ARTÍCULO ORIGINAL

Repellence of Boophilus microplus larvae in Stylosanthes humilis and Stylosanthes hamata plants

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ABSTRACT

*The aim of the study was to investigate the repellence of **B. microplus** larvae by the tropical legumes **Stylosanthes humilis** and **S. hamata**, and to identify some of the chemical compounds presents in both legumes. The effect was evaluated by a repellence bioassay in an olfactometer using extracts from stems, leaves and whole plants treated with diverse organic solvents such as hexane, acetone, chloroform and methanol. The identification of chemical compounds was done by gas chromatography -mass spectrometry on whole plant extracts. This study demonstrated that both legumes had repellence properties. Repellence ranged from 68% to 92% in **S. humilis** and from 70% to 82% in **S. hamata**. Sixteen compounds were identified in **S. humilis**, with ferrocene and beta sitosterol being the most abundant (18.3% and 14% of the total, respectively). Seventeen compounds were identified in **S. hamata**, with linolenic acid being the highest relative abundance (17.6%). The compounds identified may be considered as potential candidates for explaining the repellence effects.*

Key words: *Stylosanthes* spp, **plants**, Repellence effect, compounds, **Boophilus microplus**.

INTRODUCTION

The cattle tick, *Boophilus microplus*, is the most important ectoparasite in tropical and subtropical countries in Latin America. The use of synthetic chemical acaricides is the main method of control, however, chemical acaricides are expensive, they are harmful to the environment, and the development of resistance is common¹. It is therefore necessary to develop tick control strategies utilising a minimum amount of acaricides in combination with non-chemical

methods, such as habitat modification, biological control and anti-tick plants^{2,3}.

Several tropical pasture legumes of the genus *Stylosanthes*, as *S. scabra*, *S. viscosa* and *S. guaianensis*, have anti-tick effects. The stems and leaves of these legumes are covered with glandular trichomes, which produce a sticky secretion with a characteristic odour. Tick larvae trying to ascend the plants to await a passing host are trapped and killed in the secretion. A high density of non-glandular fine hairs also prevent larvae from ascending these stems^{4,5}. In

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This study was supported by Cosnet-SEP (Mexico), Project 606.98-P.

a previous work anti-tick effects have been demonstrated in the legumes *S. humilis* and *S. hamata*, evaluated by recovery of *B. microplus* larvae from experimental plots; however, we were not able to identify the possible causes⁶.

The purpose of this study was to investigate the repellence of *B. microplus* larvae from extracts of *S. humilis* and *S. hamata*, and to identify the chemical compounds present.

MATERIALS AND METHODS

The legumes *S. humilis* (var. Patterson) and *S. hamata* (var. Verano), were established in the experimental area of Cenid-Pavet, in Jiutepec, Morelos, Mexico. The site has an altitude of 1.350 m above sea level, the annual mean precipitation is 850 mm with most rainfall in the summer and with a tropical subhumid climate. Plots of each (4 X 2 m) were seeded in July 1999, following the management practices previously reported⁶. The plant parts for the experimental phases were collected at 90 days of age, in October, with approximately 75% flowering.

Fresh aerial parts (only stems, only leaves and stems plus leaves) of each legume were collected; 60 g of each plant part were chopped and extracted separately with one of the following organic solvents: methanol, chloroform, hexane and acetone (reagent grade) for 1 hour. Controls were extracted with water. The plant extracts were concentrated in a Soxhlet System (Hewlett Packard) at 80°C for 2 hours and stored in a brown glass vials at 4°C until his use.

B. microplus larvae were reared in the Cenid-PaVet laboratory in Jiutepec, Morelos, Mexico, as were described previously⁶. Larvae of 15 days old were used in the repellence bioassay.

A Y-shaped glass olfactometer was used for repellence bioassays^{7, 8}. A piece of cotton wool was impregnated with 1 ml of plant extract obtained previously and air dried for 24 hours. This was placed in one arm of olfactometer and another piece of cotton wool was impregnated only with the corresponding solvent, and air dried for 24 hours, was placed in the other arm. Air was sucked out by a water pump connected at the Y-junction. *B. microplus* larvae (batches with approximately 1,000 larvae) were introduced in the free end of the olfactometer and allowed to climb for 20 minutes. The number of larvae choosing either arm was counted, the repellence

were calculated as follow: number of larvae in control or treated arm / total number of climbed larvae (larvae in right arm + larvae in left arm). The bioassay was made using a random design with three replicas per treatment (plant extracts-solvents combinations). The olfactometer was cleaned after each use with soap and water and dried in hot air.

The data obtained were analysed by Analysis of Variance and Duncan's Test ($p < 0.05$). The percentage data were transformed by arcsin to normalise the data^{9,10}.

