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Evaluation of biological activities, and exploration on mechanism of action of matrine–cholesterol derivatives

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ABSTRACT

To develop new potential pesticides, a series of matrine–cholesterol derivatives were prepared by modifications of two non-food bioactive products matrine and cholesterol. Two *N*-phenylsulfonylmatrinic esters (**5i** and **5j**) showed the most potent insecticidal activity against *Mythimna separata* Walker. Two *N*-benzylmatrinic esters (**5e** and **5g**) exhibited the most promising aphicidal activity against *Aphis citricola Van der* Goot. Especially compound **5e** showed good control effects in the greenhouse against *A. citricola*. Some interesting results of their structure-activity relationships were also observed. By reverse transcription polymerase chain reaction (RT-PCR) and quantitative real-time polymerase chain reaction (qRT-PCR) analysis of *HMG-CoA reductase* in apterous adults of *A. citricola*, it demonstrated that matrine and cholesterol may be the HMG-CoA reductase inhibitors, and the hydroxyl of cholesterol or the lactam ring of matrine may be important for acting with *HMG-CoA reductase* in *A. citricola*.

1. Introduction

Over the past decade, the repeat and massive use of synthetic pesticides has led to negative effects on the environment and human health, and insect pests resistance and rerampancy [1,2]. To develop new potential alternatives for efficient control insect pests, recently, naturally non-food bioactive substances have received much attention [3–5].

Matrine (**1**, Fig. 1) is an important plant secondary metabolite, extracted and isolated as a quinolizidine alkaloid from the roots of *Sophora flavescens* (Kushen) distributed in Asia, Oceanica, and the Pacific islands [6]. Matrine and its derivatives displayed a variety of medicinal activities such as antiviral activity [7], anti-inflammatory activity [8], anticancer activity [9], and so on. Additionally, although matrine and its derivatives (registered as a botanical pesticide in China) showed pesticidal activities for crop protection [10,11], their pesticidal activities are much lower in magnitude when compared with commercially chemical pesticides. Previously, we found some cholesterol-based hydrazones exhibiting potent insecticidal activity after modification of cholesterol (**2**, Fig. 1), a type of lipid molecule, which is transported in the blood plasma of all animals [12]. Consequently, we prepared a series of matrine–cholesterol derivatives (Fig. 1) by combining two molecules, matrine and cholesterol together. Their biological activities were tested against *Aphis citricola Van der* Goot and *Mythimna separata* Walker. Furthermore, their mechanism of action against *A. citricola* was investigated.

2. Materials and methods

2.1. Preparation of matrine–cholesterol derivatives (5a–n)

A mixture of compounds **4a–n** (0.5 mmol), cholesterol (**2**, 0.6 mmol), DCC (0.5 mmol), and DMAP (0.1 mmol) in 5 mL of dry dichloromethane was stirred at room temperature. After 48–96 h, the mixture was diluted with 20 mL of dichloromethane. Then the solution was washed with 0.1 M aq. HCl (10 mL), 5% aq. Na_2CO_3 (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, concentrated in *vacuo*, and purified by preparative thin-layer chromatography (PTLC) to afford target compounds **5a–n** in 9–31% yields. Data for compound **5a**: Yield: 21%, white solid; m.p. 146–148 °C; $[\alpha]_D^{20} = -7$ (*c* 2.4 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 7.32–7.34 (m, 2H, Ph-*H*), 7.28–7.30 (m, 2H, Ph-*H*), 7.19–7.22 (m, 1H, Ph-*H*), 5.33 (s, 1H, –CH = C), 4.55–4.61 (m, 1H, –OCH), 4.10 (d, *J* = 14.0 Hz, 1H), 3.10 (d, *J* = 13.5 Hz, 1H), 2.82–2.86 (m, 2H), 2.77 (d, *J* = 11.0 Hz, 1H), 2.62 (t, *J* = 12.0 Hz, 1H), 2.31–2.34 (m, 1H), 2.24–2.26 (m, 4H), 1.90–2.03 (m, 5H), 1.80–1.84 (m, 5H), 1.65–1.74 (m, 5H), 1.47–1.60 (m, 9H), 1.34–1.44

