Chemical and Biological
Technologies in Agriculture

RESEARCH

Open Access

Chrysoeriol isolated from *Melientha suavis* Pierre with activity against the agricultural pest *Spodoptera litura*

Torranis Ruttanaphan[1], Wirin Thitathan[2], Narisara Piyasaengthong[2], Saksit Nobsathian[3*] and Vasakorn Bullangpoti[2*]

Abstract

Background: Flavonoids, a class of plant phenolic compounds, act as plant defense chemicals. Chrysoeriol is a naturally occurring flavonoid produced by *Melientha suavis* Pierre. The goal of this study was to investigate the insecticidal potential and mode of action of chrysoeriol isolated from *M. suavis* against *Spodoptera litura* (Fabricius).

Results: The effects of chrysoeriol on second-instar *S. litura* larvae were determined by topical application. Chrysoeriol was highly toxic to *S. litura* (24- and 48-h LD_{50} values of ~6.99 and 6.51 µg/larva, respectively). Moreover, mode-of-action experiments demonstrated that this compound significantly decreased the activities of both detoxification-related enzymes [carboxylesterases (CarE) and glutathione *S*-transferase (GST)] and neurological enzymes (acetylcholinesterase).

Conclusions: These results indicate that chrysoeriol isolated from *M. suavis* could be used as a potential agent with activity against S. *litura*. However, it is necessary to determine the potential side effects on nontarget species for the further development of these novel insecticides.

Keywords: Chrysoeriol, *Melientha suavis* Pierre, *Spodoptera litura*, Insecticidal activity, Detoxification enzymes

Graphical Abstract

---
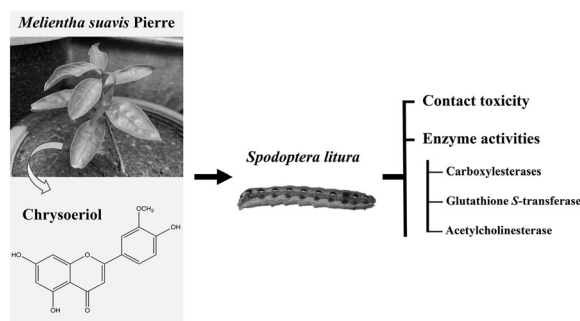
*Correspondence: saksit.nob@mahidol.ac.th; fscivkb@ku.ac.th
[2] Animal Toxicology and Physiology Specialty Research Unit, Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand
[3] Mahidol University, Nakhonsawan Campus, 402/1 Moo 5 Khaothong, Phrayuhakiri District, Nakhonsawan, Thailand
Full list of author information is available at the end of the article

Background

Spodoptera litura (Lepidoptera: Noctuidae) is a destructive agricultural insect pest that causes severe yield losses in more than 120 economically important host plant species, such as maize, cabbage, cotton, soybean, and

Ruttanaphan *et al. Chem. Biol. Technol. Agric.*　　(2022) 9:21

Page 2 of 7

tobacco [1]. Current control measures mostly rely on synthetic insecticides such as pyrethroids, carbamates, organochlorine, organophosphates, broflanilide, triflumezopyrim, and afidopyropen [2, 3]. However, intensive application of these compounds has negative impacts on nontarget organisms, contaminates the environment, and leads to insecticides resistance in pests [4, 5]. Such problems have led to a demand to identify new and safer active compounds of natural origin that are alternatives to existing synthetic insecticides [6].

Plant secondary metabolites are natural compounds originating from plants. They have long been used for various medical purposes and are recognized as safe and potent alternatives to synthetic insecticides in pest control [7, 8]. They are classified into three main groups according to their biosynthetic pathways (namely, alkaloids, terpenes, and phenolic compounds) and are known as chemical compounds of plant defence systems in response to environmental stresses, bacteria, fungi, viruses, and herbivores [9, 10]. Secondary metabolites can serve as insecticides and inhibitor agents for growth and oviposition of many pest species, especially *S. litura* [11, 12].