Gas chromatography - mass spectrometry (GC-MS) was performed with a Hewlett Packard GC System (HP6890) gas chromatograph-mass spectrometer (selective mass detector No.5973), using a plant extract from whole plant (stems + leaves) in hexane. Chromatographic separations were achieved using a capillary column (Hewlett Packard No. 19.091 - 433; 30 m x 0.2 mm ID) coated with methyl siloxane (0.25 µm thickness), with helium as the carrier gas. Compounds were identified by their electron impact mass spectral data, order of elution and relative GC retention times (RT), and by comparison of their mass spectra and GC retention times to those of authentic standards.

RESULTS

The tropical legumes, *S. humilis* and *S. hamata*, demonstrated *B. microplus* larvae repellence properties, as shown in Table 1; all parts of the plants showed repellence effects. The repellence ranged from 68% to 92% in *S. humilis* extracts and from 70% to 82% in *S. hamata*.

The plant extracts from *S. humilis* using hexane as extraction solvent had the higher repellence percentage in stems and whole plant, with 86% and 92%, respectively. There were no differences ($p > 0.05$) between hexane and others solvents from leaves extracts, except acetone. In *S. hamata*, hexane stems extracts had the higher repellence percentage ($p < 0.05$) but leaves and whole plant extracts had similar repellence in all solvents ($p > 0.05$).

The chemical compounds identified by GC-MS from whole plant extract using hexane as extraction solvent in both legumes are shown in Table 2. Sixteen compounds were identified in *S. humilis*, the highest relative abundance corresponding to two compounds with the largest

Table 1. Mean percentage of *Boophilus microplus* larvae repelled in an olfactometer bioassay with different extracts from tropical legume *Stylosanthes humilis* and *Stylosanthes hamata*

Solvent	Repellence Percentage					
		<i>S. humilis</i>			<i>S. hamata</i>	
	Stems	Leaves	Whole plant	Stems	Leaves	Whole plant
Methanol	77 ^b	82 ^a	68 ^b	79 ^b	75 ^a	82 ^a
Chloroform	78 ^b	86 ^a	78 ^b	74 ^b	73 ^a	80 ^a
Hexane	86 ^a	86 ^a	92 ^a	87 ^a	70 ^a	79 ^a
Acetone	70 ^b	75 ^b	74 ^b	71 ^b	76 ^a	76 ^a
Water	8 ^c	5 ^c	7 ^c	5 ^c	4 ^b	6 ^b

* Values within a same column followed by the same letter are not significantly different at p<0.05

Table 2. Chemical compounds identify by GC-MS from extract of whole plants from tropical legumes *Stylosanthes humilis* and *Stylosantes hamata*

Compound	RT	Relative abundance (%)
<i>S. humilis</i>		
1. Naphthalene	2.14	1.5
2. Hexadecanoic acid, methyl ester	13.16	1.2
3. Hexadecanoic acid	13.90	4.5
4. Hexadecanoic acid, ethyl ester	14.69	1.7
5. Linolenic acid, methyl ester	17.54	3.0
6. Tricosane	21.54	2.5
7. 2,4-Bis (dimethylbenzyl) phenol	24.18	2.6
8. 2,4-Bis (dimethylbenzyl) 6-t-butylphenol	24.88	3.1
9. Eneicosane	27.31	4.0
10. Gamma sitosterol	28.92	6.3
11. Octadecenamide (Z)	29.52	7.1
12. Delta. 8-tetrahydrocannabinol	33.86	8.9
13. Retinoic acid, methyl ester	34.91	9.0
14. Beta sitosterol	35.44	14.0
15. Ferrocene, 11-(acetylthio)undecil	36.47	18.3
16. Eicosane	38.69	10.6
<i>S. hamata</i>		
1. 1,2-Benzenedecarboxylic acid, bis (2-methyl propyl) ester	11.30	2.3
2. N-Isobutyl-2(E),6(z),8(E)-decatrieno	11.68	2.5
3. Hexadecanoic acid, methyl ester	13.17	10.5
4. Hexadecanoic acid, ethyl ester	14.70	5.8
5. 2,6-Bis(t-butyl)-4-(dimethylbenzyl)	16.04	4.5
6. 9,12-Octadecadienoic acid	16.70	6.1
7. Linolenic acid, methyl ester	16.78	17.6
8. Steric acid, methyl ester	17.57	2.4
9. Linolenic acid, ethyl ester	18.16	4.0
11. Tetratriacontane	19.49	4.3
12. Docosane, 7-hexyl	21.55	4.9
13. Tetracosane	23.54	6.0
14. 2,4-Bis(dimethylbenzyl)-6-t-butylph	24.88	9.6
15. Pentadecane,2,6,10,14-tetramethy pristane	25.24	3.5
16. Pentacosane	25.46	7.0
17. Eicosane	27.31	8.2

RT = Retention Time

RT, ferrocene (18.3%) and beta sitosterol (14%), the others substances ranging from 1.2% to 10.6% of relative abundance. In *S. hamata*, seventeen compounds were identified. The highest value in relative abundance was present in linolenic acid (17.6%), the others compounds ranging in relative abundance from 2.4% to 10.5%.

