Austroeupatorium inulaefolium H.B.K. Extracts in Deterring Feeding of the Rice Weevil on Wheat[1]

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ABSTRACT

Organic extracts of *Austroeupatorium inulaefolium* H.B.K., a bush common in mountain valleys of northern South America, were evaluated for their repellent or feeding deterrence qualities against adults of the rice weevil *Sitophilus oryzae* L., reared on wheat. Methanol, acetone-dichloromethane, and hexane extracts were found to be increasingly active in that order, with deterrency indexes of 0.57, 0.21 and 0.17, respectively, at 0.5% (w/w) dosage. The activity was maintained after eight weeks of treatment. This activity was found to be actual feeding deterrence rather than mere contact repellency.

Key words: *Austroeupatorium inulaefolium*, *Sitophilus oryzae*, wheat, feeding deterrence.

COMPENDIO

Los extractos orgánicos de *Austroeupatorium inulaefolium* H.B.K., una planta arbustiva común en los valles de montaña del norte de Suramérica, fueron evaluados por su actividad de repelencia o disuasión alimentaria contra ejemplares adultos del gorgojo del arroz *Sitophilus oryzae* L., cultivado sobre trigo. Los extractos de metanol, acetona-diclorometano y hexano fueron encontrados activos con índices de disuasión de 0.57, 0.21 y 0.17 respectivamente, al 0.5% (w/w) de dosificación. La actividad se preservó sin cambio luego de ocho semanas de tratamiento en el trigo. Se determinó que correspondía a la disuasión alimentaria en vez que a la repelencia por contacto.

INTRODUCTION

Stored grain, a high concentration of food energy, is subject to heavy attack by rodents, fungi and insects. Losses are worst in tropical areas due to a combination of environmental factors such as high air moisture and high temperatures throughout the year, and poor management practices, such as low quality bins, inadequate drying processes and only occasional spraying with increasingly costly pesticides.

As a consequence of rising costs, increasing ineffectiveness (10, 18) and indiscriminate toxicity of many synthetic pesticides, there has been an upsurge in the investigation of biologically active plants in recent years. This activity has revealed great potential as a source of new, biodegradable chemicals useful for the control of weed and insect pest populations. The application of some of these extracts and compounds for cereal grain protection and against other agricultural pests has been repeatedly reviewed (8, 9, 11, 12).

However, few tropical plant species, with the remarkable exceptions of *Wargurgia* and *Azadirachta* (1, 5, 7) have been examined in this context, in spite of their longstanding importance from a purely phytochemical standpoint. A program was therefore started to develop biorational allelochemicals for the control of some prevalent stored-grain insect pests from endemic plants of northern South America, taking advantage of the enormous biodiversity of this area.

The results of evaluations of methanol, acetone-methylene-chloride, and hexane extracts of the locally named worm bush, *A. inulaefolium*, against the rice weevil *S. oryzae* widespread, destructive pest of stored cereals in many parts of the world, are reported.

MATERIALS AND METHODS

Insect culture: One hundred rice weevils reared on wheat from a stock maintained in the laboratory were allowed to feed on 200 g of wheat kernels for 48 hours.

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The weevils were removed and the kernels were placed in 0.5 jars covered with cheesecloth, placed in an incubator at 26°C - 29°C, at 60+5% relative humidity (RH) and 12/12 h LD photoperiod. After 52 days, adults started to emerge. Those adults emerging from 52 - 56 days were collected, allowed to feed on wheat for an additional 18 day period under similar environmental conditions and then used for testing.

Plant material and extraction: Non-flowering *A. inulaefolium* stands approximately one year old were collected in November 1988 from an open field 5 km southwest of the city of Merida, at 1850 m above sea level. Young and intermediate leaves were clipped from stems and dried at room temperature for two weeks, and then used for extraction. A sample of this material was weighed, then dried in an oven at 60°C for 48 h, and weighed again to assess the residual moisture. This was found to be 5.9% in all samples studied. The leaf material was ground to a fine powder and divided into three 60 g portions. Each portion was suspended with mechanical stirring in 800 ml of methanol (MeOH), acetone-methylene-chloride (Ac-MC) in a 1:1 ratio, and hexane (Jx), respectively, at 30-40°C for two hours. Each extract was then filtered through glass wool and evaluated for its content of non-volatile material by evaporation under vacuum of a 10 ml aliquot and by weighing the residue. The extracts were found to contain (MeOH) 24.1%, (Ac-MC) 9.1% and (Hx) 4.3% of non-volatile matter. The extracts were stored at 5°C until used.

