Deterrent Effect of Essential Oil Extracted from *Dolichandrone cauda-felina* on Oviposition of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae)

AISHA Habita, LU Yong-yue, ZENG Ling
(Lab of Insect Ecology, South China Agric. Univ., Guangzhou 510642, China)

Abstract: Oviposition-repelling activity of essential oils of *Dolichandrone cauda-felina* (Hance) Benth. & Hook. F., behavioral tests using four-armed olfactometer and elecroantennograms (EAG) responses using EAG recording technique in *Liriomyza sativae*, were all studied. The bioassay results showed that the repellent effect of the volatile was dose-dependent; the best effect was at dosage of 5 μL, IIPC and repellent rate were 0.289, 55.14% respectively. Olfactory response of *L. sativae* indicated the strong repellent effect of the essential oil, and no patterns differences between male and female. The EAG recording produced analogous results: the essential oil elicited an EAG response in leafminers adults, and showed a dose dependent. GC/MS analysis of essential oil indicated that there were 21 known compounds; the dibutyl phthalate had the highest relative concentration (56.55%).

Key words: *Liriomyza sativae*; *Dolichandrone cauda-felina*; oviposition; deterrent effect
CLC number: S482.3 **Document code**: A **Article ID**:1001-411X(2007)01-0058-05

猫尾木挥发油对美洲斑潜蝇产卵驱避作用研究

AISHA Habita, 陆永跃, 曾 玲
(华南农业大学 昆虫生态研究室,广东 广州 510642)

摘要:采用室内生物测定和电生理学的方法研究了猫尾木挥发油对美洲斑潜蝇产卵驱避作用以及美洲斑潜蝇对该挥发油的行为反应和触角电位活性.生物测定结果表明,每棵豆苗施用1~5 μL挥发油,大部分处理的落卵量明显低于对照,其中5 μL剂量效果最好,干扰作用指数(IIPC)和驱避率分别为0.289、55.14%.这表明挥发油对美洲斑潜蝇具有明显的产卵驱避作用.四臂嗅觉仪测试中对挥发油的第一选择率明显较低,处理的停留时间大部分低于对照,进入次数仅在250 mL/min流量时处理明显降低.雌雄虫对挥发油的嗅觉反应无差异.美洲斑潜蝇对挥发油表现明显的触角电位活性,且随着挥发油浓度增大而增大.触角电位活性与挥发油量之间的关系符合模型$Y=43.414e^{0.369\,1X}$.应用GC-MS分离出猫尾木挥发油主要含有21种化合物,其中邻苯二甲酸二丁酯含量最高,占56.55%,其次为邻苯二甲酸二乙基己酯和1-壬烯-3-醇,分别占9.8%和6.64%.

关键词:美洲斑潜蝇;猫尾木;挥发油;产卵驱避

Plants contain a large reservoir of chemical structures with biological activity. Researches with alternative methods for controlling insect pests and especially food attractants, oviposition deterrents, mating disruption using sex pheromones and antifeedants have progressed greatly in recent years[1]. According to Akhtar

Received date:2005-11-12
Biographies:AISHA Habita(1978—), male, master; Corresponding author: ZENG Ling(1949—), female, professor, E-mail: zengling@scau.edu.cn
Foundation item: Natural Science Foundation of China (38770516 and 39930120)

and Isman[2], plant secondary compounds have been the subject of thorough investigation for the past 30 years in an effort to discover new sources of botanical insecticides and antifeedants. Well-documented records show that before 1850, 20 plant species belonging to 16 different families were used to control the agricultural and horticultural pests in Western Europe and China[3-4]. Actually the use of plants compounds is still under development, and only time will tell whether or not they will widely adopted[5]. Only more than 250 000 plant species on our planet have been properly evaluated for use in pest control[6]. The rich knowledge of plants with pesticide properties was not lost in China, as evidenced by a recent report stating that in China, different parts or extracts of 276 plant species are used as pesticides[7]. Some insecticides of plant origin were used on a large scale before they were out competed by synthetic insecticides; nicotine, rotenone and pyrethrins have been extensively used and were effective insecticides because they degrade rapidly, do not accumulate in the food chain[8]. In the present study the repellent activity of essential oils from *Dolichandrone cauda-felina* against *Liriomyza sativae* (Blanchard) was investigated. The olfactory and electrophysiological response was also determined, and the chemical composition of the essential oils was determined by GC/MS analysis.

