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薇苷菊提取物对桔全爪螨的产卵驱避 作用及有效组分分析

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关键词:薇苷菊; 桔全爪螨; 产卵驱避; 有效组分; 2,2'-亚甲基双(6-叔丁基-4-甲基)苯酚; 谷甾醇; 岩藻甾醇

Study on the active components of oviposition repellency of *Mikania micrantha* H. B. K. against citrus red mite, *Panonychus citri* McGregor

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Abstract: Citrus red mite Panonychus citri McGregor became a major pest of citrus in China during the late 1950s. The widespread use of synthetic pesticides since then has greatly reduced natural enemies in the citrus orchard and resulted in widespread resistance of citrus red mite, making it the most difficult citrus pest to control. New, effective control methods for citrus red mite are urgently needed. One possible avenue is the use of active components from plant secondary metabolites to control herbivorous pests. Secondary metabolites play an important role in plant defense systems. Recent studies showed that the volatiles oil from the noxious weed mile-a-minute, Mikania micrantha H. B. K., had significant biological activity on insects, plants and fungi. In this study we used different solvents to extract non-volatile secondary metabolites from M. micrantha and investigated the oviposition repellency of these extracts to citrus red mite. Methanol, ethyl acetate and ethyl ether extracts of M. micrantha all significantly reduced oviposition of citrus red mite 1d after treatment in a choice test. Methanol, the strongest polarity solvent extract resulted in the highest repellency rate of 74. 22%. A range of different solvents including ethyl ether, ethyl acetate, n-butanol methanol and water were then used to extract this methanol extract. Bioassay showed that only water, the strongest polarity solvent fraction showed significant oviposition repellency, with a repellency rate of 65.33% 1d after treatment. The methanol extract of M. micrantha was separated by chromatographic column and 6 fractions were isolated. Bioassay demonstrated that the oviposition repellency of these fractions was not as strong as the crude extract, and that the active components were in different fractions. Among them, the first fraction (F₁) was the

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most active with a repellency rate of 61% 1d after treatment, demonstrating that the active compounds were largely maintained. GC-MS analysis showed that three of the compounds in this fraction were 2, 2'-Methylenebis [6-(1, 1-dimethylethyl)-4-methyl]Phenol, β -Sitosterol and Fucosterol and they made up 70.24%,12.03% and 5.61% of the fraction respectively.

Key words: *Mikania micrantha* Kunth; citrus red mite *Panonychus citri* McGregor; oviposition repellency; active component; 2,2'-Methylenebis [6-(1,1-dimethylethyl)-4-methyl]Phenol; β-Sitosterol; Fucosterol

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桔全爪螨 $Panonychus\ citri\ McGregor\ 是柑桔的主要害虫,它以刺吸式口器吸食柑桔叶片和果实、嫩茎表皮的汁液,使受害部位呈现白色斑点,引起落叶、落花和落果,严重影响柑桔的树势和产量。这种害虫是 <math>20$ 世纪 50 年代后期有机农药大量使用后才上升为主要害虫的 $[^{1,2}]$ 。使用广谱性杀虫剂,大量杀伤天敌是这种害虫大量发生的主要原因 $[^{3}]$ 。目前在我国,桔全爪螨已发展成为柑桔最普遍、最主要和最难防治的害虫 $[^{4,5}]$ 。造成桔全爪螨难以防治的主要原因是该虫极易获得抗药性 $[^{1,6}]$ 。这样,寻找新的桔全爪螨防治的有效方法非常重要。目前,从植物次生物质中寻找有效成分防治病虫害受到广泛的关注 $[^{7-13}]$ 。植物次生物质在植物对害虫的防御中起重要作用 $[^{9-14}]$,在非嗜食植物中常常含有对害虫有驱避作用的次生物质。

