


Research Article

Evaluation of plant extracts as botanical insecticides for controlling *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

The increasing incidence of *Spodoptera frugiperda* resistance and the adverse effects of excessive synthetic insecticide use emphasize the need to develop safer and more environmentally sustainable alternative control strategies. One promising alternative is the use of botanical insecticides. This study aimed to evaluate the bioactivity of five plant extracts, namely spiked pepper (*Piper aduncum*), African marigold (*Tagetes erecta*), Mexican sunflower (*Tithonia diversifolia*), sugar apple (*Annona squamosa*), and soursop (*Annona muricata*), as candidates for botanical insecticide formulations against *S. frugiperda*. The experiments assessed toxicity, antifeedant, and ovicidal activities. Toxicity was tested using the leaf-dipping method with five concentration levels, equivalent to LC₁₅, LC₃₅, LC₅₅, LC₇₅, and LC₉₅. Antifeedant activity was tested using the choice method with three concentration levels, equivalent to LC₁₅, LC₃₅, and LC₅₅. Moreover, ovicidal activity was tested by spraying the extract on eggs with concentrations equivalent to LC₉₅ and 2×LC₉₅. The results showed a positive correlation between increasing test concentrations and higher larval mortality, antifeedant activity, and ovicidal activity. *P. aduncum* extract showed the highest toxicity, with LC₅₀ and LC₉₅ values of 0.11% and 0.70%, respectively. Among the tested extracts, *T. diversifolia* showed the highest antifeedant activity with moderate to strong effects, while the other showed very weak to moderate effects. In terms of ovicidal activity, all extracts showed strong inhibitory effects (>75%), particularly at a concentration of 2×LC₉₅, with *P. aduncum* being the most effective extract due to its highest inhibition rate. These findings indicate that each extract exhibited bioactivity in at least one parameter, supporting its potential as a botanical insecticide. *P. aduncum* emerged as the most promising candidate, with strong toxicity and ovicidal effects despite relatively weak antifeedant activity.

Keywords: Antifeedant, Choice method, Leaf-dipping method, Ovicidal, Toxicity

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda*) has continued to pose a serious threat to the success of corn cultivation in Indonesia, following the initial report in early 2019 (Sartiami *et al.*, 2020). The larvae of *S. frugiperda* attack corn at all growth stages, with the vegetative stage as the most vulnerable (Maharani *et al.*, 2019). Infestations during this stage potentially cause up to 100% damage, leading to stunted growth (Trisyono *et*

al., 2019). *S. frugiperda* infestations in several countries have been reported to significantly decline production. According to Harrison *et al.* (2019), in 2016, annual corn yield losses due to *S. frugiperda* infestations in 12 African countries were estimated at 18 million tons, with an economic loss of approximately US\$13 million. In 2018, yield losses in Kenya were estimated at 883,000 tons (de Groote *et al.*, 2020).

The significant potential for economic losses due to *S. frugiperda* infestations indicates the urgent need for effective control strategies. However, control measures to date have relied heavily on synthetic insecticides. Despite effectively controlling *S. frugiperda*, reliance on synthetic insecticides can also lead to several adverse effects, including resistance, mortality of non-target organisms, and hazardous toxic residues that pose risks to both human health and the environment (Dadang, 2023). Kumela *et al.* (2018) explained that improper use of synthetic insecticides may lead to the rapid development of resistance. In several countries, such as Brazil and Puerto Rico, genetic mutations have been identified that could lead to resistance against various insecticide active ingredients, particularly emamectin benzoate, diamides, organophosphates, spinosyns, and benzoylureas (Boaventura *et al.*, 2020), as well as spinosad (Lira *et al.*, 2020). Therefore, more environmentally friendly alternative control techniques are required than synthetic insecticides.

An environmentally friendly alternative technique for controlling *S. frugiperda* is the use of botanical insecticides, which offer several advantages, including selectivity toward target pests, rapid biodegradability, no inhibition of plant growth, and compatibility with other control technologies to enhance effectiveness (Dadang, 2023). Additionally, botanical insecticides contain various active compounds with different modes of action, which can slow the development of pest resistance (El-Wakeil, 2013).

