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Chemical composition and bioactivity of the essential oil from *Artemisia lavandulaefolia* (Asteraceae) on *Plutella xylostella* (Lepidoptera: Plutellidae)

Xing Huang¹, Si-Yan Ge¹, Jing-Hao Liu¹, Yong Wang¹, Xin-Yuan Liang¹, and Hai-bin Yuan^{1,*}

Abstract

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the dominant insect pest of cruciferous crops around the world, and is resistant to many chemical insecticides. In this study, we measured the chemical composition and bioactivity of *Artemisia lavandulaefolia* DC (Asteraceae) essential oil on *P. xylostella*. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry. A total of 35 constituents were identified. The principal compounds were: eucalyptol (35.60%), (R)-4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (16.25%), π -trimethyl-3-cyclohexene-1-methanol (6.83%), 3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one (6.63%), and (1S)-1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (4.72%). The LD₅₀ contact toxicity of the essential oil to immature *P. xylostella* was estimated at 0.045 μ L per larva. *Artemisia lavandulaefolia* oil exhibited fumigant toxicity against *P. xylostella* adults with an LC₅₀ of 0.113 mg per L after 12 h and also provided 80 to 100% repellency at a 1% v/v concentration.

Key Words: Eucalyptol; diamondback moth; fumigant toxicity; repellent; botanical insecticide

Resumen

La polilla de diamante, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), es el insecto plaga dominante en cultivos crucíferos alrededor del mundo, y es resistente a muchos insecticidas químicos. En este estudio, medimos la composición química y la bioactividad del aceite esencial de *Artemisia lavandulaefolia* DC (Asteraceae) sobre *P. xylostella*. Se obtuvo el aceite esencial por la hidrodestilación y se le analizó mediante cromatografía de gases-espectrometría de masas. Se identificó un total de 35 componentes. Los principales compuestos fueron: eucalyptol (35.60%), (R)-4-metil-1-(1-metiletil)-3-ciclohexen-1-ol (16.25%), π -trimetil-3-ciclohexeno-1-metanol (6.83%), 3-metil-6-(1-metiletil)-2-ciclohexen-1-ona (6.63%) y (1S)-1,7,7-trimetil-biciclo[2.2.1]heptano-2-uno (4.72%). Se estimó la toxicidad por contacto DL₅₀ del aceite esencial a los inmaduros de *P. xylostella* en 0.045 μ L por larva. El aceite de *Artemisia lavandulaefolia* mostró toxicidad por fumigación contra adultos de *P. xylostella* con una DL₅₀ de 0.113 mg por larva después de 12 horas y también proporcionó una repelencia del 80 para 100% a una concentración del 1% v/v.

Palabras Clave: Eucalyptol; polilla de diamante; toxicidad fumigante; repelente; insecticida botánico

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most important insect pest of cruciferous crops throughout the world (Javler 1992). The annual cost for managing this pest has been estimated to be US\$1 billion (Talekar & Shelton 1993). However, recent data on management costs combined with associated crop losses by diamondback moths have been reported to be US\$4–5 billion (Zalucki et al. 2012). Chemical control of *P. xylostella* has become less effective because of the diversity and abundance of host plants, lack or disruption of natural enemies, high reproductive potential (up to 20 generations per year), and proven ability to rapidly evolve resistance to insecticides used for its control (Magaro & Edelson 1990; Lim et al. 2002; Liang et al. 2003; Furlong et al. 2013; Lu & Lee 1984; Khan et al. 2005). Long-term use of synthetic insecticides has given rise to many ecological problems, including residues that are toxic to wildlife, and possible bioaccumulation issues associated with the environment (Shelton et al. 1993; Charleston & Kfir

2000; Isman 2006). Bioactive plant-derived compounds have been suggested to be alternative sources for insect control because many are selective to insect pests and have no or minimal adverse effects on non-target organisms and the environment (Regnault-Roger 1997; Walter 1999; Schmutterer 1990; Prakash & Rao 1996; Charleston et al. 2005).

Artemisia lavandulaefolia DC (Asteraceae) is a perennial herb with procumbent rhizomes while the aerial components are strongly aromatic. This plant species has been used in traditional medicine in many cultures for treatment of stomatitis, fever, bronchitis, chronic cervicitis, hemorrhagia, cholecystitis, including some cardiovascular diseases and liver ailments (Wang et al. 2006a; Cha et al. 2010).

