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Repellency Assays with Plant Extracts and Essential Oils from *Schinus molle* var. *areira* (L) (Anacardiaceae) and DEET against *Nezara viridula* L. (Hemiptera: Pentatomidae)

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Testes de Repelência com Extratos Vegetais e Óleos Essenciais de *Schinus molle* var. *areira* (L.) (Anacardiaceae) e DEET em *Nezara viridula* L. (Hemiptera: Pentatomidae)

RESUMO - *Nezara viridula* é considerada uma das pragas de maior importância para a cultura da soja. Neste trabalho foi avaliada a repelência de extratos etanólicos e hexânicos, óleos essenciais de folhas e frutos de *Schinus molle* var *areira* e N, N-dietil-*m*-toluamida (DEET) sobre adultos de *N. viridula* em teste de escolha em arena. Foram utilizados discos de papel de filtro de 9 cm de diâmetro que foram divididos em duas metades, sendo que os compostos foram impregnados em uma das metades e a outra metade foi tratada somente com o solvente. Cada extrato foi testado nas doses de 1.572 µg/cm^2, 3.144 µg/cm^2 e 6.288 µg/cm^2; cada um dos óleos essenciais e DEET foram avaliados a 78,6 µg/cm^2, 157,2 µg/cm^2 e 314,4 µg/cm^2. Os ensaios foram conduzidos a 1 e 24 h. Os extratos etanólicos de folhas e frutos foram neutros a 1 e 24 h. Os extratos hexânicos de folhas e frutos mostraram atividade repelente a 1 h, contudo o extrato hexânico de folhas na dose mais baixa perdeu o efeito após 24 h. Os óleos essenciais de folhas nas doses de 157,2 µg/cm^2 e 314,4 µg/cm^2 mostraram efeito repelente somente até 1 h e os óleos essenciais de frutos nessas mesmas doses mantiveram a atividade por até 24 h. Com DEET constatou-se efeito repelente em todas as doses avaliadas.

PALAVRAS-CHAVE - percevejo-verde, araguaraíba, extratos vegetais, N, N-dietil-*m*-toluamida, efeito repelente.

ABSTRACT - *Nezara viridula* L. is one of the key pests in soybean crops. The aim of this work was to study the repellent effects of ethanolic and hexanic extracts, essential oils from leaves and fruits of *Schinus molle* var. *areira* and N, N-diethyl-*m*-toluamide (DEET) against adults of *N. viridula* in a choice-test arena. Half of a circular white filter paper of 9 cm. diameter was treated with extracts, essential oils or DEET and the other half with solvent alone. Each extract was tested at 1.572 µg/cm^2, 3.144 µg/cm^2 and 6.288 µg/cm^2, each essential oil and DEET were tested at 78.6 µg/cm^2, 157.2 µg/cm^2 and 314.4 µg/cm^2. Repellency asssays were carried out at 1 and 24 h. Ethanolic extracts from leaves and fruits were neutral at 1 and 24 h. Fruits and leaves hexanic extracts showed repellent activity at 1 h; however, hexanic extracts from leaves at the lowest dose lost the effect at 24 h. Essential oils from leaves (157.2 µg/cm^2 and 314.4 µg/cm^2) showed repellent effect at 1 h and the essential oils from fruits at these same doses maintained the activity for 24 h. DEET was repellent at all doses evaluated.

KEY WORDS - **S**outhern green stink bug, Brazilian peppertree, plant extracts, N, N-diethyl-*m*-toluamide, repellent effect.

The southern green stink bug, *Nezara viridula* (L.) is a highly polyphagous pentatomid pest of worldwide distribution. It damages soybean, beans, rice, corn, cotton and tobacco (Knight & Gurr 2007). Consequently, there can be premature fruit drop, delay in crop maturity and reduced seed quality and quantity (Panizzi 2004). The pest also transmits plant pathogens (Medrano *et al.* 2007). In Argentina, this is one of the major soybean (*Glycine max* L. Merr) pests (Stadler *et al*. 2006)

Control of this pest is based largely on the intensive use of chemical pesticides, including carbamates, organophosphates and some pyrethroids (Vandekerkhove & De Clercq 2004). These kinds of products are known to be toxic to mammals and beneficial insects, and their constant use can generate the phenomenon of pest resistance by selective pressure (Stadler *et al.* 2006).