As a megadiverse country, Indonesia has many plant species with potential as botanical insecticides. Several plant species, including *Piper aduncum*, *Tagetes erecta*, *Tithonia diversifolia*, *Annona squamosa*, and *Annona muricata*, have been reported to effectively control various insect pests. The fruit extract of *P. aduncum* reportedly showed strong antifeedant and toxic activities in controlling several pests, including *Nilaparvata lugens* (Nuryanti *et al.*, 2018), *Crociodolomia pavonana* (Priyono *et al.*, 2020), and *Helopeltis antonii* (Rohimatun *et al.*, 2020). Similarly, the flower extracts of *T. erecta* and *T. diversifolia* have also shown potent insecticidal activity against *N. lugens* (Nuryanti *et al.*, 2018) and *H. antonii* (Rohimatun *et al.*, 2020). Meanwhile, the leaf and seed extracts of *A. squamosa* have shown strong toxicity against *S. litura* (Shiragave, 2018) and *C. pavonana* (Nenotek *et al.*, 2022), whereas those of *A. muricata* have exhibited toxic effects against *Bactrocera* sp. (Ningrum *et al.*, 2023) and *Aphis glycines* (Baldin *et al.*, 2023).

Many studies have reported the use of various plant species as botanical insecticides, but the majority have primarily focused on the toxicity of the extracts. However, comprehensive studies on the bioactivity of plant extracts remain limited. Dadang (2023) explained that botanical insecticides caused insect mortality and func-

tioned as antifeedants, oviposition deterrents, ovicidal agents, and growth regulators. Therefore, this study aimed to evaluate the bioactivity of five plant extracts, including *P. aduncum*, *T. erecta*, *T. diversifolia*, *A. squamosa*, and *A. muricata*, against *S. frugiperda*, with emphasis on assessing extract toxicity, antifeedant, and ovicidal activities.

MATERIALS AND METHODS

Study area

The study was conducted from January to November 2024 at the Laboratory of Insect Physiology and Toxicology, Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, West Java, Indonesia.

Extraction of plant materials

The plant materials used for extraction were obtained from various locations in West Java Province, Indonesia. The fruits of *P. aduncum* were collected from the area around the IPB University Campus, Dramaga District, Bogor Regency (6°32'56.5"S, 106°42'57.9"E). Flowers of *T. diversifolia* were collected from Cipanas District, Cianjur Regency (6°42'42.9"S, 106°59'45.4"E), and *T. erecta* flowers were purchased from PT. Bina Usaha Flora (BUF), Sukaresmi District, Cianjur Regency (6°43'44.3 "S, 107°04'31.1"E). Seeds of *A. squamosa* were obtained from the collection of the Laboratory of Physiology and Toxicology, IPB University, while seeds of *A. muricata* were collected from Singaparna District, Tasikmalaya Regency (7°20'53.3"S 108°06'40.4"E).

Extraction was conducted using the maceration method, beginning with the cutting of plant material and drying in the laboratory. All materials were dried in a room at 26 ± 2 °C and $65 \pm 5\%$ RH for 7-14 days without direct sunlight exposure and continued with oven drying at 45 °C. After drying, all materials were ground using a grinder (Retsch GmbH 5667 HAAN Type SK1 Nr. 37535) into a powder, then sieved using a 1 mm mesh, and soaked in a solvent at a ratio of 1:10 (w/v) for 48 hours (Sianturi *et al.*, 2022). The solvents used were ethanol for *T. erecta* flowers (Rohimatun *et al.*, 2020), methanol for *T. diversifolia* flowers (Firmansyah *et al.*, 2017), and hexane for *P. aduncum* fruits (Heviyanti *et al.*, 2024), *A. squamosa* seeds (Vetal and Pardeshi, 2019), and *A. muricata* seeds (Irwan *et al.*, 2021). The resulting filtrate was filtered, evaporated using a rotary evaporator (RV 10 digital pro V Complete, Germany) at 50 °C and 400–450 mmHg, and concentrated into a crude extract, which was then stored at 4 °C until further use (Agustini *et al.*, 2024).

Insect rearing

The rearing of *S. frugiperda* followed the method described by Sianturi *et al.* (2022), starting with collecting

parental insects from field populations in a cornfield at the Sawah Baru Experimental Farm, IPB University (6° 33'48.4"S 106°44'06.0"E). The larvae were placed in rearing boxes measuring 36 cm × 28 cm × 7 cm and fed with baby corn, then transferred to cups containing sawdust upon reaching the final instar for pupation. The emerging adults were moved into cages (26 cm × 26 cm × 24 cm) lined with tissue paper on the walls to serve as oviposition substrates. The adults were fed a 10% honey solution absorbed in cotton and hung inside the cage. The eggs laid by adults in tissue paper were collected and transferred to rearing boxes, and the second-instar larvae from the second generation were used as test insects.

