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Research article

Enhancing the control efficiency of plant extracts against *Spodoptera frugiperda* (Lepidoptera: Noctuoidea) through mixture formula development

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Abstract

Importance of this work: The fall armyworm, *Spodoptera frugiperda*, is a major agricultural pest, requiring effective and sustainable control methods. This study enhances control efficiency by developing a plant extract mixture that significantly increases mortality while reducing the required dosage.

Objectives: To assess the insecticidal potential of various plant crude extracts against *S. frugiperda* larvae for the future development of effective insecticide products.

Materials and methods: The insecticidal potential was investigated of various crude extracts from *Cyperus rotundus* mixed with *Piper retrofractum* against *S. frugiperda* larvae. The acute effects were determined of binary mixtures of various compounds at concentrations equal to the doses required to kill 30%, 20% and 10% of the tested population after the specified test duration (24 hr or 48 hr).

Results: The best formulation was the combination of *P. retrofractum* hexane crude extract (at a dose of 10.57 parts per million, ppm) and *C. rotundus* dichloromethane extract (at a dose of 2,504 ppm), which caused mortality rates of up to 98% compared with 20% when the individual extracts were used.

Main finding: This mixture formulation allowed the use of smaller amounts of plant substances, resulting in lower production costs. This information should be useful for the future development of effective insecticide products.

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Introduction

Thailand's exports of corn in 2023 reached USD 120 million, making it the 27th largest exporter of corn in the world (The Observatory of Economic Complexity, 2024). However, sweet corn production can be negatively affected by climate change, plant disease, and insect pest problems. Thus, domestic farmers must monitor the quality and quantity of their produce to counter diseases and pests such as *Spodoptera frugiperda (*Paredes-Sánchez et al., 2021). The current research focused on *S. frugiperda*, which has been reported to spread and cause problems for farmers, since its spread from America into Asia, including Thailand (Jing et al., 2020)

S. frugiperda is a polyphagous insect that is found globally and has been reported to feed on 80 species of plants, mainly in the grass family (Poaceae), such as corn, rice and sorghum (Jing et al., 2020), as well as destroying plants in the sunflower (Asteraceae) and bean (Fabaceae) families, among others (Jing et al., 2020). Furthermore, this insect has adapted and developed resistance to various types of pesticides (Moustafa et al., 2024). While this pest is native to the Americas and is distributed from the USA southward to Argentina, outbreaks have occurred in Africa and in Asia, where it from India to Myanmar and eventually entered Thailand (Jing et al., 2020). This species was first found along the Thailand-Myanmar border between Tak and Mae Hong Son provinces in Thailand.

At present, farmers use broadscale applications of synthetic insecticides to prevent and eliminate this pest among others. However, most chemical pesticides are imported, leading to the problem of high production costs, as well as resistance to these pesticides (Moustafa et al., 2024). Consequently, farmers must use increasing amounts of pesticides every year, causing toxic or allergic reactions in farmers, consumers, or people living near farms, along with toxic impacts on pets and the various economic problems associated with the presence of toxic residues in agriculturally exported products (Moustafa et al., 2024). Often, these chemical insecticides act on a broad toxicity spectrum, causing toxic effects on beneficial organisms, such as insect predators and parasitoids, resulting in the loss of ecological balance (Moustafa et al., 2024).

Consequently, many studies have investigated the effectiveness of new insecticides for the control of *S. frugiperda* populations, including the use of nuclear polyhedrosis virus (NPV) and microbial toxins such as *Bacillus thuringiensis* (Pavan et al., 2024; Zhou et al., 2023). The current research adopted the well-established strategy of using plant extracts to prevent and control insect pests. Plants that contain chemical components (secondary metabolites) can be used to control various pests, such as *Combretum trifoliatum* extract being used to control *S. frugiperda* (Changkeb et al., 2023); *Artocarpus lacucha* being used to control *S. litura* (Rattanaphan et al., 2023); and the phenolic secondary metabolites from *Acorus calamus* (Acorales: Acoraceae) rhizomes being used as feeding deterrents for *S. litura* (Kumrungsee et al., 2023).

