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RESEARCH ARTICLE

Insecticidal activity and phytochemical profiling of *Annona squamosa* (L.) leaf extract against the fall armyworm, *Spodoptera frugiperda* (J.E. Smith)

Durga Bhosle[1], Srinivasan Thulasy[1*], Elaiyabharathi Thiyagarajan[1], Shanmugam Pagalahalli Sankaran[1], Vellaikumar Sampathrajan[2] & Prakash Kolanchi[1]

[1]Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India

[2]Department of Agricultural Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India

*Email: entosrini@gmail.com

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Abstract

To identify an effective botanical extract for pest control, six locally available plant species were screened for their toxic effects against *Spodoptera frugiperda* (J.E. Smith) larvae. These species included *Annona squamosa* (L.), *Calotropis gigantea* (L.) W.T. Aiton, *Carica papaya* (L.), *Datura metel* (L.), *Ricinus communis* (L.) and *Rottboellia cochinchinensis* (Lour.) Clayton. Among these, *A. squamosa* demonstrated the highest larval mortality at both 5% and 10% concentrations. Based on these promising results, subsequent bioassays were conducted using a range of concentrations (4%, 6%, 8%, 10%, 12%, 14% and 16%) of *A. squamosa* to evaluate its larvicidal, antifeedant and ovicidal activities. The median lethal concentration (LC_{50}) for second instar larvae, observed 72 hours after treatment (HAT), was determined to be 8.65% (w/v). The antifeedant assay revealed a dose-dependent response, with 99.38% antifeedancy at the highest concentration tested (16%). Moreover, *A. squamosa* exhibited 100% ovicidal activity across all concentrations. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the extract identified 39 compounds, with 16-hentriacontanone, neophytadiene, caryophyllene and phytol showing the most significant peak areas. These findings suggest that *A. squamosa* leaf extract possesses substantial larvicidal, antifeedant and ovicidal properties against *S. frugiperda*, highlighting its potential as an eco-friendly, non-toxic alternative to synthetic insecticides. This botanical extract may offer valuable contributions to integrated pest management (IPM) strategies, promoting sustainable agricultural practices.

Keywords

biopesticide; botanical insecticide; custard apple; leaf extracts; phytochemicals

Introduction

Maize (*Zea mays* L.) ranks as the second most widely cultivated crop globally, following rice and is extensively grown in tropical and subtropical regions. Since its introduction to the Indian subcontinent in 2018, the invasive maize fall armyworm (*S. frugiperda* J.E. Smith) has emerged as a major pest, significantly threatening maize production (1, 2). The pest has now spread to over 90% of maize-growing regions in India (3), with reported damage intensity ranging from 9 to 62% and an estimated yield loss of approximately 34% (4). The larval stage of the fall armyworm feeds predominantly on maize leaves, with late-instar larvae often residing within the whorls, resulting in severe defoliation (5). These mature larvae are often inaccessible to insecticidal control, leading to frequent management failures (6). Presently, fall armyworm infestations are primarily controlled through the

application of chemical insecticides. However, the overreliance on these pesticides raises concerns about the development of resistance in insect populations, as well as the potential negative impacts on both environmental and human health (7). In light of these challenges, there is growing interest in exploring the potential of botanicals as an alternative, more sustainable means of managing the fall armyworm. Botanical pesticides, in contrast to synthetic insecticides, offer greater flexibility for integration with other pest management strategies, including cultural and biological control methods, as well as with integrated pest management (IPM) practices (2). Given the ongoing threat of fall armyworms to food security, particularly in regions such as sub-Saharan Africa and Asia (8), the development and optimization of botanical insecticides against this pest are crucial.

Numerous studies have demonstrated the efficacy of plant-derived compounds, including essential oils, plant extracts and secondary metabolites, in exhibiting insecticidal, antifeedant and repellent activities against *S. frugiperda* (9). For instance, methanolic extracts of *Jatropha curcas* have been shown to induce over 60% larval mortality (10). Similarly, hexane, acetone and ethanol extracts of *Tagetes erecta* resulted in larval mortality rates of 48%, 60% and 72%, respectively (11). Moreover, extracts from *Azadirachta indica*, *Schinnus molle* and *Phytolacca dodecandra* have demonstrated larval mortality rates exceeding 95% (12). Additionally, ethanolic extracts of *Cedrela odorata* and *Piper auritum*, when applied at 92 $mg.cm^{-2}$, exhibited complete (100%) mortality against fall armyworm larvae (13). The bioactivity of botanical extracts is primarily attributed to their phytochemicals, such as phenolic compounds, which interfere with the growth and development of pests like the fall armyworm (*S. frugiperda*) (14). Azadirachtin, the principal bioactive compound in neem, has shown significant insecticidal properties against *S. frugiperda*, positioning it as a promising candidate for integration into IPM strategies (9). In this study, we evaluated the efficacy of several locally available pesticidal plants, abundant in our region, against fall armyworm (FAW) larvae. We specifically assessed the larvicidal, ovicidal and antifeedant activities of the most promising botanical extracts under controlled laboratory conditions. Furthermore, a comprehensive analysis of the phytochemical composition of these extracts was conducted to identify the bioactive compounds responsible for their pest control properties.