Pak Wanpa (*Melientha suavis* Pierre), an edible plant species belonging to the Opiliaceae family, can be found in South-East Asian countries, including Laos, Vietnam, Cambodia, Malaysia, the Philippines, and Thailand. Recently, *M. suavis* extracts have demonstrated the potential for use as ingredients for the development of cosmetics [13]. Their leaves and stems contain various types of compounds, such as alkaloids, coumarins, cinnamic acid, tannins, saponins, and flavonoids [13, 14]. Chrysoeriol, a flavonoid found in various herbs, is of great interest because of its medicinal properties, including its antioxidant, antimicrobial, anti-inflammatory, neuroprotective, cardioprotective, and cancer prevention activities [15–17].

The objective of the following research was to determine whether a compound isolated from *M. suavis*, chrysoeriol, could be used for the development of a novel insecticide to control *S. litura*. Moreover, our work aimed to examine the mode of action to explore the toxicity mechanisms of this compound.

Materials and methods
Plant materials, extraction, and isolation
Leaves and twigs of *M. suavis* (Fig. 1) were collected in December 2001 from Chanthaburi, Thailand. A voucher specimen (BKF No. 17967) of *M. suavis* was deposited at the Forest Herbarium, Royal Forest Department, Bangkok 10900, Thailand.

The sun-dried leaves and twigs of *M. suavis* were crushed into powder (1.7 kg) and extracted with

Fig. 1 Morphological illustration of *M. suavis*

dichloromethane (30 L × 5 days × 5 times) at room temperature to produce crude dichloromethane (47.3 g). The bioactive crude dichloromethane extract was isolated by silica gel No 7734 (1 kg), ethyl acetate–C_6H_{14} and CH_3OH–ethyl acetate solvent gradient elution to yield fractions A_1–A_8. Fraction A6 (3.17 g) yielded chrysoeriol (451.3 mg) after Si-gel CC (CH_2Cl_2–C_6H_{14} solvent gradient elution), followed by recrystallization with EtOH–acetone.

Insects and compound treatments
An artificial diet was provided as food for larvae following Ruttanaphan et al. [18] and 20% honey solutions for adults of *S. litura*. The insects were maintained under controlled conditions [16:8 h (L:D) photoperiod, 25 ± 1 °C and $65 \pm 5\%$ RH] at the Laboratory of Department of Zoology, Faculty of Science, Kasetsart University.

The acute toxicity of chrysoeriol, *M. suavis* crude extract and cypermethrin (a commercial synthetic insecticide that is commonly used to control pest insects) to *S. litura* larvae was determined by topical application. Serial dilutions (0–40 μg/larva) of chrysoeriol were prepared with acetone (dilution factor = 0.5). Early second-instar larvae were treated with 1 μL of chrysoeriol and crude extract dilutions included in the treatment group, acetone alone (negative control group) and cypermethrin alone (positive control group), in the dorsal thoracic region using a microapplicator (Hamilton, Switzerland) (six replicates of 10 larvae per treatment; $n = 60$ per treatment) and subsequently fed artificial diets as described above. Mortality and characteristic behavioural changes were recorded at 24 and 48 h post-treatment.

Ruttanaphan *et al. Chem. Biol. Technol. Agric.* (2022) 9:21

Page 3 of 7

Mode-of-action determination

After 24 and 48 h of treatment with chrysoeriol at LD_{30}, surviving *S. litura* were used for enzyme preparation. The homogenization of ten pooled second-instar larvae was conducted using pH 7.2 phosphate buffer [ethylenediaminetetraacetic acid (EDTA, 1 mM) and potassium phosphate buffer (PPB, 100 mM)]. The supernatant obtained by centrifugation (4 °C, 10,000×*g* for 15 min) was used to measure detoxification-related and neurological enzyme activities and Bio-Rad protein assay kit was used to measure the protein content of each enzyme source [19].