The use of plants to protect crops against insect pests is not just a practice of local protection, since this approach continues to be used by farmers globally, mainly in areas where access to synthetic pesticides is more difficult, as well as in organic farming, where plants in the form of extracts, companion plants or just the harvested plants themselves are used (Belmain and Stevenson, 2001). It has been shown that complex mixtures are more efficient, with synergistic effects being reported (Yooboon et al., 2019). Thus, mixtures of plant compounds might be more durable against the evolution of resistance in insects and the development of behavioral desensitization.

The current study aimed to assess the insecticidal potential of various crude extracts of *Cyperus rotundus* mixed with those of *Piper retrofractum* against *S. frugiperda* larvae. The findings should offer valuable insights for the development of natural insecticides targeting these pests, resulting in lower production costs, which is necessary for the future development of effective insecticide products.

Materials and Methods

Insect rearing

All stages of *S. frugiperda* larvae were reared in environmental chambers (MLR-32H; Panasonic; Japan) at the Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand. They were fed an artificial diet in a temperature-controlled cabinet at 27°C, with a relative humidity of 70% and a photoperiod of 14:10 (hours of light-to-darkness). The artificial food was changed every 3 d to prevent infection. After the eggs had hatched, all larvae were moved to a new box with an artificial diet and the food was changed every 3 d. In the pupal stage, the pupae were moved to a cage (dimensions 16 cm × 21 cm × 16 cm) covered with paper. Each pupa was cleaned with 40% formaldehyde for 10 min to avoid infection and then rinsed with sterile water for 10 min and air-dried. The adults were fed 20% honey and the eggs they produced were then used for development and further experiments. The insect-rearing methods were approved by the Animal Use Ethics Committee of Kasetsart University, Bangkok, Thailand (ACKU67-SCI-017).

Plant extraction

The plant samples (*P. retrofractum* and *C. rotundus* rhizomes) were air-dried and then ground. *C. rotundus* was sequentially extracted at room temperature with hexane, dichloromethane, ethyl acetate, or ethanol. Based on other previous research on *P. retrofractum* dry fruits (Ratwatthananon, 2020), the *P. retrofractum* rhizomes were extracted using hexane only. All extracts were processed by evaporating the solvent in a rotary evaporator and then freeze-drying, after which the crude extract was in the refrigerator until the experiments were carried out to assess the toxicity of both single substances and mixtures of compounds.

Toxicity evaluation of plant extracts based on death rate of cutworm groups

This test was performed using a completely randomized design on second-instar *S. frugiperda* larvae (the stage in which larvae begin to spread within the field). Both extracts, separately or mixed at a ratio of 1:1, were tested individually according to concentration and compared with the control group. Each concentration was dissolved with acetone (analytical reagent grade) to evaluate the toxicity level in terms of the percentage of death via the topical application method using a micropipette.

The toxicity test used the topical application method, based on dropping the substance directly on the thoracic region of the worm, ensuring that all worms received a thorough wetting by the substance.

These experiments were performed five times per concentration, with 30 insects per concentration. After the test, all the treated insects were placed in the insect-rearing chamber, and the mortality percentage was recorded after exposure for 24 hr and 48 hr. Then, the doses required to kill half the members of the tested population (LD_{50}) after the specified test duration (24 hr or 48 hr) were analyzed using the StatPlus program (Version iOS; Analystsoft; US).

The acute effects of a binary mixture of the various compounds used in the study were determined as described above. As the goal was to reduce the quantity of compounds used, initially, the doses required to kill 30%, 20% and 10% of the tested population (LD_{30}, LD_{20} and LD_{10}, respectively,) after the specified test duration (24 hr or 48 hr) of each compound were chosen for pairing. Actual mortalities were compared with expected mortalities based on Equation 1, according to Trisyono and Whalon (1999):

$$E = O_a + O_b\,(1 - O_a) \qquad (1)$$

where E is the expected mortality and O_a and O_b are the observed mortalities of the pure compound A and B at the given concentration, respectively. The effects of mixtures were designated antagonistic, additive, or synergistic based on analysis using chi-squared (χ^2) comparisons (Equation 2):

$$\chi^2 = (O_m - E)^2 / E \qquad (2)$$

where O_m is the observed mortality from the binary mixture and E is the expected mortality for χ^2 with 1 degree of freedom and alpha = 0.05 having a value of 3.84. A pair with $\chi^2 > 3.84$ and greater than expected mortality was synergistic, with $\chi^2 < 3.84$ representing additive effects. The observed mortality being less than expected suggested an antagonistic effect of the mixtures.

