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PLANT EXTRACTS

Antifeedant and Repellent Effects of Extracts of Three Plants from Córdoba (Argentina) Against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

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Efeitos Antialimentar e Repelente de Extratos de Três Plantas de Córdoba (Argentina) sobre *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)

RESUMO - Extratos brutos em etanol, clorofórmio e hexano de três plantas de ocorrência freqüente na província de Córdoba, [*Aloysia polystachia* (Griseb) Moldenke (Verbenaceae), *Solanum argentinum* Bitter et Lillo (Solanaceae) e *Tillandsia recurvata* (L.)L. (Bromeliaceae)] foram avaliadas em relação à repelência e deterrência alimentar sobre *Sitophilus oryzae* (L.). O potencial antialimentar desses extratos foi determinado através do teste de deterrência ou dissuasão nutricional utilizando-se discos constituídos de trigo. Para cada extrato determinou-se o Coeficiente de Deterrência Total (T). Para a avaliação da repelência foram conduzidos testes utilizando-se papéis de filtro separados em duas partes iguais. Os extratos foram aplicados numa dessas partes em concentração de 0,31 $mg.cm^{-2}$ e posteriormente determinadas as porcentagens de repelência (PR) de cada extrato. Comparando-se todas as espécies vegetais e solventes avaliados, os extratos em clorofórmio (classe ++++) de *A. polystachia* seguido pelos extratos em etanol (classe ++++) e em hexano (classe +++) dessa mesma espécie, apresentou o maior efeito deterrente sobre *S. oryzae*. Observou-se ainda um moderado efeito de repelência dos extratos de *S. argentinum* e *A. polystachia* sobre *S. oryzae*, destacando-se o extrato em hexano de *S. argentinum* como o mais efetivo repelente (classe 4). Entre os extratos avaliados, o extrato em clorofórmio de *A. polystachia* apresentou o mayor efeito deterrente e o extrato em hexano de *S. argentinum* o maior efeito de repelência.

PALAVRAS-CHAVE - Extratos vegetais, repelência, efeito antialimentar.

ABSTRACT- Plant extracts in ethanol, chloroform and hexane of three widely distributed plants in the province of Córdoba were evaluated for their repellency and feeding deterrency to *Sitophilus oryzae* (L.). The plant species studied were, *Aloysia polystachia* (Griseb) Moldenke (Verbenaceae); *Solanum argentinum* Bitter et Lillo (Solanaceae) and *Tillandsia recurvata* (L.) L. (Bromeliaceae). Antifeedant potential of these extracts was determined by the deterrence or antifeedant test using wheat wafer disks. The Total Coefficient of Deterrence (T) was determined for each extract. Repellency tests were performed using filter-paper circles cut in halves. The extracts were applied on each half at a concentration of 0.31 $mg.cm^{-2}$. Percentual Repellency (PR) was determined for each extract. The chloroform extracts of *A. polystachia* had the strongest antifeedant effect (class ++++) followed by the ethanol extracts (class ++++) and hexane extracts of the same species (class +++). Extracts of *A. polystachia* had stronger antifeedant effect than *S. argentinum* and *T. recurvata* extracts. A moderate repellent effect of *S. argentinum* and *A. polystachia* extracts on *S. oryzae* was observed; the hexane extract of *S. argentinum* was the one with strongest repellency (class 4). Among the extracts analyzed, the strongest antifeedant effect occur with the chloroform extract of *A. polystachia* and the hexane extract of *S. argentinum* showed the strongest repellency effect.

KEYWORDS - Crude extracts, repellency, antifeedant effect.

The rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most important stored grain pest in Argentina and has been spread worldwide by commerce. Both, the adults and larvae feed on whole grains. They attack wheat, corn, oats, rye, barley, sorghum, dried beans and cereal products. Some plants of worldwide distribution in the province of Córdoba had shown, in previous observations, antifeedant an repellent effects on insects. Among this plants, *Aloysia polystachia* (Griseb) Moldenke (Verbenaceae); *Solanum argentinum* Bitter et Lillo (Solanaceae) and *Tillandsia recurvata* (L.) L. (Bromeliaceae) are highlighted. They could be used to control this pest.

