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RESEARCH PAPER

Compatibility of *Piper retrofractum* and *Annona squamosa* extract mixture against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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ABSTRACT

Dependence on synthetic insecticides for controlling *Spodoptera frugiperda* has led to various adverse effects, including pest resistance, mortality of non-target organisms, and environmental pollution. These concerns necessitate the development of alternative control strategies, such as botanical insecticides that are both effective and environmentally friendly. Therefore, this study aimed to evaluate the toxicity and potential synergistic interactions of a hexane extract mixture of *Piper retrofractum* (Pr) and *Annona squamosa* (As) against second-instar larvae of *S. frugiperda*. Toxicity assays were conducted using the feed residue method in a completely randomized design with five extract mixture ratios of Pr:As (3:1, 2:1, 1:1, 1:2, and 1:3 w/w), each replicated five times. The results showed that the 2:1 (w/w) ratio was the most effective combination, yielding the lowest LC_{50} and LC_{95} values of 0.03% and 0.12%, respectively, at 96 hours after treatment. A higher proportion of Pr in the extract mixture tended to produce faster and more pronounced lethal effects on *S. frugiperda* larvae, and vice versa. The 2:1 (w/w) ratio also exhibited strong and stable synergistic interactions at all observation times at both LC_{50} and LC_{95} levels. This enhanced effectiveness is likely attributable to synergistic interactions among the active compounds present in each extract. These findings indicate that a mixture of *P. retrofractum* and *A. squamosa* extracts at a 2:1 (w/w) ratio has strong potential as an effective and efficient botanical insecticide to support the implementation of sustainable integrated pest management strategies.

Keywords: Botanical insecticide, feed residue, mortality, sustainable, synergistic

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda*) is an invasive pest that poses a serious threat to corn cultivation in various tropical and subtropical regions, including Indonesia (Sartiami et al., 2020). This pest is known for its high fecundity, strong migration behavior, and wide host range (polyphagous), which enable rapid and widespread dispersal (Westbrook et al., 2016; Montezano et al., 2018). In addition, the larvae of *S. frugiperda* can infest corn plants at all growth stages, particularly during the vegetative stage, resulting in substantial damage and yield losses (Trisyono et al., 2019; Wilyus et al., 2022; Ginting et al., 2024). Field observations in Indonesia have shown that the population of *S. frugiperda* tends to increase during the vegetative phase of maize, indicating a critical period of infestation (Lestari et al., 2024). Under severe infestation conditions, yield losses may reach up to 80% (Adhikari et al., 2020). Furthermore, FAO & CABI (2019) reported that losses due to *S. frugiperda* infestations in Africa and Europe reached 8.3–20.6 million tons per year, equivalent to US$ 2.5–6.2 billion. Such impacts are highly detrimental and pose significant challenges to achieving food security and maintaining economic stability within affected communities (Harrison et al., 2019).

Effective control of plant pests plays a vital role in sustaining agricultural productivity, particularly for strategic food commodities such as corn. In general, the management of *S. frugiperda* still relies heavily on the use of synthetic insecticides (Paredes-Sánchez et al., 2021). Although these insecticides can provide

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effective short-term control, their unwise use has the potential to cause numerous negative impacts, including pest resistance, mortality of non-target organisms, environmental pollution, and risks to human health (Dadang, 2023). The Arthropod Pesticide Resistance Database (2025) has recorded more than 270 cases of resistance in *S. frugiperda* to various insecticides worldwide, including active ingredients that are still widely recommended. This high number of resistance cases, coupled with the adverse effects of synthetic insecticides, underscores the urgency of developing alternative control strategies that are environmentally friendly and aligned with integrated pest management (IPM) principles to support sustainable agricultural systems.