原产于中南美洲,于20 世纪80 年代初传入我国香港和华南沿海地区的薇苷菊*Mikania micrantha* H. B. K. 是一种危害性很大且繁殖速度很快的世界性有害杂草[15~18]。过去国内外对这种杂草的研究重点主要是其生物学、生态学和防治方法,尤其是生物防治方面^[19~24]。而有关薇苷菊利用方面的研究较少。近年韩诗畴等在广东省内伶仃岛调查的结果表明,在薇苷菊上几乎没有发现真正为害的昆虫,一些植食性昆虫如芫菁、蝗虫、夜蛾类在薇苷菊周围植物上取食而不取食薇苷菊,他们推测可能薇苷菊含有可以驱避昆虫或使昆虫拒食的物质^[25]。张茂新等研究发现薇苷菊的挥发性次生物质对昆虫、植物和真菌具有显著的生物活性^[26,27],本文进一步使用不同的溶剂从薇苷菊植株中提取非挥发性次生物质,并研究这些提取物对桔全爪螨产卵的驱避作用及其有效成分。

- 1 材料和方法
- 1.1 供试材料

薇苷菊,2001 年5 月采自深圳市农科中心;酸桔苗,为1 年生塑料筒栽苗,广东省杨村华侨柑桔场生产;桔全爪螨,采自杨村华侨柑桔场生产性果园;乙醇,甲醇,乙酸乙酯,乙醚,正己烷,市售化学纯试剂; $100\sim200$ 目硅胶,上海五四化学试剂有限公司生产。

- 1.2 试验方法
- 1. 2. 1 供试试样和产卵驱避作用测试 将用水蒸气蒸馏法提取完挥发油的薇苷菊晾干部分水分,放在烘箱中在 $50\,\mathrm{C}$ 条件下烘干,用植物试样粉碎机 $(\mathrm{DWF-100})$ 粉碎后称取100g 3 份分别用甲醇、乙酸乙酯、乙醚进行索氏提取。提取物用旋转蒸发仪减压蒸发浓缩成 $1\,\mathrm{gDW/ml}$ (指每 ml 水含1g 薇苷菊干物质提取物,下同),然后配成 $1\mathrm{gDW/100ml}$ 的试验浓度,并分别加入0.1% 吐温使其乳化。采用半叶法进行测试:每种提取物选用一株酸桔苗的 $10\,\mathrm{hm}$ 片叶, $1\,\mathrm{hm}$ 个重复,以叶片中脉为界,一半叶的上下两面用棉签小心涂上处理药液,另一半涂上对照溶剂,在每片叶的叶柄涂上一圈凡士林以防螨逃遁。待药液干后接上桔全爪螨雌成螨,每叶接 $10\,\mathrm{y}$ 。处理后 $24\,\mathrm{hm}$, $48\,\mathrm{hm}$ 分别在放大镜下观察一次卵量并将全部卵清除。3 种提取物的对照分别用同等浓度的溶剂加 0.1% 吐温。用 SPSS 统计软件分析各提取物处理与对照间差异是否显著,如显著则进一步计算每张叶片的产卵驱避率,计算方法为:产卵驱避率=((对照区卵量—处理区卵量)/对照区卵量)×100%,并比较各处理的产卵驱避效果。
- 1. 2. 2 葡苷菊提取物溶剂萃取组分对桔全爪螨产卵驱避作用测试 将浓度为 $1 {
 m gDW/ml}$ 的薇苷菊甲醇提取物加入等量的水,倒入分液漏斗中摇匀后加正己烷萃取 3 次除去色素,然后按溶剂极性从低-高的顺序依次用乙醚、乙酸乙酯、正丁醇分别萃取 3 次,所得 3 种萃取物以及余下的水溶物用旋转蒸发仪减压蒸发浓缩至 $1 {
 m gDW/ml}$ 备用。

测试时每毫升提取物、萃取物加入 100 ml 水,即测试浓度为 1~gDW/100 ml,再加入 0.1% 吐温使其乳化,对照为同等浓度的溶剂加 0.1% 吐温。测试方法同 1.2.1。

