BioAssay

PLANT EXTRACTS

Insecticidal Effect of Extracts from Native Plants to Mato Grosso do Sul, Brazil, on *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae)

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Efeito Inseticida de Extratos de Plantas Nativas do Mato Grosso do Sul, Brasil, sobre *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae)

RESUMO – A pesquisa e o uso de plantas inseticidas têm aumentado nos últimos anos. Sua compatibilidade com outros métodos de controle de insetos e a menor toxicidade aos mamíferos são algumas das vantagens que têm estimulado seu uso. Neste trabalho foram investigadas algumas espécies vegetais nativas de Mato Grosso do Sul quanto a seu potencial inseticida sobre *Sitophilus zeamais* Mots. Foram avaliados extratos de caules de *Tapirira guianensis* Aubl. (Anacardiaceae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Tabebuia heptaphylla* (Vell.) Toledo (Bignoniaceae) e *Gomphrena elegans* Mart. (Amaranthaceae). Os grãos de trigo foram tratados com os extratos e distribuídos em caixas de acrílico contendo 20 indivíduos adultos não-sexados de *S. zeamais*, de 10 a 20 dias de idade. No controle os grãos de trigo foram tratados somente com solventes. A avaliação foi conduzida até o décimo dia contando o número de adultos mortos. Os tratamentos com extratos de *T. heptaphylla* e *G. elegans* apresentaram efeito inseticida desde o quinto dia, enquanto os demais extratos só o exibiram no décimo dia. Os seguintes extratos causaram mortalidade dos insetos: n-butanol, hexano e diclorometano de *T. guianensis*; etanol, acetato de etila, hexano e diclorometano de *S. terebintifolius;* acetonitrila-clorofórmio, etanol, e hexano de *T. heptaphylla;* etanol, hexano, diclorometano, hidroalcoólico e acetato de etila de *G. elegans.* A última espécie vegetal foi mais efetiva.

PALAVRAS-CHAVE – Planta inseticida, *Gomphrena elegans*, biopesticida, gorgulho-do-milho, bioprospecção.

ABSTRACT – Research on insecticidal plants has increased in recent years, as has their utilization. Their compatibility with other methods of insect control and their lower toxicity to mammals are some of the advantages that have fostered their use. In the present study, selected plant species native to the state of Mato Grosso do Sul, Brazil, were investigated for their insecticidal potential against *Sitophilus zeamais* Mots. The extracts assayed were obtained from stems of *Tapirira guianensis* Aubl. (Anacardiaceae), *Schinus terebinthifolius* Raddi (Anacardiaceae), *Tabebuia heptaphylla* (Vell.) Toledo (Bignoniaceae), and *Gomphrena elegans* Mart. (Amaranthaceae). Wheat grains were treated with the extracts and distributed into acrylic containers, each holding 20 unsexed 10- to 20-day-old *S. zeamais* adults. For the control the wheat grains were treated solely with solvents. The number of dead insects was counted daily until the tenth day. *T. heptaphylla* and *G. elegans* extracts exhibited insecticidal effect since the fifth day of treatment, whereas the effect of the other extracts was not observed before the tenth day. Insect death was caused by the following extracts: *T. guianensis* in *n*-butanol, hexane, and dichloromethane; *S. terebinthifolius* in ethanol, ethyl acetate, hexane, and dichloromethane; *T. heptaphylla* in acetonitrile-chloroform, ethanol, and hexane; and *G. elegans* in ethanol, hexane, dichloromethane, ethyl acetate, and as hydroalcoholic extract. *G. elegans* extracts had the strongest insecticidal effect of all the species tested.

KEYWORDS – Insecticidal plant, *Gomphrena elegans*, biopesticide, maize weevil, bioprospection.

The maize weevil, *Sitophilus zeamais* Mots., is one of the pests that most affects stored grains in Brazil, given its bioecological features of cross infestation, high biotic potential, capability to invade stored grain mass, high number of host species, and the fact that both larvae and adult insects can cause injury to grains (Gallo *et al*. 2002).

The utilization of plants to control insect pests is a relatively ancient practice. Some of the earliest products used for this purpose were nicotine, extracted from *Nicotiana tabacum* L. (Solanaceae); ryanodine, from *Ryania speciosa* Vahl. (Flacuorticaceae); sabadile, from *Schoenocaulom officinale* A. Gray. (Liliaceae); pyrethrin, from *Chrysantemum cinerariaefolium* Vis. (Asteraceae); and rotenone, extracted from *Derris* spp. and *Lonchocarpus* spp. (Fabaceae) (Jacobson 1989).