In this context, botanical insecticides have gained increasing attention as eco-friendly alternatives. Recent analyses have shown that plant-derived insecticides can cause significant pest mortality across multiple plant families, supporting their potential as effective substitutes for synthetic chemicals. Moreover, comparative studies indicate that botanical insecticides tend to maintain arthropod diversity better than conventional insecticides, highlighting their ecological safety and compatibility with IPM-based pest management strategies (Khodijah et al., 2024; Hasibuan et al., 2025). Among the various candidates, *Piper retrofractum* and *Annona squamosa* are considered particularly promising due to their bioactive compounds, which have been proven effective as insecticides against major agricultural pests. Extracts of both plants have been reported to exhibit strong insecticidal activity against several pest species, such as *Crocidolomia pavonana, S. litura*, and *S. frugiperda* (Ente et al., 2020; Nenotek et al., 2022; Prijono et al., 2020; Ratwatthananon et al., 2020; Agustini et al., 2024; Bhosle et al., 2024).

Rather than relying on a single extract, combining two plant extracts can enhance insecticidal effectiveness, as their active compounds may inhibit the insect detoxification system. This inhibition increases pest susceptibility to toxic compounds, thereby significantly elevating mortality rates (Ullah et al., 2022).

The strategy of combining multiple plant extracts not only broadens the spectrum of insecticidal activity against various pest species but also helps overcome limitations in raw material availability and slows the development of pest resistance due to the different modes of action of the active compounds (Dadang, 2023).

Previous studies have shown that mixtures of plant extracts can produce significant synergistic interactions against target insects. For example, a mixture of ethyl acetate extract *P. retrofractum* and ethanol extract of *Curcuma xanthorrhiza* at a ratio of 2:1 (w/w) exhibited synergistic interactions against third-instar nymphs of *Helopeltis antonii,* with lower LC_{50} and LC_{95} values than those of the single extracts, namely 0.02% and 0.06% (Rohimatun et al., 2020). Similarly, a mixture of hexane extract of *P. aduncum* and methanol extract of *Aglaia odorata* at a ratio of 2:1 (w/w) also showed a synergistic interaction against second-instar larvae of *Plutella xylostella,* with LC_{50} and LC_{95} values of 0.06% and 0.15%, respectively (Heviyanti et al., 2025).

Synergistic interactions among compounds contained in plant extracts can increase toxicity at lower extract concentrations, making them more economical and environmentally friendly (Dadang, 2023; Pavela et al., 2025). Studies on synergistic interactions are therefore important for designing more effective and applicable botanical insecticide formulations for field use. Accordingly, this study aimed to evaluate the toxicity and potential synergistic interactions of a hexane extract mixture of *P. retrofractum* and *A. squamosa* against second-instar larvae of *S. frugiperda.* In this context, the term synergistic interaction refers to the combined biological effect—whether synergistic, additive, or antagonistic—resulting from the mixture of both plant extracts. The results of this study are expected to contribute to the development of botanical insecticides as part of a sustainable and environmentally friendly IPM strategy.

MATERIALS AND METHODS

Research Site. The study was conducted from May to November 2024. Plant material extraction, mass rearing of test insects, and toxicity assays of plant extract mixtures were carried out at the Insect Physiology and Toxicology Laboratory, Department of Plant Protection, IPB University.

Plant Material Extraction. The plant materials used were *P. retrofractum* fruits and *A. squamosa* seeds. Extraction was performed using the maceration method, beginning with cutting the plant materials, followed by drying for 7–14 days without direct exposure to sunlight to ensure uniform drying and prevent fungal contamination (Rohimatun et al., 2020). Once dried, the plant materials were ground into powder and sieved through a 1-mm mesh. The resulting powder was soaked in hexane solvent at a ratio of 1:10 (w/v) for 48 hours (Sianturi et al., 2022).

Hexane was selected as the extraction solvent because recent studies have shown that hexane extracts of *P. retrofractum* and *A. squamosa* exhibit higher insecticidal effectiveness than extracts obtained using polar solvents such as ethanol or methanol (Vetal & Pardeshi, 2019; Pumnuan et al., 2022; Tipsut et al., 2025). The filtrate obtained from the soaking process was filtered and evaporated using a rotary evaporator (RV 10 digital Pro V Complete, Germany) at 50 ºC and a pressure of 400–450 mmHg until a crude extract was obtained. The crude extracts were stored at 4 ºC in a refrigerator until use (Agustini et al., 2024). The crude extract yields were 4.11% for *P. retrofractum* and 22.67% for *A. squamosa,* calculated as the weight of crude extract relative to the initial dry weight of plant material.