1.2.3 薇苷菊提取物柱层析组分对桔全爪螨产卵驱避作用测试 将 2.2.1 所得的薇苷菊甲醇提取物加入适量的水后用正己烷萃取 3 次除去色素,然后用滴管慢慢滴入甲醇至不再产生沉淀为止,用减压抽滤法过滤去掉沉淀(主要成分为糖份和蛋白),清液用吸附柱层析法进行分离。层析柱的柱长为 $39\mathrm{cm}$,内径为 $1.8~\mathrm{cm}$,吸附剂为 $100\sim200$ 目硅胶,洗脱剂为甲醇和丙酮混合溶剂(体积比例为 3:1)。洗脱得到 6 个组分,按出来的先后顺序编号为 F_1 、 F_2 、 F_3 、 F_4 、 F_5 、 F_6 组分,将这些组分浓缩成固体后加入适量甲醇保存 F_7 方数 据

各组分对桔全爪螨产卵驱避的测试浓度相当于 $1 {
m gDW}/10 {
m ml}$,对照为同等浓度的甲醇。测试方法同 $1.2.1_{
m o}$

2 结果与分析

2.1 不同溶剂的薇苷菊提取物对桔全爪螨的产卵驱避作用 相同浓度薇苷菊(1gDW/100ml)的甲醇、乙酸乙酯、乙醚提取物在 处理后 1d 对桔全爪螨都有显著的产卵驱避效果,其中甲醇提取物的产卵驱避率最高,为 74.22%,显著高于乙醚提取物 (表 1)。处理后 2d 甲醇提取物与对照相比差异还达到显著水平,乙酸乙酯和乙醚提取物与对照相比差异则不显著。 3 种溶剂极性由强到弱的顺序为甲醇-乙酸乙酯-乙醚,这也说明 3 种溶剂提取物都有一定的活性,但极性大的甲醇提取物的效果较好且持续时间较长。

试验结果还发现各处理受害斑点密度都显著低于对照,说明薇苷菊提取物对桔全爪螨同时具有拒食作用。

表1 薇苷菊不同溶剂提取物对桔全爪螨的产卵驱避作用

Table 1 Oviposition repellency of different extracts of M. micrantha on citrus red mite

		处理后时间 Days after treatment			
提取物 Extract (1gDW/100ml)		1d		2d	
		* 平均每半叶卵量	* * 产卵驱避率	* 平均每半叶卵量	产卵驱避率
		Number of eggs	Oviposition	Number of eggs	Oviposition
		per half leaf	repellency rate(%)	per half leaf	repellency rate(%)
甲醇提取物	TR	7.4 \pm 1.36 a	74.22+6.54a	$20 \pm 2.95a$	38.65
Methanol extract	CK	$35.5 \pm 3.57b$	74. 22 <u> </u>	32.6 ± 1.66 b	
乙酸乙酯提取物	TR	8.4 \pm 1.51 a	60.56+6.69ab	12.8 \pm 1.58 a	
Ethyl acetate extract	CK	22.5 \pm 3.10b	00. 30 <u>1</u> 0. 09ab	14.5 ± 1.23 a	
乙醚提取物	TR	9.8±1.94 a	43.72+14.13b	9.9 \pm 2.11a	_
Ethyl ether extract	CK	$20 \pm 2.01 \text{ b}$	45.72 14.150	$12.7 \pm 1.41a$	

^{*} 同组数字后有不同字母者表示经成对数据法的 t 检验在 0.05 水平上差异显著 Within the same time group, means followed by different letters are significantly different by the pair sample t- test at $\alpha = 5\%$; *** 同列数字后有不同字母者表示经DMRT 检验在 0.05 水平上差异显著 Within the same column, means followed by different letters are significantly different by DMRT at $\alpha = 5\%$