Ethics statement

This study was approved by the Ethics Committee of Kasetsart University, Bangkok, Thailand (Approval no. ACKU67-SCI-017).

Results

Toxicity of plant extracts to S. frugiperda

Increasing concentrations of the extract from *C. rotundus* increased the mortality of *S. frugiperda*. After testing for 24 hr, the *C. rotundus* dichloromethane extract produced the highest mortality (92.33%), with an LD_{50} value of 4,582 µg/larva, followed by the methanol (81.33%), ethyl acetate (45.50%) and hexane (33.65%) extracts (Table 1), with no mortality occurring in the control.

Table 1 Comparative values of *Spodoptera frugiperda* mortality following treatment with various plant extracts after 24 hr.

Plant type	LD_{50} ± SE	LD_{10} ± SE	LD_{20} ± SE	LD_{30} ± SE	Regression equation	χ^2	*p* Value
C. rotundus EtOH extract	13941.09±5018.60	3359.70±838.61	5467.59±1093.77	7784.42±1789.47	Y=-3.57+2.06X	9.14	0.01
C. rotundus hexane extract	5109.14±418.40	1265.69±188.04	2044.06±218.67	2887.96±246.24	Y=-2.84+2.12X	3.30	0.19
C. rotundus EtOAc extract	13947.09±5018.60	3349.71±838.61	5467.59±1093.77	7784.42±1789.47	Y=-3.57+2.06X	9.14	0.01
C. rotundus DCM extract	4582.47±2793.22	1045.41±618.20	1736.80±322.80	2504.45±264.80	Y=-2.31+1.99X	8,19	0.01
P. retrofractum hexane extract	33.65±9.94	5.7±0.33	10.57±0.21	16.32±0.21	Y=2.45+1.66X	0.49	0.48

DCM =dichloromethane; EtOAc = ethyl acetate; EtOH = ethanol.

LD_{50}, LD_{30}, LD_{20} and LD_{10} = doses required to kill 50%, 30%, 20% and 10% of the tested population, respectively, after the specified test duration (24 hr).

Toxicity of mixed extracts to S. frugiperda

As shown in Table 1, *C. rotundus* crude extracts prepared with various solvents were mixed with *P. retrofractum* hexane crude extract at a ratio of 1:1 at doses of LD_{30}, LD_{20} or LD_{10}. The toxicity values are shown in Tables 2–3, revealing that the formula combining *P. retrofractum* hexane crude extract-to-*C. rotundus* dichloromethane crude extract ratio of LD_{20}:LD_{30} was the only formula that exhibited a synergistic effect, with 23 other formulas having an additive effect and another 12 formulas having an antagonistic effect.

Table 2 Toxicity values after 24 hr on *Spodoptera frugiperda* due to different mixtures of *Cyperus rotundus* extract (CR) and *Piper retrofractum* extract (PR)

