Insecticidal and Repellent Action of Pogostone Against Myzus persicae (Hemiptera: Aphididae)

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Insecticidal and repellent action of pogostone against *Myzus persicae* (Hemiptera: Aphididae)

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Abstract

Essential oil of *Pogostemon cablin* (Blanco) Bentham (Lamiaceae), known as patchouli oil, has been reported to display strong insecticidal activities, but few studies have focused on the insecticidal activity of its principal constituent, pogostone. The present work was designed to evaluate the insecticidal, repellent, and antifeedant activities of pogostone against *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). In a choice test, pogostone had strong repellent activity against *M. persicae*. Pogostone also showed pronounced contact toxicity (LC_{50} of 1,694 mg/L) to this piercing–sucking insect, as well as powerful antifeedant activity. Pogostone may partly account for the insecticidal activity of patchouli oil, but it also has other valuable properties. This study demonstrated the potential of pogostone as a promising candidate for managing agricultural insects.

Key Words: antifeedant; toxicity; honeydew production

Resumen

El aceite esencial de *Pogostemon cablin* (Blanco) Benth. (Lamiaceae), conocido como el aceite de pachuli, ha demostrado tener fuertes actividades insecticidas, pero pocos estudios se han enfocado en la actividad insecticida de su componente principal, pogostona. El presente trabajo fue diseñado para evaluar las actividades insecticidas, repelentes y de anti-alimentión de pogostona contra *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). En una prueba de elección, pogostona tuvo una actividad repelente fuerte contra *M. persicae*. Pogostona también mostró una toxicidad de contacto pronunciada (CL_{50} de 1.694 mg/L) a este insecto picador-chupador, así como una poderosa actividad de anti-alimentación. Pogostona puede explicar en parte la actividad insecticida del aceite de pachulí, pero también tiene otras propiedades valiosas. Este estudio demostró el potencial de la pogostona como un candidato prometedor para el manejo de insectos de importancia agrícolas.

Palabras Clave: anti-alimentación; toxicidad; producción de mielcilla

The family Aphididae includes some of the most destructive insect pests of crops, many of which are major vectors of viral diseases affecting many cultivated plants worldwide. Currently, the most common practice to reduce aphid populations is to apply chemical insecticides (Hansson et al. 2013; Rimaz & Valizadegan 2013). The widespread use of synthetic insecticides has adverse effects on non-target organisms and results in the contamination of natural resources with toxic residues (Bughio & Wilkins 2004). Plant-derived extracts (phytochemicals) and bio-agents represent an alternative to synthetic pesticides and could reduce these harmful effects (Goh et al. 2001; Kim & Kim 2008). In recent years, researchers have increasingly focused on the use of natural products obtained from plants to control insect pests (Andrade et al. 2012; Ebrahimi et al. 2013; Pinto et al. 2013).

Patchouli oil, the essential oil extracted from leaves of *Pogostemon cablin* (Blanco) Bentham (Lamiaceae), is an important natural material used in the perfumery and food industries. Historically, it has been used to repel clothes moths, and it is frequently applied in diverse pharmacological activities in Chinese medicine. This essential oil has also been documented to have acute toxic effects on house dust mites (Wu et al. 2010), house flies (Pavela 2008), urban ants (Albuquerque et al. 2013), and vegetable pests (Machial et al. 2010).

Pogostone ($C_{12}H_{16}O_4$; chemical structure shown in Fig. 1) is a major active ingredient of patchouli oil (Osawa et al. 1990; Hu et al. 2006; Li et al. 2012; Yi et al. 2013). The hydrolysate of pogostone was shown to have rapid acaricidal activity against the house dust mite, *Dermatophagoides farinae* Hughes (Astigmata: Pyroglyphidae) (Wu et al. 2012). Previously, we found that pogostone had strong insecticidal activity against *Spodoptera litura* F. (Lepidoptera: Noctuidae) and *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) (Huang et al. 2014). However, no data have been reported of its effect on aphids. This study

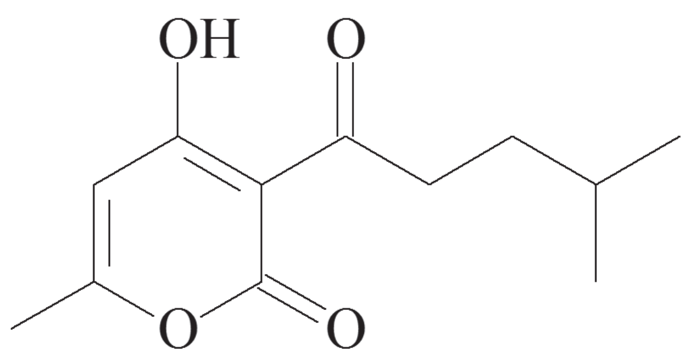

Fig. 1. Structure of pogostone.