The protocol of Ruttanaphan et al. [20] was used to determine the carboxylesterases (CarE) activity using p-nitrophenylacetate (pNPA) (Sigma-Aldrich, Germany) as a substrate. A microplate reader (Biotek PowerWave XS microplate spectrophotometer, US) was used to measure the crude enzymes in PPB (230 μL, pH of 7.4, 50 mM, and containing 10 mM pNPA in DMSO) at 410 nm for 90 s at 37 °C. 1-chloro-2,4-dinitrobenzene (Sigma-Aldrich, Germany) was used to determine glutathione *S*-transferase (GST) activities according to the method of Nobsathian et al. [21]. The mixture of PPB (110 μL, pH of 7.2, 50 mM, and containing the reduced form of 10 mM GSH in glutathione solution), 1-chloro-2,4-dinitrobenzene (100 μL, 150 mM), and supernatant (100 μL) was immediately measured at 340 nm for 3 min by a microplate reader. 5,5'-dithio-bis-(2-nitrobenzoic acid) was used to determine acetylcholinesterase (AChE) [22]. Crude enzymes were prepared from the supernatant (50 μL) and potassium phosphate buffer (pH 8.0, 100 mM). After incubation for 30 min, the mixture was added with phosphate buffer (pH of 7.2, 100 mM and containing 5,5'-dithio-bis-(2-nitrobenzoic acid) (10 mM), acetylthiocholine iodide (100 mM) and EDTA (0.1 mM). The AChE activities of the mixtures were measured by a microplate reader at 412 nm. Three biological replicates per treatment of enzyme activities were evaluated, with an extinction coefficient of 176.4705, 0.000137 and 1.36×10^4 (/M/cm) for CarE, GST and AChE, respectively.

Data analysis

The acute toxicity of chrysoeriol as determined by LD_{50} values and confidence limits was determined by probit analysis, and statistical tests of enzyme activities were performed using one-way analysis of variance (ANOVA), and Tukey's test was used for mean separation test by StatPlus Pro 7.3.0 (AnalystSoft, Inc., Canada).

Results

Isolated compounds

The chrysoeriol was isolated from leaves and twigs of *M. suavis*. The pure compound was verified by the comparison of their physical properties and spectroscopic data with those reported in the literature [23].

Chrysoeriol (Fig. 2): yellow powder from ethanol–acetone, m.p. 330–331.3 °C; UV (MeOH) λ_{max} (log ε): 269 (3.56), 340 (5.12) nm; FT-IR (KBr) $v_{max} = 3350$, 1655, 1607, 1561, 1507, 1164; ^{1}H NMR 400 MHz (DMSO-d_6):12.97 (1H, *s*, OH-5) 6.87 (1H, s, H-3), 6.18 (1H, *d*, *J* = 2.0 Hz, H-6), 6.49 (1H, *d*, *J* = 2.0 Hz, H-8), 7.55 (1H, *d*, *J* = 2 Hz-6'), 6.91 (1H, *d*, *J* = 8 Hz, H-5'), 7.55 (1H, *d*, *J* = 2 Hz, H-2') and 3.87 (3H, *s*, 3'-OCH$_3$), ^{13}C NMR (DMSO-d_6) 161.24 (C-2), 103.72 (C-3), 182.82 (C-4), 163.73 (C-5), 103.72 (C-5a), 98.89 (C-6), 164.24 (C-7), 94.12 (C-8), 157.39 (C-8a), 121.58 (C-1'), 110.26 (C-2'), 148.08 (C-3'), 150.78 (C-4'), 116.02 (C-5'), 121.58 (C-6'), 56.02 (3'-OCH$_3$); HR MS (ESI-TOF): *m/z* found 323.0521 [M + Na]$^+$, (calcd. for $C_{16}H_{12}O_6Na$, 323.0532).