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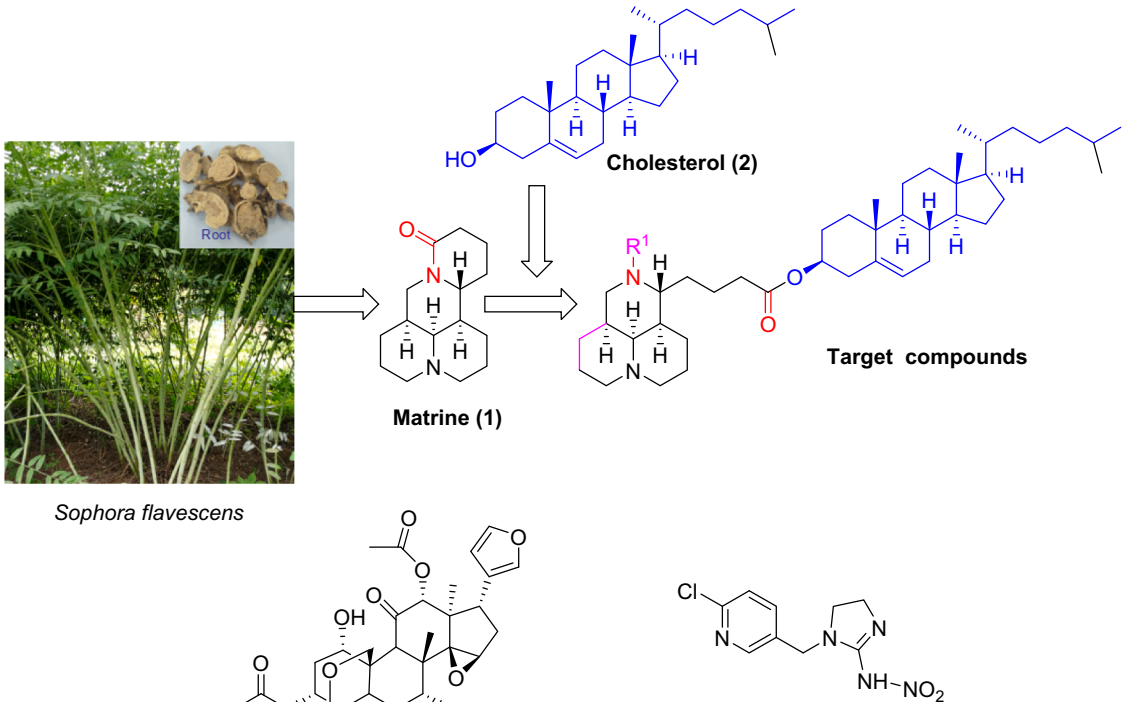


Fig. 1. Chemical structures of toosendanin, imidacloprid, matrine (**1**), cholesterol (**2**) and target compounds.

(m, 10*H*), 1.25 (s, 1H), 1.05–1.18 (m, 8H), 0.96 (s, 3H), 0.90–0.91 (m, 3H), 0.85–0.87 (m, 6H), 0.67 (s, 3H).

2.2. Biological assay

The biological activities of compounds **1**, **2** and **5a–n** were evaluated against *M. separata* and *A. citricola*, respectively [13,14].

2.3. Mechanisms of action against Aphis citricola

Acetone solutions of compounds **1**, **2**, and **5e** were prepared at 1.076, 2.878, and 0.609 mg/mL, respectively. The solution of imidacloprid (a positive control) was prepared in acetone at 0.248 μg/mL. The corresponding solution (0.04 μL) was added to the pronotum of aphids (120 healthy and size-consistency apterous adults of *A. citricola* for each compound). The aphids treated with acetone alone were used as CK. The experiment was carried out at 25 ± 1 °C and 50 ± 7% relative humidity (RH), and on 14 h/10 h (light/dark) photoperiod. After 48 h, 30 alive aphids of each treatment were selected out. The putative *HMG-CoA reductase* mRNAs of *A. citricola* were obtained from National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) and UniProt (https://www.uniprot.org/). The *N*-terminal ligand-binding conserved domain and the transmembrane regions (TM) were used by SMART program (http://smart.embl-heidelberg.de/). The primers for qRT-PCR were design using primer 3 online (http://primer3.ut.ee/). The mechanism of action of compound **5e** was explored through reverse transcription polymerase chain reaction (RT-PCR) and quantitative real-time polymerase chain reaction (qRT-PCR) analysis of the mRNAs expression in *A. citricola* [6,13].