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was designed to evaluate the possible insecticidal activity of pogostone against *M. persicae* by means of choice tests, contact toxicity assays, and antifeedant assays.

Materials and Methods

MATERIALS

Synthetic pogostone was obtained as described in Yi et al. (2013). Pogostone composition and purity were determined to be 97.4% using gas chromatography–mass spectroscopy. Adults of *M. persicae* were originally collected from organic tobacco planted at the South China Agricultural University in Guangzhou (23.16062°N, 113.34573°E) and identified by Prof. Ji-Dong Xian (entomologist at South China Agricultural University). Adults of *M. persicae* were reared on tobacco under controlled conditions at 25 ± 1 °C, 70 ± 10% relative humidity, and a photoperiod of 16:8 h L:D of artificial light (approx. 4,000 lx).

REPELLENCY ASSAY

The repellency of pogostone to *M. persicae* was tested according to the methods described by Salari et al. (2012) with some modifications. A choice test was conducted using fresh tobacco leaf discs (4 cm in diameter) divided into 2 parts by a vein, with one half dipped in pogostone solution at 0 (control), 125, 250, 500, 1,000, 2,000, or 4,000 mg/L for 3 s and air dried at room temperature (Huang et al. 2014), whereas the other half was dipped in acetone and used as a negative control, because acetone was used to dissolve the pogostone. Ten 3rd instar aphids were placed on each part of the tobacco leaf discs, and 5 replicates were maintained for each experiment (total $n = 35$ assays). After 24, 48, and 72 h, the numbers of aphids were counted on the treated and control parts of the leaves. The experiment was conducted under laboratory conditions at 25 ± 1 °C with a 14:10 h L:D photoperiod and 70 ± 10% relative humidity.

TOXICITY ASSAY

Contact toxicity was assessed by the method described by Giner et al. (2013) with some modifications. In these assays, 0.1 µL pogostone solution at 125, 250, 500, 1,000, 2,000, or 4,000 mg/L was directly applied on the thorax of *M. persicae* by using a 10 µL syringe, and 0.1 µL acetone was applied as a control. After treatment, the aphids were maintained on untreated tobacco leaves, and mortality was recorded at 24 and 48 h post treatment. The laboratory conditions were the same as in the repellency experiment. Five replicates were maintained for each treatment with five 3rd instar aphids per replicate (total $n = 35$ assays).

FEEDING ASSAY

The amount of honeydew produced by the aphids was used to determine the antifeeding effects of treatments. The ninhydrin test was used to visualize feeding as described by Kanrar et al. (2002). In brief, fresh leaf discs measuring 4 cm in diameter were dipped individually in pogostone solution at 125, 250, 500, 1,000, 2,000, or 4,000 mg/L for 3 s and air dried at room temperature (Huang et al. 2014). Leaf discs dipped in acetone were used as the control. Then a 4 cm diameter Petri dish was lined with a filter paper so that drops of honeydew would fall on it. This filter paper was removed after 24 h and sprayed with 0.1% ninhydrin reagent to detect the area of honeydew spots. The area occupied by honeydew was visually estimated using graph paper in each treatment and the control (acetone). Five replicates were maintained for each treatment, with five 3rd instar aphids per replicate (total $n = 25$ aphids). The laboratory conditions were the same as in the repellency experiment.