LD ratio	Extraction PR	method CR	Om	Om/100	Oa/100	Ob/100	E/100	χ^2 × 100	Effect	Oa	Ob	E
10:10	Hexane	Hexane	8.00	0.08	0.13	0.13	0.25		Antagonistic	13.33	13.33	24.88
10:20	Hexane	Hexane	13.33	0.13	0.13	0.23	0.34		Antagonistic	13.33	23.33	33.55
10:30	Hexane	Hexane	16.66	0.16	0.13	0.34	0.43		Antagonistic	13.33	33.66	42.50
20:10	Hexane	Hexane	36.66	0.36	0.23	0.13	0.34	0.29	Additive	23.33	13.33	33.55
20:20	Hexane	Hexane	46.66	0.46	0.23	0.23	0.41	0.72	Additive	23.33	23.33	41.21
20:30	Hexane	Hexane	50.00	0.50	0.23	0.34	0.49	0.02	Additive	23.33	33.66	49.13
30:10	Hexane	Hexane	13.33	0.13	0.30	0.13	0.39		Antagonistic	30.00	13.33	39.33
30:20	Hexane	Hexane	46.66	0.46	0.30	0.23	0.46	0.00	Additive	30.00	23.33	46.33
30:30	Hexane	Hexane	60.00	0.60	0.30	0.34	0.54	0.77	Additive	30.00	33.66	53.56
10:10	Hexane	DCM	23.33	0.23	0.13	0.15	0.26		Antagonistic	13.33	15.00	26.33
10:20	Hexane	DCM	36.66	0.36	0.13	0.23	0.34	0.29	Additive	13.33	23.33	33.55
10:30	Hexane	DCM	43.33	0.43	0.13	0.30	0.39	0.41	Additive	13.33	30.00	39.33
20:10	Hexane	DCM	38.33	0.38	0.23	0.15	0.35	0.35	Additive	23.33	15.00	34.83
20:20	Hexane	DCM	39.33	0.39	0.23	0.23	0.41	0.09	Additive	23.33	23.33	41.21
20:30	Hexane	DCM	98.00	0.98	0.23	0.30	0.46	57.62	Synergistic	23.33	30.00	46.33
30:10	Hexane	DCM	50.66	0.506	0.30	0.15	0.41	2.55	Additive	30.00	15.00	40.50
30:20	Hexane	DCM	50.66	0.506	0.30	0.23	0.46	0.40	Additive	30.00	23.33	46.33
30:30	Hexane	DCM	55.00	0.55	0.30	0.30	0.51	0.31	Additive	30.00	30.00	51.00
10:10	Hexane	EtOAC	17.00	0.17	0.13	0.17	0.28		Antagonistic	13.33	16.66	27.76
10:20	Hexane	EtOAC	23.33	0.23	0.13	0.23	0.34		Antagonistic	13.33	23.33	33.55
10:30	Hexane	EtOAC	40.00	0.40	0.13	0.37	0.45		Antagonistic	13.33	36.66	45.10
20:10	Hexane	EtOAC	45.00	0.45	0.23	0.17	0.36	2.19	Additive	23.33	16.66	36.10
20:20	Hexane	EtOAC	45	0.45	0.23	0.23	0.41	0.35	Additive	23.33	23.33	41.21
20:30	Hexane	EtOAC	56.66	0.57	0.23	0.37	0.51	0.53	Additive	23.33	36.66	51.43
30:10	Hexane	EtOAC	20	0.20	0.30	0.17	0.42		Antagonistic	30	16.66	41.66
30:20	Hexane	EtOAC	50	0.50	0.30	0.23	0.46	0.29	Additive	30	23.33	46.33
30:30	Hexane	EtOAC	60	0.60	0.30	0.37	0.56	0.34	Additive	30	36.66	55.66

Table 2 Continued

LD ratio	Extraction PR	method CR	Om	Om/100	Oa/100	Ob/100	E/100	$\chi^2 \times 100$	Effect	Oa	Ob	E
10:10	Hexane	EtOH	25	0.25	0.13	0.10	0.22	0.41	Additive	13.33	10	21.99
10:20	Hexane	EtOH	36.66	0.37	0.13	0.23	0.34	0.29	Additive	13.33	23.33	33.55
10:30	Hexane	EtOH	43.33	0.43	0.13	0.37	0.45		Antagonistic	13.33	36.66	45.10
20:10	Hexane	EtOH	33.33	0.33	0.23	0.10	0.31	0.18	Additive	23.33	10	30.99
20:20	Hexane	EtOH	46.66	0.47	0.23	0.23	0.41	0.72	Additive	23.33	23.33	41.21
20:30	Hexane	EtOH	40	0.40	0.23	0.37	0.51		Antagonistic	23.33	36.66	51.43
30:10	Hexane	EtOH	43.33	0.43	0.30	0.10	0.37	1.08	Additive	30	10	37.00
30:20	Hexane	EtOH	43.33	0.43	0.30	0.23	0.46		Antagonistic	30	23.33	46.33
30:30	Hexane	EtOH	60	0.60	0.30	0.37	0.56	0.34	Additive	30	36.66	55.66

LD ratio = lethal dose level ratio of *C. rotundus* extract (CR) mixed with *P. retrofractum* extract.