Acute toxicity and mode-of-action of chrysoeriol on S. litura larvae

A major constituent, chrysoeriol, was isolated from *M. suavis,* and its bioinsecticidal activity was demonstrated in an agricultural insect pest, *S. litura*. Our results showed that chrysoeriol was toxic to second-instar *S. litura* larvae, with LD_{50} values of 6.99 and 6.51 μg/larva at 24 and 48 h post-treatment, respectively (Table 1). The mortality rates of larvae were 28.3% and 28.3%, 35.0% and 35.0%, 56.7% and 58.3%, 75.0% and 76.7%, and 81.7% and 81.7% after 24 and 48 h treated with 2.5, 5, 10, 20 and 40 μg/larva of chrysoeriol, respectively. Moreover, this compound induced larval agitation at all dilutions. However, no significant difference was observed between
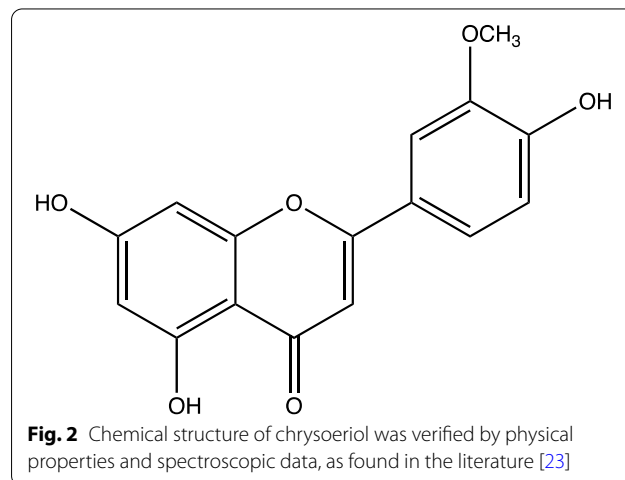

Fig. 2 Chemical structure of chrysoeriol was verified by physical properties and spectroscopic data, as found in the literature [23]

Ruttanaphan *et al. Chem. Biol. Technol. Agric.*   (2022) 9:21

Page 4 of 7

Table 1 Toxicity of chrysoeriol isolated from *M. suavis* on second-instar larvae of *S. litura* at 24 and 48 h post-treatment

Dose (µg/larva)	Chrysoeriol		*M. suavis* crude extract		Cypermethrin	
	24 h	48 h	24 h	48 h	24 h	48 h
LD_{50}[a]	6.99	6.51	7.64	6.51	0.005	0.002
Chi-square	3.52	4.25	3.21	1.56	0.261	0.383
LD_{50} LCL	5.65	5.26	7.51	6.62	0.003	0.001
LD_{50} UCL	8.75	8.15	7.84	6.29	0.007	0.003
Slope $\pm$ SE	1.21 ± 0.10	1.20 ± 0.10	0.04 ± 0.10	0.04 ± 0.10	1.12 ± 0.24	0.56 ± 0.11

In each experiment, 10 larvae/treatment in six replicates were used ($n = 60$ per treatment)

LCL lower confidence limit, *UCL* upper confidence limit

[a] LD_{50} represents lethal dosage that kills 50% of the exposed larvae, expressed in µg/larva.

chrysoeriol-treated group and crude extract-treated group ($P > 0.05$, Tukey's test).

To further investigate the mode of action of chrysoeriol in *S. litura*, detoxification-related enzyme (CarE and GST) and neurological enzyme (AChE) activities were estimated and compared with those of a control group (an acetone-only treatment). All two enzymes showed significant inhibition at 24 and 48 h post-treatment with chrysoeriol (Table 2). The detoxification-related enzyme activities (CarE and GST) of *S. litura* were significantly inhibited by chrysoeriol ($P < 0.05$, Tukey's test; Table 2). At 24 and 48 h post-treatment, the correlation factors of the CarE activity were 1.5 and 1.5, respectively. At the same time, the correlation factors of GST activity were 1.40 and 1.42 at 24 and 48 h post-treatment, respectively. Moreover, AChE activity was significantly inhibited at 24 and 48 h post-treatment, with correlation factors of 1.51 and 1.50, respectively ($P < 0.05$, Tukey's test; Table 2).