DATA ANALYSES

The repellency index (RI) was calculated by the following formula: RI = [(C−T)/C+T)] (Pascual-Villalobos & Robledo 1998). In this case, C is the number of aphids in the control area, and T is the number of aphids in the treated area. Positive and negative values indicate repellent and attractant effects, respectively. LC_{50} and LC_{90} values were calculated using probit analysis of mortality vs. concentration data in SAS® software version 9.0 (SAS Institute, Cary, North Carolina). The honeydew production or antifeedant activity (AA) was calculated by the following formula: AA = [(C–T)/C] × 100. In this case, C is the area of honeydew in the control and T the area of honeydew in the treatment. Data relating to repellent activity, contact toxicity, and feeding were analyzed using 1-way ANOVA. Differences between treatments were determined by the Duncan multiple range test and were considered statistically significant at $P < 0.05$.

Results

REPELLENCY

The results of the dual-choice bioassay, expressed as repellency index (RI) at 24, 48, and 72 h after application of pogostone at different concentrations, are shown in Table 1. Concentration ($F = 93.06$; df = 5; $P < 0.01$), but not time interval ($F = 2.77$; df = 2; $P = 0.07$), had significant effects on repellency. The interaction of concentration and time was not significant ($F = 0.13$; df = 10; $P = 0.99$). However, once a concentration of 1,000 mg/L was attained, increasing the concentration did not affect repellency.

CONTACT TOXICITY

Concentration of pogostone significantly affected toxicity ($F = 438.69$; df = 6; $P < 0.01$), as did time interval ($F = 8.61$; df = 1; $P < 0.01$). The interaction of concentration and time was not significant ($F = 0.76$; df = 6; $P = 0.60$). We found no significant differences between LC_{50} values of pogostone at 24 and 48 h, as indicated by fiducial limits overlap (Table 2). Similar LC_{90} values were also observed 24 and 48 h after exposure. Thus, the exposure time did not contribute to the acute mortality of *M. persicae*. No mortality was observed in the control group.

FEEDING ACTIVITY

The results of the ninhydrin test indicated that aphids in the pogostone treatment groups produced smaller amounts of honeydew ($F = 38.40$; df = 6; $P < 0.01$) than aphids in the control group. As shown in

Table 1. Repellent activity of pogostone against *Myzus persicae* at 6 concentrations and 3 post-treatment time intervals.

Concentration (mg/L)	Repellency index		
	24 h	48 h	72 h
125	0.230 ± 0.07aA	0.002 ± 0.17aB	0.015 ± 0.11aB
250	0.219 ± 0.05aA	0.234 ± 0.15bA	0.206 ± 0.12bA
500	0.346 ± 0.07bA	0.350 ± 0.21bA	0.245 ± 0.17bB
1,000	0.477 ± 0.14cA	0.467 ± 0.19cA	0.438 ± 0.21cA
2,000	0.493 ± 0.14cA	0.491 ± 0.07cA	0.487 ± 0.37cA
4,000	0.533 ± 0.07cA	0.531 ± 0.13cA	0.507 ± 0.30cA

Means ± SE followed by the same letter do not differ significantly according to the Duncan test ($P > 0.05$); uppercase letters represent differences among time intervals (rows), whereas lowercase letters represent differences in the tested concentrations in the same time interval (columns).

Table 2. Contact toxicity of pogostone against *Myzus persicae* at 24 and 48 h post treatment.

Time (h)	LC_{50} (mg/L)	LC_{90} (mg/L)	Toxicity regression equation	*P*	χ^2
24	1,761 (1,398–2,296)	5,010 (3,532–9,105)	$y = -9.1631 + 2.8230 \times \log_{10}(x)$	0.984	0.380
48	1,694 (1,355–2,176)	4,557 (3,285–7,916)	$y = -9.6293 + 2.9822 \times \log_{10}(x)$	0.970	0.529

Table 3, honeydew production by *M. persicae* was significantly reduced at a pogostone concentration of 125 mg/L and markedly reduced at concentrations of 500 mg/L or higher. The pogostone concentration of 2,000 mg/L resulted in more than 90% antifeedant activity. Our results suggest that the antifeedant activity of pogostone against *M. persicae* is dose dependent.

Discussion

To our knowledge, this is the first report of insecticidal activity of pogostone against aphids. The effects of pogostone on *M. persicae* included not only acute mortality, but also repellency and antifeedant activity. Previous research had shown that patchouli oil possesses insecticidal activities against different kinds of insect pests (Pavela 2008; Machial et al. 2010; Wu et al. 2010; Albuquerque et al. 2013). However, it was not clear whether pogostone, a major ingredient of patchouli oil, exhibits insecticidal effects against aphids. In this study, we demonstrated that pogostone exerts insecticidal, repellent, and antifeedant properties against *M. persicae*.