Mass Rearing of *Spodoptera frugiperda*. Mass rearing of *S. frugiperda* was conducted following the method described by Sianturi et al. (2022). Second-instar larvae from the second generation were used as test insects for the bioassays.

Toxicity Assays of Mixed Extracts. Toxicity testing began with single-extract assays of *P. retrofractum* and *A. squamosa* against second-instar larvae of *S. frugiperda*. The probit regression parameters obtained from the single-extract toxicity tests at 96 hours after treatment (HAT) reported by Siregar et al. (2025a; 2025b) were used as references to determine the concentrations for the mixed-extract treatments (Table 1).

The concentrations of extract mixtures for each ratio varied depending on the probit equations of each single extract and the expected mortality proportion (Table 2). The expected mortality proportion of the extract mixture was calculated based on the combined mortality proportions caused by *P. retrofractum* (Pr) at concentration cPr and the *A. squamosa* (As) at concentration cAs. These values were derived from the probit regression results of the single-extract assays. The expected mortality calculations were used to determine mixture concentrations expected to cause test insect mortality in the range of 15–95% (Rohimatun et al., 2020; Heviyanti et al., 2025).

The mixture of *P. retrofractum* and *A. squamosa* extracts was tested using a completely randomized design (CRD) with five concentration ratios—3:1, 2:1, 1:1, 1:2, and 1:3 (w/w)—and a control, each replicated five times. Each extract mixture was first dissolved in 1% hexane and then diluted with distilled water containing 0.2% alkyl-aryl polyglycol ether 400 L until the final volume reached 100 mL. The mixture was homogenized using a magnetic stirrer for 30 min at 750 rpm and subsequently serially diluted to obtain lower concentrations. The control treatment consisted of distilled water containing hexane and alkyl-aryl polyglycol ether 400 L at a ratio of 5:1 (v/v).

Prior to application, all extract preparations were homogenized using an ultrasonic shaker to ensure

Table 1. Toxicity of hexane extracts of *P. retrofractum* and *A. squamosa* against second-instar larvae of *S. frugiperda* at 96 HAT= Hours after treatment.

Extract	a ± SE	b ± SE	LC_{50} (CI 95%) (%)	LC_{95} (CI 95%) (%)
P. retrofractum	8.24 ± 0.41	2.70 ± 0.35	0.06 (0.05–0.07)	0.26 (0.19–0.41)
A. squamosa	6.43 ± 0.17	1.79 ± 0.26	0.16 (0.11–0.21)	1.33 (0.88–2.64)

a = Probit regression intercept; b = Probit regression slope; SE = Standard error; LC = Lethal concentration; CI = Confidence interval.

Table 2. Concentration of the hexane extract mixture of *P. retrofractum* and *A. squamosa* used in the toxicity test

Extract mixture ratio (Pr : A; w/w)	Extract mixture concentration (%)														
	LC_{15}			LC_{35}			LC_{55}			LC_{75}			LC_{95}		
	Pr	As	Mix	Pr	As	Mix	Pr	As	Mix	Pr	As	Mix	Pr	As	Mix
3 : 1	0.02	0.01	0.03	0.045	0.015	0.06	0.06	0.02	0.08	0.105	0.035	0.14	0.23	0.08	0.31
2 : 1	0.03	0.01	0.04	0.04	0.02	0.06	0.06	0.03	0.09	0.10	0.05	0.15	0.21	0.11	0.32
1 : 1	0.02	0.02	0.04	0.035	0.035	0.07	0.055	0.055	0.11	0.085	0.085	0.17	0.18	0.18	0.36
1 : 2	0.01	0.03	0.04	0.02	0.04	0.06	0.03	0.06	0.09	0.05	0.10	0.15	0.11	0.21	0.32
1 : 3	0.01	0.02	0.03	0.015	0.045	0.06	0.02	0.06	0.08	0.035	0.105	0.14	0.08	0.23	0.31