3. Results and discussion

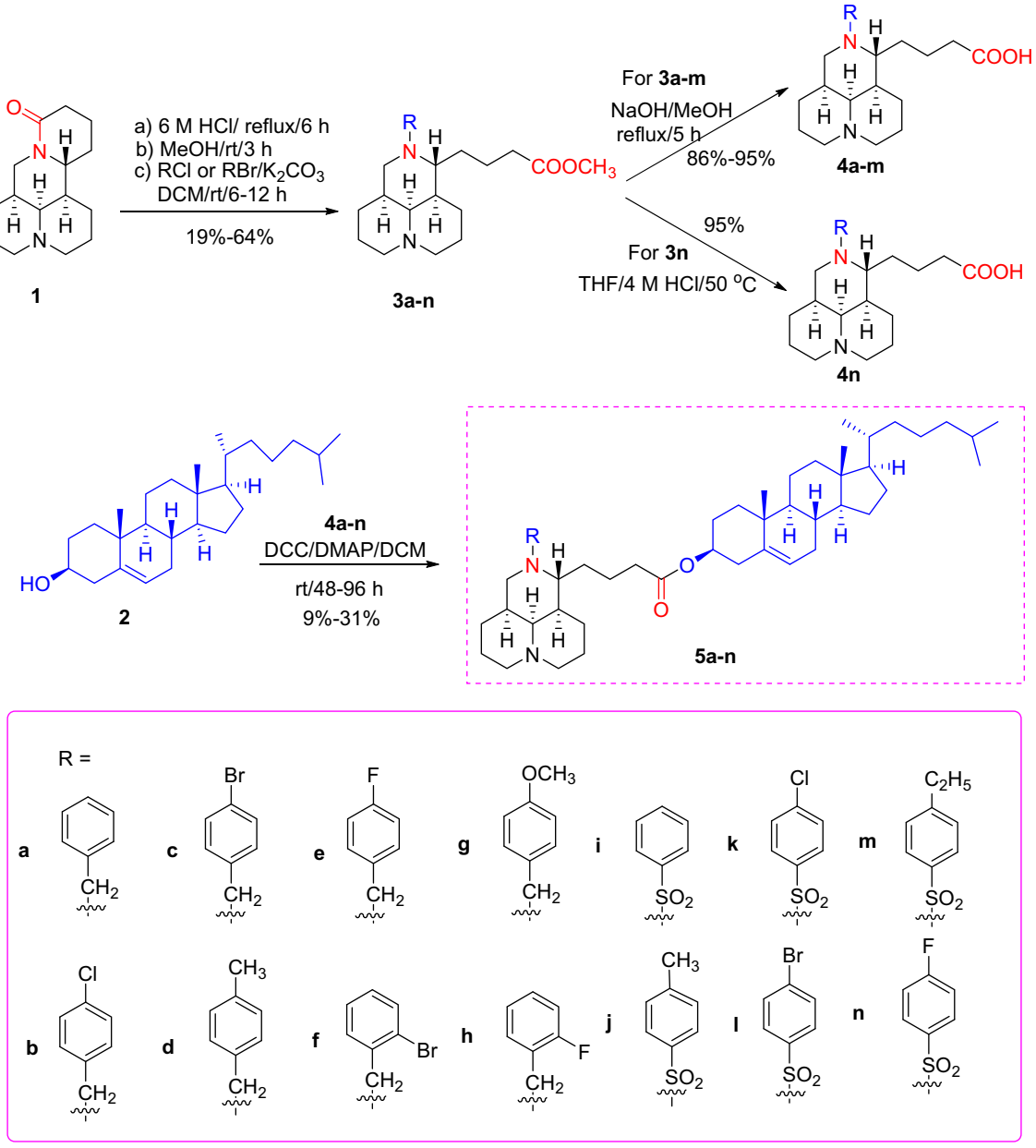
3.1. Chemistry

In Scheme 1, first, *N*-benzylmatrinic methyl esters and *N*-phenylsulfonylmatrinic methyl esters (**3a–n**) were obtained by reaction of matrine (**1**) with 6 M aq. HCl, followed by MeOH, and benzyl bromides/chlorides or phenylsulfonyl chlorides. Then hydrolysis of compounds **3a–n** in the presence of NaOH or HCl gave *N*-benzylmatrinic acids and *N*-phenylsulfonylmatrinic acids (**4a–n**). Finally, target compounds **5a–n** were obtained by reaction of compounds **4a–n** with cholesterol (**2**) in the presence of DCC and DMAP (Zhang et al., 2018). Their structures were characterized by optical rotation, IR, ^{1}H NMR, and mp (see Supplementary data).