Materials and Methods

Test insect culture

The FAW culture was maintained in an insect culture laboratory at the Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. Late instar larvae were initially collected from unsprayed maize fields on the university campus. Upon collection, the larvae were initially reared on freshly harvested young maize leaves. Following establishment, the larvae were transitioned to an artificial semi-synthetic diet, developed by CIMMYT (15). Neonate and second instar larvae were housed in 500 ml plastic containers (16 x 10 x 5 cm), each containing the artificial diet. From the third instar onwards, larvae were individually transferred to 10 ml plastic containers (3 x 2.5 x 3.5 cm), which also contained the artificial diet, to minimize cannibalism prior to pupation. Pupae were then collected and transferred to Petri dishes (9 cm diameter) and placed in adult emergence cages (45 x 45 x 45 cm). Within these cages, *Nerium oleander* (L.) plant twigs were provided to facilitate oviposition by the emerging moths. To enhance reproductive performance and fecundity, moths were supplied with a 10% honey-sucrose solution enriched with Vitamin E, zincovit and ascorbic acid (16,17). Egg masses were collected at regular intervals and placed in diet containers to continue the rearing cycle. Both larvae and egg masses obtained from this mass culturing facility were utilized in all subsequent laboratory experiments (18). The rearing conditions were carefully maintained at 25 ± 2°C, 70 ± 5% relative humidity and a 12:12-hour photoperiod (light: dark).

Plant materials

The leaves of six locally available plant species, namely *Annona squamosa* L. (Annonaceae) commonly known as custard apple, *Carica papaya* L. (Caricaceae) referred to as papaya, *Calotropis gigantea* (L.) W.T.Aiton (Apocynaceae) known as giant milkweed, *Datura metel* L. (Solanaceae) or thorn-apple, *Ricinus communis* L. (Euphorbiaceae) recognized as castor bean and *Rottboellia cochinchinensis* (Lour.) Clayton (Poaceae), termed itch grass, were collected from the Tamil Nadu Agricultural University, Coimbatore campus farm in December 2023. These plant species were selected based on their previously documented insecticidal properties, as reported in the literature (2).

Collection and extraction

The leaves were thoroughly washed with tap water, shade-dried and subsequently ground into a fine powder. For the preliminary bioassay, the cold maceration extraction method was employed (19). A total of 30 g of the leaf powder was immersed in 300 ml of ethyl acetate solvent and left at room temperature (27 ± 2°C) and 75 ± 5% relative humidity for a period of 72 hours. After this incubation period, the extract was filtered through Whatman filter paper No. 4 and the solvent was evaporated using a rotary vacuum evaporator under reduced pressure at 50°C, with a rotation speed of 90 rpm. The resulting crude extracts were stored in separate glass vials at 4°C for subsequent use.

Following the preliminary bioassay, *A. squamosa* was identified as the most potent plant extract. The powdered leaves of *A. squamosa* were placed in the thimble of a Soxhlet apparatus and extracted with ethyl acetate solvent at a 1:10 (w/v) ratio. The extraction process was conducted for approximately 5-6 hours at 50-60°C (20) until the compounds were fully extracted from the leaf material. The extract was then filtered and the solvent was removed using a rotary vacuum evaporator, yielding a crude residue. A standard stock solution of the crude residue was prepared by diluting it in acetone. Concentrations ranging from 4 -16% (w/v) were formulated from this stock solution to assess larvicidal, ovicidal and antifeedant activities. These concentrations were determined based on the results of the preliminary bioassays. All bioassay experiments were carried out with a positive control (emamectin benzoate 5 SG at 0.4 g L^{-1}) and a negative control (acetone + water). Emamectin benzoate was selected as the positive control due to its established efficacy as a recommended synthetic insecticide for the control of *S. frugiperda* larvae (4).

Preliminary bioassay of ethyl acetate leaf extracts on S. frugiperda larvae

Crude extracts obtained through the cold maceration technique were subsequently diluted in acetone to prepare 5% and 10% concentrations, in addition to positive and negative controls. For the bioassays, second instar larvae were utilized, with 10 larvae per replication. Each larva was placed in a Petri dish lined with filter paper and the extracts were directly applied by spraying onto the larvae (21). After treatment, the larvae were left undisturbed for approximately one minute before being transferred individually into 12-well bioassay plates, each containing fresh diet. Larval mortality was observed at 24, 48 and 72 HAT. The botanical extract inducing the highest mortality rate was selected for subsequent experiments. The experiment was performed in triplicate.