LC = Lethal concentration; Pr = *Piper retrofractum*; As = *Annona squamosa*; Mix = Mixture.

uniform suspension in distilled water. Toxicity tests were conducted individually in separate trays to prevent data distortion caused by the cannibalistic behavior of *S. frugiperda* larvae, following the feed residue method. The feed consisted of fresh, insecticide-free corn leaf pieces measuring 2 cm × 2 cm. Each leaf piece was dipped into the extract solution according to the assigned treatment concentration until uniformly wetted and then air-dried. After drying, one leaf piece and one second-instar *S. frugiperda* larva were placed in each tray compartment.

Each replicate consisted of 10 larvae, resulting in a total of 50 larvae per treatment. This sample size is considered statistically sufficient for probit analysis to estimate LC_{50} and LC_{95} values with reliable confidence intervals and is commonly used in *S. frugiperda* bioassays (Sianturi et al., 2022; Agustini et al., 2024). After 48 hours of exposure, treated leaves were replaced with fresh, untreated leaves. Larval mortality was recorded at 24, 48, 72, and 96 HAT.

Compatibility Analysis of Extract Mixtures. Compatibility analysis for each extract mixture ratio was performed by calculating the combination index (CI) values at the 50% lethal concentration (LC_{50}) and 95% lethal concentration (LC_{95}) levels following the method of Chou & Talalay (1984).

$$CI = \left[\left(\frac{LC_x^{Pr(mix)}}{LC_x^{Pr}}\right) + \left(\frac{LC_x^{As(mix)}}{LC_x^{As}}\right)\right] + \left[\left(\frac{LC_x^{Pr(mix)}}{LC_x^{Pr}}\right) \times \left(\frac{LC_x^{As(mix)}}{LC_x^{As}}\right)\right]$$

LC_x^{Pr} and LC_x^{As} represent the LC_x values of each extract obtained from the single-extract toxicity tests (Table 1), whereas $LC_x^{Pr(mix)}$ and $LC_x^{As(mix)}$ represent the LC_x values from the extract mixture tests multiplied by the proportion of each extract in the mixture that caused x% mortality (e.g., 50% or 95%) (Table 3). The nature of interactions between extract mixtures was determined based on CI value criteria: strong synergistic (CI < 0.5), weak synergistic (0.5 < CI ≤ 0.77), additive (0.77 < CI ≤ 1.43), and antagonistic (CI

Table 3. Toxicity of hexane extract mixtures of *P. retrofractum* (Pr) and *A. squamosa* (As) against second-instar larvae of *S. frugiperda* at different observation times