A number of factors appear to limit the success of insecticidal plants, including the availability of competitive products (new synthetic compounds, materials derived from microbial fermentation) that are not only viable, but also somewhat safer than earlier synthetic insecticides. In the context of Integrated Pest Management (IPM), plant-derived insecticides constitute an alternative now widely used in organic production in industrialized countries. Their role, however, may be more relevant in the production and protection of stored grains in developing countries, because of their lower cost (Isman 2006).

Knowledge on the negative impacts of the indiscriminate use of chemicals and the requirements of consumers on the quality of foodstuffs have motivated the search for new alternatives to control insects with lower environmental impact. Botanical insecticides for the control of stored grain pests are particularly promising, given the ease of management of treated materials (Tavares & Vendramim 2005).

Considering these aspects and the need for conducting improved investigations on the phytochemical potential of native plant species of the state of Mato Grosso do Sul, the Biochemical Laboratory of the Federal University of Mato Grosso do Sul (UFMS) has been carrying out laboratory tests of the insecticidal activity of plant extracts obtained from selected species native to the Pantanal and Cerrado regions of the state.

Material and Methods

Biological material. The experiments were performed at 25 ± 2.2 °C, under relative humidity of 60 ± 10% and natural photoperiod—the same conditions under which the *S. zeamais* colony on wheat grains was maintained. *G. elegans* Mart., vulgar name ‘gonfrena,’ was collected along the rivers Sucuri and Baía Bonita (Bonito county, MS). *T. guianensis* Aubl., ‘tapirira,’ *S. terebinthifolius* Raddi, ‘aroeira-mansa,’ and *T. heptaphylla* (Vell.) Toledo, ‘ipê-rosa,’ were collected from the Biological Reserve of UFMS, located in the Cerrado biome. Plant identification was performed by Ubirazilda Rezende, MSc., of UFMS, and exsiccates have been deposited at the UFMS Herbarium.

Preparation of plant extracts. Plant materials, ranging in weight from 0.8 to 1.4 kg for each species, were air-dried, cut into small pieces, and powdered in a Wiley-type mill. The resulting matter was extracted with ethanol for seven days, with occasional stirring. The procedure was followed by filtration and concentration of the filtrate by rotaevaporation, and the residues were placed in a desiccator for dehydration. The solvents employed for extraction, partition, and chromatography were of analytical grade.

The ethanolic extract was sequentially partitioned with hexane / dichloromethane / ethyl acetate / hydroalcoholic solution / *n*-butanol / acetonitrile-chloroform, leaving an insoluble residue. For each species and fraction, an amount of extract equivalent to that obtained from 10 g of dried stems was reserved for the experiments.

Biological assays of plant extracts. Using a laminar flow hood and a glass nebulizer coupled to a vacuum pump, wheat grains were nebulized with an amount of each extract fraction corresponding to 10 g of dried plant material. The fractions were prepared by diluting 10 g of extract with the appropriate solvents for every 100 g of wheat grains. To evaluate the insecticidal effect of the solvents alone, preliminary tests were conducted in which wheat grains were left for about 72 h in a hood at 38 °C after nebulization with solvents (methanol, ethanol, chloroform, and hexane). The procedure did not affect insect survival. The temperature at 38 °C was chosen because it preserves the chemical characteristics of organic compounds in plant extracts.

After drying, 10 g of grains each were transferred to round acrylic containers. Controls consisted of untreated grains. Twenty unsexed 10- to 20-day-old adults of *S. zeamais* were place in each acrylic container. Evaluations were done on the first, second, fifth, and tenth days by counting the number of dead insects. The extract fractions were distributed according to a random experimental planning of ten repetitions for each treatment. For the purpose of analysis, data were cumulative from the first to the tenth day.

The results were subjected to analysis of variance (F-test). Whenever a significant difference was found between the means at the 5% level of error probability, the analysis was supplemented by applying Tukey’s test to compare the means.

Results and Discussion

Results of extracts obtained from 10 g of dried stems, tested for insecticidal activity against *S. zeamais* are in Table 1.

Table 1. Extracts obtained from 10 g of dried stems, tested for insecticidal activity against *S. zeamais*.