3.2. Pesticidal activities

3.2.1. Growth inhibitory activity of compounds *1, 2* and *5a–n* against *M. separata*

As shown in the Table 1, the final mortality rates (FMRs) of these derivatives against *M. separata* after 35 days were generally higher than those after 10 or 20 days, so these derivatives displayed the delayed insecticidal activity against *M. separata*. On the other hand, the symptoms for all treated *M. separata* were observed as the same as our previous papers [6,13,14]. For instance, the thin and wrinkled dead larvae appeared at the larval stage (Fig. 2); some malformed and dead pupae were observed at the pupation period (Fig. 3); some malformed moths were found during the adult stage (Fig. 4). When compared with a botanical insecticide toosendanin (Fig. 1), compounds **5d**, **5g**, **5i**, **5j** and **5n** exhibited potent insecticidal activity against *M. separata*. The final



Scheme 1. Preparation of target compounds **5a–n**.

mortality rates (FMRs) of compounds **5d**, **5g**, **5i**, **5j** and **5n** were 51.7%, 51.7%, 58.6%, 55.1%, and 51.7%, respectively. Especially compounds **5i** and **5j** showed the most promising insecticidal activity; whereas the FMRs of their lead compounds (matrine (**1**) and cholesterol (**2**)) were only 24.1% and 20.7%, respectively (Table 1). To *N*-benzylmatrinic esters (**5a–h**), compounds **5d** (R = 4-methylbenzyl) and **5g** (R = 4-methoxybenzyl) showed potent insecticidal activity. It suggested that compounds containing the electron-donating groups on their phenyl usually exhibited more potent insecticidal activity than those containing the electron-withdrawing ones. To *N*-phenylsulfonylmatrinic esters (**5i–n**), compounds **5i** (R = phenylsulfonyl), **5j** (R = 4-methylphenylsulfonyl) and **5n** (R = 4-fluorophenylsulfonyl) displayed pronounced insecticidal activity, however, the electron effect of the substituents R on the insecticidal activity was not obvious.

*3.2.2. Aphicidal activity of compounds **1**, **2** and **5a–n** against Aphis citricola*

As described in Table 2, compounds **5c**, **5e**, **5g** and **5h** showed potent aphicidal activity against *A. citricola*. The 48 mortality rates (MRs) of compounds **5c**, **5e**, **5g** and **5h** were 48.8%, 56.9%, 50.5%, and 44.1%, respectively. Especially compound **5e** (R = 4-fluorobenzyl) displayed the most pronounced aphicidal activity with the 48 h MR of 56.9%; whereas the 48 h MRs of compounds **1** and **2** were only 31.8% and 19.3%, respectively. In general, derivatives **5a–h** (R as the (un) substituted benzyl) exhibited more promising aphicidal activity than **5i–n** (R as the (un)substituted phenylsulfonyl). To compounds **5a–h**, introduction of the fluorine atom at the 4-position on the phenyl was important for the aphicidal activity (e.g., **5e** (4-F) Vs. **5c** (4-Br); **5e** (4-F) Vs. **5h** (2-F)).

Table 1
Growth inhibitory activity of compounds **1**, **2** and **5a–n** against *M. separata* at 1 mg/mL.

