Chemical and Biological Technologies in Agriculture

Antifeedant Activity and Biochemical Responses in *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) Infesting Broccoli, *Brassica oleracea* var. alboglabra exposed to *Piper ribesioides* Wall Extracts and Allelochemicals

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Abstract

Background: *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) is a widely occurring insect pest of several crops conventionally controlled by pyrethroids and organophosphates hazardous for the environment and human health. Thus, the alternatives are biocide-based phytochemicals. Accordingly, the *Piper ribesioides* Wall. (Piperales: Piperaceae) plant, well distributed in the northern regions of Thailand (Nan Province), was used due to its known bioactivity against insects. The objective was to determine the feeding deterrent activity of *P. ribesioides* extracts and isolated allelochemicals under laboratory conditions and correlate the efficacy under greenhouse conditions after the extracts were applied to *S. exigua* larvae infesting potted *Brassica oleracea* var. *alboglabra* (Bailey) Musil plants. Another objective was to look at the impact of spray applications on detoxification enzymes to check the possibility of resistance development against such natural extracts.

Results: Ethyl acetate extract deterred feeding of larvae better than other extracts with the concentrations causing 50% feeding inhibition (FI_{50}) of 26.25 $\mu g/cm^2$ and feeding deterrence index (FDI) of 91.8%, which was slightly lower than the positive control (cypermethrin, FDI = 100%; FI_{50} 0.027 $\mu g/cm^2$). The most effective feeding deterrent compounds against *S. exigua* were pinostrobin and pinocembrin with FDI range of 77 to 90% and FI_{50} values of 14.39 and 19.38 $\mu g/cm^2$. In the greenhouse, the larvae treated on potted *B. oleracea* at FI_{50} concentrations (determined in laboratory experiments), ethyl acetate extract gave the highest mortality of 63.33% within 24 h of first spray and total of 73.33% after 24 h of the second spray. Impact on detoxification enzymes (24 h post-treatment) was determined from survived 3rd instars of *S. exigua* using spray applications. Inhibition of carboxylesterase (CE) was 1.94-fold after hexane extract treatment. However, ethyl acetate extract inhibited glutathione-s-transferase (GST) 1.30-fold.

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Conclusions: Ethyl acetate extract of *P. ribesioides* twigs and isolated pinostrobin and pinocembrin compounds were potential antifeedants against *S. exigua* larvae. The data obtained also showed that such antifeedant levels of treatment could be used in greenhouse or field trials directly as an extract after establishing the efficacy of extracts and the active compounds therein under laboratory conditions.

Keywords: Antifeedants, Beet armyworm, *P. ribesiodes*, Phytochemicals, Biocides, Resistance