Toxicity test

The toxicity test of the plant extract was conducted in two stages, namely a preliminary and an advanced test. The experiment was arranged in a completely randomized design (CRD) with five concentration levels equivalent to LC₁₅, LC₃₅, LC₅₅, LC₇₅, and LC₉₅, determined from the preliminary test results (Nuryanti *et al.*, 2018). The preliminary test used three replicates, and the advanced test used five replicates, with each replicate consisting of ten *S. frugiperda* larvae. Before testing, the plant extract was dissolved in 1% solvent (the same type of solvent as during extraction), then a mixture consisting of distilled water and 0.2% Alkyl-aryl polyglycol ether 400 L was added to reach a total volume of 100 mL (Agustini *et al.*, 2024). Extract solutions were homogenized using a magnetic stirrer at 750 rpm for 30 minutes and then serially diluted to make lower concentrations. Control was distilled water added with solvent and Alkyl-aryl polyglycol ether 400 L at a ratio of 5:1 (v/v) (Sianturi *et al.*, 2022). The test was conducted using the leaf-dipping method, with each *S. frugiperda* larva placed individually in separate wells of a tray to prevent cannibalism and ensure accurate results. The test insect feed consisted of 2 cm × 2 cm pieces of fresh corn leaves, free of insecticide, sourced from corn plants cultivated without the use of insecticides. Initially, the corn leaf pieces were dipped into the extract solution according to the treatment concentration for approximately five seconds until evenly coated, then drained and placed in each tray well with a single *S. frugiperda* larva. After 48 hours, the treated leaves were replaced with fresh untreated leaves and larval mortality was observed at 24, 48, 72, and 96 hours after treatment (HAT) (Agustini *et al.*, 2024).

Antifeedant test

The antifeedant test was conducted using the choice method in a residual feeding test. Extract concentrations tested were equivalent to LC₁₅, LC₃₅, LC₅₅, and control based on toxicity test results. The experiment was arranged in a CRD with five replications. Each tray

well contained a single *S. frugiperda* larva, then one treated leaf, and one control leaf was provided and placed side by side. Observations were conducted by measuring the leaf area consumed 24 HAT using the *BioLeaf* application (Machado *et al.*, 2016). The percentage of antifeedant activity (AP) was calculated using the formula:

$$AP = \left(\frac{CT - ET}{CT + ET} \right) \times 100\% \quad \text{Eq. 1}$$

CT and ET represent the leaf area consumed in the control and treatment groups.

Ovicidal test

The ovicidal test was conducted by spraying the extract solution onto groups of *S. frugiperda* eggs using a hand sprayer (Krinski *et al.*, 2018). The egg criteria used were one day old after oviposition, normal morphology (intact shape and uniform color), and consisted of at least fifty eggs in a group. The spraying was carried out evenly until the entire egg surface was wet. Furthermore, the eggs were air-dried and placed in tissue-lined petri dishes, with one group per dish. The experiment was arranged using a CRD with three replications. Each replication consisted of one group of insect eggs. The extract concentrations tested were LC₉₅, 2×LC₉₅, and a control based on toxicity test results. These concentrations were chosen to assess the maximum effectiveness of the extract against insect eggs, which are more resistant than the larval stage due to the presence of a protective chorion. After treatment, the eggs were stored in a room maintained at 26 ± 2 °C and 65 ± 5% RH. Observations were carried out daily until all eggs hatched or failed to hatch. The percentage of ovicidal activity (OP) was calculated using the formula:

$$OP = \left(\frac{\left(\frac{A}{N_1} \times 100 \right) - \left(\frac{B}{N_2} \times 100 \right)}{\left(\frac{A}{N_1} \times 100 \right)} \right) \times 100\% \quad \text{Eq. 2}$$

A, B, N₁, and N₂ represent the number of eggs hatched in the control, the number of eggs hatched in the treatment, the total number of eggs in the control, and the total number of eggs in the treatment group, respectively.