Choice tests are considered to be a reliable method for measuring the repellent effect on *M. persicae* (Salari et al. 2012), and the repellency index (RI) can effectively be used to reflect repellent effects on aphids (Kanrar et al. 2002). Repellency of *M. persicae* has been reported by several authors. For example, Gutiérrez et al. (1997) tested bisabolene, farnesol, and geraniol as aphid repellents against *M. persicae* in assays using apterous aphids and leaf discs embedded in agar. These compounds affected the insects' probing behavior and acted as repellents for apterous aphids. A similar result was obtained by Salari et al. (2012), who found that *Peganum harmala* L. (Tetradiclidaceae) acetonic seed extract acted as a strong repellent on *M. persicae*. In addition, allyl esters (except allyl hexanoate) had high to moderate repellency to beetles and aphids (Giner et al. 2013).

In this study, pogostone had strong repellent activity against aphids in concentrations equal to or greater than 1,000 mg/L; patchouli oil was previously shown to have similar effects at higher concentrations (Zeng et al. 2006a,b). This suggests that pogostone may be the active constituent responsible for the repellent effect of patchouli oil on insects. We found no significant differences among RI values as the concentration of pogostone increased. Also, time of exposure was not a significant factor. The RI values did not increase or decrease with time within the 72 h of observation; this was also observed by other authors when assessing aphid repellents (Lowery & Isman 1993; Bruce et al. 2005; Zapata et al. 2010).

Pogostone also exhibited contact toxicity against *M. persicae* and at concentrations lower than those found for other plant oils. For example, Ebrahimi et al. (2013) reported LC_{50} values of neem, eucalyptus, and laurel essential oils on cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), of 5,389, 9,515, and 13,730 mg/L, respectively, which were higher than the LC_{50} values obtained in our study. Our results suggest that pogostone is a major active constituent responsible for the toxic effects of patchouli oil on aphids.

Aphids produce honeydew when they feed. The ninhydrin test used here to evaluate honeydew production is widely used for antifeedant activity studies on aphids (Sadeghi et al. 2009). Liu et al. (2010) similarly reported that beta-caryophyllene and alpha-pinene, isolated from *Vitex negundo* L. (Lamiaceae) seed extract, had significant adverse effects on the honeydew excretion frequency and honeydew production of cotton aphid. Clearly, pogostone functions as an antifeedant and might provide additional protection to plants.

The molecular structure of pogostone is composed principally of the parent structure of alpha-pyrone. Accumulated evidence suggests compounds having a parent structure of alpha-pyrone exert an excellent controlling effect on insects (Arimori et al. 2011). Supratman et al. (2001) reported that all bufadienolides having an orthoacetate and alpha-pyrone moiety showed strong insecticidal activity. In addition, Hidayat et al. (2014) reported that both orthoacetate and alpha-pyrone moieties were probably essential structural elements for insecticidal activity against 3rd instar larvae of silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). Hence, the insecticidal activity of pogostone against *M. persicae* may partly be attributed to its alpha-pyrone moiety.

In conclusion, pogostone exerts significant repellency, contact toxicity, and antifeedant activity against *M. persicae*. Pogostone may account, at least in part, for the purported insecticidal efficacy of patchouli oil. This study has provided important baseline information for the potential use of pogostone as a promising, safe insecticidal agent with low environmental toxicity. Nonetheless, the precise underlying repellent, toxic, and antifeedant mechanisms merit further investigation.

Table 3. Honeydew production by *Myzus persicae* and antifeedant activity of pogostone when applied at several concentrations.

Concentration (mg/L)	Honeydew area (mm^2)[a]	Antifeedant activity (%)
125	24.200 ± 9.203 b	48.35
250	21.000 ± 7.746 b	55.12
500	6.300 ± 5.729 c	86.35
1,000	6.300 ± 5.461 c	86.14
2,000	2.300 ± 1.956 c	94.24
4,000	1.100 ± 1.084 c	95.95
Control	46.900 ± 6.097 a	0.00

[a]Means ± SE within columns followed by the same letter do not differ significantly according to the Duncan test ($P > 0.05$).

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