2.2 薇苷菊甲醇提取物的不同萃取组分对桔全爪螨的产卵驱避作用

薇苷菊甲醇提取物处理后 1d 对桔全爪螨有显著的产卵驱避效果,产卵驱避率为 73.62%,而其乙醚、乙酸乙酯、正丁醇萃取组分处理的着卵量仅略低于对照,与对照相比差异都不显著 (表2),说明这 3 种溶剂萃取组分对桔全爪螨都没有显著的产卵驱避效果。但几种溶剂萃取后余下的水溶物却有较好的驱避效果,处理后 1d 产卵驱避率为 65.33%,处理后 2d 为 58.40%,显著降低桔全爪螨的卵量,与甲醇提取物的驱避效果无显著差异。几种溶剂中水的极性最强,说明驱避作用活性物质主要存在于极性强的组分。

表 2 薇苷菊提取物及其萃取组分对桔全爪螨的产卵驱避作用

Table 2 Oviposition repellency of different fractions of extract of M. micrantha on citrus red mite

•		处理后时间Days after treatment					
提取物/萃取物 Extract /Fraction (1gDW/100ml)		1d		2d			
		* 平均每半叶卵量	* * 产卵驱避率 Oviposition	平均每半叶卵量 Number of eggs	* * 产卵驱避率 Oviposition		
		Number of eggs					
		per half leaf	repellency rate(%)	per half leaf	repellency rate(%)		
甲醇提取物	TR	9.7±1.56a	72 60 4 07	7.9 ± 1.55 a	66.93±7.64a		
Methanol extract	CK	$41.6 \pm 4.22b$	73.62 \pm 4.97 a	28.7 \pm 3.85b			
乙醚萃取物	TR	28.3 \pm 3.89 a	_	_	_		
Ethyl ether	CK	35.8 $\pm 2.95a$		_			
乙酸乙酯萃取物	TR	$19 \pm 3.10a$		_	_		
Ethyl acetate	CK	27.4 ± 3.46 a	_	_			
正丁醇萃取物	TR	8.5 \pm 1.30 a		_	_		
N-butanol methanol	CK	11.3 ± 1.74 a	_	_			
水萃取物	TR	20.5 \pm 3.52 a	a= 00 a 05	$27.2 \pm 4.99a$	58.40±6.88a		
Water	CK	$64.2 \pm 6.04b$	65.33 \pm 6.97 a	68.1 \pm 10.01b			

^{*} 同组数字后有不同字母者表示经成对数据法的 t 检验在 0.05 水平上差异显著 Within the same time group, means followed by different letters are significantly different by the pair sample t-test at $\alpha = 5\%$; *** 同列数字后有不同字母者表示经 DMRT 检验在 0.05 水平上差异显著 Within the same column, means followed by different letters are significantly different by DMRT at $\alpha = 5\%$

2.3 薇苷菊甲醇提取物柱层析组分对桔全爪螨的产卵驱避作用

除去挥发油的薇苷菊甲醇提取物经柱层析分离所得的 6 个组分中,在处理后 1d F_1 、 F_2 、 F_4 有显著的产卵驱避效果,处理后 2d 只有 F_1 处理与对照相比卵量差异达显著水平(表3)。各组分的使用浓度为1g 薇苷菊干物质/10ml 水,相当于表1,2 中分离前 的甲醇提取物浓度的10倍,但产卵驱避率仍低于甲醇提取物,说明分离后组分的驱避效果都不如提取物,薇苷菊甲醇提取物对 桔全爪螨的产卵驱避作用是各组分联合作用的结果。

表 3 薇苷菊甲醇提取物柱层析组分对桔全爪螨的产卵驱避作用试验结果

Table 3 Oviposition repellency of different fractions of methanol extract of M. micrantha on citrus red mite