Insecticidal activity of A. squamosa leaf extract

Larvicidal activity bioassay

The larvicidal bioassay for *A. squamosa* extract was conducted following the methodology outlined in the initial experiment, utilizing seven distinct concentrations (treatments), each replicated three times. In each replication, ten second-instar larvae were exposed to the respective concentrations. The larvae were individually placed in 12-well bioassay plates, each well containing a fresh artificial diet. Mortality observations were recorded at 24-hour intervals for a duration of up to 72 hours following treatment. The percentage of mortality was determined using the formula provided in Equation 1 (22). The LC50 value of the extract was subsequently derived using Finney's Probit analysis (23).

$$\text{Percent mortality} = \frac{\text{Number of larvae died}}{\text{Total Number of larvae treated}} \times 100 \quad \text{(Eq. 1)}$$

Antifeedant activity bioassay

The antifeedant activities of plant extracts were evaluated using a no-choice bioassay method with *Z. mays* leaves (24). Fresh leaves (3 × 3 cm) of *Zea mays* were treated with varying concentrations of *A. squamosa* extracts. After application, the treated leaves were allowed to air-dry for approximately one minute to facilitate solvent evaporation, following which they were placed in individual 6-well bioassay plates. The control treatment involved spraying the leaves with a mixture of acetone and water. All treatments were replicated three times, with five larvae per replication. To maintain leaf freshness, each well was lined with moist cotton and filter paper. In each well, a single, pre-starved second instar larva of *S. frugiperda* was introduced and allowed to feed on the treated leaf for 72 hours. Observations were made at 24, 48 and 72 HAT using the graph paper method (25). The percent antifeedant activity was calculated using the following formula (Eq. 2) (26).

$$\text{Percent antifeedant activity} = \frac{C-T}{C+T} \times 100 \quad \text{(Eq. 2)}$$

Where C is the area of the leaf disc (mm^2) consumed by the larva in control and T is the area of the leaf disc (mm^2) consumed by the larva in treatment

Ovicidal activity bioassay

Egg masses, aged one day, were collected from the oviposition cage, along with *Nerium* leaves, as one-day-old eggs are particularly susceptible to external agents (27). To ensure uniform coverage of the sprayed extract, a single layer of eggs was selected. The egg count was approximated at fifty per mass, verified under a stereo microscope, with any excess eggs removed using a fine camel hairbrush to maintain consistency. The egg masses were subsequently treated with varying concentrations of leaf extract and placed in 9 cm diameter Petri plates for hatching. The plates were sealed with paraffin film to prevent the escape of hatched larvae. The bioassay was conducted with three replicates, alongside positive (emamectin benzoate 5SG at 0.4 g l^{-1}) and negative (acetone + water) controls. Observations were recorded until larvae had completely hatched in the negative control. The percent ovicidal activity (POA) was calculated using the formula provided in Equation 3 (28).

$$\text{POA} = \frac{\text{No. of eggs hatched in control} - \text{No. of eggs hatched in treatment}}{\text{No. of eggs hatched in control}} \times 100 \quad \text{(Eq. 3)}$$

GC- MS Analysis

The phytochemical compounds present in the ethyl acetate leaf extract of *A. squamosa* were analyzed via gas chromatography-mass spectrometry (GC-MS) using the Shimadzu GCMS-TQ8040 NX system under the following conditions. The injector temperature was maintained at 280°C and the samples were injected in split mode with a split ratio of 1:25. A 1 µL injection volume was employed. The analysis utilized an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 µm, composed of 5% diphenyl and 95% dimethyl polysiloxane). Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The oven temperature program began at 60°C, held for 2 minutes, then increased to 260°C at a rate of 10°C per minute, with a final hold at 260°C for 10 minutes. The mass spectrometer was operated with an ionization potential of 70 eV and the interface and ion source temperatures were set at 260°C and 280°C, respectively. The mass scan range was 40–550 m/z. The identification of specific compounds was achieved by comparing their molecular masses (m/z values) to established standards and literature data. The phytochemicals were identified using the National Institute of Standards and Technology (NIST) 14 library, with mass spectra compared to known compound profiles (29).

Statistical analysis

The data were subjected to arc sin transformation and analyzed using one-way analysis of variance (ANOVA) in R software. Percent mean values were calculated using MS Excel 2021. Lethal concentration for larval mortality was determined using Finney's method of probit analysis. Significant differences between treatments were determined using Tukey's HSD test ($P \leq 0.05$).