1 Materials and methods

1.1 Insect collection

Infested leaves with *L. sativae* larvae were collected from the countryside in Guangzhou. After larvae were changed into them, pupae were collected in small tubes, and new emerged adults were ready to tests.

1.2 Extracting method of essential oils

Fresh leaves of *D. cauda-felina* were collected from the campus of South China Agricultural University, and cut to small pieces then steam distilled. The mixture of oil and water obtained was separated using ethyl ether, then dried by anhydrous Na_2SO_4 and the solvent was removed using evaporator rotator. The crude of essential oils was stored in a refrigerator at 3-4 ℃ until used.

1.3 Bioassay methods

Kidney bean plants *Phaseolus vagaris* L. were used as tested host plants, with two seedlings in a cup each seedling has two leaves. A known quantity of essential oils was applied at a filter paper beside the seedlings placed in a bioassay cage, adult leafminers were introduced in the cage, and 10% (φ) of honey was provided as a supplement food to the adults. After 24 h number of eggs was checked under the microscope. The experiment was conducted at (27 ± 1) ℃.

1.4 Behavioral test

Four-armed olfactometer was used to test the behavior of the both sex of the adult leafminer, one arm was selected as the treated arm, and the other 3 arms were untreated. The humidified air stream, drained through a hole in the center of device, was set at 60, 100, 200 and 250 mL/min successfully for each arm, in each test flow, 20 adults were individually introduced into the chamber through the exit hole and test time was 10 min for every adult; the adult was moved freely in the chamber, the time spent in each arm, the number of entries and the first choice of the test adult were recorded through the software OLFA.

1.5 Electrophysiological recording

The elecroantennograms (EAG) technique and the odor delivery system are as describing by Zhao and Kang [9]. The tested insect's head was removed, and the tip of the arista cut off, the reference electrode was positioned into the haemocoel of the cranial cavity, while the recording electrode was connected with the cut arista. A constant flow (0.1 L/min) of charcoal-filtered and humidified compressed air was passed over the antenna through a disposable nozzle positioned 1 cm from the antenna. Test substances were introduced into the air stream by inserting the tubing of the cartridge into the hole of delivery tube and injecting the odor stimulation was 0.2 s, the signal was amplified 10 × by a universal AC/DC Un-06 and viewed in the computer screen. EAG's were recorded with a Syntech EAG cartridge recording system for windows. Different concentrations were prepared by dilution of *D. cauda-felina* essential oils with paraffin oil; they were applied to filter paper, which placed inside Pasteur pipettes (l = 15 cm), a standard stimulus cis-3-hexen-1-ol was tested. For each stimulus EAGs were recorded from 10 antennae of the leafminer flies, the negative deflection values were recorded in the computer. These values were converted by measuring the percentage of the response to

the accompanying cis-3-hexen 1-ol standard.

2 Results

2.1 Oviposition deterrent of *D. cauda-felina* against *L. sativae* adults

The data in the table 1 showed that all dosages (except dosage 3 μL) had a significant repellency effect on the leafminers adults, and the best effects were similar in dosage of 1,2,4 and 5 μL. The repellency rate was 57.28%, 77.38%, 65.32% and 66.83% respectively.