Graphical Abstract
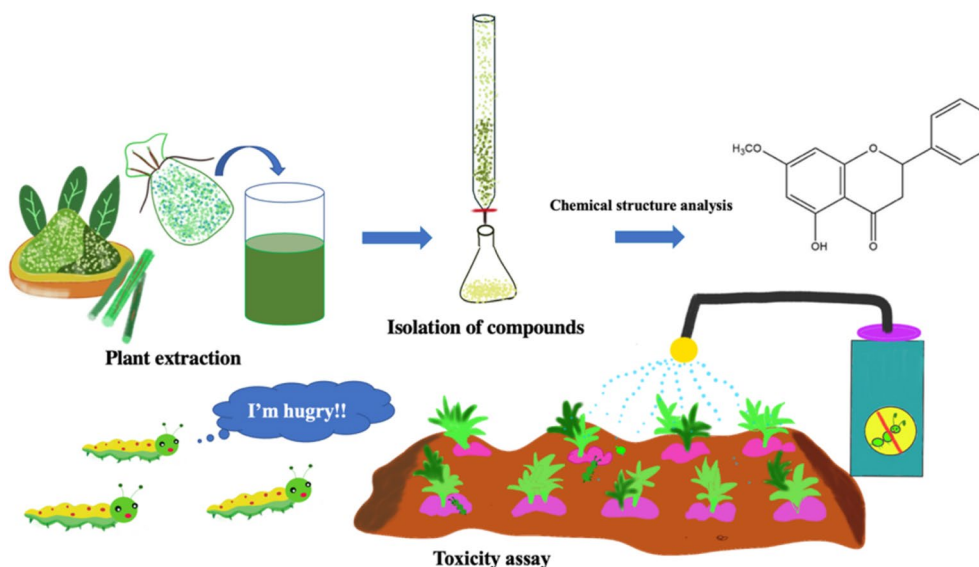
Introduction

The beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), has global existence in Europe, Africa, Australia, America and Asia, including Thailand. This insect is an economically important pest of several crops like cabbage, potato, legumes and tobacco [1, 3]. Conventional insecticides like organophosphates, carbamates and pyrethroids are used for *S. exigua* control [37]. However, the insect resistance to these synthetic molecules [34] has impacted global ecology via the food chain, toxic residues in the environment, harm to mammals, and loss of biodiversity [26]. The alternative strategy is to use phytochemicals that comprise the secondary metabolites used as self-defence from plants' herbivory. Such products have been evaluated as agrochemicals, pesticides, pharmaceuticals and botanical insecticides in organic crops [11, 20]. Biological activities of botanicals as insecticides, feeding deterrents, growth regulators, growth inhibitors, repellents, ovicides and nanobiopesticides have been comprehensively documented [12–14]. Several plant extracts from *Jatropha gossypifolia* Linnaeus, *Houttuynia cordata* Thunb., *Melia azedarach* Linnaeus, *Ricinus communis* Linnaeus and *Derris elliptica* (Wall.) Benth. have been reported as toxins, feeding and detoxification enzyme inhibitors of *S. exigua* [13, 19]. Similarly, compounds like linalool, pulegone, thymol are insecticidal against this insect [18]. The number of volatile oils is toxic to *S. exigua* [22]. Despite the multiple studies on phytochemical insecticides, commercial products are limited. Therefore, the need for botanical biopesticides should be a priority in research endeavors. However, from environmental, traditional agriculture or small farm point of view, plant allelochemicals have received broad and due considerations for exploitation as insecticides [25].

Several factors support the argument that botanical insecticides would benefit developing countries, including Thailand, because of indigenous knowledge of using plants and plant extracts for pest control [10]. In addition to the economic benefits of local use of botanical extracts, the least impact on human health would be enormous, preventing farmers from insecticide poisoning. Accordingly, *Piper* species (Piperaceae), having about 700 species in Asian and African continents, is an economically important plant source for insecticidal metabolites deleterious to various agriculture and post-harvest crop pests [6, 29, 35]. Several allelochemicals have been isolated from *Piper* species and reported as insecticides, antifeedants, ovicides and growth deterrents [12]. Earlier studies have also shown that such compounds have the

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most negligible impact against pollinator bees, predator insects and other natural enemies [7, 20]. In the current study, we chose *Piper ribesioides* Wall. *(*Piperales: Piperaceae) plant because of its local occurrence in the rain forests of southern regions of Thailand [27] and its fruit and stem use in traditional medicines [27]. Cytotoxic, fungicidal, insecticidal and molluscicidal activities reported for *P. Ribesioides* are well known. We recently reported the toxicity of *P. ribesioides* extracts against *S. exigua* under laboratory conditions [24]; that study was based on topical application assays to determine acute toxicity against *S. exigua*. However, the present study assessed the impact on the feeding of *S. exigua* in the laboratory and subsequent control under greenhouse conditions. The current research correlated the efficacy under greenhouse conditions after the extracts were sprayed on potted *Brassica oleracea* var *alboglabra* infested with *S. exigua* larvae. Allelochemicals interfere with detoxification enzymes via activation or inhibition in beet armyworms is known [8, 24]; therefore, one cannot summarily ignore resistance against plant extracts and phytochemicals from *P. ribesioides* under field conditions. Accordingly, the other objective was to study the impact of extracts on detoxification enzymes of *S. exigua* were reported in Table 5, specifically from greenhouse experiments, to determine the possibility of resistance development under field conditions.

Materials and methods

Plant specimens
Twigs of *Piper ribesioides* were used for the study and obtained from Nan Province, Northern part of Thailand (19.49° N, 100.90° E). Botanists carried out the specimen identification from the Department of National Park, Wildlife and Plant Conservation, Bangkok, Thailand, and recorded it under voucher number BK068730 [25].

Extraction of plant extract
Twigs of *P. ribesioides* (10 kg) were dried and powdered before extraction. The powder was then soaked in organic solvents at room temperature (37 ± 2 °C) for 7 days. The solvents used were hexane, dichloromethane, ethyl acetate, and methanol, respectively. The crude extracts were concentrated using a vacuum pump and rotary evaporator (BUCHI R-215). The percentage yield of each extract in organic solvents was recorded (Table 1). Finally, extracts were kept at 4 °C until further use for the bioassays. Compounds were reported in Nobsathian et al. [24].

Isolation of compounds
Initial assays with the extracts against *S. exigua* larvae showed that ethyl acetate extract was most efficacious among all the extracts evaluated. Accordingly, ethyl

Table 1 Characteristics of *P. ribesioides* extracts and yield obtained in respective solvents after extraction

Solvents used for extraction	Characteristics	Yield of crude extract (w/w) %[A]
Hexane	Yellow oil	1.1663
Dichloromethane	Sticky gray solid	0.6794
Ethyl acetate	Sticky dark brown solid	0.8390
Methanol	Sticky dark green solid	0.1299

[A] Yield calculated as $W/W_0 \times 100$ ($W =$ total weight of dried extract; $W_0 =$ weight of the sample of *P. ribesioides* after extractions)

acetate extract was further purified, compounds isolated and identified by spectral analysis as reported earlier [24].