Data analysis

Data processing started with data tabulation using Microsoft Excel 2021. Extract toxicity was analyzed using probit analysis to determine the relationship between concentration and insect mortality based on LC₅₀ and LC₉₅ values through the *Poloplus* software. Antifeedant

and ovicidal activities were classified according to specific criteria. Antifeedant activity was classified into five criteria: strong ($AP \geq 80\%$), moderate ($60\% \leq AP < 80\%$), weak ($40\% \leq AP < 60\%$), very weak ($0\% < AP < 40\%$), and no activity ($AP = 0\%$) (Rohimatun *et al.*, 2020). Similarly, ovicidal activity was also classified into five criteria: strong ($OP \geq 75\%$), moderate ($50\% \leq OP < 75\%$), weak ($25\% \leq OP < 50\%$), very weak ($0\% < OP < 25\%$), and no activity ($OP = 0\%$) (Apriality *et al.*, 2021).

RESULTS AND DISCUSSION

Toxicity of extract against *Spodoptera frugiperda* larvae

The toxicity of the five plant extracts showed a positive correlation between increasing concentrations and the percentage of *S. frugiperda* larval mortality. During the assessment, larval mortality increased from the first observation at 24 HAT until 96 HAT. The highest mortality rates for each extract at 96 HAT, in order, were 100% *P. aduncum* (concentration 0.73%), 98% *A. squamosa* (1.47%), 88% *T. erecta* (1.87%), 74% *T. diversifolia* (5.79%), and 72% *A. muricata* (2.30%) (Fig.1).

The relationship between concentration and larval mortality percentage showed the toxicity level of each plant extract. According to probit analysis, the plant extract with the highest toxicity was *P. aduncum*, which had lower LC_{50} and LC_{95} values than the other extracts, at 0.11% and 0.70%, respectively. This was followed by *A. squamosa*, *T. erecta*, *A. muricata*, and *T. diversifolia* (Table 1). A previous study by Widayani *et al.* (2023) reported that the methanol extract of *P. aduncum* exhibited LC_{50} and LC_{95} values of 0.53% and 4.66%, respectively, against second-instar larvae of *S. frugiperda*. In comparison, the present study demonstrated significantly higher toxicity, with LC_{50} and LC_{95} values approximately 4.82 and 6.66 times lower, respectively, than previously reported values. Besides cultivar variation and geographical conditions, factors such as the specific plant parts used, tissue maturity, and post-harvest handling practices (drying, grinding, and storage) can significantly influence the phytochemical composition and bioactivity of plant extracts (Raya *et al.*, 2015; Firmansyah *et al.*, 2017; Oszmiański *et al.*, 2018). Moreover, the choice of solvent is also important, as it affects the polarity and determines the types of bioactive compounds that can be efficiently extracted (Tipsut *et al.*, 2025). Numerous reports have shown that extracting plant materials with hexane solvent was more effective against test insects than other solvents such as dichloromethane, ethyl acetate, methanol, and ethanol (Lucena *et al.*, 2017; Pumnuan *et al.*, 2022; Wanna *et al.*, 2023). The choice of solvent in plant material extraction determines the compounds obtained, depending on polarity (Pumnuan *et al.*,

2022). The basic principle is "*like dissolves like*," meaning that when the target compounds are polar, a polar solvent should be used, and vice versa (Dadang, 2023). In general, plants of the Piperaceae family, including *P. aduncum*, have long been recognized as potential sources for botanical insecticides due to the abundance of potential phytochemicals with various insecticidal activities, including toxicity, antifeedant, and anti-oviposition effects (Dadang, 2023; Assunção *et al.*, 2024). About 400 compounds with diverse structures and bioactivities have been isolated from this genus, primarily belonging to the alkaloid, lignan, flavone, chalcone, phenylpropanoid, and kava-pyrone groups (Xiang *et al.*, 2016).

Dillapiol is a phenylpropanoid compound and a major component of *P. aduncum* (Morais *et al.*, 2023). This compound is valuable and has great potential as an insecticide due to its strong insecticidal activity against various insect species (Lucena *et al.*, 2017; Rohimatun *et al.*, 2020; Heviyanti *et al.*, 2024). It produces neurotoxic effects by inhibiting acetylcholinesterase enzymes and modulating octopamine synapses and GABA receptors, leading to loss of control over movement, seizures, paralysis, and ultimately, death (Fazolin *et al.*, 2022). In addition, the presence of methylenedioxy rings in the dillapiol structure can also inhibit the activity of cytochrome P450 enzymes, which are responsible for xenobiotic metabolism, including insecticide detoxification (Fierascu *et al.*, 2020). This mechanism increases insect susceptibility to insecticides while enhancing effectiveness.