Feeding deterrency

Grain treatment: Wheat used in rearing and in the bioassays was collected from a farm near Mucuchies, Merida, Mex. at 26 50 m above sea level. The grain was free from pesticide or fertilizer use during cultivation, and devoid of insect damage owing to prevailing low temperatures. Wheat was selected as a testing cereal because rice weevil is a primary pest of wheat which breeds there more successfully than in local strains of rice. Grain was acclimated to constant humidity for six weeks before the tests, and moisture content was determined weekly 14% before this period ended. This was found to be in the 13.0% to 14.5% range. 700 g clean kernels were placed in equal amounts in three 500 m round-bottomed flasks and mixed with 750 ml of extract solution containing 3.5 g of non-volatile material so as to yield a 0.5% (w/w) extract dose. Solvents were distilled to dryness in a rotary evaporator at 40°C. The kernels were thus covered with an uniform layer of extract. The treated grain was spread in trays and allowed to acclimate in the environmental chamber under the aforementioned conditions, for 72 h before use.

Two choice-test: The modified method of Mikolajczak and Reed (13) was performed as follows: Four 4.0 cm x 4.0 cm plastic cups filled to the rim with 43 g of treated kernels were placed in a wooden 21 cm x 21 cm x 6 cm box in a circular fashion. Four additional cups filled with control kernels were placed interspaced within the former cups, thus forming a circle of approximately 15 cm in diameter. Paper strips bridging contiguous cups were added to facilitate insect dispersion. Four boxes were prepared in a similar fashion and were acclimated in the testing chamber under the aforementioned conditions for 24 h before use. Then, at time zero, 128 unsexed, 18-22 day-old adult weevils were released in the center of each box. The boxes were immediately covered with a glass lid to prevent the insects from escaping. Few insects remained outside the cups after 5 h - 10 h of exposure.

Weevils in each cup were counted after 24 hours. No significant mortality was recorded (%). Four replicates of this experiment were performed on consecutive days. The tests were repeated two, four, and eight weeks after treatment of the kernels with plant extracts, using fresh insects each time. Insect counts were converted to percentages, and frequency distribution histograms were constructed to calculate mean population values in treated and control cups. Both average values were compared using Duncan's multiple range test. Deterrency indexes (DI) were calculated using the formula DI= % insects in treated cups/ percentage insects in control cups, based on Mikolajczak and Reed (13).

Split arena test: A 10 cm Whatman No. 1 filter paper was sprayed on one half with 0.5% (w/w) extract solution of the test plant, while the other half was covered with a glass plate. The process was repeated by spraying the second half of the filter paper with solvent only. The filter paper was allowed to dry overnight at room temperature and was placed in a Petri dish inside the environmental chamber. At time zero, 10 weevils were released in the center of the dish, which was quickly covered. Insects standing on both surfaces were counted every 15 min for the next four hours in four parallel Petri dishes similarly prepared.

RESULTS AND DISCUSSION

A. inulaefolium was chosen for this study based on three criteria: 1) This bush is a prevalent, second-generation growth of secondary forests in cleared areas of mountain valleys 900 - 2000 masl, in the northern Andean ranges of South America. In spite of its relative abundance, arthropod and mammal herbivores display little interest in feeding on this plant, probably because of its bitter taste (to humans); 2) some farmers in the Venezuelan Andes spray *A. inulaefolium* extracts to protect their cabbage and cauliflower crops from attack by well-adapted lepidopteran pests such as *Pieris arypa*; 3) in the Merida area, we have been able to detect only one insect species that uses the worm bush to a significant extent as its host plant, from which it derives its common name. This insect is the larvae of *Actinotes thalia anteas* (Lepidoptera) which feeds actively on its leaves. The host association of adapted insects has been taken as an indication of the probable presence in the plant of allelochemicals with potent general anti-insect properties, a product of evolutionary pressure.

Figures 1a, 1b, 1c show population distributions of rice weevils in the two-choice test. The methanol extract showed the smallest effect (DI=0.57), in spite of being the major contributor to whole plant extract (24.1%) of dry weight, the medium- and low-polarity extracts showed the strongest feeding deterrence (see Table 1).