E = expected mortality; O_m = the observed mortality from the binary mixture; Oa = the observed mortalities of the pure compound A at the given concentration; Ob = the observed mortalities of the pure compound B at the given concentration.

Table 3 Mortality after 24 hr of *Spodoptera frugiperda* due to different mixtures of *Cyperus rotundus* extract (CR) and *Piper retrofractum* extract (PR)

LD ratio of PR to CR + their extraction methods	*P. retrofractum* (ppm)	*C. rotundus* (ppm)	Mortality (%)	Effect
(CR) Hexane	-	1,265	13.33 ± 0.57	-
	-	2,044	23.33 ± 0.57	-
	-	2,887	33.66 ± 0.57	-
(CR) DCM	-	1,265	15 ± 0.57	-
	-	2,044	23.33 ± 0.57	-
	-	2,887	30.66 ± 0.57	-
(CR) EtOAC	-	1,265	16.66 ± 0.57	-
	-	2,044	23.33 ± 0.57	-
	-	2,887	36.66 ± 0.57	-
(CR) EtOH	-	1,265	10.00 ± 0.57	-
	-	2,044	23.33 ± 0.57	-
	-	2,887	36.66 ± 0.57	-
(PR) Hexane	5.7	-	13.33 ± 0.57	
	10.57	-	23.33 ± 0.57	
	16.32	-	30.0 0.57	
10:10 (PR) Hexane:(CR) Hexane	5.7	1,265	8.00 ± 1.00	Antagonistic
10:20 (PR) Hexane:(CR) Hexane	5.7	2,044	13.33 ± 0.57	Antagonistic
10:30 (PR) Hexane:(CR) Hexane	5.7	2,887	16.66 ± 0.57	Antagonistic
20:10 (PR) Hexane:(CR) Hexane	10.57	1,265	36.66 ± 0.57	Additive
20:20 (PR) Hexane:(CR) Hexane	10.57	2,044	46.66 ± 0.57	Additive
20:30 (PR) Hexane:(CR) Hexane	10.57	2,887	50.00 ± 0.00	Additive
30:10 (PR) Hexane:(CR) Hexane	16.32	1,265	13.33 ± 0.57	Antagonistic
30:20 (PR) Hexane:(CR) Hexane	16.32	2,044	46.66 0.57	Additive
30:30 (PR) Hexane:(CR) Hexane	16.32	2,887	60.00 0.00	Additive
10:10 (PR) Hexane:(CR) Dichloromethane	5.7	1,045	23.33 ± 0.57	Antagonistic
10:20 (PR) Hexane:(CR) Dichloromethane	5.7	1,736	36.66 ± 0.57	Additive
10:30 (PR) Hexane:(CR) Dichloromethane	5.7	2,504	43.33 ± 1.15	Additive
20:10 (PR) Hexane:(CR) Dichloromethane	10.57	1,045	39.33 ± 0.57	Additive
20:20 (PR) Hexane:(CR) Dichloromethane	10.57	1,736	98.33 ± 0.57	Additive
20:30 (PR) Hexane:(CR) Dichloromethane	10.57	2,504	98.00 ± 2.00	Synergistic
30:10 (PR) Hexane:(CR) Dichloromethane	16.32	1,045	50.66 ± 0.57	Additive
30:20 (PR) Hexane:(CR) Dichloromethane	16.32	1,736	50.66 ± 0.57	Additive
30:30 (PR) Hexane:(CR) Dichloromethane	16.32	2,504	55.00 ± 0.00	Additive
10:10 (PR) Hexane:(CR) Ethyl acetate	5.7	3,449	17.00 ± 0.00	Antagonistic
10:20 (PR) Hexane:(CR) Ethyl acetate	5.7	5,467	23.33 ± 0.57	Antagonistic
10:30 (PR) Hexane:(CR) Ethyl acetate	5.7	7,784	40.00 ± 1.00	Antagonistic
20:10 (PR) Hexane:(CR) Ethyl acetate	10.57	3,449	45.00 ± 0.57	Additive
20:20 (PR) Hexane:(CR) Ethyl acetate	10.57	5,467	45.00 ± 0.00	Additive