Plants have developed for 400 million years and have acquired effective defense mechanisms that ensure survival under rough environmental conditions and in the presence of natural enemies. Besides a number of morphological protective mechanisms, plants have developed subtile chemical defense mechanisms against insects and other organisms; these defense mechanisms do not generally produce immediate death but do affect common biochemical and physiological functions (Prakash & Rao 1997). Until a few decades ago, plant secondary metabolites were considered substances with no specific function, which only reflected an aspect of biodiversity (Pérez Izquierdo & Ocete 1994). Recent chemical ecology studies have shown that many of these secondary compounds play an important role in plant-insect relations. Some compounds, either separately or synergically, make up a chemical defense barrier in the plant against certain pests and diseases.

Synthetic insecticides have been widely developed and are extensively used because of their effectiveness and easy application and storage. However, their extensive use has brought about severe disadvantages, like environmental disturbances, pest recovery, pest resistance, lethal effects on non-target organisms, and toxicity to users and consumers (Prakash & Rao 1997). Evaluating and using botanical pesticides, either as crude or formulated extracts, is an alternative strategy. Botanical pesticides tend to have broad-spectrum activity, are relatively specific in their mode of action and easy to process and use. Furthermore, they would be safe for higher animals and the environment and could be easily produced by farmers and small-scale industries (Talukder & Howse 1994). According to Prakash & Rao (1997) these pesticides do not contribute to resistance development or pest resurgence, nor do they cause negative effects on non-target organisms; also, they do not affect plant growth, seed viability or food quality of products.

The international literature on the biological properties of crude extracts and isolated secondary substances of plants against different insects and other organisms is abundant. Jilani & Su (1983) and Jilani *et al.* (1988) conducted insect repellency assays using extracts of different plants on stored-product pests. Talukder & Howse (1994) mentioned the toxic and repellent properties of extracts of *Aphanamixis polystachya* against *S. oryzae*.

The effect of a crude extract of *Melia azedarach* fruits on *Tribolium castaneum* was studied by Del Tío *et al.* (1996). Also, Ocete & Pérez (1996) evaluated the effect of extracts of *Daphne gnidium* and *Anagyris foetida* on several insect pests. Boeke *et al.* (2004) evaluated the efficiency of 23 different plant extracts on *Callosobruchus maculatus* and found repellency of volatile oils.

Prakash & Rao (1997) conducted an extensive revision of plants containing active secondary substances against insects. These authors provide information on 866 plant products with insecticidal activity –repellent, antifeedant, or regulatory of insect growth– used against agricultural pests. Pretheep-Kumar *et al.* (2004) observed promising results in the protection of rice against *S. oryzae* using protein-enriched pea flour extract. Rahman & Talukder (2006) studied the bioefficacy of seven plant derivatives on *C. maculatus* in Bangladesh. They obtained better results with the vegetal oils, mainly of *Azadirachta indica*. In Brazil, Tavares & Vendramim (2005) studied the bioactivity of *Chenopodium ambrosioides* on *S. zeamais*, whereas Oliveira & Vendramim (1999) observed repellent properties of essential oils and powders from three plant species on *Zabrotes subfasciatus*.

In Argentina this subject has received less attention. Peñaloza (1995) studied the biological effects of crude extracts of six plants on *Tribolium castaneum*. Novo *et al.* (1997, 1998) observed the repellent activity of several crude extracts of four native plants against *T. castaneum* and the antifeedant effect on *Anticarsia gemmatalis*. Valladares *et al.* (2003) examined the antifeedant activity of an extract of senescent leaves of *Melia azedarach* on nine insect species, including *S. oryzae*. This work explores the presence of secondary substances with biological properties against insects in wild plants of central Argentina with the aim of including them in integrated pest management programs. The aim of this work was to evaluate the repellent and antifeedant properties of crude extracts of common plants from the province of Córdoba, Argentina, against *S. oryzae*, one of the most important cereal pests of world-wide distribution.

Materials and Methods

Preparation of Plant Extracts. Crude extracts of the following plant species were used: *Aloysia polystachia* (Griseb) Moldenke (Verbenaceae) (leaves); *Solanum argentinum* Bitter et Lillo (Solanaceae)(leaves), and *Tillandsia recurvata* (L.) L. (Bromeliaceae) (whole plant). Plant material was air-dried (in shade conditions) and fragmented. Maceration was performed in solvents of different polarity (ethanol, chloroform and hexane) for 72 h with the aim of extracting different plant components. Each extract was then concentrated to dryness using a rotary vacuum evaporator until constant weight was obtained. The extracts obtained were dissolved in the corresponding pure solvent until a 10% (w/v) stock solution was obtained.