In order to avoid these problems, alternative tools of control are being investigated. Essential oils and plant extracts have been shown to possess insecticidal and repellent properties (Venkatachalam & Jebanesan 2001, Ferrero *et al.* 2006, Sánchez Chopa *et al*. 2006, Ferrero *et al.* 2007, Nathan 2007). The compound N, N-diethyl-*m*-toluamide (DEET) is a common insect repellent and is still the most widely used mosquito repellent (Peterson & Coats 2001).

In the current study we describe the repellency of ethanolic and hexanic extracts, essential oils from leaves and fruits of *Schinus molle* var. *areira* and DEET against adults of *N. viridula.*

Materials and Methods

Leaves and fruits from *Schinus molle* L. var. *areira* (Anacardiaceae) were collected during the summer season, in Bahía Blanca, Argentina, and identified at the Herbarium of Departamento de Biología, Bioquímica y Farmacia, UNS (Herbarium Vaucher Number, BBB 10444). Fresh leaves (600 g) and fruits (700 g) were separately extracted with solvent (4 l), either hexane or ethanol, by maceration at room temperature in the dark over 48 h. Filtration and solvent evaporation under reduced pressure yielded the corresponding extracts. The yields of dried hexane extracts were 4.8% for leaves and 1.1% for fruits. The yields of dried ethanol extract were 6.8% for leaves and 10.7% for fruits. The essential oils were prepared by hydrodistillation using a Clevenger type apparatus for 3 h. The oils were dried over anhydrous sodium sulphate and stored at 4ºC under N_2. DEET (N, N-diethyl-*m*-toluamide; 98% purity; Aldrich, Milwaukee, USA) was used. Adults of *N. viridula* were obtained from a colony reared in our laboratory. The ethanolic extracts were dissolved in ethanol; the hexanic extracts, the oils and DEET, in hexane. Repellency test (Scheffer & Dombrowski 1992) was performed using a circular white filter paper #1 (9 cm diameter, Whatman International Ltd., Maidstone, England) divided in two halves. One of the halves was treated with 0.5ml of solvent alone and the other one with 0.5ml solvent solutions of plant extracts or DEET. After solvent evaporation (10 minutes) both halves of the filter paper were fitted together to make a single layer and used to cover the floor of Petri dishes. Ten insects were released in the centre of each Petri dish and their distribution was recorded at 1 and 24 h later. The doses tested for extracts were 1.572 µg/cm², 3.144 µg/cm² and 6.288 µg/cm²; for essential oils and DEET the doses were 78.6 µg/cm², 157.2 µg/cm² and 314.4 µg/cm². Each experiment was replicated 4 times. A Repellency Proportion (RP) was calculated as:

$$R.P. = A/B$$

Where: A= number of insects on the untreated zone and B= number of total insects in each Petri dish.

A statistical Z was calculated to standardize an originating proportion of a binomial distribution:

$$Z = n\,(RP\text{-}P_0) / \sqrt{nP_0\,(1\text{-}P_0)}$$

Where: n= number of total insects used for each concentration; P_0 = expected proportion (P_0: 0.5) The Z value was compared with t $critic_{0.05}$ (t = 1.96, df = ∞; P < 0.05) or t $critic_{0.01}$ (t = 2.57; df = ∞; P < 0.01) (Zar 1999).

Results and Discussion

The time may have come for natural pesticides based on plant extracts and essential oils which may represent alternative crop protectants. All these products have been used in the control of several pests (Isman 2000). However, there is lack of information on the effectiveness of repellents against *N. viridula* and other phytophagous hemipterans (Hoffman-Campo *et al*. 2003).

In the present study, hexanic extracts from leaves and fruits showed repellent effect at one 1 and 24 h (Tables 1 and 2). Only the hexanic extract from leaves at the lowest dose

Table 1. Repellent effect of ethanolic and hexanic extracts and essential oils from leaves and fruits of *Schinus molle* var. *areira* and DEET against adults of *N. viridula* with 1 h exposure.