Insect
S. exigua larvae obtained from National Center for Genetic Engineering and Biotechnology were reared using the modified procedure of Nobsathian et al. [24]. The artificial diet used to feed larvae comprised green bean powder (120 g) (Raithip Brand), gelatin powder (10 g), ascorbic acid (1.5 g), sorbic acid (1 g), methylparaben (2 g), yeast (8.5 g), diverse vitamin (18 mL), formaldehyde (1.5 mL), and distilled water (700 mL). The larvae were placed in plastic boxes (30 cm × 40 cm) until pupation occurred; subsequently, pupae were kept in net cages (50 × 50 cm) for adult emergence. Adults were fed on 10% glucose-soaked cotton swabs placed in small plastic cups 6 cm diameter in the moth net cages. Broccoli leaves were used for oviposition in the net cages. Egg colonies were collected daily and dipped in 5% formaldehyde to avoid bacterial infection. Neonates were reared further under 27 ± 2 °C, 60–70% relative humidity and 14:10 h light:dark photoperiods in the insect culture room. Further experiments were carried out with the larvae obtained from this new generation.

Antifeedant assay
Modified leaf disc choice assays of Koul et al. [15] and Akhtar et al. [2] were used to determine the antifeedant activity of *P. ribesioides* extracts and the isolated compounds. Broccoli, *Brassica oleracea* var *alboglabra* leaves were collected from the greenhouse 20 days after sowing, cleaned with water and dried at room temperature. Leaf discs of 1.5 × 1.5 cm size were punched out from the leaves and used in the choice test. The concentration of each crude extract and isolated compound were prepared in acetone (0.5–10.0 mg/mL). Leaf discs were applied with each concentration of extracts and compounds (10 μL solution on each side of the disc) and allowed to air dry. Acetone alone was used for controls. One control and one treated leaf disc were placed approximately

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2 cm apart in Petri dishes (9 cm diameter). One third instar larva of *S. exigua* was placed in the center of the Petri dish in each case. Each treatment was replicated 10 times. Larvae were allowed to feed for 5 h and subseqently FI_{50} value (concentrations causing 50% feeding inhibition) were calculated. A feeding deterrence index (FDI) was calculated by using the formula

$$FDI(\%) = \left(\frac{\text{Consumptionincontrols} - \text{consumptionintreatments}}{\text{Consumptionincontrols} + \text{consumptionintreatments}} \right) \times 100,$$

where consumption means area of the leaf disc consumed by *S. exigua* larvae.

Green house experimental design

Greenhouse experiments were carried out at Banphoromyen (13.40° N, 99.94° E) in Amphawa District, Samutsongkram Province, Thailand; prominent broccoli, *Brassica oleracea* var *alboglabra* crop-growing region. The procedure followed for greenhouse experiments was modified from Wang et al. [36], Seffrin et al. [30] and Zhao et al. [38]. The greenhouse conditions were 31 ± 2 °C (day) and 22 ± 2 °C (night) temperature, $75 \pm 5\%$ relative humidity and natural photoperiod amplified by sodium lamp (Philips; Son-T E40). The broccoli plants were grown in plastic trays (45×65 cm) in a horticultural greenhouse until 20 days (i.e., 4–5 leaf stage). These were then replanted singly in each pot ($n = 60$) for futures studies. The pots were arranged in a completely randomized design in the greenhouse (1.5 m $\times$ 2.5 m) and separated from each other by 1.5 m buffer zones. The treatments applied were hexane, ethyl acetate and methanol extracts, controls (only solvent), and positive control (cypermethrin). Each extract spray solution was a FI_{50} concentration determined in laboratory experiments. The basis for this was significant larval mortality recorded at this concentration in greenhouse preliminary spray assay. Each extract was dissolved in a small quantity of the respective solvents (3 mL); to this 4 mL of 2% of Tween 80 (surfactant) was added and made to final volume of 1 L in water to obtain the FI_{50} concentration. Cypermethrin (Sigma-Aldrich) was used as a positive control at the prescribed application rate of 0.08 mg/L of water. In controls, only water + tween 80 mixture and solvent (amount used in extract dilutions) were used. In each case, three biological pots 150 larvae/plant were released and allowed to adopt the replicates per treatment were estimated. The third instars of *S. exigua* were starved for 4 h before being released onto 25-day-old broccoli potted plants. In greenhouse conditions for 3 days. After 3 days the respective extracts and control sprays were applied using a backpack sprayer (Nexos Knapsack). Each potted plant received FI_{50} concentration of each treatment (200 mL of spray/pot). Overall, there were 5 sets of treatments, i.e., 3 extracts, control and positive control. Mortality was calculated based on randomized count in each pot after 24 h of the spray. This 24 h count was as prescribed for cypermethrin. Accordingly, the second spray was applied after 7 days of first spray, based on similar information and followed for the application of extracts. The mortality data were again recorded 24 h after the second spray. The survived larvae in the greenhouse experiment were collected and used further for detoxification enzyme analysis.

Detoxification enzyme activity

This activity was determined for the survived larvae from greenhouse experiments only to observe the possible impact under field conditions. The action was determined on similar lines as done earlier under laboratory conditions for both extracts and active allelochemicals against *S. exigua* larvae [24].