Another extract that showed strong toxicity was *A. squamosa*. The strong insecticidal activity can be attributed to the presence of the main active compounds, namely, acetogenins (Durán-Ruiz *et al.*, 2024). Several compounds classified as acetogenins include annonain, squamosin, annonacin, asimicin, cohibinsin, squamostatin-A, and bullatacin (Chen *et al.*, 2012). Acetogenins act on a specific target by inhibiting mitochondrial respiration at NADH-ubiquinone oxidoreductase (mitochondrial complex I). This inhibition disrupts electron transfer, preventing the formation of a proton gradient and ultimately halting ATP production through oxidative phosphorylation (González-Coloma *et al.*, 2002; Yabunaka *et al.*, 2003; Durán-Ruiz *et al.*, 2024). Consequently, cells experience energy deficiency, leading to metabolic disruption. In insects, this condition causes paralysis and physiological impairment, ultimately resulting in mortality due to the failure of ATP-dependent cellular systems.

Antifeedant effects of the extracts on *Spodoptera frugiperda* larvae

All plant extracts showed variation in inhibiting the feeding activity of *S. frugiperda* larvae, with effectiveness depending on the type and concentration. Extracts of *P.*

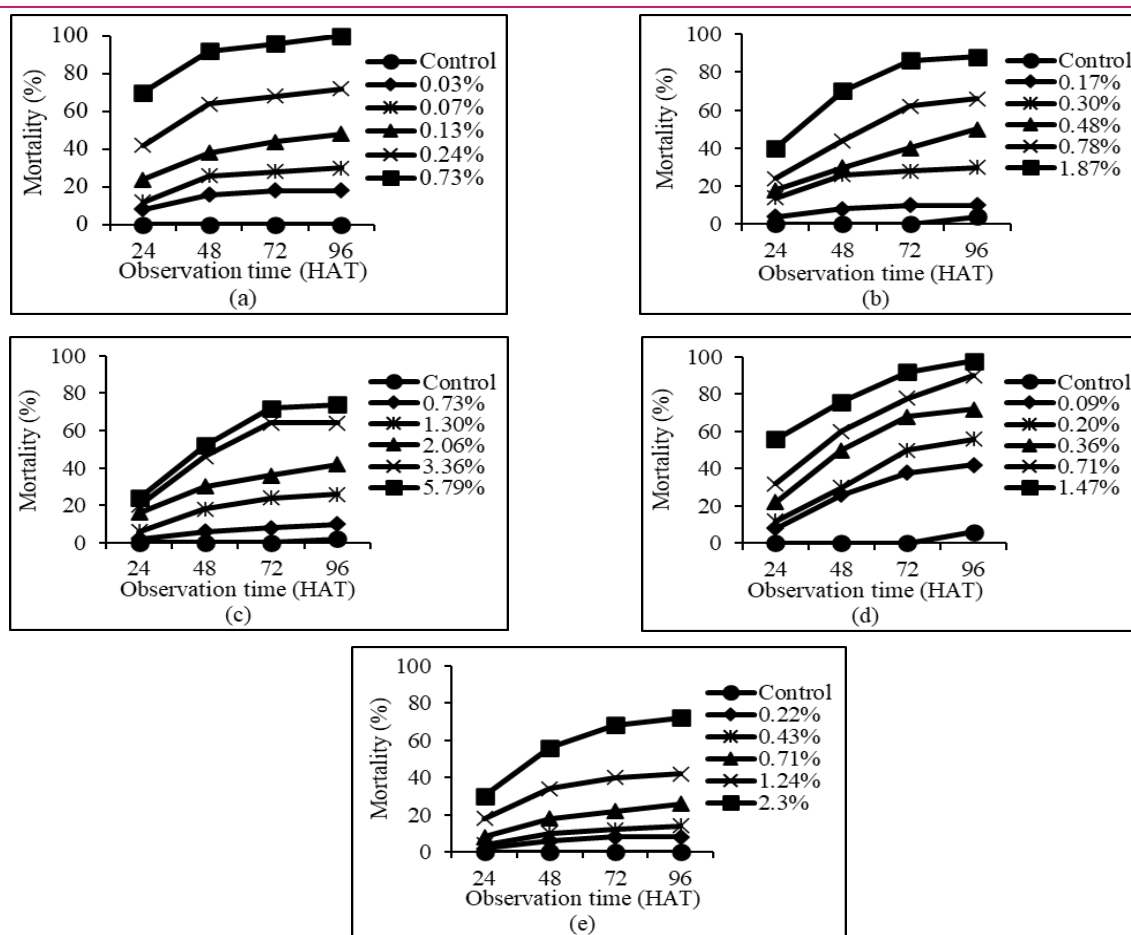


Fig. 1. Mortality rate progression of second-instar larvae of *Spodoptera frugiperda* treated with five types of plant extract: a = *Piper aduncum*; b = *Tagetes erecta*; c = *Tithonia diversifolia*; d = *Annona squamosa*; e = *Annona muricata*

Table 1. Toxicity of five plant extracts against second-instar larvae of *Spodoptera frugiperda*

