IN VITRO NEMATICIDAL ACTIVITY OF AQUEOUS PLANT EXTRACTS ON CHILEAN POPULATIONS OF *XIPHINEMA AMERICANUM SENSU LATO*

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ABSTRACT

Insunza, V., E. Aballay, and J. Macaya. 2001. *In vitro* nematicidal activity of aqueous plant extracts on Chilean populations of *Xiphinema americanum sensu lato*. Nematropica 31:47-54.

In a search for alternatives to chemicals in nematode management, the nematicidal activity of 30 plant species (9 native and 21 naturalized in Chile) was tested on a Chilean population of *Xiphinema americanum sensu lato*, an economically important pathogen in grapevine. Aqueous extracts were tested in a standard concentration (S) at 1:4 W/V of fresh plant material, or 1:20 W/V of air-dried plant parts; and 21 plants, were also tested at 25% of S. Distilled water was used as the control. Nematicidal activity was evaluated by nematode immobility after 24-48 h of immersion in the extracts, followed by 24 h in distilled water. At the standard concentration, all the extracts showed nematicidal or nematostatic activity after 24 h of exposure, but this effect decreased in the dilute treatment of 33% of the extracts, mostly top extracts. Nematode immobility was observed at 25% of S, after 24 h, with plant extracts in 18 out of 21 plant species including: *Tagetes erecta*, *T. patula nana* and *Zinnia elegans* (tops and roots); *Asparagus officinalis*, *Brassica campestris*, *Calendula officinalis*, *Melissa officinalis*, *Plantago major*, *Ruta graveolens* (roots); *Thymus serpyllum* (leaves); and the native Chilean plants *Aristotelia chilensis* (leaves); *Cestrum parqui* (bark); *Oenothera affinis*, *Oxalis rosea*, *Stachys albicaulis* (whole plants); *Quillaja saponaria* (leaves and flowers); and *Vestia lycioides* (top). After 48 h of exposure, nematode immobility was observed at 25% of S in *Chenopodium ambrosioides* (top and root) and *M. officinalis* (top). These results confirm earlier reports on nematicidal properties of the 30 plant species tested. Some of these plants could have practical application in the management of *Xiphinema* in vineyards.

Key words: antagonistic plants, Chile, grapevine, native plants, nematicidal plants, nematode management, plant extracts, *Xiphinema americanum sensu lato*.

RESUMEN

Insunza, V., E. Aballay, y J. Macaya. 2001. Actividad nematicida *in vitro* de extractos acuosos de plantas en poblaciones chilenas de *Xiphinema americanum sensu lato*. Nematrópica 31:47-54.

En la búsqueda de alternativas a los productos químicos en el manejo de nematodos, se evalúo la actividad nematicida de 30 especies de plantas (9 nativas y 21 naturalizadas en Chile), en una población chilena de *Xiphinema americanum sensu lato*, un patógeno económicamente importante en la vid. Se probaron extractos acuosos de una concentración standard (S) de 1:4 P/V en material vegetal fresco, o de 1:20 P/V en material vegetal secado al aire; en 20 de esas plantas, se probó, además, la dilución del 25% de S. Como testigo se usó agua destilada. La actividad nematicida se evaluó según la movilidad de los nematodos después de 24-48 horas de exposición a los extractos, seguido de 24 horas en agua destilada. En la concentración standard, todos los extractos resultaron ser nematicidas o nematostáticos después de 24 horas de exposición, pero dicho efecto decreció en los tratamientos del 25% de S en el 33% de los extractos, en la mayoría extractos de las partes aéreas. La inmovilidad de los nematodos se observó hasta el 25% de S, después de 24 horas, con extractos de 18 de las 20 plantas, incluyendo: *Tagetes erecta*, *T.patula nana* y *Zinnia elegans* (partes aéreas y raíces); *Asparagus officinalis*, *Brassica campestris*, *Calendula officinalis*, *Melissa officinalis*, *Plantago major*, *Ruta graveolens* (raíces); *Thymus serpyllum* (hojas); y de las plantas nativas chilenas *Aristotelia chilensis* (hojas); *Cestrum parqui*

(corteza); *Oenothera affinis, Oxalis rosea, Stachys albicaulis* (toda la planta); *Quillaja saponaria* (hojas y flores); *Vestia lycioides* (partes aéreas). Después de 48 horas de exposición, la inmobilidad de los nematodos se observó al 25% de S, en *Chenopodium ambrosioides* (parte aérea y raíz) y en *M. officinalis* (parte aérea). Estos resultados confirman los obtenidos anteriormente sobre las propiedades nematicidas de las 30 plantas evaluadas. Algunas de estas plantas podrían tener aplicación práctica en el control de *Xiphinema* en vides.

Palabras claves: Chile, extractos vegetales, manejo de nematodos, plantas antagónicas, plantas nativas, plantas nematicidas, vid, *Xiphinema americanum sensu lato*.