Treatment	Doses (µg/cm²)	R.P.[1]	Z	Significance Level	Biological Activity
Ethanolic	1572	0.53	0.36	ns	Neutral
extracts	3144	0.53	0.36	ns	Neutral
from leaves	6288	0.60	1.09	ns	Neutral
Ethanolic	1572	0.50	0.00	ns	Neutral
extracts	3144	0.63	1.46	ns	Neutral
from fruits	6288	0.60	1.09	ns	Neutral
Hexanic	1572	0.96	5.11	**	Repellent
extracts	3144	0.96	5.11	**	Repellent
from leaves	6288	0.96	5.47	**	Repellent
Hexanic	1572	0.73	2.55	**	Repellent
extracts	3144	0.73	2.55	**	Repellent
from fruits	6288	0.96	5.11	**	Repellent
Essential	78.6	0.47	-0.36	ns	Neutral
oil	157.2	0.93	4.74	**	Repellent
from fruits	314.4	0.96	5.11	**	Repellent
Essential	78.6	0.43	-0.73	ns	Neutral
oil	157.2	0.70	2.19	*	Repellent
from leaves	314.4	0.70	2.19	*	Repellent
	78.6	0.86	4.01	**	Repellent
DEET	157.2	1.00	5.47	**	Repellent
	314.4	0.96	5.11	**	Repellent

[1]: repellent proportion
*Student´s t test. ns: no significant differences (p>0.05); *: significant differences (p<0.05); **: highly significant differences (p<0.01).

Table 2. Repellent effect of ethanolic and hexanic extracts and essential oils from leaves and fruits of *Schinus molle* var. *areira* and DEET against adults of *N. viridula* with 24 h exposure.

Treatment	Doses (μg/cm^2)	R.P.[1]	Z	Significance Level	Biological Activity
Ethanolic	1572	0.46	-0.36	ns	Neutral
extracts	3144	0.46	-0.36	ns	Neutral
from leaves	6288	0.63	1.46	ns	Neutral
Ethanolic	1572	0.46	-0.36	ns	Neutral
extracts	3144	0.60	1.09	ns	Neutral
from fruits	6288	0.50	0.00	ns	Neutral
Hexanic	1572	0.53	0.36	ns	Neutral
extracts	3144	0.96	5.11	**	Repellent
from leaves	6288	1.00	5.47	**	Repellent
Hexanic	1572	0.76	2.93	**	Repellent
extracts	3144	0.83	3.65	**	Repellent
from fruits	6288	0.76	2.93	**	Repellent
Essential	78.6	0.36	-1.46	ns	Neutral
oil	157.2	0.73	2.55	*	Repellent
from fruits	314.4	0.93	4.74	**	Repellent
Essential	78.6	0.36	-1.46	ns	Neutral
oils	157.2	0.43	-0.36	ns	Neutral
from leaves	314.4	0.43	-0.36	ns	Neutral
	78.6	0.80	3.28	**	Repellent
DEET	157.2	0.76	2.92	**	Repellent
	314.4	0.90	4.38	**	Repellent

[1]: repellent proportion.
*Student´s *t* test. ns: no significant differences ($p>0.05$); *: significant differences ($p<0.05$); **: highly significant differences ($p<0.01$).

was neutral at 24 h. This effect could be attributable to the volatilization of the compounds of small molecular weight. Similar results were reported by Rajkumar & Jebanesan (2005) using apolar extracts from leaves of *Solanum trilobatum* against the malarial vector *Anopheles stephensi*. Previous work by our team demonstrated that hexanic extracts from leaves and fruits of *S. molle* had repellent effects on neonate larvae of *Cydia pomonella* (Chirino *et al*. 2001) and nymphs of *Triatoma infestans*, a hematophagous hemipteran (Ferrero *et al*. 2006). Ferrero *et al.* (2007), using petroleum ether extracts of leaves and fruits from this plant, also observed the same activity on *Blattella germanica*. Both ethanolic extracts did not produce repellent effect at the times assayed. By using either apolar or polar solvents, different compounds are obtained (Novo *et al.* 1997) and this differential extraction may be the reason by which ethanolic extracts failed to produce repellency (Tables 1 and 2).

Essential oils from fruits maintained repellent effects at the highest doses for 24 h (Tables 1 and 2). Essential oils from leaves only showed repellency at highest doses at 1 h; however, the effect was neutral at 24 h at all doses. In support of our results, Murray *et al.* (2005), who analyzed the main constituents of these essential oils, reported that different compounds were present in each oil.

The repellency of DEET has been evaluated previously on other hematophagous hemipterans (Buescher *et al*. 1985, Alzogaray *et al*. 2000, Ferrero *et al.* 2006). Results in the present work demonstrated that DEET showed repellent effect against *N. viridula* at all doses and times evaluated (Tables 1 and 2).

The results present herein indicate that apolar extracts from leaves and fruits, essential oils from fruits of *Schinus molle* var. *areira* and DEET have good repellent properties against *N. viridula* and therefore they can be used in the development of agriculture repellents. This research is an attempt to find effective and affordable extracts containing natural products to be used in the control of this pest.

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