Discussion

Chrysoeriol, a 3'-methoxy derivative of luteolin, is present in many plant species; the present study is the first report of chrysoeriol isolated from *M. suavis*. Chrysoeriol is little known for its insecticidal potential compared with its medicinal properties [24]. The bioactivity of the crude extract does not significantly differ from that with the pure compound, indicating that it would be better to use a crude extract for controlling this insect than to make the effort to isolate the active principle, which is more convenient and economical for insecticide production. Our study provides essential information for the further development of novel compounds for use in agricultural production.

Detoxification mechanisms involve enzymes that catalyse reactions to make xenobiotics easier to excrete from an insect's body. Phase I enzymes catalyse oxidation, hydrolysis or reduction reactions, and phase II enzymes catalyse conjugation reactions. Finally, xenobiotics are excreted via phase III excretion [5]. Insects resist insecticides by presenting increased activities of CarE (a phase I enzyme) and GST (a phase II enzyme) [25, 26]. Our results indicated that chrysoeriol decreased CarE and GST activities, which might associate with toxic activity the high insecticidal activity of this compound [21]. Acetylcholinesterase hydrolyses the neurotransmitter acetylcholine to terminate synaptic transmission in insects' cholinergic nervous systems [27]. This enzyme has been exploited as a target of insecticides for insect pest control because the inhibition of AChE activity leads to increased insect mortality as a result of nervous system failure [28]. The present study demonstrated that *S. litura* larvae exposed to chrysoeriol exhibited decreased AChE activity and agitation, similar to the application of carbamate and organophosphate, indicating the occurrence of neurotoxic effects [28]. However, there are many enzymes associated with insect's mortality such as cytochrome P450 monooxygenases, UDP-glycosyl transferases, antioxidant and peroxidation enzymes, further studies are needed to explain the insecticidal activity of this compound [29, 30].

Conclusions

Spodoptera litura has developed resistance to many insecticides, so there is an urgent need to identify promising candidates for IPM to reduce the reliance on synthetic insecticides [31]. Chrysoeriol is highly toxic and inhibits the activity of enzymes critical to insect survival and has potential as a novel insecticidal agent against *S. litura*. Moreover, a keto group at C-4 of chrysoeriol exhibited strong antifeedant activity on *Mythimna unipuncta* [32]. However, further studies are needed to examine its potential in field trials and toxicity effects on nontarget organisms.

Table 2 Enzyme activities of *S. litura* after 24 and 48 h (h) treated with chrysoeriol and *M. suavis* crude extract

Treatments	CarE[a] (CF)[b]		Activity		GST[a] (CF)[b]		Activity		AChE[a] (CF)[b]		Activity	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control	3.71 ± 0.09^a	3.72 ± 0.09^a	–	–	2.06 ± 0.06^a	2.11 ± 0.06^a	–	–	1.76 ± 0.12^a	1.78 ± 0.07^a	–	–
Crude extract	3.58 ± 0.19^b (1.03)	3.53 ± 0.09^b (1.05)	Inhibition	Inhibition	1.70 ± 0.02^b (1.21)	1.64 ± 0.02^b (1.28)	Inhibition	Inhibition	1.23 ± 0.01^b (1.44)	1.27 ± 0.04^b (1.41)	Inhibition	Inhibition
Chrysoeriol	3.21 ± 0.08^b (1.15)	3.23 ± 0.09^b (1.15)	Inhibition	Inhibition	1.47 ± 0.03^b (1.40)	1.48 ± 0.04^b (1.42)	Inhibition	Inhibition	1.17 ± 0.06^b (1.51)	1.19 ± 0.01^b (1.50)	Inhibition	Inhibition

The mean values followed by the same letter within the same column of each treatment are not significantly different ($P > 0.05$) using Tukey's test

[a] Unit of enzymes: CarE activity (nM *p*-nitrophenol/min/mg), GST activity (mM glutathione conjugated product/min/mg) and AChE activity (acetylcholinesterase/mg protein/min).

[b] *CF* the correlation factor (acetone-treated group/chrysoberyl-treated group).