Tab. 1 The effect of oviposition deterrent of plant volatile on adult leafminers *L. sativae*[1)]

dosage/μL	mean number of eggs	repellency rate/%
1	8.50 ± 0.80b	57.28
2	4.50 ± 0.50b	77.38
3	13.00 ± 5.45ab	34.67
4	6.91 ± 2.61b	65.32
5	6.66 ± 3.27b	66.83
0	19.92 ± 3.19a	

1) Means in the same column, followed by the same letter are not significantly different at level 0.05 (DMRT)

2.2 Olfactory response of *L. sativae* in various flow rates of plant volatiles

The results of bioassays in the olfactometer are shown in the table 2 and table 3. In the control, the leafminers spent more time than in the treated arms. The time spent was significantly different in different air flux, it was the maximum at 60 mL/min, and 250 mL/min of air current in the control arms was the lowest in treated arms. The number of entries was significantly different at 250 mL/min between control and treated arms. The first choice rate was significantly different in all different air current flux. The results in the tests showed that essential oil had obvious deterrent effect on the *L. sativae* adults. Olfactory response patterns of male and female leafminers were similar in the bioassay (table 3).

2.3 Electrophysiological response of *L. sativae* to plant volatiles

The chemical senses of insects are characterized by an extremely high sensitivity and are key factors for the insect's behavior. A variety of electrophysiological and behavioral bioassays have been developed to use these features for scientific and practical applications. EAG is a kind of technique for recording the sensory information. It involves measuring changes in electrical potential across the whole antenna. It has the advantage of exposing volatile components within a mixture of chemicals to all the receptors on the antenna[10]. The data of EAGs recorded in the experiment indicated that the essential oil of *D. cauda-felina* excite the sensory cells of olfactory sensilla, which can be as messenger to insect's brain to regulate its behavior and avoid oviposition of *L. sativae*. The results showed also that EAG were dose-dependent, and the model $Y = 43.414e^{0.3691X}$ constructed can describe the relationship between the *D. cauda-felina* essential oil and reduction ratio of EAG response to the standard (Fig. 1).

Tab. 2 Olfactory response of *L. sativae* adults in various flow rates of plant volatiles[1)]

air current flux/	*t*/min		number of entries		first choice	
(mL · min^{-1})	CK	treatment	CK	treatment	CK	treatment
60	2.80 ± 0.23a	1.00 ± 0.54b	2.40 ± 0.30a	2.15 ± 0.50a	0.95 ± 0.10a	0.05 ± 0.10b
100	2.67 ± 0.25a	1.64 ± 0.73a	2.10 ± 0.60a	1.70 ± 0.95a	0.80 ± 0.15a	0.20 ± 0.15b
200	2.60 ± 0.27a	0.65 ± 0.49b	3.06 ± 0.70a	2.15 ± 0.82a	0.90 ± 0.13a	0.10 ± 0.13b
250	2.72 ± 0.15a	0.07 ± 0.05b	2.68 ± 0.32a	0.40 ± 0.20b	0.95 ± 0.10a	0.05 ± 0.10b

1) Means in the same column, followed by the same letter are not significantly different at level 0.05 (DMRT)

Tab. 3 Olfactory responses of female and male of leafminer adults in various flow rates of plant volatiles[1)]

air current flux	*t*/min		number of entries		first choice	
/(mL · min^{-1})	female	male	female	male	female	male
60	1.61 ± 0.36a	2.18 ± 0.54a	2.35 ± 0.39a	2.20 ± 0.44a	0.50 ± 0.00 a	0.50 ± 0.00a
100	2.03 ± 0.55a	2.29 ± 0.57a	2.48 ± 1.06a	1.32 ± 0.32a	0.50 ± 0.10a	0.50 ± 0.10a
200	1.81 ± 0.54a	1.44 ± 0.35a	2.28 ± 0.57a	2.93 ± 0.92a	0.50 ± 0.00a	0.50 ± 0.00a
250	1.42 ± 0.33a	1.37 ± 0.32a	1.52 ± 0.40a	1.56 ± 0.35a	0.50 ± 0.00a	0.05 ± 0.00a

1) A pair of means in the same row in the same index, followed by the same letter are not significantly different at level 0.05 (DMRT)