Compound	Corrected mortality rate (mean ± SE, %)		
	10 days	20 days	35 days
1	6.7 ± 3.3	16.6 ± 3.3	24.1 ± 3.3
2	3.3 ± 3.3	13.3 ± 3.3	20.7 ± 6.6
5a	13.3 ± 3.3	13.3 ± 3.3	34.5 ± 6.6
5b	6.7 ± 6.6	6.7 ± 6.6	27.6 ± 5.7
5c	23.3 ± 6.6	26.6 ± 8.8	44.8 ± 8.8
5d	6.7 ± 3.3	20.0 ± 5.7	51.7 ± 8.8
5e	16.6 ± 6.6	23.3 ± 8.8	48.2 ± 5.7
5f	6.7 ± 6.6	13.3 ± 3.3	44.8 ± 3.3
5g	20.0 ± 5.7	23.3 ± 8.8	51.7 ± 3.3
5h	30.0 ± 5.7	43.3 ± 8.8	48.2 ± 5.7
5i	33.3 ± 6.6	46.6 ± 3.3	58.6 ± 5.7
5j	6.7 ± 3.3	16.6 ± 8.8	55.1 ± 8.8
5k	20.0 ± 5.7	30.0 ± 10.0	44.8 ± 3.3
5l	10.0 ± 5.7	13.3 ± 3.3	44.8 ± 8.8
5m	10.0 ± 5.7	16.6 ± 6.6	34.5 ± 3.3
5n	20.0 ± 5.7	20.0 ± 5.7	51.7 ± 6.6
toosendanin	16.6 ± 3.3	23.3 ± 8.8	48.2 ± 5.7

Fig. 2. The representative abnormal larvae pictures of compounds **5i (XJW-26)**, **5k (XJW-30)**, **5h (XJW-33)**, **5g (XJW-23)**, **5n (XJW-28)**, **5c (XJW-32)**, and **5m (XJW-35)** against *M. separata* during the larval period (CK: blank control group).

Fig. 3. The representative malformed pupae pictures of compounds **5d (XJW-21)**, **5g (XJW-23)**, **5l (XJW-24)**, **5j (XJW-25)**, **5k (XJW-30)**, **5f (XJW-31)**, and **5m (XJW-35)** against *M. separata* during the pupation period (CK: blank control group).

Fig. 4. The representative malformed moth pictures of compounds **5d (XJW-21)**, **5g (XJW-23)**, **5j (XJW-25)**, **5i (XJW-26)**, **5e (XJW-27)**, **5n (XJW-28)**, and **5h (XJW-33)** against *M. separata* during the adult emergence period (CK: blank control group).

The 48 h LD_{50} values of some potent derivatives against *A. citricola* were shown in Table 3. The LD_{50} values of **5c**, **5e**, **5g** and **5h** were between 0.041 and 0.047 μg/larvae. Compounds **5e** and **5g** exhibited > 2.4 folds more pronounced aphicidal activity than compound **1** (LD_{50} value: 0.101 μg/larvae), and compounds **5e** and **5g** exhibited > 4.5-fold more potent aphicidal activity than compound **2** (LD_{50} value: 0.191 μg/larvae). Moreover, compounds **1** and **5e** were tested for their control effects against *A. citricola* at 1.0 mg/mL in the greenhouse (Table 4). Compound **5e** exhibited good control effects when compared with that of compound **1**. The control effects of compound **5e** on the 3rd, 5th and 7th day were 55.0%, 58.7%, and 59.1%, respectively; whereas the control effects of compound **1** were 26.5%, 32.9%, and 34.4%, respectively. The pictures of control efficiency of compounds **1** and **5e** against *A. citricola* were shown in Fig. 5.

3.3. Mechanisms of action against A. citricola

HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase) is the rate-controlling enzyme for cholesterol biosynthesis [15]. In insects pest, HMG-CoA reductase can regulate insect juvenile hormones (JH) biosynthesis [16,17]. Cholesterol derivaties alone or in combination with statin can be used as inhibitors reducing HMG-CoA reductase protein accumulation in CHO-7 cells to treat cardiovascular disease in mammals [18]. So compounds **1**, **2**, and **5e** were investigated targeting on HMG-CoA reductase of *A. citricola*. The primers of HMG-

Table 2
Aphicidal activity of compounds **1**, **2** and **5a–n** against apterous adults of *A. citricola* treated at 0.04 μg/larvae [a]At 0.8 ng/larvae.

Compound	Corrected mortality rate (mean ± SE, %)	
	24 h	48 h
1	13.4 ± 2.9	31.8 ± 5.0
2	6.7 ± 1.1	19.3 ± 2.9
5a	8.0 ± 1.1	27.0 ± 2.9
5b	3.5 ± 0	29.4 ± 1.9
5c	25.0 ± 1.9	48.8 ± 4.0
5d	6.9 ± 1.9	35.2 ± 2.9
5e	13.6 ± 2.2	56.9 ± 2.9
5f	9.2 ± 1.1	34.1 ± 2.9
5g	13.7 ± 1.9	50.5 ± 3.8
5h	19.3 ± 4.0	44.1 ± 1.9
5i	3.4 ± 1.1	32.5 ± 1.1
5j	14.9 ± 2.9	32.9 ± 3.3
5k	8.0 ± 1.9	19.7 ± 1.9
5l	11.4 ± 2.9	37.6 ± 4.0
5m	11.3 ± 3.8	31.3 ± 2.9
5n	6.8 ± 1.1	29.0 ± 2.2
imidacloprid[a]	64.0 ± 1.1	88.6 ± 1.1