Preparation of enzyme source

Detoxification enzyme reactions were determined by a modified procedure of Kumrungsee et al. [18]. Surviving larvae of *S. exigua* from both lab and greenhouse assay (15 larvae/replicate) were used for enzyme sources (3 replicates). The larvae were macerated using a piston in a microtube with phosphate buffer (100 mM, pH 7.2) and Triton X-100 (0.5%). The homogenate was centrifuged at 10,000 *g* at 4 °C for 5 min. The supernatants were collected (stored at − 20 °C) and used as an enzyme source.

Carboxylesterase

Carboxylesterase (CE) reaction was determined using 50 µL of enzyme solution from the enzyme source that was homogenized with the substrate (50 µL) comprising 0.12 M of paranitrophenylacetate (pNPA) and 0.1 M phosphate buffer with pH 7.5 (2.9 mL) in dimethyl sulfoxide (DMSO). The homogenates were transferred to a 96-well plate and analyzed for pNPA in kinetic mode using a microplate reader (400 nm) at 25 °C for 3 min. Enzyme activity for each treatment was replicated thrice, and the extinction coefficient of pNPA was used for calculation.

Glutathione-*S*-transferase

Glutathione-*S*-transferase (GST) reaction was determined by a modified method of Kumrungsee et al. [18]. Amounts

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used from enzyme source from lab, greenhouse and controls were 50 μL each. The homogenates of enzyme and substrate were prepared for measurements that comprised 0.1 M phosphate buffer (1150 μL, pH 7.3), 10 μL GST solution, and 150 mM 1-chloro-2,4-dinitrobenzene (CDNB) (10 μL). The reaction of CDNB was measured at 340 nm using 96-well microplate reader for 3 min at 25 °C. The GST activity was calculated by the extinction coefficient of CDNB, which was 0.0096 from three biological replicates.

Acetylcholinesterase

Enzyme source (50 μL) was mixed with the substrate using 100 mM (50 μL) phosphate buffer with pH 7.2, DTNB (5,5′-dithio-bis (2-nitrobenzoic acid) at 10 mM level mixed in 0.1 M EDTA (ethylenediaminetetraacetic acid), and 100 mM of acetylthiocholine iodide. The mixture was incubated at 30 °C for 30 min. The acetylcholinesterase (AChE) reaction was measured using a microplate reader in kinetic mode at a wave length of 412 nm.

Data and statistical analysis

The feeding deterrence index (FDI %) based on leaf discs consumed by insects in treated and control experiments was calculated by using WinDias 3 DeltaT-Device Program. The concentrations causing 50% feeding inhibition (FI_{50}) of plant crude extracts and isolated
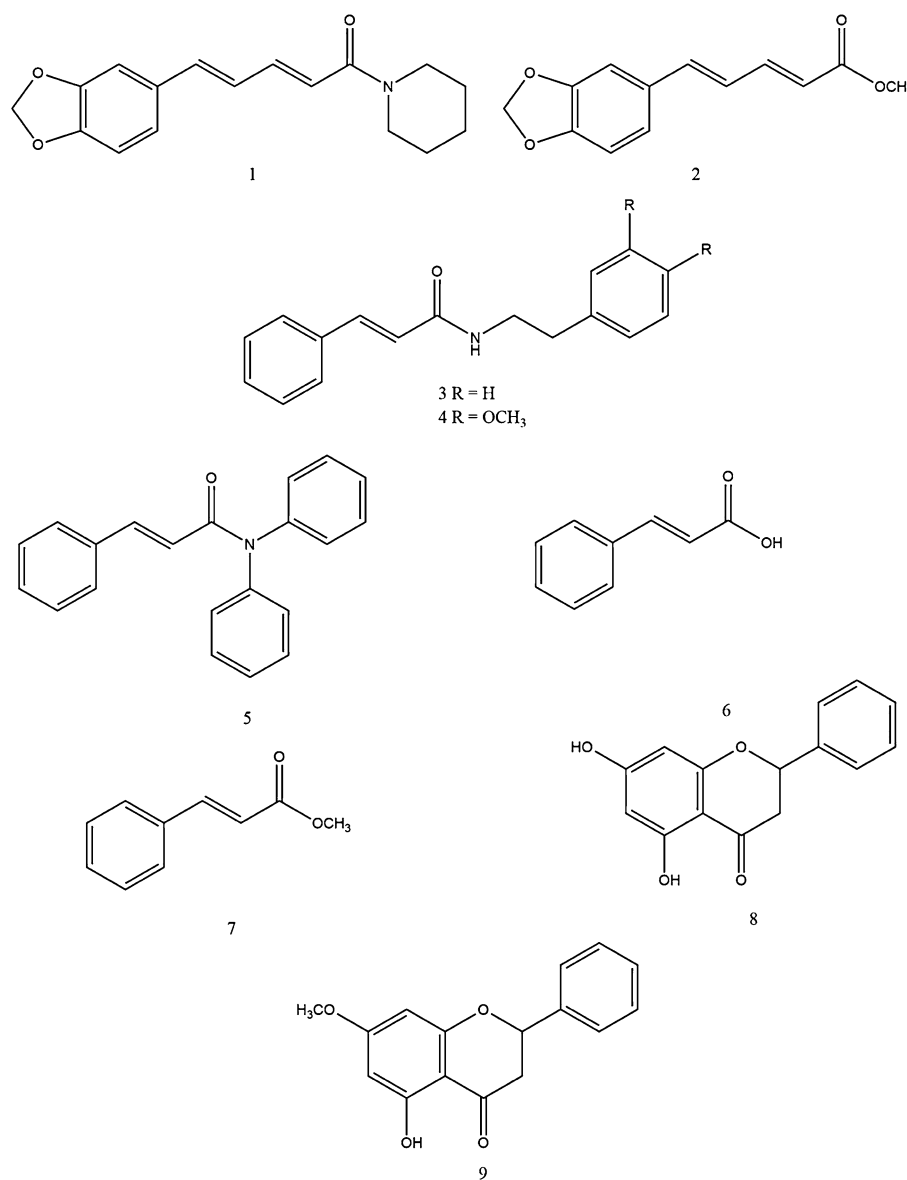