Plant extract	Observation time (HAT)	a ± SE	b ± SE	LC ₅₀ (CI 95%) (%)	LC ₉₅ (CI 95%) (%)
<i>Piper duncum</i>	24	5.75 ± 0.18	1.55 ± 0.21	0.33 (0.25-0.48)	3.80 (1.91-12.04)
	48	6.44 ± 0.21	1.75 ± 0.21	0.15 (0.10-0.23)	1.31 (0.63-6.34)
	72	6.66 ± 0.22	1.85 ± 0.22	0.13 (0.08-0.20)	0.98 (0.48-5.24)
	96	6.97 ± 0.25	2.07 ± 0.24	0.11 (0.07-0.19)	0.70 (0.34-5.73)
<i>Tagetes erecta</i>	24	4.44 ± 0.11	1.25 ± 0.27	2.83 (1.67-9.33)	59.09 (14.80-1798.90)
	48	5.06 ± 0.11	1.70 ± 0.26	0.92 (0.72-1.28)	8.57 (4.47-27.66)
	72	5.51 ± 0.12	2.23 ± 0.28	0.59 (0.50-0.72)	3.24 (2.19-6.03)
	96	5.61 ± 0.13	2.42 ± 0.31	0.56 (0.47-0.67)	2.67 (1.87-4.71)
<i>Tithonia diversifolia</i>	24	3.38 ± 0.19	1.35 ± 0.36	15.91 (8.10-122.72)	262.94 (52.77-44732.00)
	48	3.86 ± 0.15	1.73 ± 0.30	4.55 (3.51-6.96)	40.75 (19.37-180.41)
	72	3.99 ± 0.14	2.25 ± 0.30	2.80 (2.35-3.44)	15.05 (9.87-30.41)
	96	4.04 ± 0.15	2.24 ± 0.31	2.69 (2.24-3.30)	14.54 (9.49-30.01)
<i>Annona squamosa</i>	24	4.82 ± 0.12	1.33 ± 0.23	1.36 (0.93-2.60)	23.39 (8.44-180.54)
	48	5.45 ± 0.12	1.16 ± 0.20	0.41 (0.30-0.59)	10.73 (4.39-63.45)
	72	6.03 ± 0.14	1.36 ± 0.22	0.17 (0.11-0.24)	2.81 (1.58-8.03)
	96	6.43 ± 0.17	1.79 ± 0.26	0.16 (0.11-0.21)	1.33 (0.88-2.64)
<i>Annona muricata</i>	24	3.90 ± 0.11	1.61 ± 0.36	4.85 (2.88-16.51)	50.93 (15.41-1023.04)
	48	4.44 ± 0.09	1.79 ± 0.29	2.05 (1.54-3.23)	16.93 (8.19-66.91)
	72	4.64 ± 0.09	1.96 ± 0.28	1.54 (1.22-2.11)	10.59 (5.96-28.85)
	96	4.72 ± 0.09	2.01 ± 0.28	1.38 (1.11-1.83)	9.03 (5.31-22.37)

HAT = hours after treatment; a = intercept of probit regression line; b = probit regression slope; SE = standard error; LC = lethal concentration; CI = confidence interval.