Results and Discussion

Preliminary bioassay of ethyl acetate leaf extracts on S. frugiperda larvae

In the preliminary screening bioassay, the leaf extracts of various plants demonstrated differing degrees of larval mortality (*Table 1*). The highest mortality rates, 33% and 40%, were observed in *A. squamosa* at concentrations of 5% and 10%, respectively. Similarly, *Cissampelos gigantea* exhibited 40% mortality at 10%, but only 10% mortality at 5% concentration. Moderate mortality was recorded for *D. metel* (20-26%) and *R. communis* (16-20%), with mortality rates below 30% at both concentrations. The leaf extracts of *C. papaya* and *Rottlera cochinchinensis* demonstrated lower mortality, with 13.33% and less than 20%, respectively. Based on these observations, *A. squamosa* was selected for further bioassays. While the insecticidal properties of the other plants have been documented in previous studies (30-33), the observed low efficacy could be attributed to variations in the extraction method, solvent used, plant part employed, or application technique.

Insecticidal activity of A. squamosa leaf extract

Larvicidal bioassay

The *A. squamosa* leaf extracts exhibited varying levels of larvicidal activity against *S. frugiperda* in contact toxicity assays (*Fig. 1*). Mortality in second instar larvae ranged from 23.33% at a 4% concentration to 76.66% at a 16% concentration after 72 HAT. The LC_{50} value was calculated to be 8.65% (w/v) at 72 HAT (*Table 2*). Larval mortality showed a concentration-dependent increase, with higher concentrations of the leaf extract leading to greater mortality. A similar dose-dependent mortality response was noted in the *A. squamosa* seed extracts, which were prepared with various solvents (methanol, ethyl acetate, hexane and chloroform) and tested against *Spodoptera litura* (34). Previous studies have also reported the insecticidal activity of *A. squamosa* extracts against the fall armyworm (*S. frugiperda*). For example, topical bioassay tests using 25 mg/ml ethanolic leaf extracts of *A. squamosa*, *Azadirachta indica* and *Artocarpus heterophyllus* resulted in 95-100% mortality in second instars and 85-95% mortality in third instars of *S. frugiperda* (35). Additionally, the insecticidal efficacy of *A. squamosa* extracts has been demonstrated against various pests, including *Spodoptera litura* (34), *Spodoptera exigua* (36) and *Tribolium castaneum* (37).

Table 1. Evaluation of ethyl acetate leaf extracts against second instar larvae of *S. frugiperda*

Treatments	Conc.	24 HAT	48 HAT	72 HAT
A. squamosa	5%	16.66 ± 0.58 (23.86)^{bc}	30.00 ± 2.00 (32.22)^{b}	33.33 ± 1.53 (34.93)^{b}
	10%	26.66 ± 1.53 (30.29)^{B}	30 ± 1.00 (33.00)^{B}	40 ± 1.15 (38.85)^{B}
C. gigantea	5%	3.33 ± 0.58 (6.14)^{cd}	10.00 ± 1.00 (15.00)^{bcd}	10 ± 1.00 (15.00)^{cde}
	10%	33.33 ± 1.53 (34.93)^{B}	40 ± 1.73 (39.06)^{B}	40 ± 1.73 (39.06)^{B}
C. papaya	5%	13.33 ± 0.58 (21.14)^{bc}	13.33 ± 0.58 (21.14)^{bcd}	13.33 ± 0.58 (21.14)^{bcde}
	10%	13.33 ± 0.58 (21.14)^{BC}	13.33 ± 0.58 (21.14)^{BC}	13.33 ± 0.58 (21.15)^{BC}
D. metel	5%	23.33 ± 0.58 (28.78)^{b}	23.33 ± 0.58 (28.78)^{bcd}	26.66 ± 1.00 (31.00)^{bc}
	10%	16.66 ± 0.58 (23.86)^{BC}	16.66 ± 1.00 (26.07)^{BC}	20 ± 0.58 (28.78)^{BC}
R. communis	5%	16.66 ± 0.58 (23.86)^{bc}	16.66 ± 0.58 (23.86)^{bcd}	16.66 ± 0.58 (23.86)^{bcd}
	10%	20 ± 0.67 (26.07)^{BC}	20 ± 1.00 (26.07)^{BC}	20 ± 1.00 (26.07)^{BC}
R. cochinchinensis	5%	3.33 ± 0.58 (6.14)^{cd}	3.33 ± 0.58 (6.14)^{cd}	3.33 ± 0.58 (6.14)^{de}
	10%	13.33 ± 1.15 (17.71)^{BC}	16.66 ± 1.53 (19.93)^{BC}	20 ± 1.73 (22.14)^{BC}
Positive control (C+)	Emamectin benzoate	96.66 ± 0.58 (83.86)^{aA}	96.66 ± 0.58 (83.86)^{aA}	96.66 ± 0.58 (83.86)^{aA}
Negative control (C-)	Acetone + water	0 ± 0 (0.00)^{cC}	0 ± 0 (0.00)^{dE}	0 ± 0 (0.00)^{eD}

Values are mean of three replications ± standard deviation of means. Figures in the parentheses are arc sine transformed values.