Extract mixture ratio (Pr : As, w/w)	Observation time (HAT)	a ± SE	b ± SE	LC_{50} (95% CI) (%)	LC_{95} (95% CI) (%)
3 : 1	24	2.82 ± 0.37	2.22 ± 0.31	0.05 (0.02–0.09)	0.30 (0.15–5.94)
	48	2.81 ± 0.39	1.97 ± 0.32	0.04 (0.01–0.06)	0.26 (0.14–2.71)
	72	3.00 ± 0.42	1.99 ± 0.34	0.03 (0.01–0.05)	0.21 (0.12–1.21)
	96	2.99 ± 0.42	1.93 ± 0.35	0.03 (0.02–0.04)	0.20 (0.14–0.40)
2 : 1	24	2.90 ± 0.39	2.25 ± 0.34	0.05 (0.04–0.06)	0.28 (0.20–0.49)
	48	3.97 ± 0.63	2.65 ± 0.52	0.03 (0.02–0.04)	0.13 (0.10–0.22)
	72	3.80 ± 0.62	2.42 ± 0.52	0.03 (0.01–0.04)	0.13 (0.10–0.23)
	96	3.93 ± 0.67	2.47 ± 0.55	0.03 (0.01–0.04)	0.12 (0.09–0.21)
1 : 1	24	1.49 ± 0.27	1.58 ± 0.27	0.11 (0.09–0.15)	1.26 (0.67–4.37)
	48	2.26 ± 0.32	1.76 ± 0.30	0.05 (0.04–0.07)	0.45 (0.29–1.04)
	72	2.59 ± 0.35	1.96 ± 0.33	0.05 (0.03–0.06)	0.33 (0.23–0.65)
	96	2.90 ± 0.39	2.19 ± 0.35	0.05 (0.02–0.07)	0.27 (0.17–1.05)
1 : 2	24	1.03 ± 0.28	1.58 ± 0.28	0.22 (0.17–0.36)	2.47 (1.08–13.31)
	48	1.56 ± 0.29	1.63 ± 0.28	0.11 (0.09–0.14)	1.13 (0.60–3.85)
	72	1.73 ± 0.30	1.63 ± 0.28	0.09 (0.07–0.11)	0.89 (0.49–2.82)
	96	1.76 ± 0.30	1.63 ± 0.28	0.08 (0.06–0.10)	0.85 (0.47–2.65)
1 : 3	24	0.53 ± 0.30	1.54 ± 0.31	0.46 (0.29–1.14)	5.36 (1.80–62.20)
	48	1.15 ± 0.28	1.67 ± 0.27	0.21 (0.16–0.31)	1.98 (0.95–7.89)
	72	1.30 ± 0.27	1.57 ± 0.26	0.15 (0.12–0.21)	1.67 (0.81–6.69)
	96	1.45 ± 0.28	1.64 ± 0.26	0.13 (0.10–0.18)	1.31 (0.68–4.38)

HAT = Hours after treatment; a = Probit regression intercept, b = Probit regression slope, Pr = *Piper retrofractum*, As = *Annona squamosa*, SE = Standard error, LC = Lethal concentration, CI = Confidence interval.

> 1.43) (Kosman & Cohen, 1996).

Data Analysis. Larval mortality data were analyzed using probit analysis with the PoloPlus program to determine LC_{50} and LC_{95} values for each extract mixture ratio at each observation time point.

RESULTS AND DISCUSSION

Toxicity of *P. retrofractum* and *A. squamosa* Extract Mixtures. The effectiveness of a botanical insecticide, whether applied singly or in combination, is strongly influenced by the composition of its active compounds and their interactions with the physiological systems of the target insect. The results of toxicity testing of hexane extract mixtures of *P. retrofractum* (Pr) and *A. squamosa* (As) against second-instar larvae of *S. frugiperda* showed a consistent increase in larval mortality with increasing observation time and extract concentration. In general, all extract mixture ratios exhibited lethal effects from 24 hours after treatment (HAT), with mortality continuing to increase up to 96 HA (Figure 1).

The Pr:As ratios of 2:1 and 3:1 (w/w) were the most effective combinations, causing high larval mortality within a relatively short period. At the highest concentration, these ratios achieved 100% mortality within 48 HAT. Similarly, the Pr:As ratio of 1:1 (w/w) also demonstrated considerable effectiveness, although it required a longer time to reach maximum mortality compared with the 2:1 and 3:1 ratios. In contrast, the Pr:As ratios of 1:2 and 1:3 (w/w) resulted in lower mortality and did not achieve 100% mortality even after 96 HAT at the highest concentration.

A higher proportion of *P. retrofractum* in the extract mixture resulted in faster and more lethal effects on *S. frugiperda* larvae, and vice versa. This pattern was consistently observed for the 2:1 and 3:1 (w/w) ratios, which produced lower LC_{50} and LC_{95} values than the other ratios from 24 to 96 HAT. Probit analysis indicated that the 2:1 (w/w) ratio was the most toxic mixture, with the lowest LC_{50} and LC_{95} values of 0.03% and 0.12%, respectively, at 96 HAT. The 3:1 and 1:1 (w/w) ratios also exhibited high toxicity, but slightly lower than that of the 2:1 ratio, particularly as reflected by higher LC_{95} values at each observation interval. Conversely, the 1:2 and 1:3 (w/w) ratios, in which *A. squamosa* extract was dominant, it showed lower toxicity, as shown by highe LC_{50} and LC_{95} values (Table 3).