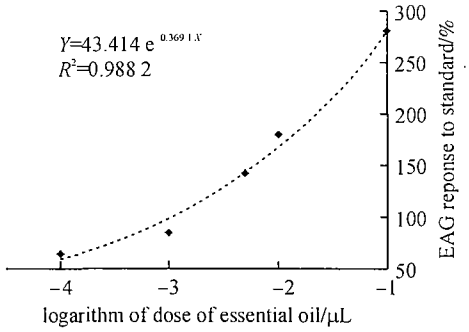

Fig. 1 Dose-response of *L. sativae* to *D. cauda-felina* essential oil

2.4 Chemical analysis of *D. cauda-felina* essential oil

The analysis of essential oil of *D. cauda-felina* using gas chromatography/mass spectrometric showed the majority of compounds are some oily esters; the dibutyl phthalate had the highest relative concentration (56.55%), followed by di- (2-ethylhexyl) phthalate (9.8%) and 1-octen-3-ol (6.64%) (table 4).

Tab. 4 Chemical composition of *D. cauda-felina* essential oil

retain time /min	compound name	molecular formula	probability	relative content/%
3.96	3-hexen-1-ol, (Z)- (CAS)	$C_6H_{12}O$	30.23	2.24
4.50	hexanal (CAS)	$C_6H_{12}O$	29.21	2.39
6.75	(+-)-5-cyyclohexyidene-3-hydroxy-1-pentene	$C_{11}H_{18}O$	62.05	0.87
7.67	1-octen-3-ol	$C_8H_{16}O$	48.56	6.64
10.72	linalool oxide(2)	$C_{10}H_{18}O_2$	48.25	0.49
11.65	linalool	$C_{10}H_{18}O$	63.21	1.67
14.34	benzoic acid, 2-hydroxy methyl ester (CAS)	$C_8H_8O_3$	44.86	0.43
18.64	10,12-otadecadiynoic acid	$C_{18}H_{28}O_2$	6.73	0.68
19.69	ά-damascenone	$C_{13}H_{18}O$	78.09	1.41
20.86	ά-ionone	$C_{13}H_{20}O$	38.63	0.73
23.31	nonadecane(CAS)	$C_{19}H_{40}$	12.44	0.34
27.88	hexadecane, 2,6,10,14- tetramethyl- (CAS)	$C_{20}H_{42}$	7.39	1.18
33.50	dibutyl phthalate	$C_{16}H_{22}O_4$	55.18	56.55
39.15	2-hexadecen-1-ol,3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	$C_{20}H_{40}O$	43.25	0.99
47.15	di- (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	46.72	9.80
3.96	3-hexen-1-ol, (Z)- (CAS)	$C_6H_{12}O$	30.23	2.24
4.26	(Z)-3-hptene	C_7H_{14}	10.35	2.81
5.39	benzene, methoxy- (CAS)	C_7H_8O	94.3	2.07
19.96	10-(acetylmethyl)-(+)-3-carene	$C_{13}H_{20}O$	19.6	1.31
30.73	1,2-benzenedicarboxylic acid bis (2-methylpropyl) ester (CAS)	$C_{16}H_{22}O_4$	39.25	1.02

3 Discussion

D. cauda-felina (Hance) belongs to the family of Bignoniaceae, which posses a wide of interesting chemicals structures and pharmacological activities[11]. *D. cauda-felina* is a tree with 5-15 m tall, young branches, leaves and peduncles yellow-brown, flowers yellow or yellow red, leaves 30-55 cm, leaflets 4-8 pairs. This species is found in sparse forests, humid places 900-1 200 m, distributed in South and South East Asia. It has been used for medicine and pharmacology.