Letters followed by transformed values indicate the significant difference between the treatments by Tukey's HSD test ($P < 0.05$): Small letters and capital letters represent the significant difference between the treatments at 5% and 10% concentration respectively.

HAT = Hours After Treatment

Table 2. Dosage mortality response of *S. frugiperda* second instar larvae to *A. squamosa* after 72 hours of treatment

Leaf extract	Chi-square	LC_{50} (w/v %)	Fiducial Limits (50%)		LC_{95} (w/v%)	Fiducial Limits (95%)		Slope
			Upper limit	Lower limit		Upper limit	Lower limit	
Larval mortality	2.37	8.65	10.47	7.15	36.43	79.87	16.62	2.675961

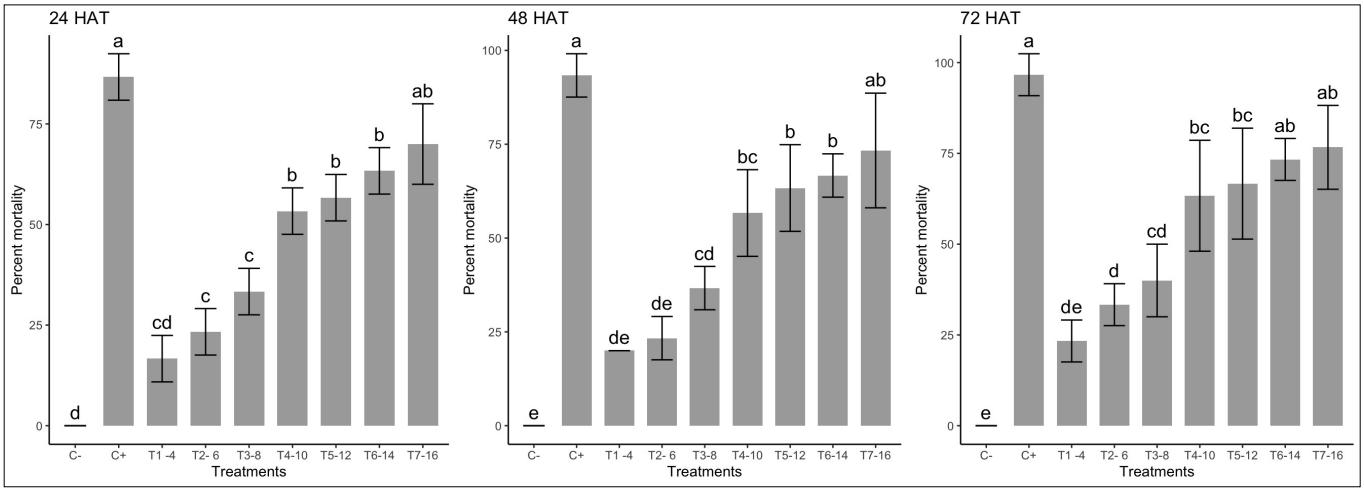

Fig. 1. Mortality of *S. frugiperda* larvae after treatment with *A. squamosa* leaf extract at concentrations of 4-16% (T-4 to T-16), with positive (C+) and negative (C-) controls. Bars with same letter are not significantly different at $P < 0.05$ analysed using Tukey's HSD test. Error bars indicate the standard deviation of means.

Antifeedant activity bioassay

The leaf extract of *A. squamosa* significantly inhibited feeding by *S. frugiperda* larvae on maize leaves at all tested concentrations (*Fig. 2*). In a no-choice assay, the maximum antifeedant activity of 82.37% was observed at the highest concentration of 16% at 72 HAT. Antifeedant activity was also noted at 41.53%, 66.81% and 74.28% at concentrations of 4%, 8% and 10%, respectively. The observed antifeedant activity was concentration-dependent, with higher concentrations leading to greater inhibition of feeding. In the positive control (emamectin benzoate), 99.28% antifeedant activity was recorded compared to the negative control. The antifeedant properties of *A. squamosa* extracts have been previously reported against a range of lepidopteran pests, including *Plutella xylostella* (80%) (38), *Helicoverpa armigera* (>90%) (39) and *Crypsiptya coclesalis* (40), further supporting the current findings of antifeedant activity against *S. frugiperda*.

