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Screening for extracts with insect antifeedant properties in native plants from central Argentina

[Evaluación de la actividad antialimentaria de insectos en extractos de plantas nativas del centro de Argentina]

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Abstract: Ethanol extracts obtained from aerial parts of 64 native plants from Central Argentina were tested for their insect antifeedant activity against *Epilachna paenulata* (Coleoptera: Coccinellidae) by choice test. Extracts derived from *Achyrocline satureioides* (Asteraceae), *Baccharis coridifolia* (Asteraceae), *Baccharis flabellata* (Asteraceae), *Ruprechtia apetala* (Polygonaceae) and *Vernonanthura nudiflora* (Asteraceae), showed more than 97% inhibition of the feeding of *E. paenulata* at 100 μg/cm^2. These active extracts were further evaluated for their effectiveness against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). All these extracts except for that derived from A. satureioides, negatively influenced the feeding behavior of *S. frugiperda* at 100 μg/cm^2.

Keywords: Native plants to Argentina; insect antifeedants; *Vernonanthura nudiflora*; *Baccharis flabellata*; *Baccharis coridifolia*; *Ruprechtia apetala*; *Achyrocline satureioides*.

Resumen: Se evaluaron los extractos etanólicos obtenidos de las partes aéreas de 64 plantas de la región Central de Argentina, como antialimentarios de insectos mediante ensayos de elección, contra *Epilachna paenulata* (Coleoptera: Coccinellidae). Los extractos derivados de *Achyrocline satureioides* (Asteraceae), *Baccharis coridifolia* (Asteraceae), *Baccharis flabellata* (Asteraceae), *Ruprechtia apetala* (Polygonaceae) y *Vernonanthura nudiflora* (Asteraceae) mostraron mas de 97% de inhibición de la alimentación de *E. paenulata* a 100 μg/cm2. Estos extractos fueron posteriormente evaluados en su efectividad contra *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Todos ellos, con excepción del extracto de *A. satureioides*, afectaron negativamente el comportamiento alimentario de *S. frugiperda* a 100 μg/cm2.

Palabras clave: Plantas nativas de Argentina; antialimentarios de insectos; *Vernonanthura nudiflora*, *Baccharis flabellata*; *Baccharis coridifolia*; *Ruprechtia apetala*; *Achyrocline satureioides*

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INTRODUCTION

Chemical control of pest insects has given rise to several problems including the pollution of the environment by insecticide residues (de Vlaming *et al.*, 2004), the selection of resistant pest populations (Storer *et al.*, 2012), the negative side effects on beneficial parasites and predators (Cordero *et al.*, 2007) and the deleterious effects on human health (de Jong, 1991, Sulak *et al.*, 2005). It is therefore necessary to design efficient insect control agents presenting reduced environmental consequences (Diaz Napal *et al.*, 2009).

Plants synthesize a wide array of compounds that are involved in plant-insect interactions. Such compounds of secondary metabolism as alkaloids, terpenoids, phenols, flavonoids, steroids, etc. confer some resistance against phytophagous insects (Carpinella *et al.*, 2002; González-Coloma *et al.*, 2002; Urzúa *et al.*, 2010a; Urzúa *et al.*, 2010b; Urzúa *et al.*, 2011; Céspedes *et al.*, 2013). These compounds act as antifeedant and toxic substances as well as growth regulators affecting several physiological processes of insects (González-Coloma *et al.*, 2002).

In this context, we are studying the insecticidal potential of the flora of Central Argentina (Palacios *et al.*, 2007, Palacios *et al.*, 2009), searching for environmentally-friendly pesticides. As a result of this ongoing study, we have identified several plants as sources of efficient pest controllers (Defagó *et al.*, 2006, Diaz Napal *et al.*, 2009, Diaz Napal *et al.*, 2010, Palacios *et al.*, 2010, Defagó *et al.*, 2011). In this report, we examine the insect antifeedant properties of another group of 64 plants from Central Argentina against *Epilachna paenulata*, as part of this program for the selection of highly active plant species for natural insecticide development.

E. paenulata Germar (Coleoptera: Coccinellidae) is a phytophagous insect that affects species from the Cucurbitaceae family, which was extensively used in our laboratory as model insect for insecticide studies (Diaz Napal *et al.*, 2010). The results were then confirmed testing the most active extracts in another pest insect: the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). This insect comprises a pest of economic importance for basic grain production and for many other crops in North, Central and South America (Wyckhuys and O'Neil, 2006). Being the main corn pest in Brazil and in Argentina (Tavares *et al.*, 2009), *S. frugiperda* has developed resistance against many synthetic insecticides (Tomquelski and Martins, 2010) and against transgenic BT-maize (Storer *et al.*, 2012).