Table 2. Inhibition of the feeding activity of five plant extracts against second-instar larvae of *Spodoptera frugiperda*

Plant extract	Concentration (%)	Antifeedant activity (%) \pm SE	Criteria
<i>Piper aduncum</i>	LC ₁₅ (0.04)	15.24 \pm 2.91	Very weak
	LC ₃₅ (0.07)	43.54 \pm 3.99	Weak
	LC ₅₅ (0.13)	54.25 \pm 3.36	Weak
<i>Tagetes erecta</i>	LC ₁₅ (0.21)	43.02 \pm 5.25	Weak
	LC ₃₅ (0.39)	64.33 \pm 2.01	Moderate
	LC ₅₅ (0.63)	70.95 \pm 3.61	Moderate
<i>Tithonia diversifolia</i>	LC ₁₅ (0.93)	78.54 \pm 3.73	Moderate
	LC ₃₅ (1.81)	84.81 \pm 1.30	Strong
	LC ₅₅ (3.06)	87.22 \pm 4.47	Strong
<i>Annona squamosa</i>	LC ₁₅ (0.04)	38.49 \pm 13.06	Very weak
	LC ₃₅ (0.10)	46.35 \pm 10.98	Weak
	LC ₅₅ (0.19)	65.46 \pm 2.44	Moderate
<i>Annona muricata</i>	LC ₁₅ (0.42)	34.57 \pm 4.12	Very weak
	LC ₃₅ (0.89)	44.94 \pm 3.90	Weak
	LC ₅₅ (1.59)	53.86 \pm 3.71	Weak

LC = lethal concentration; SE = standard error.

Table 3. Inhibition of *Spodoptera frugiperda* egg hatching by treatment with five plant extracts

Plant extract	Concentration (%)	Ovicidal activity (%) \pm SE	Criteria
<i>Piper aduncum</i>	LC ₉₅ (0.70)	87.62 \pm 9.99	Strong
	2 x LC ₉₅ (1.40)	98.61 \pm 0.70	Strong
<i>Tagetes erecta</i>	LC ₉₅ (2.67)	57.55 \pm 17.09	Moderate
	2 x LC ₉₅ (5.34)	91.64 \pm 4.18	Strong
<i>Tithonia diversifolia</i>	LC ₉₅ (14.54)	68.22 \pm 11.95	Moderate
	2 x LC ₉₅ (29.08)	89.66 \pm 4.56	Strong
<i>Annona squamosa</i>	LC ₉₅ (1.33)	81.53 \pm 11.89	Strong
	2 x LC ₉₅ (2.66)	92.69 \pm 4.92	Strong
<i>Annona muricata</i>	LC ₉₅ (9.03)	73.47 \pm 7.46	Moderate
	2 x LC ₉₅ (18.06)	92.92 \pm 1.55	Strong

LC = lethal concentration; SE = standard error.

aduncum and *A. muricata* showed relatively low anti-feedant activity percentages, with inhibition criteria ranging from very weak to weak. In contrast, *A. squamosa* and *T. erecta* extracts showed higher antifeedant activity, particularly at higher concentrations, with moderate inhibition criteria. Although most extracts caused relatively weak feeding inhibition, the potential as anti-feedants became stronger at higher concentrations. This shows that antifeedant activity increases with treatment concentration (Rohimatun *et al.*, 2020). Several previous studies reported that increasing the test concentration significantly inhibited the feeding activity of *S. frugiperda* larvae. *P. aduncum* extract at the highest concentration (0.25%) showed strong antifeedant activity against second-instar *S. frugiperda* larvae, reaching 94.35% (Lina *et al.*, 2023). Similarly, *A. squamosa* extract at the highest concentration (16%) also showed strong antifeedant activity, reaching 82.37% (Bhosle *et al.*, 2024).

Among all the tested extracts, *T. diversifolia* showed the highest effectiveness in inhibiting the feeding activity of second-instar *S. frugiperda* larvae, with LC₃₅ and LC₅₅ concentrations treatments, reaching 84.81% and 87.22%, respectively (Table 2). The presence of anti-feedant compounds influences the high feeding inhibition of *T. diversifolia* extract. Antifeedant compounds are chemical substances that inhibit or suppress insect feeding activity by affecting specific sensory receptors, leading to deterrent or suppressant effects on plant consumption (Purrington, 2017). Furthermore, Mossa (2016) explained that antifeedant compounds influence insect feeding behavior through peripheral sensilla, ultimately reducing food intake. A review by Kerebba *et al.* (2019) identified several antifeedant compounds present in *T. diversifolia*, including 6-methoxyapigenin, tagitinins (A, B, C, and F), diversiform, tirotundin, tithonine, sulphurein, hispidulin, and sesquiterpene lactones.