CoA reductase gene of *A. citricola* used for qRT-PCR were described in Table 5, and the schematic diagram of *HMG-CoA reductase* in *A. citricola* was shown in Fig. 6a. The mRNA expressions changes of *HMG-CoA reductase* in *A. citricola* against compounds **1**, **2**, **5e** and imidacloprid (a positive control) were tested by qRT-PCR and RT-PCR (Fig. 6b–c). Compounds **1** and **2** down-regulated *HMG-CoA reductase* to 0.08 and 0.13 folds, respectively; whereas imidacloprid up-regulated *HMG-CoA reductase* to 11.43 folds. It suggested that compounds **1** and **2** may be inhibitors of HMG-CoA reductase. The result of compound **2** was the same as the previous reports [18]. Interestingly, cholesterol-matrine derivative **5e** had no effect on the mRNA expression changes of *HMG-CoA reductase* in *A. citricola*. That is, opening the lactam ring of compound **1** or esterization of hydroxyl of compound **2** may lead to no action with *HMG-CoA reductase* in *A. citricola*. It demonstrated the hydroxyl of compound **2** or the lactam ring of compound **1** may be necessary for acting with *HMG-CoA reductase* in *A. citricola*.

4. Conclusion

A series of matrine–cholesterol derivatives were prepared by modifications of two non-food bioactive products, matrine and cholesterol. Especially two *N*-phenylsulfonylmatrinic esters (**5i** and **5j**) showed the most promising insecticidal activity against *M. separata*. Two *N*-benzylmatrinic esters (**5e** and **5g**) exhibited the most pronounced aphicidal activity against *A. citricola*. It demonstrated that *N*-benzylmatrinic esters generally showed more promising aphicidal activity than *N*-phenylsulfonylmatrinic ones; on the contrary, *N*-phenylsulfonylmatrinic esters generally exhibited more potent insecticidal activity than *N*-benzylmatrinic ones. Compound **5e** displayed good control effects against *A. citricola*. By RT-PCR and qRT-PCR analysis of *HMG-CoA reductase* in apterous adults of *A. citricola*, it suggested that compounds **1**

Table 3
LD_{50} values of some compounds at 48 h against *A. citricola*.[a]

Compound	Regression equation	LD_{50} (μg/larvae)	Confidence interval 95% (μg/larvae)	r
1	Y = 1.411 + 1.417X	0.101	0.079–0.159	0.979
2	Y = 1.711 + 2.381X	0.191	0.168–0.229	0.944
5c	Y = 4.181 + 3.102X	0.045	0.041–0.050	0.988
5e	Y = 3.254 + 2.342X	0.041	0.036–0.047	0.987
5g	Y = 3.926 + 2.848X	0.042	0.038–0.047	0.987
5h	Y = 2.980 + 2.247X	0.047	0.041–0.057	0.990
imidacloprid	Y = 3.822 + 0.869X	0.00004	0.000026–0.000062	0.972

[a] Regression analysis by IBM SPSS Statistics 23.0, $P < 0.05$.

Table 4
Control efficiency of compounds **1** and **5e** against *A. citricola* in greenhouse tested at 1.0 mg/mL.

Compound	Control efficiency (mean ± SE, %)			
	1st day	3rd day	5th day	7th day
1	7.7 ± 1.6	26.5 ± 1.4	32.9 ± 1.7	34.4 ± 1.2
5e	11.0 ± 1.2	55.0 ± 1.6	58.7 ± 1.7	59.1 ± 2.6
Imidacloprid[a]	68.2 ± 1.6	98.0 ± 0.4	99.4 ± 0.4	100.0 ± 0

[a] At 10 mg/L.