Ruttanaphan *et al. Chem. Biol. Technol. Agric.*   (2022) 9:21

Page 6 of 7

Authors' contributions
VB, SN and TR designed the experiment. TR, WT, NP and SN conducted the experiments. TR, VB and SN analysed the data, and wrote the manuscript. All authors read and approved the final version of the manuscript.

Availability of data and materials
All data are presented in Tables 1 and 2.

Declarations

Ethics approval and consent to participate
The Animal Ethics Committee of Kasetsart University approved all the methods of insect rearing (ACKU64-SCI-017).

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Division of Biology, School of Science, Walailak University, Nakhon Si Thammarat, Thailand. [2] Animal Toxicology and Physiology Specialty Research Unit, Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand. [3] Mahidol University, Nakhonsawan Campus, 402/1 Moo 5 Khaothong, Phrayuhakiri District, Nakhonsawan, Thailand.

References
1. Acharya R, Yu YS, Shim JK, Lee KY. Virulence of four entomopathogenic nematodes against the tobacco cutworm *Spodoptera litura* Fabricius. Biol Control. 2020;150:104348.
2. Sparks TC, Crossthwaite AJ, Nauen R, Banba S, Cordova D, Earley F, Ebbinghaus-Kintscher U, Fujioka S, Hirao A, Karmon D, Robert Kennedy R, Nakao T, Popham HJR, Salgado V, Watson GB, Wedel BJ, Wessels FJ. Insecticides, biologics and nematicides: updates to IRAC's mode of action classification—a tool for resistance management. Pestic Biochem Physiol. 2020;167:104587.
3. Tharamak S, Yooboon T, Pengsook A, Ratwatthananon A, Kumrungsee N, Bullangpoti V, Pluempanupat W. Synthesis of thymyl esters and their insecticidal activity against *Spodoptera litura* (Lepidoptera: Noctuidae). Pest Manag Sci. 2020;76:928–35.
4. Coats JR. Risks from natural versus synthetic insecticides. Annu Rev Entomol. 1994;39:489–515.
5. Hilliou F, Chertemps T, Maïbèche M, Le Goff G. Resistance in the genus *Spodoptera*: Key insect detoxification genes. Insects. 2021;12:544.
6. Le Goff G, Nauen R. Recent advances in the understanding of molecular mechanisms of resistance in noctuid pests. Insects. 2021;12:674.
7. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol. 2006;51:45–66.
8. Lengai GMW, Muthomi JW, Mbega ER. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Sci Afr. 2020;7:e00239.
9. Chomel M, Guittonny-Larchevêque M, Fernandez C, Gallet C, Des Rochers A, Paré D, Jackson BG, Baldy V. Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. J Ecol. 2016;104:1527–41.
10. Ruttanaphan T, de Sousa G, Pengsook A, Pluempanupat W, Huditz HI, Bullangpoti V, Le Goff G. A novel insecticidal molecule extracted from *Alpinia galanga* with potential to control the pest insect *Spodoptera frugiperda*. Insects. 2020;11:686.
11. Junhirun P, Pluempanupat W, Yooboon T, Ruttanaphan T, Koul O, Bullangpoti V. The study of isolated alkane compounds and crude extracts from *Sphagneticola trilobata* (Asterales: *Asteraceae*) as a candidate botanical insecticide for lepidopteran larvae. J Econ Entomol. 2018;111:2699–705.
12. Tlak Gajger I, Dar SA. Plant allelochemicals as sources of insecticides. Insects. 2021;12:189.
13. Sansomchai P, Jumpatong K, Lapinee C, Utchariyajit K. *Melientha suavis* Pierre. extract: antioxidant and sunscreen properties for future cosmetic development. Chiang Mai Univ J Nat Sci. 2020;20:e2021008.
14. Somsub W, Kongkachuichai R, Sungpuag P, Charoensiri R. Effects of three conventional cooking methods on vitamin C, tannin, myo-inositol phosphates contents in selected Thai vegetables. J Food Compos Anal. 2008;21:187–97.