Table 3 Continued

LD ratio of PR to CR + their extraction methods	*P. retrofractum* (ppm)	*C. rotundus* (ppm)	Mortality (%)	Effect
20:30 (PR) Hexane:(CR) Ethyl acetate	10.57	7,784	56.66 ± 0.57	Additive
30:10 (PR) Hexane:(CR) Ethyl acetate	16.32	3,449	20.00 ± 1.00	Antagonistic
30:20 (PR) Hexane:(CR) Ethyl acetate	16.32	5,467	50.00 ± 0.00	Additive
30:30 (PR) Hexane:(CR) Ethyl acetate	16.32	7,784	60.00 ± 0.00	Additive
10:10 (PR) Hexane:(CR) Ethanol	5.7	3,359	25.00 ± 0.57	Additive
10:20 (PR) Hexane:(CR) Ethanol	5.7	5,467	36.66 ± 0.57	Additive
10:30 (PR) Hexane:(CR) Ethanol	5.7	8,7784	43.33 ± 0.57	Antagonistic
20:10 (PR) Hexane:(CR) Ethanol	10.57	3,359	33.33 ± 0.57	Additive
20:20 (PR) Hexane:(CR) Ethanol	10.57	5,467	46.66 ± 1.53	Additive
20:30 (PR) Hexane:(CR) Ethanol	10.57	8,7784	40.00 ± 2.65	Antagonistic
30:10 (PR) Hexane:(CR) Ethanol	16.32	3,359	43.33 ± 0.57	Additive
30:20 (PR) Hexane:(CR) Ethanol	16.32	5,467	43.33 ± 0.57	Antagonistic
30:30 (PR) Hexane:(CR) Ethanol	16.32	87,784	60.00 ± 0.00	Additive

DCM = dichloromethane; ppm = parts per million.

LD ratio of PR to CR = LD_{30}, LD_{20} and LD_{10} = doses required to kill 30%, 20% and 10% of the tested population, respectively, after the specified test duration (24 hr).

PR (extraction method): CR (extraction method) = extraction method used for *P. retrofractum* and for *C. rotundus*, respectively.

Discussion

The plant extract compounds from *C. rotundus* and *P. retrofractum* were extracted using various solvents, with the goal of farmers being able to use this knowledge to develop the components of an insecticide to control *S. frugiperda*, as well as potentially reducing the cost of pest control. All the crude extracts were evaluated via topical application to second-instar *S. frugiperda* larvae. Based on the results of the toxicity test, there was a clear effect on the worms.

P. retrofractum and *C. rotundus* are used as alternative medicines and supplements in primary health care settings worldwide (Farnsworth and Bunyapraphatsara, 1992; Pasam et al., 2021). They are less toxic to nontarget organisms and mammals. For example, one study by Wiwattanawanichakun et al. (2018) showed that *P. retrofractum* was moderately toxic to guppy fish compared with the known data available for synthetic pesticides. In addition, methanol extracts of *C. rotundus* rhizomes given orally at doses of 250 mg/kg body weight (b.w.) and 500 mg/kg b.w. showed significant antidiarrheal activity in mice with castor oil-induced diarrhea (Uddin, 2006).

Based on the results from the current study, after direct exposure to the mixture of plant extract compounds, the treated *S. frugiperda* began to move abnormally and then stopped at 24 and 48 hours, respectively. The percentage of dead worms did not significantly differ between the two periods. This finding may indicate that the crude *C. rotundus* extract, after being mixed with the *P. retrofractum* extract, was acutely toxic to *S. frugiperda* within 24 hours.

Similarly, Singh and Bapatla (2022) reported that *C. rotundus* rhizome extract prepared by soaking in methanol was toxic to *S. litura*, causing 60% mortality, with a Median lethal concentration (LC_{50}) value of 5.17 μg/larva. In addition, important compounds identified in this extract included alkaloids, flavonoids, phenols and terpenoids.

Similar results were reported by Visetson and Milne (2001), where the essential oil of *C. rotundus* controlled *Plutella xylostella*, with notable contents, namely, 4,11-selinnadien-3-one or α-cyperone, which are sesquiterpenes that can help control moth caterpillars.