The essential oil of *A. lavandulaefolia* contains various chemicals proven to inhibit the mycelial growth of *Pyricularia grisea* (Pyriculariaceae) and *Rhizoctonia solani* (Ceratobasidiaceae) fungi (Jiang et

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al. 2008) as well as possessing antimicrobial activity against obligate anaerobic bacteria (Cha et al. 2005). The chemical composition of *A. lavandulaefolia* essential oil has been characterized in several studies and whole plant extracts were found to have insecticidal activity on *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) (Yuan et al. 2010; Liu et al. 2010a). Also, numerous studies have reported the bioactivity of essential oils derived from various *Artemisia* species against stored-product insects (Kordali et al. 2006; Liu et al. 2006, 2010b; Wang et al. 2006b; Goel et al. 2007; Negahban et al. 2007; Tripathi et al. 2000; Chu et al. 2010, 2012; You et al. 2015). However, no studies have evaluated the bioactivity of *A. lavandulaefolia* against *P. xylostella*. Therefore, we report here on the contact and fumigant toxicity, as well as repellent properties of *A. lavandulaefolia* essential oil, on *P. xylostella* in laboratory trials.

Materials and Methods

PLANT MATERIAL AND ESSENTIAL OIL EXTRACTION

Fresh aerial parts of *A. lavandulaefolia* were collected in Sep 2015 at the flowering stage in Changchun (43.8170°N, 125.3235°E), China. Plant samples were dried in the shade at ambient temperature, then crushed and soaked in water for 12 h with a solid: liquid ratio of 1:1. Afterwards, the crushed aerial parts were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate and stored in a sealed vial in a refrigerator at 4 °C.

INSECT REARING

Plutella xylostella were reared from larvae and pupae obtained from cabbage in an experimental field of Jilin Agricultural University, Changchun, China. Larvae were reared on individual cabbage (*Brassica oleracea* var. *capitata*) plants that had never been exposed to pesticides, and maintained in a screened cage (40 × 29 × 17 cm) at 25 ± 1 °C and 75% RH, and a 12:12 h (L:D) photoperiod. After moth emergence, the adults were fed a 10% honey solution. Cabbage leaves were used for oviposition. Third instars and 3-day-old adults were used in bioassays.

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ANALYSES

The essential oil of *A. lavandulaefolia* was analyzed using a gas chromatograph (Agilent 6890N, Agilent Technologies Incorporated, California, United States), and the oil constituents were identified using a mass spectrometer (MS, Agilent 5975N, Agilent Technologies Incorporated, California, United States). The gas chromatograph apparatus was equipped with an HP-5 capillary column (30 m × 0.25 µm inside diameter, film thickness of 0.25 µm). Settings were as follows: initial column temperature held at 60 °C for 3 min, then ramped at 10 °C per min intervals to 180 °C and held isothermally for 1 min, and finally raised to 280 °C at 20 °C per min and maintained for 5 min. The injector temperature was maintained at 280 °C. A diluted 1 µL sample of essential oil was injected at a split ratio of 50:1. Helium was used as the carrier gas at a flow rate of 1.0 mL per min. The mass spectrometer spectra used an electron ionization source (70 eV ionization, source temperature of 230 °C). The scan range was 20-650 m/z at 2 scans per s. Constituents of the essential oils were identified by comparing the results with the mass spectra libraries (National Institute of Standards and Technology, Gaithersburg, Maryland, USA: NIST databases), and component relative percentages were expressed as percentages by peak area normalization (Adams 1989).

BIOASSAYS

Third instar *P. xylostella* were used to evaluate the contact toxicity of the essential oil. Five concentrations (0.025, 0.05, 0.075, 0.1 and 0.125 µL per larva) were diluted in acetone. All treatments used a 0.5-µL dose to the dorsal thoracic region. Acetone was used as a control. Ten larvae were treated per concentration, and the study was repeated 3 times. Treated and control insects were placed separately in Petri dishes (90 mm diam) and kept in incubators at 29 ± 1 °C and 75 ± 5% RH for 24 h, after which mortality was recorded during 2, 4, 6, 8, 10, 12, and 24 h. Mortality was calculated as follows:

$$\text{MR (\%)} = N_d / N_t \times 100$$

$$\text{CM (\%)} = (\text{MR}_t - \text{MR}_c) / (1 - \text{MR}_c) \times 100$$

Where MR is the mortality rate, N_d is the number of dead insects and N_t is the total number of insects treated. CM is corrected mortality, MR_t is mortality rate on the insecticide-treated plants and MR_c is mortality rate on the acetone-treated (control) plants.

Three-day-old adults of *P. xylostella* were used to evaluate the fumigant toxicity of the essential oil. Serial dilutions of the *A. lavandulaefolia* essential oil were treated with acetone (0.1, 0.2, 0.3, 0.4, and 0.5 mg per L). Acetone was used as a control. Ten µL of the appropriate concentration of the essential oil was added to filter paper (8.0 cm × 1.5 cm). The solvent was allowed to evaporate for 30 s before the cap was placed on the glass bottle (60 mL, with 10 insects) to form a sealed chamber. All treatments and controls were maintained in incubators (29 ± 1 °C, 75 ± 5% RH). The mortality was recorded during 2, 4, 6, 8, 10, and 12 h.