Fig. 2. Ovicidal effects of the five plant extracts on *Spodoptera frugiperda* eggs at six days after treatment. a = *Piper aduncum*; b = *Tagetes erecta*; c = *Tithonia diversifolia*; d = *Annona squamosa*; e = *Annona muricata*.

These results showed that *T. diversifolia* extract has potential as a candidate for botanical insecticide development despite the relatively lower toxicity compared to other plant extracts. Blessing *et al.* (2010) explained that plant extract with strong feeding inhibition activity can cause insects to gradually suffer mortality from starvation, thereby reducing feeding activity and minimizing damage to cultivated plants. Therefore, the use of botanical insecticides should not rely solely on toxicity, but also apply alternative approaches that effectively suppress insect activity, such as antifeedant compounds (Pavela *et al.*, 2025).

Ovicidal effects of the extracts on *Spodoptera frugiperda* eggs

Ovicidal activity of all tested plant extracts was in the strong category, particularly at a concentration of $2 \times LC_{95}$ (Table 3). Extracts of *P. aduncum* and *A. squamosa* showed strong ovicidal activity at both tested concentrations. At LC_{95} , these two extracts had ovicidal percentages of 87.62% and 81.53%, respectively, which significantly increased to 98.61% and 92.69% at $2 \times LC_{95}$. Meanwhile, extracts of *A. muricata*, *T. erecta*, and *T. diversifolia* showed moderate ovicidal activity at LC_{95} , with values of 73.47%, 57.55%, and 68.22%, respectively. At $2 \times LC_{95}$, these three extracts showed a significant increase in effectiveness, falling into the strong category, with ovicidal percentages of 92.92%, 91.64%, and 89.66%, respectively. This increase showed that higher concentrations can enhance the ovicidal potential of plant extract, thereby improving the inhibition of egg development.

In general, all tested plant extracts were effective as ovicidal agents, inhibiting at least 75% of egg hatching, particularly at the $2 \times LC_{95}$ concentration (Krinski *et al.*, 2018). An ovicidal effect was demonstrated by the darkening of eggs, which failed to hatch beyond the normal incubation period (Bhosle *et al.*, 2024) (Fig. 2). Russizani *et al.* (2021) explained that *S. frugiperda* eggs generally hatch simultaneously within each egg group, occurring 2–3 days after oviposition. Meanwhile, observations showed that up to six days after treatment

(DAT), most eggs did not hatch, and a few hatched within 2–3 DAT.

One of the causes of egg-hatching failure was the presence of essential oils (EOs) in the extract, which can suppress the respiratory rate. The ovicidal properties of EOs were due to their ability to block oxygen supply to the developing embryo or to the toxicity of specific chemical compounds, thereby inhibiting development and causing egg mortality (Riedl *et al.*, 1995). Krinski *et al.* (2018) reported that several EOs from the Piperaceae, suspected to have bioactivity against *Anticarsia gemmatilis* eggs, were asaricine, myristicin, spathulenol, (E)-caryophyllene, germacrene B, dillapiol, (E)- β -ocimene, limonene, (E)-nerolidol, piperitone, 1-epi-cubenol, cadalene, 4,6-dimethyl-5-vinyl-1,2-benzodioxide, eudesm-7(11)-en-4-ol, cyclocolorone, α -copaene, (E, E)- α -farnesene, and alloaromadendrene.

Conclusion

All plant extracts showed varying effectiveness against *S. frugiperda*, depending on the type and concentration tested. Increasing the concentration was positively correlated with higher larval mortality as well as antifeedant and ovicidal activity. *P. aduncum* extract showed the highest toxicity, while *T. diversifolia* showed the strongest antifeedant activity. Furthermore, all extracts effectively acted as ovicidal agents, inhibiting more than 75% of egg hatching at the highest concentration ($2 \times LC_{95}$). *P. aduncum* was the most effective, due to its highest inhibition rate.

Conflict of interest

The authors declare that they have no conflict of interest.

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