather than as the simple sum of the effects of its constituents.

This characteristic also makes it impossible to distinguish, under the conditions of this study, whether the effects resulted from synergistic, additive, or predominantly individual actions of specific compounds. Confirmation of these relationships requires experiments with isolated constituents and controlled combinations. Similarly, the cellular and neurophysiological mechanisms discussed are based on the literature and were not directly measured in this study. Therefore, the relationships between chemical composition and activity should be considered associations and hypotheses for future studies.

The potential action on multiple physiological targets also raises the hypothesis of a lower likelihood of rapid resistance selection in *S. frugiperda* populations, since adaptation might require simultaneous changes in different biochemical pathways (Monteiro et al. 2021; Lemus de la Cruz et al. 2022). However, this possibility still needs to be confirmed by multigenerational selection studies.

The results demonstrate that essential oils from *Piper* species exhibit insecticidal potential against *S. frugiperda* eggs and larvae, with activity dependent on the concentration and chemical profile of each species. Among the oils evaluated, *P. marginatum* and *P. fuligineum* exhibited the most consistent set of desirable characteristics, considering both ovicidal activity and larvicidal potency.

The activity observed at higher concentrations, combined with the presence of different classes of bioactive compounds, reinforces the potential of essential oils as plant-based alternatives for pest management. However, the volatility of certain constituents and the chemical complexity of the oils pose challenges for their application. Formulation strategies, including approaches based on nanotechnology, have been investigated to improve the stability and availability of essential oils and may help overcome some of these limitations (Luneja and Mkindi 2025; de Souza et al. 2025; Maleski et al. 2025).

Thus, the results obtained expand the evidence regarding the potential of *Piper* species in the management of *S. frugiperda* and indicate that research on these oils should progress toward studies that relate chemical composition, mechanisms of action, and formulation, thereby enabling the transformation of the activity observed in the laboratory into more consistent integrated pest management strategies (Pavela and Benelli 2016; Devrnja et al. 2022).

CONCLUSIONS

The essential oils from the seven *Piper* species exhibited ovicidal and larvicidal activity, with responses that were predominantly concentration-dependent, highlighting the potential of this plant group as a source of bioactive compounds against *S. frugiperda*.

Among the species evaluated, *Piper fuligineum, P. marginatum*, and *P. aduncum* exhibited the greatest insecticidal potential, standing out for their high larvicidal activity and lower lethal concentrations, with LC_{50} values ranging from 1.02% to 1.11% and LC_{90} values ranging from 2.67% to 3.59%. These oils were also the only ones to exceed 50% larval mortality at a concentration of 1%, indicating greater relative potency and the ability to produce a significant effect at lower concentrations.

Piper tuberculatum and *P. divaricatum* also exhibited significant insecticidal activity, especially at higher concentrations, while P. hispidum and P. arboreum demonstrated lower larvicidal potency within the evaluated concentration range; it was not possible to determine the LC_{90} for these species.

The differences in activity among the essential oils were associated with their distinct chemical profiles. The presence of germacrene B and germacrene D among the main constituents of the most active oils suggests a possible association between the sesquiterpene-rich composition and the observed toxicity. However, the occurrence of these compounds in species with different levels of activity indicates that the biological response likely results from the interaction among multiple constituents, rather than from the isolated action of a single compound.

Taken together, *P. fuligineum, P. marginatum*, and *P. aduncum* stand out as the most promising species for the development of bioinsecticides intended for the management of *S. frugiperda*. Their activity at relatively low concentrations, combined with their effect on eggs and larvae, reinforces the potential of these species for management strategies targeting the early stages of the pest's development, during which population suppression can help reduce subsequent crop damage.

The results therefore provide an experimental basis for the development of botanical products based on *Piper* OEs; however, additional studies are needed to elucidate the mechanisms of action, evaluate the individual and combined contributions of the constituents, improve the stability and formulation of the oils, and determine their efficacy and selectivity under field conditions.