The repellent activity of the essential oil to individual *P. xylostella* adults was measured using a “Y” glass tube olfactometer. The essential oil was tested at different volume fractions (0.25, 0.5, 1, 2, and 4% v/v) in acetone. Each tube was connected to an aromatic-source bottle where 10 µL of the appropriate concentration was added to a 25 × 10 mm filter paper, then placed in an aromatic-source bottle after the solvent evaporated for 30 s. Acetone was used as a control. A fluorescent light was set parallel above the Y-tube to avoid light interference. Both arms of the tube were filled with pure humidified air at a rate of 400 mL per min.

A single adult diamondback moth was placed at the entrance of the olfactometer and after 10 min, its position in the tube was recorded (Wang et al. 2016). Moth response criteria were determined as follows: if the moth climbed to more than half the length into one of the tubes and remained for 1 min or more, it was deemed the insect chose this path; if the moth made no choice after 5 min, it was deemed no choice. Ten adults were exposed to each concentration and each concentration was replicated 3 times. The olfactometer tube was cleaned with ethyl alcohol after each concentration. The percent repellency (PR) values were determined as follows:

$$\text{PR (\%)} = [(N_c - N_r) / N_c] \times 100$$

Where N_c is the number of insects in the essential oil-containing areas, and N_r is the number of insects in the areas lacking essential oil.

STATISTICAL ANALYSES

Statistical procedures for all analyses used SPSS Statistics 17.0 (IBM, New York, New York, USA). Results from all bioassays were subjected to probit analysis to determine the LC_{50} and LD_{50} values, fiducial limits, and slopes (Alarie 1988; Sakuma 1998). To determine if differences ($P < 0.05$) existed between treatments and controls in repellent bioassays, data were analyzed using Student's *t*-test. These data were plotted by Prism 6.0 (GraphPad Software, La Jolla, California, USA).

Results

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ANALYSES

The essential oil yield of *A. lavandulaefolia* was 4.50×10^{-3} L per kg (v/w). A total of 35 components were identified, accounting for 97.37% of the total oil (Table 1). The main compounds were eucalyptol (35.59%), (R)-4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol (16.25%), π -trimethyl-3-cyclohexene-1-methanol (6.82%), 3-methyl-6-(1-methylethyl)-2-cyclohexen-1-one (6.63%), and (1S)-1,7,7-trimethyl-bicyclo[2.2.1]heptan-2-one (4.71%).

BIOASSAYS

In the contact toxicity study, the LD₅₀ of *A. lavandulaefolia* essential oil was 0.07 μ L per *P. xylostella* larva at 2 h and 4 h post application then subsequently decreased to 0.05 μ L per larva at 24 h (Table 2). For the fumigation toxicity study, the LC₅₀ of the essential oil was greatest at 0.25 mg per L for adult diamondback moths at 2 h exposure (Table 3). The effectiveness of this concentration decreased with time to an LC₅₀ of 0.113 mg per L after 12 h of continuous exposure. *Artemisia lavandulaefolia* oil produced its greatest repellency to adult *P. xylostella*

at the 4% concentration. However, only the repellency of the 0.25% concentration proved to be significantly different from controls (Fig. 1).

Discussion

The chemical compositions of the essential oil reported here are in partial agreement with previous reports, as Deng et al. (1987) reported that the main constituents of *A. lavandulaefolia* essential oil to be eucalyptol (36.54%), borneol (3.50%), and 4-terpineol (2.59%). Yuan et al. (2010) reported that the principal compounds of the essential oil from this plant species when extracted by steam distillation contained eucalyptol (10.74%), $\alpha,\alpha,4$ -trimethyl-3-cyclohexene-1-methanol (5.26%), and 4-carene (4.00%). Zhang et al. (2012) reported that eucalyptol (20.62%), borneol (15.32%), and eudesm-7(11)-en-4-ol (13.81%). Indeed, variation in chemical composition of essential oils may be due to geographic and seasonal factors. For example, the main compounds of the essential oil of *A. lavandulaefolia* collected from Jiangxi Province (Northern China) were caryophyllene (15.53%), (1R)-1,7,7-trimethyl-bicyclo[2.2.1]heptane-2-one (10.37%), α -caryophyllene (8.8%), camphor (6.89%) and D-myrcene (6.48%) (Xiong 2011). In addition, the main compounds of the essential oil obtained from Beijing (Central

Table 1. Chemical constituents of *Artemisia lavandulaefolia* essential oil.