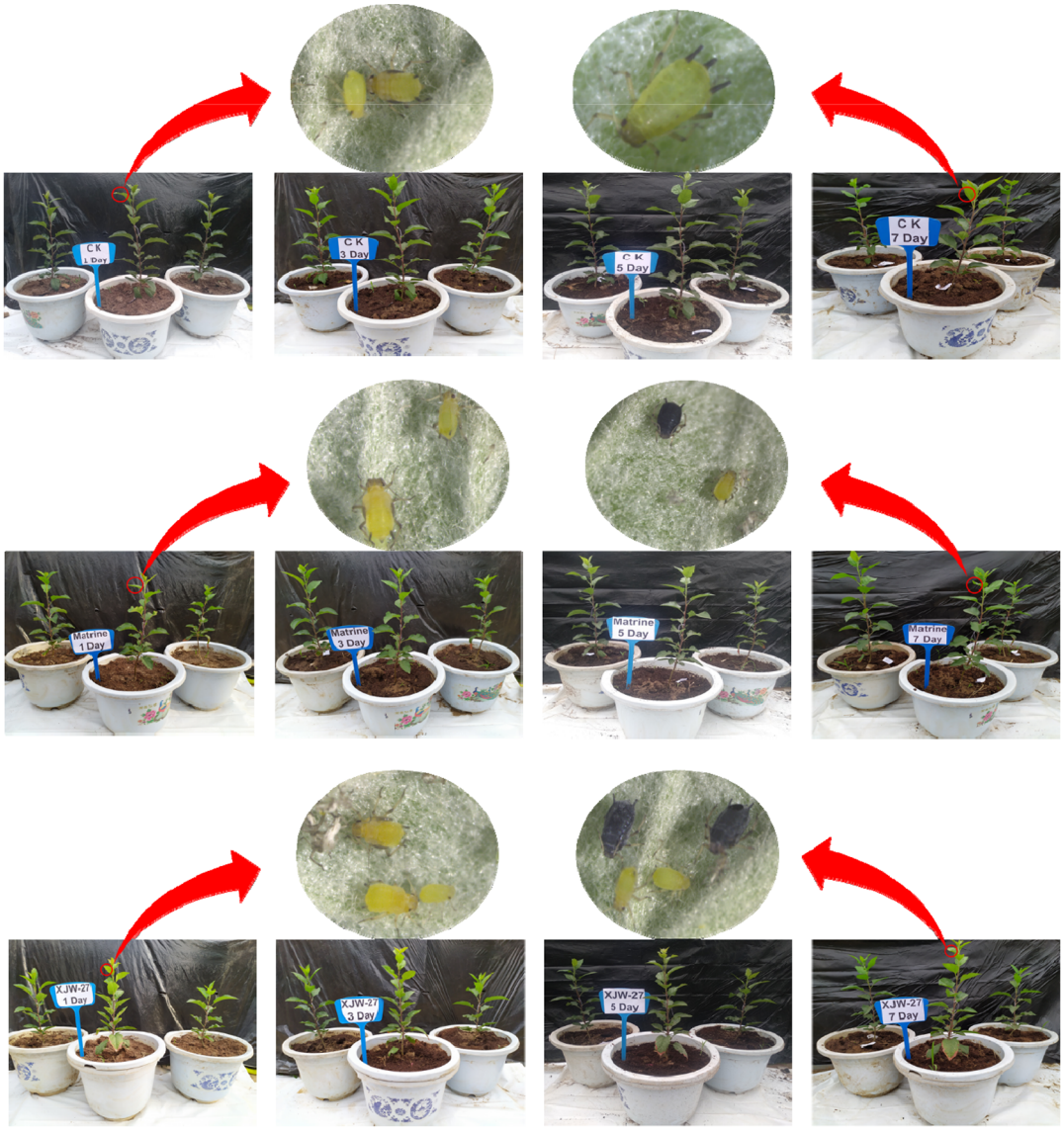


Fig. 5. Pictures of control efficiency of matrine (**1**), and compound **5e** (**XJW-27**) against *Aphis citricola* in the greenhouse.

Table 5
Primers of *HMG-CoA reductase* of *A. citricola* used for qRT-PCR.

Gene	Sequence (5′–3′)[b]	Annealing temperature (°C)	Product size (bp)
Ef1-α	F-agcctggtatggttgtcgtt R-ctgtgaaatcagcagctccc	60	207
HMG-CoA reductase[a]	F-cagagagggtgttggtctt R-gtccagcaactccaacaggt	60	165

[a] *HMG-CoA reductase*: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. [b]F: forward primer; R: reverse primer.