Fig. 1 Chemical structures of allelochemicals from *P. ribesioides* ethyl acetate extract (Nobsathian et al., 2021) [24]

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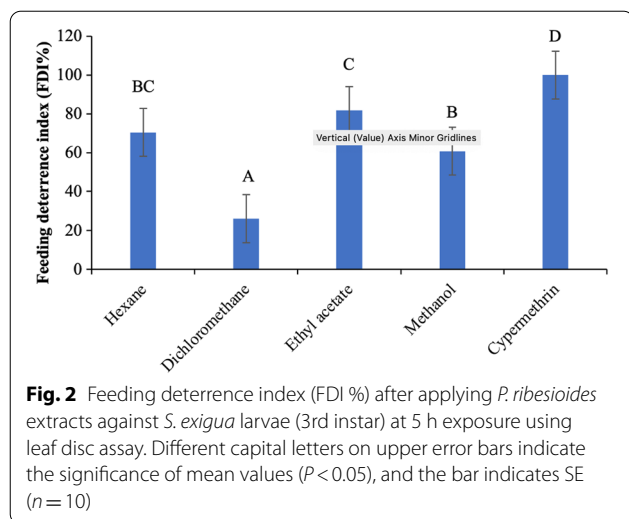
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Fig. 2 Feeding deterrence index (FDI %) after applying *P. ribesioides* extracts against *S. exigua* larvae (3rd instar) at 5 h exposure using leaf disc assay. Different capital letters on upper error bars indicate the significance of mean values ($P < 0.05$), and the bar indicates SE ($n = 10$)

Table 2 The concentration inhibiting feeding by 50% (FI_{50}) after the application of *P. ribesioides* extracts against *S. exigua* larvae (3rd instar) at 5 h exposure using leaf disc assay

Crude extracts	FI_{50} ($\mu g/cm^2$)	LCL-UCL[A]	df	χ^2	P-value
Hexane	49.21	37.80–69.02	3	5.47	0.14
Dichloromethane[B]	>100	>100	3	1.85	0.60
Ethyl acetate	26.25	21.24–32.60	3	2.84	0.42
Methanol	82.17	62.40–120.46	3	7.42	0.06
Cypermethrin[C]	0.027	0.02–0.51	3	0.28	0.96

[A] Lower confidence limit–upper confidence limit ($\mu g/cm^2$)

[B] FI_{50} values > 100 $\mu g/cm^2$ were not determined

[C] Positive control

compounds were determined involving 5 concentrations (0.5–10.0 mg/mL and 0.1–5 mg/mL, respectively) by probits using the Stat plus program (Version 2021). The crude extracts at FI_{50} concentrations were used as sprays in greenhouse experiments. Significant differences of sample mean variance of larvicidal activity in greenhouse, detoxification enzyme activities and feeding deterrence index were compared by statistical software (SPSS; version 2021) using one-way analysis of variance (ANOVA). Tukey's HSD was used for mean separation.

Results
Extracts
Piper ribesioides twig powder was extracted with organic solvents hexane, dichloromethane, ethyl acetate, and methanol in a sequence. Quantitatively, the highest amount was hexane extract, followed by ethyl acetate, dichloromethane, and methanol extracts (Table 1).

Isolated compounds
The initial bioassay-guided evaluation showed that ethyl acetate extract was a more active feeding deterrent than other extracts. In our earlier study, nine compounds were isolated from this extract and evaluated as chronic toxins against *S. exigua* [24]. The compounds (Fig. 1) were piperine (**1**), methyl piperate (**2**), *N*-cinnamoyl-(2-phenylethyl) amine (**3**), lemairamin (**4**), *N,N*-diphenyl cinnamamide (**5**), cinnamic acid (**6**), methyl cinnamate (**7**), pinostrobin (**8**) and pinocembrin (**9**). In the present study, these compounds were assessed for antifeedant activity under laboratory conditions and correlated with the efficacy of extracts in the greenhouse.