15. Lan JE, Li XJ, Zhu XF, Sun ZL, He JM, Zloh M, Gibbons S, Mu Q. Flavonoids from *Artemisia rupestris* and their synergistic antibacterial effects on drug-resistant *Staphylococcus aureus*. Nat Prod Res. 2021;35:1881–6.
16. Takemura H, Nagayoshi H, Matsuda T, Sakakibara H, Morita M, Matsui A, Ohura T, Shimoi K. Inhibitory effects of chrysoeriol on DNA adduct formation with benzo[a]pyrene in MCF-7 breast cancer cells. Toxicology. 2010;274:42–8.
17. Wu JY, Chen YJ, Bai L, Liu YX, Fu XQ, Zhu PL, Li JK, Chou JY, Yin CL, Wang YP. Chrysoeriol ameliorates TPA-induced acute skin inflammation in mice and inhibits NF-κB and STAT3 pathways. Phytomedicine. 2020;68:153173.
18. Ruttanaphan T, Pluempanupat W, Bullangpoti V. Cypermethrin resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) from three locations in Thailand and detoxification enzyme activities. Agric Nat Resour. 2018;52:484–8.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.
20. Ruttanaphan T, Pluempanupat W, Aungsirisawat C, Boonyarit P, Le Goff G, Bullangpoti V. Effect of plant essential oils and their major constituents on cypermethrin tolerance associated detoxification enzyme activities in *Spodoptera litura* (Lepidoptera: Noctuidae). J Econ Entomol. 2019;112:2167–76.
21. Nobsathian S, Ruttanaphan T, Bullangpoti V. Insecticidal effects of triterpene glycosides extracted from *Holothuria atra* (Echinodermata: *Holothuroidea*) against *Spodoptera litura* (Lepidoptera: Noctuidae). J Econ Entomol. 2019;112:1683–7.
22. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88–95.
23. Bashyal P, Parajuli P, Pandey RP, Sohng JK. Microbial biosynthesis of antibacterial chrysoeriol in recombinant *Escherichia coli* and bioactivity assessment. Catalysts. 2019;9:112.
24. Kim MH, Kwon SY, Woo SY, Seo WD, Kim DY. Antioxidative effects of chrysoeriol via activation of the Nrf2 signaling pathway and modulation of mitochondrial function. Molecules. 2021;26:313.
25. Armes NJ, Wightman JA, Jadhav DR, Ranga Rao GV. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh. India Pestic Sci. 1997;50:240–8.
26. Karuppaiah V, Srivastava C, Padaria JC, Subramanian S. Quantitative changes of the carboxylesterase associated with pyrethroid susceptibility in *Spodoptera litura* (Lepidoptera: Noctuidae). Afr Entomol. 2017;25:175–82.
27. Thany SH, Tricoire-Leignel H, Lapied B. Identification of cholinergic synaptic transmission in the insect nervous system. Adv Exp Med Biol. 2010;683:1–10.
28. Lee SH, Kim YH, Kwon DH, Cha DJ, Kim JH. Mutation and duplication of arthropod acetylcholinesterase: implications for pesticide resistance and tolerance. Pestic Biochem Physiol. 2015;120:118–24.

29. Amezian D, Nauen R, Goff GL. Comparative analysis of the detoxification gene inventory of four major *Spodoptera* pest species in response to xenobiotics. Insect Biochem Mol Biol. 2021;138:103646.
30. Moustafa MAM, Awad M, Amer A, Hassan NN, Ibrahim EDS, Ali HM, Akrami M, Salem MZM. Insecticidal activity of lemongrass essential oil as an eco-friendly agent against the black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae). Insects. 2021;12:737.
31. Xu L, Mei Y, Liu R, Chen X, Li D, Wang C. Transcriptome analysis of *Spodoptera litura* reveals the molecular mechanism to pyrethroids resistance. Pestic Biochem Physiol. 2020;169:104649.
32. Medeiros J, Lima E, Medeiros E. Relationships between the structure of flavonoids and antifeedant activity against *Mythimna unipuncta* (Haworth) (Lepidoptera: Noctuidae). Arquipelago. 1994;12:63–6.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.