DECLARATIONS

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Author Contributions

William Cardoso Nunes, as lead author of the study, collected the plant material, reared the insects, and conducted all experiments, being responsible for all stages of the work. Nicole Cristina Martins Rocha was responsible for the interpretation and organization of the chromatographic data. Beatriz Martins de Oliveira performed the laboratory activities and maintained the insect colonies throughout the study. Ricardo José da Silva supervised the experimental design, the statistical analyses, and the cataloging of the data. Daniela Hartwig supervised and performed the chromatographic analyses. Diones Krinski co-supervised the study and participated in all of its stages. Mônica Josene Barbosa Pereira supervised the study as advisor. All authors contributed to the writing and read and approved the final version of this manuscript.

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Ethics approval

Not applicable.

Competing interests

The authors declare no competing interests. This manuscript is based on the results of the master's thesis of the first author (William Cardoso Nunes), submitted to the Graduate Program in Environment and Agricultural Production Systems at the State University of Mato Grosso (UNEMAT). Use of Artificial Intelligence was used exclusively to assist with spelling corrections and adherence to English language conventions. The authors reviewed the entire text and assume full responsibility for the content of the manuscript.

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Figures

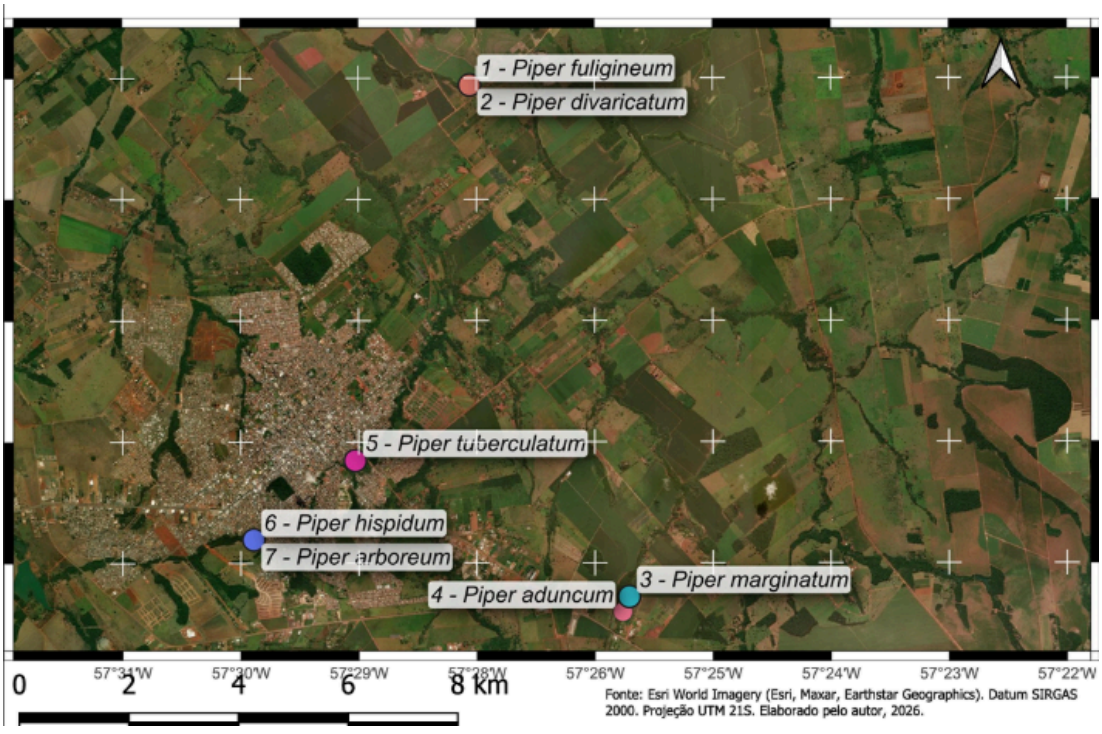


Figure 1

Location of collection sites for species of the genus *Piper* in the municipality of Tangará da Serra, Mato Grosso. The geographic distribution of the seven collected species is indicated by colored markers: 1. *Piper fuligineum;* 2.*Piper divaricatum*; 3. *Piper marginatum*; 4. *Piper aduncum;* 5 *Piper tuberculatum;* 6. *Piper hispidum* e 7. *Piper arboreum.*

Source: Qgiz