Insect. A stored-product pest species, the weevil *Sitophilus oryzae* (L) (Coleoptera: Curculionidae) was used for the bioassays. Weevils were reared in growth chambers at 27±2°C and relative humidity of 75±5% with alternating light and dark periods of 12 h. Jars covered with a fine piece of cotton cloth were used to allow the passage of air; weevils were fed on wheat grains.

Adults of *S. oryzae* were placed in the rearing medium for 10-15 days; then they were removed and placed in a new medium to obtain a new progeny and avoid generation overlapping. The medium containing the eggs was placed in the growth chamber until adult emergence. This process was successively repeated with the aim of obtaining homogenous generations.

Feeding Deterrent Activity Bioassays. The potential of the antifeedant effect of the extracts was determined by the feeding deterrency test described by Talukder & Howse (1994). Wheat wafer disks were used as test food; the disks were saturated by dipping into either solvents only (control C) or into 10mg.mL^{-1} solution of each extract (treatment T) and were air-dried for 20 h. Wheat disks were then weighed and 10 weevils were placed on them; the disks were reweighed after 6 days. Food consumption of weevils was recorded under three conditions: 1) on pure food, composed of two untreated disks (CC); 2) on food with possibility of choice between one treated (T) and one untreated (C) disk: choice test, and 3) on food with two treated (TT) disks: non-choice test.

Blank disks treated with the solvent but not offered to weevils were prepared. The experiment was arranged in a randomized complete design with five replications. Disks were dipped for two different time periods: 15 and 30 seconds. Once dried, wheat disks may increase in their weight as a result of water absorption from the surrounding air which was humidified for the normal growth and development of weevils. Hence, a correction procedure was applied.

Disk weight loss, which was estimated as the amount of food consumed (FC), was calculated by the formula of Serit *et al*. cited by Talukder & Howse (1994):

$$FC = IW_s - [(FW_s * IW_b)/FW_b]$$

where: IW= initial weight of disk after being treated with extract or solvent, FW= final weight of disk, subscript b= blank disk (treated with solvent and not offered to weevils), and subscript s = treated and control disks, on which weevils were released.

According to the amount of food consumed in the three different tests (CC, TT, and CT) three feeding deterrent activity coefficients were calculated:

- Absolute coefficient of deterrency A= (CC - TT/CC + TT)*100
- Relative coefficient of deterrency R= (C - T/C + TO)*100
- Total coefficient of deterrency T= A + R

The values of total coefficient of deterrency serve as an index of antifeedant activity expressed on a scale between 0 and 200. The index zero (0) stands for an inactive compound and 200 for a compound with maximum activity. An index T of 150-200 was designated ++++; of 100-150 +++; of 50-100 ++, and of 0-50 +. Mean separation of antifeedant activity of extracts were made using non parametric Kruskall-Wallis's test (P≤0,05). Contrasts analyses between plant species, solvents and time of exposure were made using the same test (INFOSTAT, 2001).

Repellent Activity Bioassays. Repellency tests were conducted following the method proposed by Talukder & Howse (1993, 1994). Filter-paper circles of 9 cm in diameter were cut in half. Extracts were applied on one half at a concentration of 10mg.mL^{-1}. One mL of solution was uniformly applied with a pipette, in such a way as to have a treated substrate of 0.31 mg.cm^{-2} extract. The treated half-circles were air-dried until the solvent was totally evaporated. The treated and the untreated half-circles were placed contiguously on the Petri dishes and 10 adult weevils were released on each dish. Weevils present in each half circle were counted at hourly intervals for 5 h after treatment. Data were converted to express percentage repulsion (PR) using the following formula: **PR (%) = (N_c – 50) x 2,** where N_c is the percentage of weevils present in the control half. Positive values (+) indicated repellency and negative values (-) attractancy. Five replications were made of each treatment. Data were analyzed using a two factor completely randomized design using the different plant species and solvents as the two factors. Mean separation of repellent activity of the different extracts and the comparisons analyses between plant species and solvents were made using Fisher's least significant difference (LSD) test (P≤0,05) (INFOSTAT, 2001). Mean values were classified according to the following scale:

Class	Repellency rate (%)
0	>0.01 to < 0.1
1	0.1 to 20
2	20.1 to 40
3	40.1 to 60
4	60.1 to 80
5	80.1 to 100

Results and Discussion

Antifeedant Activity. The extract with the strongest antifeedant effect on *S. oryzae* was the chloroform extract of *A. polystachia*, in both treatment times (class ++++). An important antifeedant effect was also observed in the ethanol extracts of *A. polystachia* (class ++++), and with the hexane extracts of the same species to a lesser degree (class +++) (Table 1).