The high effectiveness observed at the 2:1 and 3:1 (w/w) ratios can be largely attributed to the dominance of active compounds from *P. retrofractum*, particularly piperamides, which are known to possess strong neurotoxic activity with rapid knockdown effects (Miyakado et al., 1989; Sholehah & Ulya, 2024). Ratwatthananon et al. (2020) reported that hexane extract of *P. retrofractum* fruit exhibited high toxicity against *S. litura* larvae, with an LD_{50}value of 0.87 µg/larvae at 24 HAT, indicating rapid lethal activity. extract mixtures with ratios of 1:1, 1:2, and 1:3 (w/w) showed reduced effectiveness compared with the 2:1 and 3:1 ratios. At the 1:1 ratio, the balanced proportions of *P. retrofractum* and *A. squamosa* extracts resulted in the

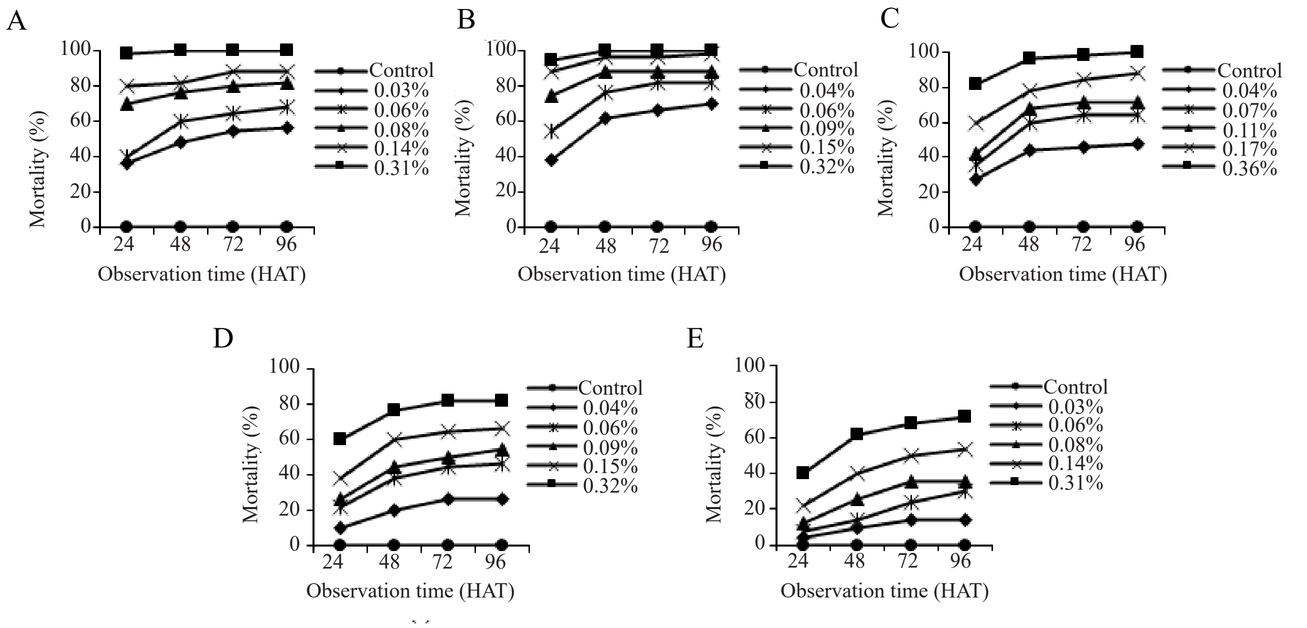

Figure 1. Mortality development of *S. frugiperda* larvae treated with mixtures of *P. retrofractum* and *A. squamosa* extract. A. 3:1; B. 2:1; C. 1:1; D. 1:2; E. 1:3 (w/w).

simultaneous action of different modes of action. The decline in effectiveness became more pronounced at the 1:2 and 1:3 ratios, presumably due to the increasing dominance of active compounds from *A. squamosa*.