Antifeedant activity
Highly significant feeding deterrence was recorded after ethyl acetate extract application in the laboratory experiments ($FI_{50} = 26.25$ $\mu g/cm^2$) with a feeding deterrence index (FDI) of 81.80% (Fig. 2). However, this was slightly lower than the positive control (cypermethrin, FDI = 100%) that required a low concentration of 0.027 $\mu g/cm^2$ to deter feeding of 50% population of *S. exigua* third instars. Hexane and methanol extracts were similar in inhibiting the larval feeding, where FI_{50} values were statistically identical as the overlap of fiducial limits was observed. Dichloromethane extract was the least active (Table 2).

Allelochemicals, pinostrobin and pinocembrin assayed for feeding deterrence were significantly similar in activity, and FDI ranged between 77 and 90% (Fig. 3). FI_{50} values recorded were 14.39 and 19.38 $\mu g/cm^2$, respectively (Table 3). In the case of cinnamic acid and *N*-cinnamoyl-(2-phenyl ethyl) amine, FDI ranged between 70 to 72% (Fig. 3), which was statistically similar. Other compounds deterred feeding by 50 to 60%, except lemairamin and methyl piperate that could only inhibit the feeding of *S. exigua* larvae by about 40% (Fig. 3).
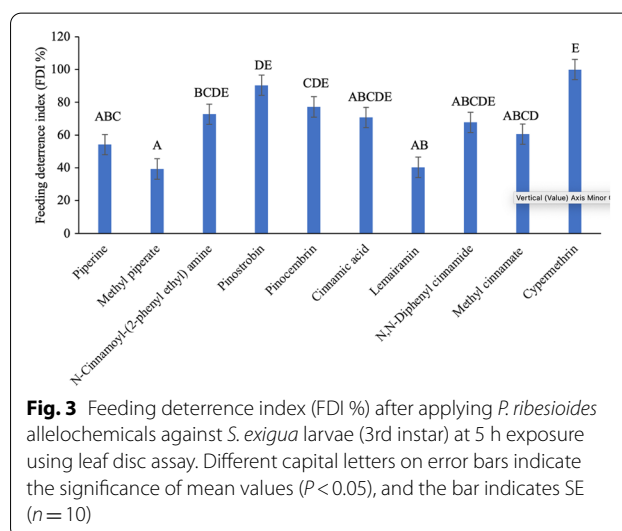

Fig. 3 Feeding deterrence index (FDI %) after applying *P. ribesioides* allelochemicals against *S. exigua* larvae (3rd instar) at 5 h exposure using leaf disc assay. Different capital letters on error bars indicate the significance of mean values ($P < 0.05$), and the bar indicates SE ($n = 10$)

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Table 3 The concentration inhibiting feeding by 50% (FI_{50}) after applying *P. ribesioides* allelochemicals from ethyl acetate fraction against *S. exigua* larvae (3rd instar) after 5 h exposure using leaf disc assay

Compounds[A]	FI_{50} ($\mu g/cm^2$)	LCL-UCL[B]	df	χ^2	P-value
Piperine	88.62	>100	3	14.71	0.002
Methyl piperate [C]	>100	>100	3	2.02	0.568
N-Cinnamoyl-(2-phenylethyl) amine	25.11	*19.50*–34.12	3	3.71	0.295
Pinostrobin	14.39	10.7 – 52.19	3	32.91	0.001
Pinocembrin	19.38	8.32 – 86.77	3	12.77	0.005
Cinnamic acid	26.28	20.03–36.80	3	5.95	0.114
Lemairamin[C]	>100	>100	3	0.81	0.846
N,N-Diphenyl cinnamide	49.74	>100	3	28.71	0.001
Methyl cinnamate	56.38	>100	3	10.66	0.013
Cypermethrin [D]	0.027	0.02–0.51	3	0.28	0.96

[A] Compounds were isolated earlier (Nobsathian et al., 2021) [24]

[B] Lower confidence limit–upper confidence limit *(*$\mu g/cm^2$)

[C] FI_{50} values > 100 $\mu g/cm^2$ were not determined

[D] Positive control

Greenhouse spray assay

In the greenhouse experiment, the larval mortality was highest after spraying ethyl acetate extract on potted plants infested with early third instar *S. exigua* larvae at FI_{50} concentration levels (determined in laboratory experiments). After the first spray of ethyl acetate extract on the 29th day of *B. oleracea* var *alboglabra* planting, the mortality recorded was 63.33% post-24 h treatment and subsequently after the second spray (i.e., after 36 days of planting), the cumulative mortality was 73.33% after 24 h of the second spray. Comparatively, methanol and hexane extracts were significantly similar, and cumulative mortality of the larvae ranged between 36.67 and 56.67% (Table 4). Cypermethrin application, however, was better than plant extracts (cumulative mortality = 93.33%). A dichloromethane extract FI_{50} under laboratory conditions was > 100 $\mu g/cm^2$; therefore, this extract was not evaluated under greenhouse conditions. Slight mortality in controls (13.3% after the second spray) was corrected by Abbot's formula and adjusted in treatments accordingly (Table 4).