MATERIALS and METHODS

Plant material

Plants were collected in the hills of Córdoba, Argentina, from November 2010 to December 2011. Voucher specimens have been deposited in the "Marcelino Sayago" Herbarium of the School of Agricultural Science, Catholic University of Córdoba and were authenticated by the botanist, Gustavo Ruiz. Plants were selected according to their availability and accessibility and emphasing those for which there was no information about their activity or chemical contents. The vegetative material was air-dried at room temperature, crushed and extracted by 48 h maceration with ethanol. Yields of each viscous extract, obtained after solvent removal, were expressed as percentage weight of air-dried plant material.

Insects

E. paenulata and *S. frugiperda* larvae were obtained from a laboratory colony, reared on a natural diet of *Cucurbita maxima* Duch. leaves and artificial diet (Céspedes *et al.*, 2000), respectively. The insects were maintained in a growth chamber at 24 ± 1º C and 70-75% relative humidity, with a photoperiod of 16/8 h light-dark cycle and periodically renewed with field specimens (Diaz Napal *et al.*, 2010).

Test solution preparation

Ten mg of each extract were dissolved in 1 mL of acetone immediately before the corresponding assay.

Feeding choice assay

Two cotyledon leaves from a *C. maxima* seedling were placed in a Petri dish; a glass disk with two 1 cm^2 diameter holes was placed on top. A third-instar *E. paenulata* larva was placed equidistant from both a treated and an untreated leaf disk, with 10 μl of test solution and 10 μl of acetone, solvent control, respectively. The insect was allowed to feed for 24 h. Ten replicates were run for each test. In the case of the assays against *S. frugiperda*, two circular sections of *Lactuca sativa* seedling were placed in a Petri dish. A third-instar *S. frugiperda* larva was placed equidistant from both a treated (with 10 μl of test solution) and an untreated (with 10 μl of acetone, solvent control) leaf disk, and allowed to feed until 50 percent of the food was eaten. The relative amounts (recorded in percentages from 0 to 100) of the treated and untreated substrate areas eaten in each test were estimated visually by dividing the food area into imaginary quarters. An antifeedant index (AI) was calculated as [1- (T/C)] x 100 (Diaz Napal *et al.*, 2009), where T and C represent consumption on treated and untreated food, respectively.

A rank for each of the most active plants was estimated considering an index (I_n) calculated for that plant extracts by the following equation:

$$I_n = y_n \,.\, AI\ (\textit{E. paenulata})\ .\ AI\ (\textit{S. frugiperda})$$

where I_n is the rank of the species n, y_n is the yield extract of plant n base on 100 g of plant material, and the AI is the antifeedant index of extract of plant n at 100 μg/cm^2 for each insect. The plant extract with the highest I_n is the most active one and consequently received the lowest rank number (Rank = 1), with lower I_n receiving consecutive rank numbers (Rank > 1).

Statistical analysis

Results from feeding choice assays were analyzed for statistical significance using the Wilcoxon signed ranks test.

RESULTS and DISCUSSION

The feeding inhibition properties of each extract obtained from the 64 plant species were evaluated in a choice feeding assay against *E. paenulata*. Most of the species were native to our environment although some of them (*Cotoneaster glaucophylla*, *Dipsacus fullonum*, *Marrubium vulgare*, *Melissa officinalis* and *Podranea ricasoliana*) are in fact naturalized. The results of this screening are presented in Table 1.

Table 1

Insect antifeedant activity of extracts from native plants against *Epilachna paenulata*.

Plant species	AI(%)[a]
Achyrocline satureioides (Lam.) DC.	97.14
Achyrocline tomentosa Rusby	27.00
Aloysia citriodora Palau	66.90
Aloysia gratissima (Gill.& Hook.) Tronc.	75.70
Ambrosia elatior L.	-7.20
Amphilophium cynanchoides (DC.) L.G. Lohmann	21.00
Angelphytum aspilioides (Griseb.) H. Rob.	92.26
Argemone subfusiformis G. B. Ownbey	91.80
Baccharis artemisioides Hook. & Arn.	76.60
Baccharis coridifolia DC.	98.20
Baccharis flabellata Hook. & Arn.	97.00
Baccharis salicifolia (Ruiz & Pav.) Pers.	27.00
Bidens pilosa L.	57.14
Capparis atamisquea Kuntze	26.00
Condalia microphylla Cav.	63.50
Cortaderia rudiuscula Stapf	-2.20
Cotoneaster glaucophylla Franch.	4.30
Croton lachnostachyus Baill.	5.00
Dipsacus fullonum L.	8.71
Dolichandra unguis-cati (L.) L.G. Lohmann	5.00
Dysphania ambrosioides (L.) Mosyakin & Clemants	2.00
Eryngium horridum Malme	46.40
Eupatorium hookerianum Griseb.	29.00