These compounds are thought to be important substances for the control *S. frugiperda* and were found in the *C. rotundus* dichloromethane crude extract. However, the *C. rotundus* ethyl acetate crude extract had the weakest effect on controlling *S. frugiperda* in the current experiment, possibly because most of the compounds had little effect on controlling this worm. This was consistent with the chemical composition separation described by Masfria and Permata (2018), who reported that the components in *C. rotundus* extracts made from ethyl acetate were mostly flavonoids and anthraquinone glycosides; very few reports have described the effects of these compounds on insect control.

Other studies involving *P. retrofractum* have shown that hexane extracts always have the greatest potential for controlling insects, such as *S. litura* (Ratwatthananon et al., 2020), *Culex quinquefasciatus* (Wiwattanawanichakun et al., 2018) and *S. frugiperda* (Sianturi et al., 2022), because its active compounds (piperine and piperanine) were the most abundant alkaloid compounds found in the crude hexane extract (Musthapa et al., 2018).

The synergistic effects of complex mixtures are thought to be important in plant defenses against herbivory. Plants usually present defenses on the basis of a group of compounds, not individual compounds. Although mortality was noted with the *P. retrofractum* extract or *C. rotundus* extract alone in the current study, the combination of the two extracts in a binary assay produced greater toxicity.

Based on the results from the current study, the extracts from *C. rotundus* were able to increase the control efficiency against *S. frugiperda* (Tables 1–2). This was especially true when the *P. retrofractum* hexane crude extract and *C. rotundus* dichloromethane crude extract were mixed at a ratio of LD_{20}:LD_{30}.

This finding was similar to that of Yooboon et al. (2019), who reported an increase in the efficacy of a mixture of *P. retrofractum* with *A. calamus* at all doses. Among all their tested combinations, the mixture of *P. retrofractum* and *A. calamus* (LD_{30}:LD_{10}) was the best mixture for controlling *S. frugiperda*. Both combinations had synergistic effects and exhibited greater antifeedant activity (82.43%) than any of the other combinations.

Fujiie et al. (2008) reported the robust efficacy of mixtures of *P. retrofractum* with *Annona squamosa* and *Aglaia odorata*, which resulted in 100% and 94% mortality, respectively, in *Crocidolomia pavonana* after treatment for 48 hr with a 0.05% extract mixture. The extract mixture of *A. odorata* and *A. squamosa* yielded a synergistic combination with multiple mechanisms of action, such as feeding inhibition and insecticidal activity.

In conclusion, the current study provided initial information to aid decision making in the development of a plant mixture formula to control one of corn's most important economic pests (*S. frugiperda*). This information should encourage farmers to use plant extracts for pest control and further increase the efficiency of plant extracts, which will reduce agricultural costs for farmers and may provide economic benefits.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Belmain, S., Stevenson, P. 2001. Ethnobotanicals in Ghana: Reviving and modernising age-old farmer practice. Pestic Outlook. 12: 233–238. doi.org/10.1039/B110542F

Changkeb, V., Nobsathian, S., Goff, L.G., Coustau C., Bullangpoti V. 2023. Insecticidal efficacy and possibility of *Combretum trifoliatum* Vent. (Myrtales: Combretaceae) extracts in controlling *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Pest. Manag. Sci. 79: 4868–4878.

Farnsworth NR., Bunyapraphatsara. N. 1992. Thai medicinal plants. Recommended for primary health care system. Prachachon, Bangkok.

Fujiie, A., Sawas, E.A., Hadi, M.A., Sawas, A., Ueda, S., Natsuaki, K.T. 2008. Insecticidal activity of extract mixtures of four plant species against *Crocidolomia pavanana* (F.) (Lepidoptera: Pyralidae) Larvae.

Jing DP, Guo JF, Jiang YY, Zhao JZ, Sethi A, He KL, Wang ZY. 2020. Initial detections and spread of invasive Spodoptera frugiperda in China and comparisons with other noctuid larvae in cornfields using molecular techniques. Insect Sci. 27(4):780-790.

Kumrungsee, N, Wiwattanawanichakun, P, Phankaen, P, Saiyaitong, C, Koul, O, Nobsathian, S, Bullangpoti, V, Dunkhunthod, B. 2023. Phenolic secondary metabolites from *Acorus calamus* (Acorales: Acoraceae) rhizomes: the feeding deterrents for *Spodoptera litura* (Lepidoptera: Noctuidae). J. Econ. Entomol. 116: 1613–1620.