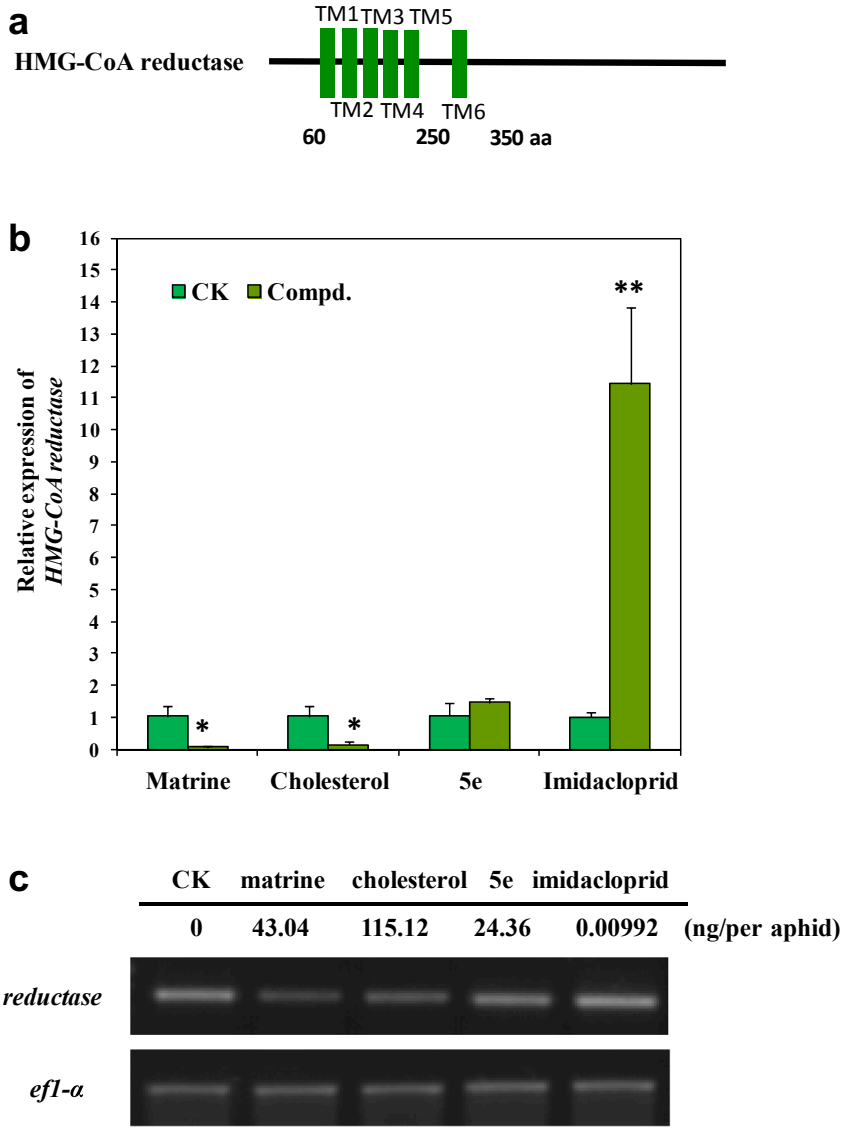


Fig. 6. The expression patterns of *HMG-CoA reductase* in apterous adults of *A. citricola* collected 48 h post-treatment with matrine (43.04 ng/larvae), cholesterol (115.12 ng/larvae), compound **5e** (24.36 ng/larvae), and imidacloprid (0.00992 ng/larvae), respectively. (a): The schematic diagram of the *HMG-CoA reductase* in *A. citricola.* TM: the transmembrane regions. (b): The *HMG-CoA reductase* mRNA expressions were evaluated by qRT-PCR. *HMG-CoA reductase* mRNA expressions were normalized to *ef1-α* expression (mean ± SD, n = 3). Asterisks indicate significant differences (*$P < 0.05$; **$P < 0.01$) compared with CK. CK: blank control group. (c): *HMG-CoA reductase* was evaluated through RT-PCR.

and **2** may be the HMG-CoA reductase inhibitors, and hydroxyl of compound **2** or the lactam ring of compound **1** may be necessary for acting with *HMG-CoA reductase* in *A. citricola.*

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103439.

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