DISCUSSION

In a previous work, the anti-tick effects detected in *S. humilis* and *S. hamata* plants were related, as in others *Stylosanthes* plants, with several factors as the presence of glandular trichomes in stems and leaves, their stickiness, density of non-glandular hairs and effect of toxic volatile agents produced by sticky secretion^{4,5,6}. In the present study we have demonstrated that extracts from both tropical legumes had repellence effects. The values observed in the bioassay can be considered higher in both legumes, more important in *S. humilis* than *S. hamata* (Table 1). In our previous work *S. humilis* showed the better anti-tick effects⁶. The repellence per-centages obtained in this work are similar to those reported in others plants with tick repellence properties such as *Gynandropsis gynandra* (East African shrub) and Molasses grass, *Melinis minutiflora*^{3,7,8,11}.

The compounds identified has not been reported previously in the literature, they are sixteen or seventeen non-polar lipophilic compounds. The relative abundance of each compound were estimated in a short range and his individual contribution were moderate or scarce, no more than 18% (Table 2).

In conclusion, whole extract of the legumes *S. humilis* and *S. hamata*, demonstrated repellence properties. The compounds identified may be considered as potential candidates for explaining the plant repellent effects.

RESUMEN

El objetivo de este estudio fue investigar la repelencia de larvas de *B. microplus* presente en las leguminosas tropicales *Stylosanthes humilis* y *S. hamata*, e identificar algunos de los compuestos químicos presentes en ambas leguminosas. El efecto fue evaluado mediante un bioensayo de repelencia con un olfactómetro utilizando extractos de hojas, tallos y de la planta

completa, tratados con diferentes solventes orgánicos como hexano, acetona, cloroformo y metanol. La identificación de los compuestos químicos se realizó mediante cromatografía de gases - espectrofotometría de masas en extractos de planta completa. Este estudio demostró que ambas leguminosas tropicales tienen importantes propiedades de repelencia, ésta se ubicó en un rango de 68% a 92% en *S. humilis*, y entre 70% y 82% en *S. hamata*. Dieciséis compuestos químicos fueron identificados en *S. humilis*, siendo ferroceno y beta sitosterol los más abundantes (18,3% y 14%). Diecisiete compuestos fueron identificados en *S. hamata* siendo el ác. Linolenico el que tuvo la más alta abundancia relativa (17,6%). Los compuestos identificados pueden ser considerados posibles candidatos para explicar el efecto repelente de estas plantas.

REFERENCES

- 1.- KUNZ S E, KEMP D H. Insecticides and acaricides: Resistance and environmental impact. Rev Sci Tech Off Int Epiz 1994; 13: 1249-86.
- 2.- KAAYA G P. Non chemical agents and factors capable of regulating tick populations in nature: a mini review. Insect Sci Applic 1992; 13: 587-94.
- 3.- KAAYA G P. The potential for anti-tick plants as component of an integrated tick control strategy. Ann NY Acad Sci 2000; 916: 576-82.
- 4.- SUTHERST R W, JONES R J, SCHNITZERLING H J. Tropical legumes of the genus *Stylosanthes* immobilize and kill cattle ticks. Nature 1982; 295: 320-1.
- 5.- SUTHERST R W, REID L J, KERR R. A survey of the ability of tropical legumes in the genus *Stylosanthes* to trap larvae of cattle tick, *Boophilus microplus* (Ixodidae). Aust J Exp Agric 1988; 28: 473-9.
- 6.- FERNANDEZ-RUVALCABA M, CRUZ-VAZQUEZ C, SOLANO-VERGARA J, GARCIA-VAZQUEZ Z. Anti-tick effects of *Stylosanthes humilis* and *Stylosanthes hamata* on plots experimentally infested with *Boophilus microplus* larvae in Morelos, Mexico. Exp Appl Acarol 1999; 23: 171-5.
- 7.- MALONZA M M, DIPELOU O O, AMOO A O, HASSAN S M. Laboratory and field observations on anti-tick properties of the plant *Gynandropsis gynandra* (L.) Brig. Vet Parasitol 1992; 42: 123-36.
- 8.- MWANGI E N, ESSUMAN S, KAAYA G P et al. Repellence of the tick *Rhipicephalus appendiculatus* by the grass *Melinis minutiflora*. Trop Anim Hlth Prod 1995; 27: 211-6.
- 9.- PAGANO M, GAUVREAU K. Principles of Biostatistics, p.257-71. Duxbury Press. USA. 1993.
- 10.- SAS INSTITUTE. SAS user's guide: statistics, version 6.0. SAS Institute, Cary, NC. USA. 1994.
- 11.- LWANDE W, NDAKALA A J, HASSANALI A, et al., *Gynandropsis gynandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry 1999; 50: 401-5.