The split-arena test indicated that this effect is not simple repellency to contact with plant chemicals, as insects did not show a meaningful preference for the untreated area. Weevils and other stored product pests tend to settle on their food supply, hence the observed distributions should be interpreted in terms of food preference for the untreated wheat. This conclusion is underscored by the accumulation of over 40% (51 insects) of the weevils in only one of the four untreated wheat cups presented to them in two of the recorded experiments, while several cups containing treated kernels remained essentially unpopulated, as seen in Figure 1c.

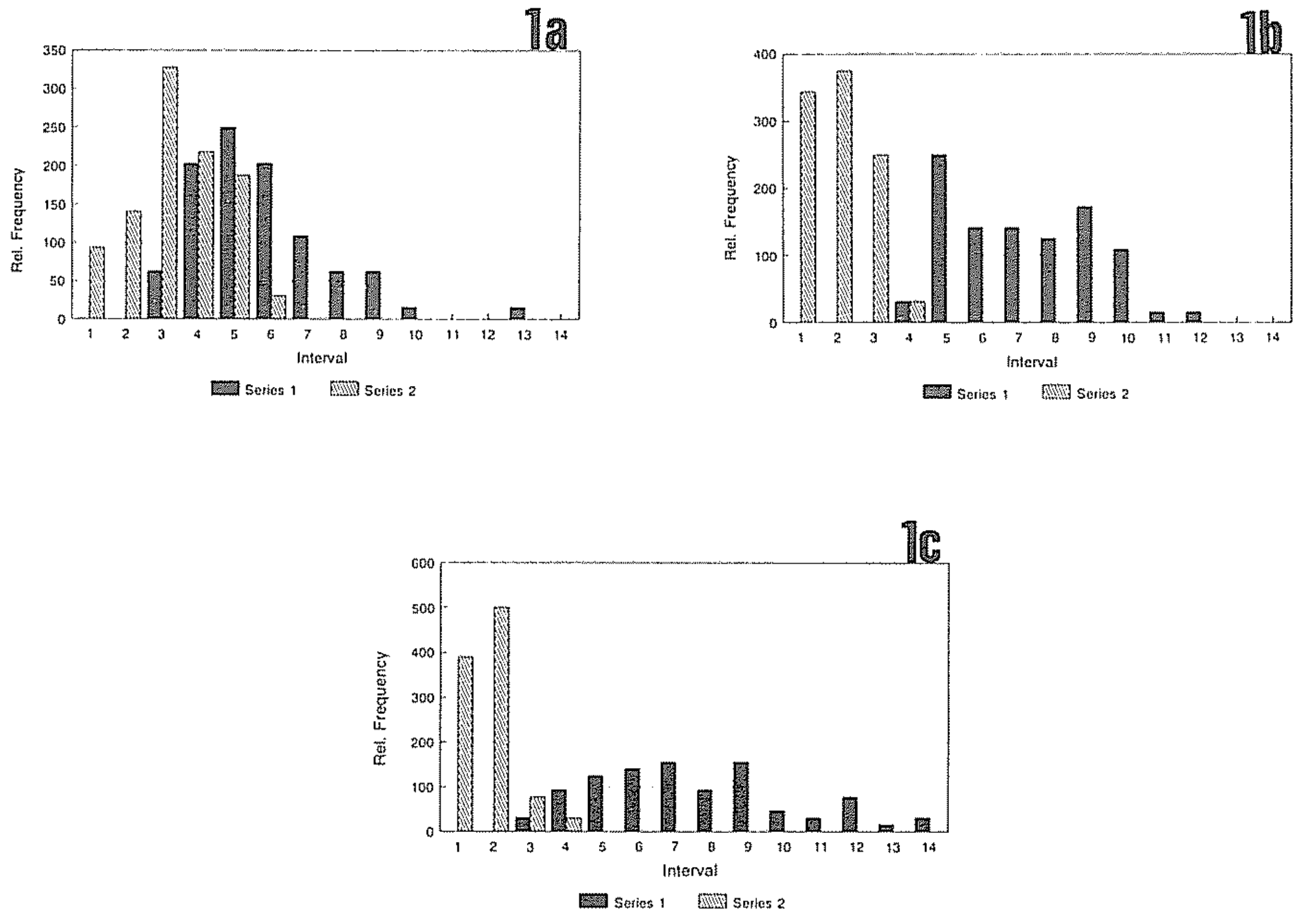

Fig. 1abc. Frequency distribution histograms of *S. oryzae* populations in treated and control wheat kernels, based on 128 insects per experiment in two-choice tests, over 64 replicates. Grain treatment with *A. inulaefolium* extracts at 0.5% (w/w) dosage. See Table 3 for mean percentage values of each interval. 1a: MeOH=methanol extract. 1b: Ac-MC= acetone-methylene chloride 1:1 extract. 1c: Hx= Hexane extract. Series 1= controls. Series 2= treated grain