组份(按出来先后顺序) Fraction (1gDW/10ml)		处理后时间 Days after treatment					
		10	1	2d			
		* 平均每半叶卵量 Number of eggs per half leaf	产卵驱避率Oviposition repellency rate(%)	* 平均每半叶卵量 Number of eggs per half leaf	产卵驱避率 Oviposition repellency rate(%)		
F1	TR	3.9±0.67a	61.00	2.2±0.42a	51.11		
	CK	$10 \pm 2.28b$	01.00	4.5 \pm 0.73b	31.11		
F2	TR	7.9 \pm 1.32a	57.30	3.7 \pm 0.62a	_		
	CK	18.5 \pm 1.89b	57.30	5.3 \pm 0.78a			
F3	TR	4.1 \pm 1.00a		_			
	CK	$5 \pm 0.95a$		_			
F4	TR	3. 4 ± 0 . $64a$	50.00	6.6 \pm 1.74a			
	CK	6.8 \pm 0.77b	50.00	$9\pm2.53a$	_		
F5	TR	$3.3 \pm 0.97a$		_	_		
	CK	4.6±0.90a	_	_			
F6	TR	2.1 \pm 0.60a		_			
	CK	3.3 \pm 0.92a	_	_	_		

* 同组数字后有不同字母者表示经成对数据法的 t 检验在 0.05 水平上差异显著 Within the same time group, means followed by different letters are significantly different by the pair sample t-test at $\alpha = 5\%$

2.4 薇苷菊对桔全爪螨产卵驱避的有效成分

对产卵驱避作用持续时间较长的 F1 组分进行气相色谱-质 谱(GC-MS)分析,结果发现2,2'-亚甲基双(6-叔丁基-4-甲基)苯 酚是其主要成分,含量达70.24%,同时含少量的β-谷甾醇和岩藻 甾醇,含量分别为 12.03% 和 5.61% (图 1),前者是典型的酚类化 合物,后两者均为甾醇类化合物(图2)。

3 讨论

薇苷菊从 20 世纪 40 年代传入东南亚后,由于缺少原产地的 天敌而成为最危险的有害杂草。东南亚和太平洋地区各国投入 了大量的人力、物力和财力防治这种杂草[18]。在我国广东省内伶 仃岛国家级自然保护区和深圳等华南沿海地区也已造成严重的 危害,被称为"植物杀手"[17]。薇苷菊生长迅速,研究其利用价值

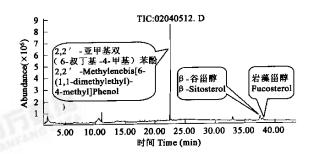


图 1 薇苷菊甲醇提取物柱层析 F1 组分的化学成分

Result of GC test of F1 fraction of methanol extract of M. Fig. 1 micrantha

-亚甲基双 (6-叔丁基-4-甲基) 苯酚 2,2' -Methylenebis[6-(1,1-dimethylethyl) 4-methyl]Phenol C23H32O2

岩藻甾醇 Fucosterol C29H48O

图 2 薇苷菊柱层析 F1 组分中的 3 种化合物结构

草[30]。 薇苷菊提取物还具有抗菌性,其挥发油对植物、真菌和细菌具有生物活性,对植物和水稻稻瘟病的抑制活性尤其显 著[27.31]。最近的研究结果发现薇苷菊挥发油对蔬菜主要害虫小菜蛾、黄曲条跳甲和猿叶甲有显著的产卵驱避作用,同时也具有 一定的毒杀作用[26]。本研究结果表明,用甲醇、乙酸乙酯、乙醚提取的薇苷菊非挥发性次生物质对桔全爪螨具有显著的产卵驱 避作用和拒食作用,其中甲醇提取物的效果最好,产卵驱避作用有效成分主要存在于极性强的组分,从最有效的组分中检测出 一种酚类化合物 2,2'-亚甲基双(6-叔丁基-4-甲基)苯酚和 2 种甾醇类化合物,其中苯酚的含量最高。据报道,酚类化合物在植物 对害虫的防御中起重要作用,一些酚类化合物对害虫有驱避、拒食甚至毒杀作用[28-29]。关于这3种化合物的活性将在今后进一

具有重要意义。据报道,薇苷菊的叶片和根的提取物具有化学他感作用,能够抑制一些杂草的生长,因此可用来控制这些些杂

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步研究。本研究和前人的研究结果表明,薇苷菊次生化合物在病虫害防治中可能具有重要的应用前景。

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