Table 1. Feeding deterrent coefficients and efficacy of extracts of three plant species on adults of *Sitophilus oryzae*.

Name of extract	Solvent	Immersion time[1]	Coefficient of deterrency[2]			Efficacy of extract
			Absolute	Relative	Total	
A. polystachia	Ethanol	15	53.42	98.72	152.14 f	++++
		30	59.03	96.09	155.12 ef	++++
	Chloroform	15	95.89	68.54	164.43 f	++++
		30	91.21	71.78	162.99 f	++++
	Hexane	15	18.41	89.65	108.06 abcd	+++
		30	16.57	96.08	112.65 abcde	+++
S. argentinum	Ethanol	15	21.56	75.42	96.98 ab	++
		30	15.01	71.23	86.24 ab	++
	Chloroform	15	18.74	79.78	98.53 abc	++
		30	19.07	89.67	106.74 abcd	+++
	Hexane	15	54.57	71.68	126.25 bcdef	+++
		30	42.09	98.82	140.91 def	+++
T. recurvata	Ehtanol	15	44.41	92.47	136.88 def	+++
		30	47.32	96.85	144.17 ef	+++
	Chloroform	15	41.23	92.81	134.04 cdef	+++
		30	36.64	88.32	124.96 bcdef	+++
	Hexane	15	25.73	65.42	91.15 a	++
		30	52.36	46.84	99.20 ab	++

Values followed by the same letter are not significantly different according to Kruskal Wallis's test (P≤0,05).
[1] Expressed in seconds
[2] Absolute coefficient of deterrency A= (CC - TT/CC + TT)*100
Relative coefficient of deterrency R= (C - T/C + TO)*100
Total coefficient of deterrency T= A + R

The analysis of antifeedant effect of each plant, regardless of the solvent or immersion time used, shows that *A. polystachia* was significantly stronger than *S. argentinum* and *T. recurvata*. However, no significant differences were observed between the extracts of *T. recurvata* and *S. argentinum* (Table 2). Contrasts between solvents indicate that the hexane extracts were significantly less potent than chloroform and ethanol extracts; however, no significant differences were detected between the two latter extracts (P=0,9429). Contrast between immersion times indicated that they were not significantly different (P=0,6601), suggesting that using either of them renders no difference (Table 2).

Similar results of antifeedant effect on *S. oryzae* and *T. castaneum* were observed by Talukder & Howse (1993, 1994) with four different extracts of *Aphanamixis polystachya*, acetone extract being the most significant with a total deterrency coefficient of 159.5. Valladares *et al.* (2003) also indicated an antifeedant effect of ethanol extract of senescent leaves of *Melia azedarach* on *S. oryzae*, with an antifeedant index of 100%.

Table 2. Probability values for Kruskal Wallis's test of total coefficients of deterrency of extracts of three plant species on adults of *Sitophilus oryzae*.

Contrasts between plants	Total coefficients of deterrency[1]		Probability
	Mean(1)	Mean(2)	
A. polystachia vs *T. recurvata*	142,56	121,74	0,0357
T. recurvata vs *S. argentinum*	121,74	109,92	0,1627
A. polystachia vs. *S. argentinum*	142,56	109,92	0,0005
Contrasts between solvents			
Chloroform vs. Ethanol	131,95	129,23	0,9429
Ethanol vs. Hexane	129,23	113,04	0,0301
Chloroform vs. Hexane	131,95	113,04	0,0250
Contrast between time period of dipping			
30 sec vs. 15 sec	126,32	123.16	0,6601

[1]Absolute coefficient of deterrency A= (CC - TT/CC + TT)*100
Relative coefficient of deterrency R= (C - T/C + TO)*100
Total coefficient of deterrency T= A + R