Detoxification enzyme assay
Carboxylesterase (CE)

Impact on detoxification enzymes from survived 3rd instar *S. exigua* after spray application of *P. ribesioides* extracts in greenhouse after 24 h post-treatment showed that hexane extract inhibited CE 1.94-fold compared to the induction by cypermethrin (0.58-fold) (Table 5). There was no significant impact on CE in larvae that were treated with ethyl acetate and methanol extracts.

Table 4 Mortality of *S. exigua* larvae by *P. ribesioides* extract spray applications after 24 h exposure under greenhouse conditions at FI_{50} concentrations

Treatments	Mortality (%) ± SE	
	1st application[B]	2nd application[C]
Dichloromethane extract[A]	> 100 $\mu g/cm^2$	> 100 $\mu g/cm^2$
Hexane extract	46.67 ± 5.77[bc]	56.67 ± 5.77[bc]
Ethyl acetate extract	63.33 ± 5.77[c]	73.33 ± 5.77[cd]
Methanol extract	30.00 ± 10.00[b]	36.67 ± 11.55[b]
Cypermethrin[D]	83.33 ± 5.77[d]	93.33 ± 11.55[d]
Control	9.33 ± 1.15[a]	13.33 ± 3.06[a]
P-value	< 0.0001	< 0.0001
F-value	61.35	42.46

[A] Dichloromethane extract FI_{50} values > 100 $\mu g/cm^2$ were not determined for greenhouse experiments

[B] 1st application means 29 days of *Brassica oleracea* var *alboglabra* planting and mean values within a column followed by same letter were not significantly different ($P < 0.05$) by Tukey's test

[C] 2nd application means 36 days of *Brassica oleracea* var *alboglabra* planting and mean values within a column followed by same letter were not significantly different ($P < 0.05$) by Tukey's test

[D] Positive control

Glutathione-S-transferase (GST)

In the case of GST reactions in insects, ethyl acetate extract inhibited the enzyme 1.30-fold. All other treatments had a similar impact, and the variation in enzyme levels was statistically identical to controls (Table 5).

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Acetylcholinesterase (AChE)

After spraying ethyl acetate extract of *P. ribesioides* to *S. exigua* larvae, AChE activity recorded in larvae between the control (0.107 µM/min/mg) and the ethyl acetate treatment (0.088 µM/min/mg) was not significantly different. However, methanol extract and cypermethrin (positive control) significantly induced enzyme reactions by 0.54- and 0.74-fold, respectively (Table 5).

Discussion

Extracts from plant sources, essential oils from aromatic plants or the secondary metabolites from various plant parts are well known for their toxic and antifeedant activities against insects [13]. The beet armyworm, *S. exigua* is no exception where several such products have been reported as toxins or physiological metabolism inhibitors of this species [13, 22, 23]. Similarly, Piperaceae family and various allelochemicals therein have also been reported to impact various insects in different ways [17, 19]. *P. ribesioides*, which also belongs to the Piperaceae family, has significant potential for insect control and could be equally productive against susceptible and resistant insects [21, 24]. The strategy was to develop farmer-friendly products that could replace conventional hazardous insecticides by using extracts/ allelochemicals that play a self-defence role in plants from insect herbivory via feeding deterrence, growth inhibition or regulation, repellency, or as specific physiological toxins [11, 15, 24].

The results obtained suggest that *P. ribesioides* extracts and the allelochemicals from the ethyl acetate extract are not only acute toxins [24], but also potential feeding deterrents as well when assayed for antifeedant activity using leaf disc tests. The ethyl acetate fraction with the concentration inhibiting feeding by 50% (FI_{50}) = 26.25 µg/cm^2 leaf discs was about 1.87-fold more deterrent than hexane extract and 3.2-fold more than methanol extract. Ethyl acetate extract of *P. ribesioides* as an acute toxin reported earlier [24] does induce feeding deterrent effects as well in *S. exigua*. Acute toxicity of ethyl acetate extract against *S. exigua* was due to cinnamic acid, piperine, and *N,N*-diphenylcinnamamide [24]. However, the antifeedant effect was due to pinostrobin and pinocembrin from the same extract, and both compounds were significantly similar as feeding deterrents (FDI ranged between 77 to 90%). Two other compounds, *N*-cinnamoyl-(2-phenyl ethyl) amine and cinnamic acid, were also feeding deterrents but comparatively lesser (FDI ranged between 70 to 72%). This shows that different compounds within the same extract may play different roles, such as cinnamic acid and piperine from *P. ribesioides* as acute toxins [24] and pinostrobin and pinocembrin reported here are potential feeding deterrents. Obviously, the use of a multicomponent strategy would be more useful in any integrated pest management system where dual activity of toxicity and feeding deterrence could be exploited for better control. It is well known that allelochemicals in a mixture have more potential as anti-insect agents that provide the slightest chance of resistance against such compositions [16, 31].