In Thai's traditional medicine, the leaves and barks are externally used for treating skin diseases as well as being internally used for analgesic effect. In investigation done by Joshi et al [12] for this species, they isolated some compounds like dehydro-alfa-lapachone, lapachol, dehydro-iso-alfa-lapachone, B-sitosterol, B-lapachone, tectol, paulowin and paulwinin and palmitone from the alcoholic extract of stem- heart wood. Early, Tripetch et al. [13] isolated 19 compounds, 5 were new verbacoside derivatives (markhamiosides A-E); one was a new hydroquinone derivative (markhamioside F) along with 13 known compounds from the leaves and branches of this plant. Six classes of compounds have been identified: quinines, flavonoids, terpenoids, iridoids, a phthalate and a phenolic ester, these compounds seem to be widespread in this family[11]. Urrea-

bulla et al[14]. indicated the biological activity of phenolic glycosides, which were isolated from *Alchornea glandulosa* and showed an antifeedant and larval growth effect against *Spodoptera frugiperda*.

There was no interest investigation for its effect on insect pests. The results of this study confirm that some occurring botanical materials have definite ovipositional deterrence against the vegetable leafminer *L. sativae*. The material tested produced a significant reduction in the number of eggs deposited by the insect on the treated kidney bean leaves.

The behavioral and electrophysiological response of the *L. sativae* toward the essential oils of this plant confirms the previous results obtained. The experiments showed that the essential oil of *D. cauda-felina*, when applied at high flow rate 250 mL/min significantly reduced the time spent and the number of entries in the treatment area, and applied at concentration of 0.1 mL/mL elicit a distinct EAG response.

The chemical constituent of the *D. cauda-felina* essential oils analyzed by the GC/MS, showed a total of twenty essential compounds that the dibutyl phthalate as a major compound with concentration of 56.55%.

This study is only the starting point in the long process of development of these chemicals as a promising component of leafminers management program and served as the foundation for more detailed investigations of the effects of selected extracts on the control effect of *Liriomyza*.

References:

[1] MENSAH R K, VEMEAU S, FREROT B. Deterrence of oviposition of adults *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae) by a natural enemy food supplement Envirofeast on maize in France[J]. International Journal of Pest Management, 2000, 46(1):49-53.

[2] AKHTAR Y, ISMAN M B. Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species[J]. Journal of Applied Entomology, 2004, 128: 32-38.

[3] NEEDHAM J. Science and civilization in China[M]. Cambridge: Cambridge University press, 1986.

[4] SMITH A E, SECOY D M. Plants used for agricultural pest control in Western Europe before 1850[J]. Chemistry and Industry, 1981, 1: 12-17.

[5] PEDIGO L P. Entomology and pest management[M]. Iowa: Iowa State University, 1999.

[6] CANCELADO R E. Botanical insecticides[M]. Minnesota: University of Minnesota, 2004.

[7] YANG R Z, TANG C S. Plants used for pest control in China, a literature review[J]. Econ Bot, 1988, 42: 376-406.

[8] SCHOONHOVEN L M, JERMY T, VAN LOON J J A. Insect-plant biology: From physiology to evolution[M]. London: Chapman and Hall, 1998.

[9] ZHAO Y X, KANG L. Role of plant volatiles in host plant location of the leafminer, *Liriomyza sativae* (Diptera: Agromizydae) [J]. Physiological Entomology, 2002, 27: 103-111.

[10] LUSZNIAK M, PICKETT J. Self-defense for plants[M]. Cambridge: Royal Society of Chemistry, 1998.

[11] RASADAH M A. Studies in the chemistry and biological activity of some Malaysian Bignoniceae[M]. London: University of London, 1997.

[12] JOSHI K C, SINGH P, PARADASANI R T. Chemical constituents of the stem heart wood of Markhamia stipulata [J]. Planta Medica, 1978, 34: 219-221.

[13] TRIPETCH K, RIJOJI K, KAZUO Y. Phenolic glycosides from Markhamia stipulata[J]. Phytochemistry Journal, 2002, 59: 557-563.

[14] URREA-BULLA A, SUAREZ M M, MORENO-MURILLO B. Biological activity of phenolic compounds from *Alchornea glandulosa*[J]. Fitoterapia, 2004, 75(3-4): 392-400.

【责任编辑 周志红】