Masfria, Permata, Y.M. 2018. Total phenolic content and antibacterial activity of nut grass (*Cyperus rotundus* L.) extract. Indonesian J. of Pharmaceutical and Clinical Res. 1: 28–36.

Moustafa, M.A.M., El-Said, N.A., Alfuhaid, N.A., Abo-Elinin, F.M.A., Mohamed, R.M.B., Aioub, A.A.A. 2024. Monitoring and Detection of Insecticide Resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae): Evidence for Field-Evolved Resistance in Egypt. *Insects*, *15*, 705

Musthapa, I., Gumilar, G.G., Dara, F. 2018. Isolation of metyhl-piperate from n-hexane extract of fruit of cabe jawa (*Piper retrofractum* Vahl.). Pertanika J. Trop. Agric. Sci. 41: 1489–1495.

Paredes-Sánchez, F.A., Rivera, G., Bocanegra-García, V., Martínez-Padrón, H.Y., Berrones-Morales, M., Niño-García, N., Herrera-Mayorga, V. 2021. Advances in Control Strategies against *Spodoptera frugiperda*. A Review. Molecules. 26: 5587.

Pavan S., Patel, N., Raghunandan BI, Baldaniya A, Bhatt N. 2024. Comparative efficacy of nucleopolyhedrovirus (NPV) alone and in conjunction with chemical insecticides against fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Noctuidae: Lepidoptera) under laboratory conditions. Int. J. Trop. Insect Sci. 10.1007/s42690-024-01258-w.

Ruttanaphan, T., Songoen, W., Pluempanupat, W., Bullangpoti, V. 2023. Potential insecticidal extracts from *Artocarpus lacucha* against *Spodoptera litura* (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 116: 1205–1210.

Sianturi, Y.P.P.A., Dadang, D., Her mana, D., Sartiami, D. 2022. Bioactivity of extracts from *Syzygium aromaticum*, *Annona muricata*, and *Piper retrofractum* against *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae). IOP Conf. Ser.: Earth Environ. Sci. 1083 012075

Singh, D., Bapatla, K.G. 2022. Toxicity and lethal effects of herbaceous plant crude extracts against *Spodoptera litura*. J. Basic. Appl. Zool. 83: 8.

The Observatory of Economic Complexity. 2024. Corn in Thailand. https://oec.world/en/profile/bilateral-product/corn/reporter/tha. Available online 26 September 2024

Trisyono, A., Whalon, M.E. 1999. Toxicity of neem applied alone and in combinations with *Bacillus thuringiensis* to Colorado Potato Beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 92: 1281–1288.

Visetson, S., Milne, J. 2001. Toxicity of 4, 11-selinnadien-3-one from nutsedge (*Cyperus rotundus* L.) tuber extracts to diamondback moth larvae (*Plutella xylostella* L.), detoxification mechanisms and toxicity to non target species. Agr. Nat. Resour. 35: 284–292.

Wiwattanawanichakun, P, Ratwatthananon, A, Poonsri, W, Yooboon, T, Pluempanupat, W, Piyasaengthong, N, Piyasaengthong, N., Nobsathian, S., Bullangpoti, V. 2018. The possibility of using isolated alkaloid compounds and crude extracts of *Piper retrofractum* (Piperaceae) as larvicidal control agents for *Culex quinquefasciatus* (Diptera: Culicidae) Larvae. J. Med. Entomol. 55: 1231–6.

Yooboon, T., Anchulee, P., Atcharee, R., Wanchai, P., Bullangpoti, V. 2019. A plant-based extract mixture for controlling *Spodoptera litura* (Lepidoptera: Noctuidae). Chem. Biol. Techn. Agric. 6: 5.

Uddin, S.J., Mondal, K., Shilpi, J.A., Rahman. M.T. 2006. Antidiarrhoeal activity of *Cyperus rotundus*, Fitoterapia 77: 134–136.

Zhou, Y., Huang, C., Chen, Y., Han, L., Xie, J., Chen, X. 2023. Sensitivities of Fall Armyworm (*Spodoptera frugiperda*) Populations in Different Regions of China to Four Bt Proteins. *Agronomy*. *13*, 2415.