Ovicidal bioassay

The ethyl acetate extract of *A. squamosa* demonstrated complete mortality (100%) of one-day-old *S. frugiperda* eggs at concentrations ranging from 4-16% (w/v) (Fig. 3). After 72 HAT, the eggs treated with *A. squamosa* extract turned black and remained intact on the substrate, likely due to the immature nature of the egg shells, which failed to prevent the ingress of external agents (27). In the positive control (emamectin benzoate), egg hatchability was reduced to 1.33% at the recommended concentration of 0.4 g l^{-1}. The negative control, consisting of acetone and water, exhibited 87.33% egg hatchability after 72 HAT. Additionally, the leaf extract of *A. squamosa* showed a pronounced effect on egg hatchability, corroborating findings from a recent study where the ethanolic leaf extract of *A. squamosa* caused 100% egg mortality in FAW at concentrations of 1%, 2.5% and 5% (35). Similar results, showing 100% egg mortality, were reported for ethanol extracts of *Psychotria goyazensis* stem and *Psychotria capitata* leaves against newly deposited and one-day-old FAW eggs, respectively (41). Another study observed altered and reduced hatchability of FAW eggs when treated with essential oils from *Lippia origanoides*, *Cymbopogon winterianus* and *C. citratus* at concentrations ranging from 0.1% to 5.0%. The average ovicidal effect of *L. origanoides* and *C. citratus* was 97.8%, while that of *C. winterianus* was 78.02% (42).

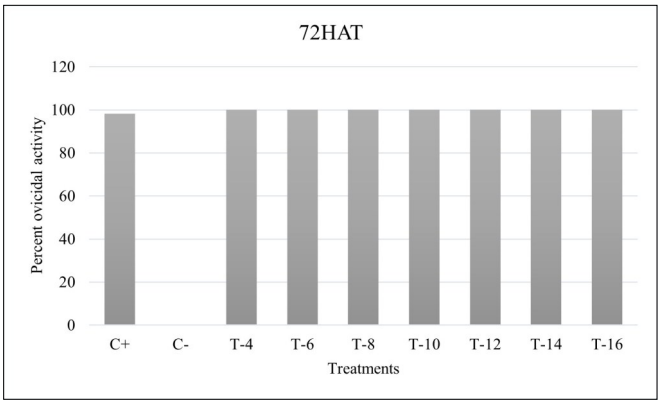

Fig. 3. Mortality of S. frugiperda eggs treated with A. squamosa leaf extract at concentrations of 4-16%, with positive (C+) and negative (C-) control.

GC-MS analysis

Phytochemicals present in plant extracts play a pivotal role in mediating various biological activities, including insecticidal, ovicidal and antifeedant properties. A total of 39 compounds were identified in the ethyl acetate extract of *A. squamosa* (Table 3). The GC-MS chromatogram (Fig. 4) revealed peak areas corresponding to various compounds, with the major components identified as 16-hentriacontanone (20.34%), neophytadiene (9.82%), γ-sitosterol (9.14%), vitamin E (8.30%), campesterol (4.29%), caryophyllene (4.13%), germacrene D (4.09%) and squalene (0.36%). Minor compounds included linoleic acid (0.99%) and nonacosanal (1.27%). The bioactive chemicals identified in *A. squamosa* contribute significantly to its biological activities. Notably, 16-hentriacontanone, which accounted for the highest peak area (20.34%), exhibits neurotoxic effects against *Sitophilus oryzae* and *Tribolium castaneum* (43). Phytol, a terpene derivative, is known for its insecticidal properties against *Bemisia tabaci* (44). Caryophyllene derivatives are reported to exhibit high contact toxicity against *Megoura japonica* and *Plutella xylostella* (45), while squalene has been shown to possess insecticidal activity against *Melanaphis sacchari* (46). The presence of these compounds in the GC-MS profile of *A. squamosa* further supports the observed insecticidal, ovicidal and antifeedant effects of its leaf extract when tested against FAW.