Species	Extracts (g)							
	Hexane	Acetonitrile-chloroform	Dichloro-methane	Ethyl acetate	*N*-butanol	Ethanol	Hydro-alcoholic	Residue
Tapirira guianensis Aubl.	0.015	-	0.020	0.130	0.680	1.100	-	0.030
Schinus terebinthifolius Raddi	0.098	-	0.064	0.280	1.410	2.700	-	0.850
Tabebuia heptaphylla (Vell.)	0.030	0.100		1.040	0.010	1.900	-	-
Gomphrena elegans Mart.	0.041	-	0.031	0.141	-	0.360	0.010	-

Because no mortality attributable to any of the extracts was observed during the first two days of the experiment, the corresponding data were not included in the analysis. From the fifth day onward, significant differences from controls were observed for some extracts. Only on the tenth day did these differences become more pronounced (Tables 2-5). Insect death caused by extracts of *G. elegans* was observed only after the fifth day (Table 5) - a reason why the experiment was carried out for ten days, given the likelihood that a cumulative insecticidal effect may have occurred which was not obvious until the fifth day.

The dichloromethane, hexane, and *n*-butanol extracts from *T. guianensis* stems exhibited weak activity (below 20%) on the fifth day ($F = 22.08$, df = 6, $P = 0.0001$) and tenth day ($F = 44.26$, df = 6, $P = 0.0001$) (Table 2). No previous data were available on the insecticidal activity of this species, although the presence of tannins, flavonoids, and triterpenoids has been reported for several parts of this plant (Jardim *et al*. 2005). These classes of compounds are generally associated with antifeedant activity against insects, being thus defined as deterrents (Sharma & Norris 1994, Murthy *et al.* 1998, Prasad *et al*. 1998, Park *et al*. 2000, Calcagno *et al.* 2002, Morimoto *et al*. 2002, Piubelli *et al*. 2003).

Table 2. Mortality rates (% ± EP) in *S. zeamais* adults feeding on wheat grains treated with extracts of *T. guianensis* stems.

Treatment (extracts)	Mortality rates			
	1st day[1]	2nd day[1]	5th day[2]	10th day[2]
Dichloromethane	0.00 ± 0.00	0.00 ± 0.00	13.00 ± 3.50 a	13.00 ± 3.50 a
Hexane	0.00 ± 0.00	0.00 ± 0.00	12.50 ± 2.64 a	12.50 ± 2.64 a
N-butanol	0.00 ± 0.00	0.00 ± 0.00	12.00 ± 4.83 a	12.00 ± 4.83 a
Ethyl acetate	0.00 ± 0.00	0.00 ± 0.00	5.00 ± 4.08 b	5.00 ± 4.08 b
Ethanol	0.00 ± 0.00	5.00 ± 1.58	4.50 ± 4.38 b	4.50 ± 4.38 b
Residue	0.00 ± 0.00	0.00 ± 0.00	1.50 ± 3.50 b	1.50 ± 3.50 b
Controls	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 2.11 b	1.00 ± 2.11 b

[1] Means not analyzed.
[2] Means followed by the same letter in the same row do not differ significantly (Tukey's test, 5% significance).

Tannins are inhibitors of proteases, affecting insect growth and survival, since they inactivate digestive enzymes and form a tannin-protein complex not easily degradable (Strong *et al*. 1984, Mello & Silva-Filho 2002). Flavonoids may act as feeding inhibitors at high concentrations and as phagostimulants at low concentrations, as demonstrated for *Heliothis zea* Boddie and *Helicoverpa armigera* (Hubner) in the final stages of development (Simmonds 2001). Terpenoids are toxic and deterrent to many insects and herbivorous mammals. The best known examples are pyrethroids, which are monoterpene esters with strong insecticidal activity, and essential oils with recognizable repellent action. Phytoecdisones are terpenes that share a basic chemical structure with hormones of juvenile insects, affecting their growth. Azadirachtin, a terpenic compound, is also highly effective, thanks to its low toxicity to mammals and its noxious effect on insects. It is extracted from a species native to India, *Azadirachtina indica*, popularly known as 'neem' (Viegas Júnior 2003, Taiz & Zeiger 2006).

Although the results of most treatments with *S. terebinthifolius* differed from those of controls, mortality was considerably low, not achieving 20% ($F = 23.87$, df = 6, $P = 0.0001$) (Table 3). The dichloromethane and hexane fractions were the most effective. Other species of this genus have demonstrated insecticidal activity against dipterans (Laurent *et al*. 1998).

Table 3. Mortality rates (% ± EP) in *S. zeamais* adults feeding on wheat grains treated with extracts of *S. terebinthifolius* stems.