Eupatorium viscidum Hook. & Arn.	42.00
Flourensia campestris Griseb.	85.16
Gomphrena pulchella Mart.	77.50
Grindelia pulchella Dunal	96.12
Jarava ichu Ruiz & Pav.	1.50
Jodina rhombifolia (Hook. & Arn.) Reissek	0.00
Ligaria cuneifolia (Ruiz & Pav.) Tiegh.	54.90
Lippia turbinata Griseb.	37.25
Lithrea molleoides (Vell.) Engl.	11.00
Mandevilla laxa (Ruiz & Pav.) Woodson	-83.00
Marrubium vulgare L.	71.30
Melinis repens (Willd.) Zizka	52.50
Melissa officinales L.	42.00
Microliabum candidum (Griseb.) H. Rob.	29.00
Minthostachys verticillata (Griseb.) Epling	56.41
Morrenia brachystephana Griseb.	64.13
Ophryosporus charrua (Griseb.) Hieron.	51.23
Pavonia aurigloba Krapov. & Cristóbal	34.00
Podronea ricasoliana (Tanfani) Sprague	80.00
Porlieria microphylla (Baill.) Descole, O'Donell & Lourteig	75.00
Pterocaulon alopecuroides (Lam.) DC.	95.90
Pyrostegia venusta (Ker Gawl.) Miers	55.00
Ruprechtia apétala Wedd.	97.75
Schizachyrium condensatum (Kunth) Nees	-18.00
Senecio madagascariensis Poir.	67.36
Senecio vira-vira Hieron.	78.00
Senna aphylla (Cav.) H.S. Irwin & Barneby	39.00
Sida rhombifolia L.	87.80
Solanum argentinum Bitter & Lillo	26.00
Solanum palinacanthum Dunal	39.20
Solanum sisymbriifolium Lam.	80.30
Sphaeralcea bonariensis (Cav.) Griseb.	14.00
Spharalcea cordobensis Krapov. (mutant)	49.00
Tagetes minuta L.	85.00
Tripodanthus flagellaris (Cham. & Schltdl.) Tiegh.	67.00
Verbesina encelioides (Cav.) Benth. & Hook. f. ex A. Gray	0.00
Vernonanthura nudiflora (Less.) H. Rob. f. nudiflora	99.00
Vernonia mollissima Hook. & Arn.	47.00
Viguiera tucumanensis (Hook. & Arn.) Griseb. var. tucumanensis	25.40
Wedelia glauca (Ortega) O. Hoffm. ex Hicken	93.80
Zanthoxylum coco Hook.f. & Arn.	83.00
Azadiracthin[b]	100.00

a: data represent the mean of ten replicates. b: reference natural insecticide at 4 μg/cm^2

Those extracts that possess an AI between 50-75% were previously classified as moderate antifeedants (Hassanali and Lwande, 1989). Only those with values greater than 75% were considered to have high values. According to this criteria, 20% of the plant extracts showed moderate antifeedant properties (13/64 = 20%) against *E. paenulata* at 100 μg/cm^2, whereas 13 extracts (13/64 = 20%) had activity between 75% and 95% and only 7 extracts (7/64 = 11%) exhibited an AI superior to 95%. This last group is composed of the extracts derived from *Achyrocline satureioides*, *Baccharis coridifolia*, *Baccharis flabellata*, *Grindelia* *pulchella, Pterocaulon alopecuroides, Ruprechtia apetala,* and *Vernonanthura nudiflora.* The rest of the plant extracts were divided between the non-active (27/64 = 42%) and the phagostimulant extracts with negative AI (4/64 = 6%).

Within the group with the greatest antifeedant activity, the most active ones were also tested against *S. frugiperda*, a polyphagous pest that is possibly more tolerant to allelochemicals due to its ability to detoxify xenobiotics (Ali and Agrawal, 2012). Four extracts also showed high antifeedant activity against *S. frugiperda*, *B. coridifolia* and *V. nudiflora* were the most active (Table 2). The effectiveness of these extracts suggests that they can be used as sources of natural antifeedants to control harmful pests. These results also suggest that these extracts could contain highly active secondary metabolites. If we assume that such activity is due to a single metabolite and taking into account that these compounds do not normally exceed 10% of the extract, the results suggest that active ingredients would exert the antifeedant effect at a concentration approximately of 10 $\mu g/cm^2$. This level of activity make these compounds especially promising antifeedants, as compared with the vast majority of the plant antifeedants which are effective only at doses greater than 20 $\mu g/cm^2$ (Koul, 2005). The well-known antifeedants azadirachtin or meliartenin (Carpinella *et al.*, 2006) have equivalent activities against *E. paenulata* and *S. frugiperda* at 4 and 1 $\mu g/cm^2$, respectively.