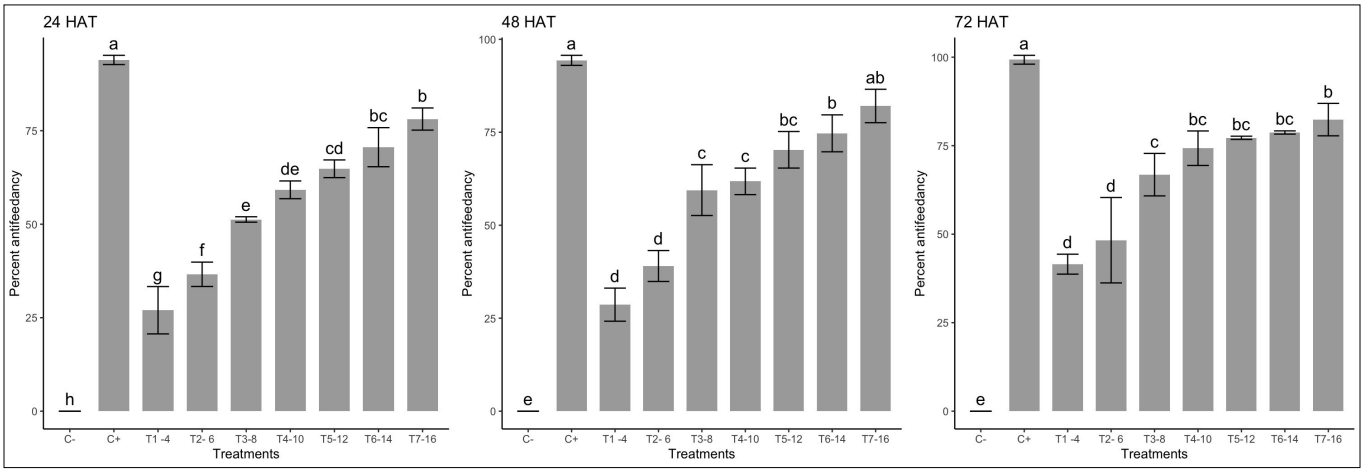

Fig. 2. Antifeedant activity of *A. squamosa* leaf extract against second instar larvae of *S. frugiperda* at concentrations of 4-16% (T-4 to T-16), with positive (C+) and negative (C-) controls. Different letters on bars indicate significant differences at $p < 0.05$ analysed using Tukey's HSD test. Error bars indicate the standard deviation of means.

Table 3. List of compounds identified in *A. squamosa* leaf extract (RT: Retention time)

S.No.	RT (min)	Compound name	Molecular formula	Molecular weight
1	14.050	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethhyl)-	$C_{15}H_{24}$	204
2	15.451	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-met thylethenyl)-	$C_{15}H_{24}$	204
3	15.840	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydr o-1,4,9,9-tetramethyl-	$C_{15}H_{24}$	204
4	16.243	Caryophyllene	$C_{15}H_{24}$	204
5	17.131	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-	$C_{15}H_{24}$	204
6	17.756	Germacrene D	$C_{15}H_{24}$	204
7	18.110	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	$C_{15}H_{24}$	204
8	18.376	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206
9	18.524	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-meto-7-methyl-4-methylene-1-(1 -methylethyl)-	$C_{15}H_{24}$	204
10	20.052	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodecaa-2,7-dienol	$C_{15}H_{26}O$	222
11	20.330	1-Nonadecene	$C_{19}H_{38}$	266
12	21.538	tau-Cadinol	$C_{15}H_{26}O$	222
13	25.636	Neophytadiene	$C_{20}H_{38}$	278
14	25.759	3,7,11,15-Tetramethylhexadec-2-ene	$C_{20}H_{40}$	280
15	29.311	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetrmethyl-, (E,E)	$C_{20}H_{34}O$	290
16	30.944	Phytol	$C_{20}H_{40}O$	296
17	32.436	Octacosanol	$C_{28}H_{58}O$	410
18	40.345	dl-Laudanosine	$C_{21}H_{27}NO_4$	357
19	40.988	Tetracosane	$C_{24}H_{50}$	338
20	42.725	Dotriacontane	$C_{32}H_{66}$	450
21	44.153	1,6,10,14,18,22-Tetracosahexaen-3-o	$C_{30}H_{50}O$	426
22	44.482	Squalene	$C_{30}H_{50}$	410
23	45.844	Tetrapentacontane	$C_{54}H_{110}$	758
24	47.185	Ergost-5-en-3-ol, acetate, (3.beta.,24R)-	$C_{30}H_{50}O_2$	442
25	47.916	Phenol-TMS	$C_9H_{14}OSi$	166
26	48.111	Cholesta-4,6-dien-3-ol	$C_{27}H_{44}O$	384
27	48.409	beta.-Sitosterol acetate	$C_{31}H_{52}O_2$	456
28	48.735	1-Hexacosanol	$C_{26}H_{54}O$	382
29	48.950	Vitamin E	$C_{29}H_{50}O_2$	430
30	49.922	Triacontane	$C_{30}H_{61}Br$	500
31	49.982	Linoleic acid	$C_{38}H_{64}O_2$	552
32	50.247	Campesterol	$C_{28}H_{48}O$	400
33	50.613	Stigmasterol	$C_{29}H_{48}O$	412
34	51.064	16-Hentriacontanone	$C_{31}H_{62}O$	450
35	51.485	gamma.-Sitosterol	$C_{29}H_{50}O$	414
36	51.819	1,6,10,14,18,22-Tetracosahexaen-3-ol	$C_{30}H_{50}O$	426
37	53.571	gamma.-Sitostenone	$C_{29}H_{48}O$	412
38	53.706	Nonacosanal	$C_{29}H_{58}O$	422
39	54.591	2,3-Nonadecanediol	$C_{19}H_{40}O_2$	300