No.	Compounds	RI ^a	Composition (%)
1	Santolina triene	901	0.33
2	β -Pinene	922	1.05
3	Camphene	943	0.35
4	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	961	0.66
5	β -Phellandrene	964	0.76
6	Ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-	970	0.16
7	3,3,6-Trimethyl-1,4-heptadien-6-ol	987	0.53
8	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	1008	0.95
9	Benzene, 1-methyl-3-(1-methylethyl)-	1010	1.81
10	Eucalyptol	1023	35.60
11	1,5-Heptadien-4-one, 3,3,6-trimethyl	1041	0.27
12	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	1047	1.58
13	3,3,6-Trimethyl-1,5-heptadien-4-ol	1068	0.22
14	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	1078	0.31
15	Thujone	1096	2.95
16	Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl	1099	2.41
17	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans	1123	0.82
18	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	1146	4.72
19	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis	1151	1.14
20	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1 π 2 π 5 π)-	1157	0.47
21	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	1165	16.25
22	3-Cyclohexene-1-methanol, π -trimethyl	1172	6.83
23	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-	1228	6.63
24	Bicyclo[3.1.1]hept-2-en-6-ol, 2,7,7-trimethyl-, acetate, [1S-(1 π 5 π 6 π)-	1242	1.50
25	Copaene	1376	0.83
26	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2 π 4 π 8 π 8 π)-	1411	0.59
27	Caryophyllene	1424	1.12
28	β -Caryophyllene	1456	0.53
29	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a	1457	1.32
31	β -Elemene	1465	0.34
	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1 π 4 π 8 π 8 π)-	1480	0.32
32	Caryophyllene oxide	1601	0.51
33	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	1605	1.42
34	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis	1630	1.52
35	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methylethylidene)-, [1R-(1 π 4 π 8 π 8 π)-	1631	0.60

^aRetention index (RI) as determined on an HP-5 MS capillary column using the homologous series of *n*-hydrocarbons.

Table 2. Contact toxicity of *Artemisia lavandulaefolia* essential oil to *Plutella xylostella* larvae.

Time (h)	LD ₅₀	Slope ± SE	95% FL ^a
2	0.072	2.661 ± 0.285	0.056–0.096
4	0.072	2.959 ± 0.296	0.057–0.092
6	0.066	3.324 ± 0.304	0.046–0.091
8	0.066	3.101 ± 0.295	0.045–0.095
12	0.059	3.363 ± 0.299	0.018–0.108
24	0.045	3.161 ± 0.290	0.012–0.071

^aFiducial limits.**Table 3.** Fumigation toxicity of *Artemisia lavandulaefolia* essential oil to *Plutella xylostella* adults.

Time (h)	LC ₅₀	Slope ± SE	95% FL ^a
2	0.249	4.304 ± 0.343	0.082–0.179
4	0.191	4.819 ± 0.373	0.107–0.268
6	0.180	4.630 ± 0.363	0.087–0.264
8	0.119	9.003 ± 1.069	0.112–0.128
12	0.113	9.150 ± 1.373	0.106–0.122

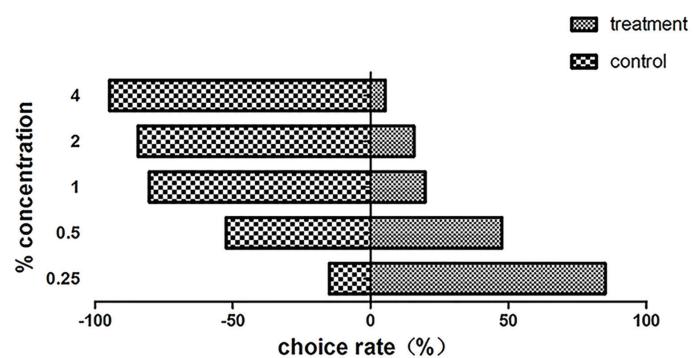
^aFiducial limits.

China) were caryophyllene (15.5%), β-thujone (13.8%), eucalyptol (13.1%), and β-farnesene (12.3%) (Liu et al. 2010a). The compositions in the oil from Guizhou Province (Southern China) were caryophyllene (25.39%), 7,11-dimethyl-3-methylene-1,6,10-dodecatriene (13.21%) and ryanthenone (7.75%) (Ma et al. 2012).

In summary, our study showed that extracts of the aerial portion of *A. lavandulaefolia* possessed contact toxicity, fumigant, and repellent activity against *P. xylostella*. Additionally, we believe that the essential oil from this plant species has potential for development as a novel bioactive product against *P. xylostella*. Further studies are required to characterize those components of the essential oil with the greatest bioactivity for additional screening, so that their potential application in controlling pests can be fully exploited.

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**Fig. 1.** Repellent activity of *Artemisia lavandulaefolia* essential oil to *Plutella xylostella*.

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