Treatment (extracts)	Mortality rates			
	1st day[1]	2nd day[1]	5th day[1]	10th day[2]
Dichloromethane	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	18.00 ± 3.50 a
Hexane	0.50 ± 1.58	0.50 ± 1.58	0.50 ± 1.58	16.50 ± 5.80 b
Ethyl acetate	0.00 ± 0.00	0.50 ± 1.58	1.50 ± 3.37	14.00 ± 3.94 c
Ethanol	0.00 ± 0.00	0.50 ± 1.58	2.50 ± 4.25	13.00 ± 5.38 c
Residue	0.00 ± 0.00	0.50 ± 1.58	0.50 ± 1.58	10.00 ± 1.50 d
N-butanol	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 3.50	5.00 ± 2.36 e
Controls	0.50 ± 1.58	0.50 ± 1.58	0.50 ± 1.58	1.50 ± 2.42 f

[1] Means not analyzed.
[2] Means followed by the same letter in the same row do not differ significantly (Tukey's test, 5% significance).

With regard to *T. heptaphylla*, only its hexanic extract showed insecticidal effect (23%) on the fifth day (F = 7.26, df = 6, P = 0.0001). The ethanolic and hexanic extracts led to similar mortality rates, of nearly 35%, on the tenth day (F = 6.23, df = 6, P = 0.0001) (Table 4). The ethyl acetate, *n*-butanol, and acetonitrile-chloroform fractions caused mortality rates of intermediate value (Table 4). Other species of the genus *Tabebuia* are known for their insecticidal effect (Dória *et al*. 2004, Singh & Saratchandra 2005).

All extracts of *G. elegans* stems led to mortality rates ranging from 36% to 46.55% only on the tenth day, though not differing from controls before that time point (F = 97.34, df = 5, P = 0.0001) (Table 5). No phytochemical data for this species were found in the literature. Studies of this type have been conducted at the Biochemistry Laboratory of UFMS.

The mortality effect observed in the extracts of the plants selected for this study can be attributed to the presence of secondary metabolites, thus termed for not playing a direct role in essential metabolic processes such as photosynthesis, respiration, transport of solutes, translocation, or protein and lipid synthesis. Moreover, they occur in restricted families, and diversely from primary metabolites (Taiz & Zeiger 2006).

The results achieved in the present investigation warrant more extensive studies to be conducted with *G. elegans*, with the purpose of isolating compounds with insecticidal activity.

Table 4. Mortality rates (% ± EP) in *S. zeamais* adults feeding on wheat grains treated with extracts of *T. heptaphylla* stems.

Treatment (extracts)	Mortality rates			
	1st day[1]	2nd day[1]	5th day[2]	10th day[2]
Hexane	0.50 ± 1.58	2.50 ± 2.63	23.00 ± 13.58 a	35.50 ± 15.54 a
Ethanol	0.50 ± 1.58	2.00 ± 2.58	10.00 ± 11.30 b	35.50 ± 27.73 a
Acetonitrile-chloroform	0.50 ± 1.58	1.50 ± 2.41	10.00 ± 9.72 b	24.00 ± 12.43 ab
N-butanol	0.00 ± 0.00	0.00 ± 0.00	6.50 ± 5.30 b	20.00 ± 14.91 ab
Ethyl acetate	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 3.50 b	12.50 ± 15.14 b
Controls	0.50 ± 1.58	0.50 ± 1.58	0.50 ± 1.58 b	0.50 ± 1.58 b

[1] Means not analyzed.
[2] Means followed by the same letter in the same row do not differ significantly (Tukey's test, 5% significance).

Table 5. Mortality rates (% ± EP) in *S. zeamais* adults feeding on wheat grains treated with extracts of *G. elegans* stems.

Treatment (extracts)	Mortality rates			
	1°[1]	2°[1]	5°[1]	10°[2]
Dichloromethane	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	46.50 ± 5.30 a
Hexane	0.00 ± 0.00	1.00 ± 2.11	1.00 ± 2.11	40.00 ± 4.08 a
Ethyl acetate	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	39.50 ± 4.97 a
Hydroalcoholic	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	39.00 ± 6.15 a
Ethanol	0.00 ± 0.00	5.00 ± 1.58	5.00 ± 1.58	36.00 ± 6.58 a
Controls	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 2.58 b

[1] Means not analyzed.
[2] Means followed by the same letter in the same row do not differ significantly (Tukey's test, 5% significance).

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