Greenhouse experiment is a piece of potential evidence in support where insect mortality (73.33%) was recorded after ethyl acetate extract sprays at FI_{50} concentration levels (determined in laboratory experiments) against early 3rd instars of *S. exigua.* This could be an outcome of both acute toxicity and the starvation caused by antifeedant components of the extract. This implies that using ethyl acetate extract as a mixture is better than individual compounds with variable activity levels. Higher efficacy of cypermethrin (positive control) could be attributed to direct neurotoxic action of pyrethroids via voltage-gated sodium channels in insect neuronal systems, thereby breaking

Table 5 Detoxification enzyme activities after *P. ribesioides* extracts were sprayed on 3rd instar *S. exigua* larvae in greenhouse after 24 h exposure

Compounds	Carboxylesterase activity^A	CF^B	Glutathione-*S*-transferase activity^A	CF^B	Acetylcholinesterase activity^A	CF^B
Control	0.062 ± 0.013^{bc}	–	0.184 ± 0.003^{bc}	–	0.107 ± 0.003^a	–
Hexane extract	0.032 ± 0.004^a	1.94	0.163 ± 0.006^b	1.13	0.092 ± 0.005^a	1.16
Ethyl acetate extract	0.055 ± 0.007^b	1.13	0.141 ± 0.008^a	1.30	0.088 ± 0.012^a	1.22
Methanol extract	0.077 ± 0.004^c	0.81	0.176 ± 0.003^{bc}	1.05	0.197 ± 0.015^c	0.54
Cypermethrin	0.107 ± 0.005^d	0.58	0.195 ± 0.004^c	0.94	0.144 ± 0.008^b	0.74
P-value	< 0.0001		< 0.0001		< 0.0001	
F-value	43.947		52.880		65.368	

^{A} Mean values within a column followed by same letter were not significantly different ($P < 0.05$) by Tukey's test. Carboxylesterase (CE) measured as p-nitrophenol/min/mg esterase in nM. Glutathione-*S*-transferase (GST) measured as glutathione conjugated product/min/mg in nM. Acetylcholinesterase (AChE) measured as µM/min/mg of protein

^{B} Correction factor (CF) of detoxification enzyme activities calculated as enzyme activity in controls / enzyme activity in treatments

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electrical signaling in the neuro-transmitter [33]. This provides an upper edge for using plant-based products, like ethyl acetate extract of *P. ribesioides* in the present case, that will prevent farmers from insecticide poisoning on one hand and insecticide resistance on the other.

Comparison of the efficacy of *P. ribesioides* extracts and the active allelochemicals therein with cypermethrin (a conventional pyrethroid insecticide) activity showed that cypermethrin was about 900-fold more potent in terms of the feeding deterrent activity to early third instar *S. exigua* larvae. However, evidence shows a trend of induction of detoxification enzymes in lepidopterans due to indiscriminate use of cypermethrin and expanding resistance to this pyrethroid [28]. Therefore, using plant-based products becomes more crucial for the discovery of novel insecticides. It was, therefore, inevitable to look at the impact of *P. ribesioides* extracts on detoxification enzymes like carboxylesterase (CE), glutathione-*S*-transferase (GST) and acetylcholinesterase (AChE), which insects use to defend themselves from biocides [4, 32, 38]. Hexane extract inhibited CE (1.94-fold) compared to the induction by cypermethrin (0.58-fold). Similarly, ethyl acetate extract inhibited GST (1.30-fold) and methanol extract and cypermethrin (positive control) significantly induced AChE reactions by 0.54- and 0.74-fold, respectively, in greenhouse experiments. It is known that GST activity leads to the catalytic conjugation of electrophilic xenobiotics with the thiol group of reduced glutathione reactions [5, 9]. Ethyl acetate extract of *P. ribesioides* inhibited GST reaction in *S. exigua*, which suggests the slightest chance of resistance against this extract. Therefore, ethyl acetate extract of *P. ribesioides* could become a safe biopesticide for insect control. Comparatively, induction of detoxification enzymes by cypermethrin indicates every chance for insect resistance development against this pyrethroid.

Conclusion
Ethyl acetate extract from *P. ribesioides* deterred feeding of *S. exigua* larvae, and these antifeedant concentrations, when used as sprays in the greenhouse on potted plants, caused significant larval mortality. The present study also suggests that it would be appropriate to study the efficacy of individual allelochemicals obtained from a plant (*P. ribesioides* in the present case) to initiate the correlation between the active compounds vis-a-vis the type of activity in an extract. The use of total extract under greenhouse or field trials will be safe, more viable and economically feasible at farmers' level.

Abbreviations
FI$_{50}$: Concentrations causing 50% feeding inhibition; FID: Feeding deterrence index; CE: Carboxylesterase; GST: Glutathione-*S*-transferase; AChE: Acetylcholinesterase; CF: Correction factor.

Availability of data and materials
All data are presented in this article.

Declarations

Ethics approval and consent to participate
All bioassays in this article were performed with the approval of an appropriate animal ethics committee of Rajamangala University of Technology Thanyaburi, Pathumthani, Thailand.

Consent for publication
This article has been confirmed for publication in the journal.

Competing interests
The authors have no conflicts of interest.

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