Acetogenins produced by *A. squamosa* exert their insecticidal effects by inhibiting mitochondrial complex I in the electron transport chain, thereby disrupting ATP production and inducing cell death through apoptosis (Hidalgo et al., 2018; Durán-Ruiz et al., 2024). This mode of action differs from that of piperamides, which act more rapidly by inhibiting acetylcholinesterase, leading to acute neural disruption and rapid insect mortality. Consequently, the dominance of acetogenins in the extract mixture contributes to slower toxic effects on larvae due to their more gradual and specific mode of action. This hypothesis is supported by previous reports indicating that acetogenins from the Annonaceae family generally exhibit delayed but effective insecticidal activity. For example, Souza et al. (2017) reported that rolliniastatin-1, an acetogenin isolated from *A. mucosa*, caused complete mortality of of *Helicoverpa armigera* larvae only after 120 hours of exposure.

Compatibility of *P. retrofractum* and *A. squamosa* Extract Mixtures. Evaluation of combination index (CI) values revealed that mixtures of *P. retrofractum* and *A. squamosa* hexane extract against second-instar larvae of *S. frugiperda* exhibited varying interaction patterns depending on the mixture ratio and observation time. Among all tested ratios, the 2:1 (w/w) mixture was the most effective and stable, consistently producing strong synergistic interactions at all observation times at both LC_{50} and LC_{95} levels. The low CI values at these concentrations indicate a high level of toxicity, while the stability of the interaction suggests that this mixture maintains enhanced effectiveness over prolonged exposure periods. In contrast, other mixture ratios exhibited less stable interaction patterns, with CI values tending to increase as exposure time increased (Table 4).

The strong synergistic interaction observed

Table 4. Interaction effects of *P. retrofractum* and *A. squamosa* hexane extract mixtures on second-instar larval mortality of *S. frugiperda*

Extract mixture ratio (Pr : As, w/w)	Observation time (HAT)	Combination index value			
		LC_{50}	Criteria	LC_{95}	Criteria
3 : 1	24	0.32	SS	0.17	SS
	48	0.45	SS	0.57	LS
	72	0.44	SS	0.64	LS
	96	0.41	SS	0.65	LS
2 : 1	24	0.28	SS	0.14	SS
	48	0.36	SS	0.26	SS
	72	0.36	SS	0.35	SS
	96	0.34	SS	0.35	SS
1 : 1	24	0.49	SS	0.51	LS
	48	0.49	SS	0.69	LS
	72	0.58	LS	0.73	LS
	96	0.59	LS	0.67	LS
1 : 2	24	0.73	LS	0.73	LS
	48	0.83	AD	1.24	AD
	72	0.97	AD	1.60	AN
	96	0.93	AD	1.99	AN
1 : 3	24	1.32	AD	1.35	AD
	48	1.44	AN	1.78	AN
	72	1.63	AN	2.78	AN
	96	1.45	AN	2.95	AN

Pr = *Piper retrofractum*; As = *Annona squamosa*; HAT = Hours after treatment; SS = Strongly synergistic; LS = Less synergistic; AD = Additive; AN = Antagonistic.

at the 2:1 (w/w) ratio is likely due to the combined action of the active compounds present in each extract. Although piperamides from *P. retrofractum* and acetogenins from *A. squamosa* differ in their primary modes of action, their combination may enhance toxicity through metabolic interactions. Kulkarni & Hodgson (1980) reported that synergism can occur when one compound inhibits metabolic detoxification enzymes, thereby increasing the toxicity of other compounds. In this context, piperamides containing methylenedioxyphenyl groups act as synergists by inhibiting detoxification enzymes, particularly cytochrome P450 monooxygenases (Scott et al., 2008; Xiang et al., 2016). Inhibition of these enzymes reduces the metabolic degradation of acetogenins, allowing them to persist longer within the insect body and reach their mitochondrial targets more effectively. This disruption of detoxification processes enhances toxin accumulation from both extracts, resulting in greater toxicity than when either extract is applied alone. Furthermore, combining compounds with different modes of action can broaden pest control spectra and slow the development of insecticide resistance (Dougoud et al., 2019; Dadang, 2023).