Repellency Effects. Among all the combinations of plant species and solvents tested, the hexane extract of *S. argentinum* was the one with the strongest repellent effect on *S. oryzae* (class 4) with PR of 63%, followed by the ethanol extract of this species (class 3). Other extracts with significant repellency activity were the chloroform extract of *A. polystachia* (class 3) and the ethanol extract of *T. recurvata* (class 3) (Table 3). The percentage of repellency observed during the 5 hours of the test did not show a defined behaviour either between each hour. The factorial analysis indicate significative differences between plant species (P=0,0004), solvents (P=0,0396) and in the interaction plant species vs. solvents (P=0,0006). The analysis of the repellency effect of each plant species, regardless of the solvent used, shows that the extracts of *A. polystachia* and *S. argentinum* did not differ significantly but were significantly stronger than *T. recurvata* (Table 3). Comparisons between solvents used indicate that the repellency effect of ethanol extracts was significantly higher than chloroform extracts but was not significantly different of hexane extracts (Table 3).

In general, the extracts of *A. polystachia* and *S. argentinum* had a moderate repellency effect on S. *oryzae*. These results agree with those obtained by Talukder & Howse (1993, 1994) with extracts of *A. polystachya* on *S. oryzae* (1994) and *T. castaneum* (1993), although the latter species showed a higher repellency effect; the authors also found a higher repellency effect of extracts on *S. oryzae*, mainly extracts of methanol (67%), acetone (67%), and ethanol (57%). Jilani & Su (1983) reported the repellent effects of extracts of three common plants in Pakistan on three stored-product pests, including *Sitophilus granarius*. The powdered extract of *Curcuma longa* had the strongest repellency effect on the three species studied. Oliveira & Vendramim (1999) observed repellency to vegetal oils and powders on *Zabrotes subfasciatus* in bean seeds. The strongest repellency was obtained with the oils of *Cinnamomum zeylanicum* (96.2% repellency) and of *Azadirachta indica* (89.4% repellency). Pretheep-Kumar *et al.* (2004) found that an extract of protein-enriched bean flour had a high level of repellency on *S. oryzae*. They found 76.3% and 91,2% of repellency with a concentration of 0.1% and 1% of the extract, respectively, after 48 h after the assay was initiated. In contrast, Tavares & Vendramim (2005) reported the insecticide activity but a lack of repellent activity of extracts of *Chenopodium ambrosioides* on *S. zeamais*; these findings do not agree with the results observed in the present work, in which extracts showed repellent activity but no toxic effects.

Table 3. Repellency of extracts of three plant species in different solvents on adults of *Sitophilus oryzae*, using the filter paper test.

Extract	Solvent	Repellency (%)[1] at:					Mean repellency (%)[1]	Class repellency
		1 h	2 h	3 h	4 h	5 h[2]		
S. argentinum	Ethanol	35	45	65	75	55	55 e	3
	Chloroform	5	30	15	45	35	26 b	2
	Hexane	15	60	70	80	90	63 e	4
A. polystachia	Ethanol	20	30	20	60	20	30 b	2
	Chloroform	30	70	65	40	55	52 cde	3
	Hexane	10	30	35	35	55	33 bc	2
T. recurvata	Ethanol	35	40	30	40	60	41 bcd	3
	Chloroform	5	0	5	0	0	2 a	1
	Hexane	20	15	10	45	15	21 ab	2
Plant species								
S. argentinum							48.00 b	
A. polystachia							38.33 b	
T. recurvata							21.33 a	
Solvents								
Ethanol							42.00 b	
Chloroform							26.67 a	
Hexane							39.00 ab	

Values followed by the same letter are not significantly different according to the Fisher's LSD test (P≤0,05).
[1] Percentage of repellency PR(%) = (N_c – 50)*2, where N_c is the percentage of weevils present in the control half.
[2] hours after treatment

Among the extracts analyzed, the strongest antifeedant effect occur with the chloroform extract of *A. polystachia*, followed by the ethanol extracts and, to a lesser degree, the hexane extract of the same species. Regardless of the solvent and immersion time used, the extracts of *A. polystachia* show a stronger antifeedant effect than the extracts of *S. argentinum* and *T. recurvata*. The results of the repellency bioassays indicate that the hexane extract of *S. argentinum* is the one with strongest repellency effect on *S. oryzae*, with a PR of 63% (class 4). In general, the extracts of *S. argentinum* and *A. polystachia* showed a moderate repellency effect on *S. oryzae*.

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