Table 1. Deterrency Indexes (DI) calculated for methano (MeOH), acetone-methylene chloride (Ac-MC) 1:1, and hexane (Hx) extracts of *A. inulaefolium* in twochoice tests against *S. oryzae* on wheat at 0.5% (W/W) dosage.

Extract	Insects per cup group (%)		DI	Net effect
	Treated	controls		
MeOH	8.58 ± 6.33	14.9 ± 8.12[a]	0.57	weak
Ac-MC	4.41 ± 4.19	20.2 ± 10.3	0.21	strong
Hx	3.75 ± 3.6	21.24 ± 13.4	0.17	very strong

a Mean values not significantly different.

Feeding deterrence by *S. oryzae* did not decrease significantly for at least 8 weeks after treatment with *A. inolaefolium* extracts of low and medium polarity (see Table 2). This fact suggested that those compounds responsible for feeding discouragement are not readily hydrolyzed or oxidized by exposure to the atmosphere, even as thin layers over the kernel surface.

Table 2. Variation of deterrency index (DI) of *A. inulaefolium* extracts to *S. oryzae* adults on wheat with time elapsed since grain treatment.

	Time		
Extract	4 days	4 weeks	8 weeks
Ac MC	0.21	0.23	0.20
Hx	0.17	0.19	0.20

Table 3. Actual value of intervals of figures 1 a, b, c as porcentage of insects.

Interval (num.)	Mean value of interval (%)
1	1.5
2	4.5
3	7.5
4	10.5
5	13.5
6	16.5
7	19.5
8	22.5
9	25.5
10	28.5
11	31.5
12	34.5
13	37.5
14	40.5

A few new compounds have been isolated in the recent past from *A. inulaefolium* (2, 3, 14, 15). However, to our knowledge, none of these substances has ever been evaluated for its anti-insect activity. In addition, the known organic chemistry of the worm bush remains largely unreported, not to mention of its chemodynamics. At this point, our studies are aimed at shedding some light on these matters. Meanwhile, the results presented here point towards *A. inulaefolium* as a source of chemicals with potential for protecting cereal grains in storage.

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RESEÑA DE LIBROS

SOIL COLLOIDS AND THEIR ASSOCIATIONS IN AGREGATES. 1990. M. F. de Boodt, M. H. Hayer , A. Herbillon (Eds.). New York, Plenum Press. 598 p.

Excelente libro de referencia con más información de lo que promete el título. En él los 32 autores de los 21 capítulos presentan revisiones actualizadas sobre cinco de los aspectos fundamentales de la ciencia del suelo desde el punto de vista de las asociaciones coloidales.

La primera parte ocupa más de un tercio del volumen y está dedicada a los coloides inorgánicos y las técnicas modernas para su estudio. Se indica en estos capítulos un resumen crítico de la información y de las bibliografías que hay en el ámbito mundial.

Los coloides orgánicos son el tema de la segunda parte, con una orientación especial hacia los puntos de vista que la escuela inglesa tiene en este campo.

Las interacciones y estructuras que resultan de los contactos arcillas-agua y su influencia en la microestructura de suelos son examinadas en la tercera parte del libro. Esta información no es fácil de obtener y es útil para tomar decisiones sobre el manejo físico sostenible de suelos.

Los agregados en suelos se discuten en la cuarta parte. Ahí se sintetiza gran cantidad de la información de los capítulos previos y se presenta la aplicación de los principios previamente examinados.

La última parte informa sobre acondicionadores de suelos y los agregados que resultan de su aplicación. Analiza, además, las posibilidades y las limitaciones que resultan de su uso. Un breve capítulo de esta sección se dedica al control de la erosión en los trópicos.

Es un volumen de referencia muy útil, tanto por la alta calidad de sus autores, como por las opiniones novedosas que se exponen. Se considera que, para toda biblioteca que apoye los trabajos en suelos, ya sean de aplicación agrícola o de protección del medio ambiente, este texto puede ser de considerable provecho.

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