A shift in interaction patterns became evident at the 1:2 (w/w) ratio, in which the proportion of *A. squamosa* extract exceeded that of *P. retrofractum* extract. At this ratio, strong synergistic interactions were no longer observed. From the initial observation period, both LC_{50} and LC_{95} values exhibited weak synergistic interactions, which gradually shifted toward additive effects as exposure time increased. Additive interactions indicate that the combined effect of the two extracts is equivalent to the sum of their individual effects, without enhanced toxicity. At later observation times, particularly at LC_{95} from 72 to 96 HAT, the interaction shifted to antagonistic, indicating reduced effectiveness of the mixture. This trend became more pronounced at the 1:3 (w/w) ratio, where antagonistic interaction were observed as early as 48 HAT at both LC_{50} and LC_{95}. High CI values (>1.43) indicate that the extracts reduce each other's effectiveness, resulting in lower toxicity of the mixture compared with single-extract applications.

These findings highlight the potential of *P. retrofractum* and *A. squamosa* extract mixtures—particularly at the 2:1 ratio—for development as practical botanical insecticides. From a technological perspective, these extracts could be formulated into emulsifiable concentrates, wettable powders, or nanoemulsions to improve stability, ease of handling, and field applicability. For farmers, such formulations would offer safer and more environmentally friendly alternatives to synthetic insecticides, reducing risks of resistance development, non-target effects, and pesticide residues. Moreover, the strong synergistic effect observed at the 2:1 ratio suggests that effective pest control could be achieved at lower application rates, thereby reducing production costs and increasing feasibility for smallholder farmers. These advantages are particularly relevant for sustainable maize production systems in regions severely affected by *S. frugiperda* infestations.

Despite these promising results, several limitations of this study should be acknowledged. All experiments were conducted under controlled laboratory conditions, and field performance may vary due to environmental factors. Potential phytotoxic effects on maize plants were not evaluated and should be examined in future studies. In addition, variability in plant material quality and extraction yield may affect consistency, underscoring the need for standardized extraction and formulation protocols for large-scale application. Addressing these limitations will require further research on formulation stability, quality control, and field validation. Future studies should also assess the economic feasibility of developing these extracts into commercial products to ensure that they are effective, affordable, and accessible to farmers.

CONCLUSION

In conclusion, the hexane extract mixture of *P. retrofractum* and *A. squamosa* at a ratio of 2:1 (w/w) proved to be the most effective in controlling *S. frugiperda*, as indicated by the lowest LC_{50} and LC_{95} values and strong, stable synergistic interactions. This effectiveness can be attributed to synergistic interactions between the active compounds in each extract, which possess different but complementary modes of action. The combination increased toxicity at low concentrations and short exposure durations, highlighting its potential to reduce reliance on synthetic insecticides and to provide an environmentally friendly alternative for sustainable integrated pest managemen. Further studies are recommended to develop stable botanical insecticide formulations based on the most effective extract ratios, followed by evaluations of field efficacy, formulation stability, and ecological safety, including potential impacts on natural enemies. These steps are essential to ensure the practical applicability of this botanical insecticide in agricultural system.

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AUTHORS' CONTRIBUTIONS

DD, DS, and IWW conceived and supervised the research design. HMS, SE, and GI conducted the toxicity assays of the extract mixtures, analyzed larval mortality data, and drafted the manuscript. All authors contributed to refining the research design, data analysis and interpretation, and provided critical feedback to improve the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST

The authors declare that they have no competing interests regarding the publication of this manuscript.

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