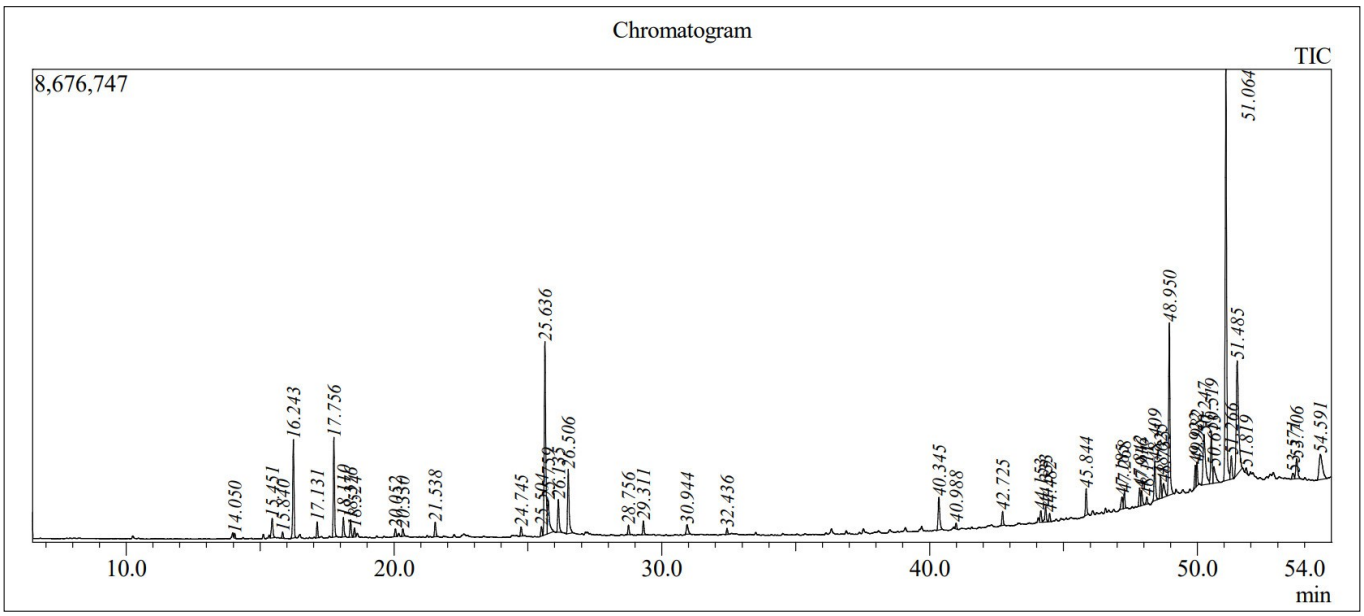

Fig. 4. GC-MS chromatogram for A. squamosa leaf extract. Major compounds were 16-Hentriacontanone (20.34%; RT-51.064 min), Neophytadiene (9.82%; RT-25.636 min), γ-Sitosterol (9.14%; RT–51.485min) and Vitamin E (8.30%; RT-48.95min).

Conclusion

Among the six local plant species screened for activity against *S. frugiperda*, *A. squamosa* leaf extract exhibited significant insecticidal and ovicidal effects. The bioassays revealed that treatment with the ethyl acetate leaf extract of *A. squamosa* resulted in a substantially higher mortality rate in FAW larvae and eggs compared to the control (acetone + water). The insecticidal activity of *A. squamosa* exhibited a dose-dependent response, with considerable larval mortality and antifeedant activity observed at higher concentrations. At all tested concentrations, *A. squamosa* extract achieved 100% egg mortality in *S. frugiperda* eggs. Given these promising results, there is potential to develop this extract into a botanical formulation. However, the consistent availability of raw materials and the high cost of extracting active compounds should be considered. Further studies are required to assess the stability of the extract, its efficacy under field conditions and its impact on natural enemy populations before it can be integrated into pest management programs.

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Authors' contributions

All authors made equal contributions to the development of the research concept, the experimental design and the provision of laboratory facilities for analysis. They also played a key role in supervising the study, interpreting the data and revising the final manuscript, ensuring its accuracy and scientific rigor.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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