Table 2
Most active plant extracts against *Epilachna paenulata* and *Spodoptera frugiperda*

Plant species	Yield (g%)	AI (%) *E. paenulata*	AI (%) *S. frugiperda*	Rank[a]
Achyrocline satureioides	4.5	97.14	33.60	5
Baccharis coridifolia	2.2	98.20	91.22	3
Baccharis flabellata	7.5	97.00	80.36	2
Ruprechtia apetala	1.9	97.75	81.30	4
Vernonanthura nudiflora	7.2	99.00	98.61	1

a: for rank calculation, see Materials and Methods section.

In anticipation of the application of these extracts to control insects in domestic or organic agriculture, we propose to categorize each extract as an insect control product taking into account the antifeedant activity as well as the extract yield of each species. With this aim in mind, we established a rank of the most active extracts, using their yields and their activity on both insects, which are listed in Table 2. *V. nudiflora*, *B. flabellata, B. coridifolia, R. apetala* and *A. satureioides* had ranks from 1 to 5, respectively.

Based on the aforementioned analysis, it is clear that extracts from *V. nudiflora, B. coridifolia* and *B. flabellata* constitute the most promising insect control products.

V. nudiflora (Asteraceae) is a herbaceous plant with blue-purple flowers that is considered a weed in many crops. Neither antifeedant reports nor bioguided studies have been found in the literature for its ethanolic extract. Aerial parts of *V. nudiflora* furnished glaucolides A and B, flavonoids, hirsutinolide and cadinanolides derivatives (Bardón *et al.*, 1992). The antifeedant activity of *V. nudiflora* could be attributed to glaucolide A, which has previously been reported as a feeding deterrent against lepidopteran larvae (Burnett Jr *et al.*, 1974).

B. coridifolia (Asteraceae) is a low-growing woody shrub known as “mio-mio”. This plant has an extensive distribution that includes the north-east, center and north of Argentina. No reference about antifeedant activity was found for this species. As far as we know, this is the first report on this matter, although the plant is toxic to livestock (Rizzo *et al.*, 1997). The toxins of *B. coridifolia* are macrocyclic trichothecenes, including roridin A, D, and E and verrucarin A and J (Rizzo *et al.*, 1997). The trichothecins in this plant are generally considered to be produced by entophytic fungi that live within the plant, although some soil microbes, primarily *Myrothecium roridum* and *M. verrucaria*, may also make them, they are no longer considered to be absorbed by the plants (Busam and Habermehl, 1982) but rather produced within them.

From the aerial parts of *B. flabellata* (Asteraceae), neo-clerodane diterpenoids such as flabeloic acid, 5,10-seco-clerodane diterpenoid derivative (Saad *et al.*, 1988), 2,19;15,16-diepoxyneo-clerodan-3,13,14-trien-18-oic acid, 15,16-epoxy-5,10-seco-clerodan-1(10),2,4,13(16),14-pentaen-18, 19-olide and 15,16-epoxy-neo-clerodan-1,3,13(16),14-tetraen-18,19-olide were isolated (Juan Hikawczuk *et al.*, 2002). Some related neo-clerodanes isolated from different species have been reported as effective antifeedant compounds (Muñoz *et al.*, 1997, Bremner *et al.*, 1998, Cifuente *et al.*, 2002) although this plant was not previously reported as antifeedant against insects.

R. apetala (Polygonaceae), known as "manzanito del campo", is a small tree with pale pink flowers. Not many activities have been reported for the ethanolic extract, however a potent anticholinesterase inhibitory effect has been found (Carpinella *et al.*, 2010). This property could be connected with the anti-insect activity observed in this screening.

A. satureiodes (Asteraceae) (common name "marcela") is a low-growing shrub with a woody base or suffrutex with grayish leaves. Infusions from this plant are used today in southern Brazil, Paraguay and Argentina for the treatment of different illnesses. Bactericidal properties of this species have been demonstrated (Joray *et al.*, 2011a, Joray *et al.*, 2011b), however, no information about its antifeedant properties has been reported.

In conclusion, the plants with good ranking, especially *V. nudiflora*, *B. coridifolia* and *B. flabellata* may be considered as potential insect control materials for different agricultural practices, due to their potencies and yields. It could also be highly advisable to study both the responsible compounds for the antifeedant effect and their mechanisms of action.

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