INTRODUCTION

Several species of *Xiphinema* are serious pathogens on table and wine grape (*Vitis vinifera* L.) in Chile. The most damaging of these are members of the *Xiphinema americanum*-group and *X. index* Thorne and Allen, which are widespread in all the viticultural areas of the country. These nematodes cause problems as root pathogens and as virus vectors (Aballay *et al.*, 1998; Valenzuela *et al.*, 1992). Nematode control is based on nematicides, which present potential risks to non-target organisms and the environment. In the search for more benign and acceptable alternatives to chemicals, possibilities are being investigated to exploit nematode-antagonistic plants for *Xiphinema* control. The work started with the screening of selected plant species for nematicidal activity against, a.o. *X. americanum sensu lato*. *X. americanum* Cobb was reported in Chile by González and Valenzuela (1968) and by Allen *et al.,* (1971), but was re-identified by Lamberti *et al.*, (1988) as a complex of species which comprises *X. californicum, X. floridae, X. inaequale, X. pachtaicum, X. peruvianum* and *X. utahense*. Lamberti *et al.* (1988) concluded that *X. americanum sensu stricto* does not occur in Chile. However, the taxonomy and identification of these populations continues to be uncertain. Therefore, in our work we refer to these species as *X. americanum sensu lato*.

Some information about *in vitro* nematicidal activity of plant extracts on *X. americanum sensu stricto* were given by Halbrendt and Jing (1994); and in field studies, by Good *et al.* (1965), Halbrendt (1991), and on Chilean populations of *X. americanum s. l.* by Insunza and Aballay (1995). In previous *in vitro* tests in Chile, using other plant parasitic nematodes, 50 of 80 plants of the Chilean flora appeared to possess nematicidal activity (Insunza and Eriksson, 1989; Insunza, 1994).

The objective of this work was to evaluate the *in vitro* nematicidal activity of selected plants against Chilean populations of *Xiphinema americanum sensu lato* Cobb. Partial results of this work were presented at the XXX Annual Meeting of ONTA (Insunza *et al.*, 1998).

MATERIALS AND METHODS

The work was conducted as a laboratory study at the Departamento de Sanidad Vegetal, Universidad de Chile, Santiago de Chile. Extracts were derived from 30 plant species (8 native and 22 naturally occurring in Chile), belonging to 14 botanical families (Table 1). The plants were previously reported to be nematicidal. The plants were obtained from various sources including farms, glasshouses, wild vegetation, and herbal shops. Aqueous extracts were prepared from either fresh or air-dried plant material, with aerial parts and roots prepared separately. The plant material was finely chopped and soaked in distilled water, at the rates of 1:4 W/V for fresh material, and 1:20 W/V, after infusion, for air-dried material. After 1 day, the

Table 1. Toxicity of aqueous extracts of selected plant species to *Xiphinema americanum sensu lato*.[v]

Plant family/species[w]	Plant part and type of extract[x]	Percentage of immobile nematodes	
		Concentration	
		Standard (S)	25% of S
Asteraceae			
Calendula officinalis L.	leaf, flower, a	100.0*[y]	47.1[z]
	root, a	100.0*	92.4*
Cosmos bipinnatus Cav.	leaf, a	94.7*	3.7[z]
	root, a	64.3*	51.7[z]
Chamomilla recutita (L.) Rausch.	flower, b	100.0*	48.3[z]
Gaillardia aristata Pursh.	leaf, flower, a	95.8*	21.6[z]
	root, a	100.0*	49.7#
Matricaria discoidea DC.	leaf, flower, a	100.0*	—
	root, a	100.0*	—
Tagetes erecta L.	leaf, a	100.0*	89.3*
	root, a	100.0*	91.7*
Tagetes patula nana L.	leaf, flower, a	100.0*	66.9*
	root, a	100.0*	81.5*
Zinnia elegans Jacq.	leaf, a	100.0*	100.0*
	root, a	89.8*[z]	100.0*
Brassicaceae			
Brassica campestris L.	leaf, flower, a	100.0*	3.8[z]
	root, a	100.0*	96.0*
Raphanus sativus L.	root, a	100.0*	—
Chenopodiaceae			
Chenopodium ambrosioides L.[w]	top, a	100.0*	88.2*#
	root, a	100.0*	70.9*#
Elaeocarpaceae			
Aristotelia chilensis (Mol.) Stuntz[w]	leaf, a	100.0*	96.7*
Gentianaceae			
Centaurium cachanlahuen (Mol.) Rob.[w]	whole plant, a	100.0*	—
	whole plant, b	100.0*	—
Lamiaceae			
Melissa officinalis L.	leaf, a	100.0*	75.3*#
	root, a	100.0*	84.0*
Mentha citrata Ehrh.	leaf, flower, a	100.0*	28.5
	root, a	100.0*	—
Ocimun basilicum L.	leaf, a	100.0*	—
Stachys albicaulis Lindl.[w]	whole plant, a	100.0*	81.6*
Thymus serpyllum L.	leaf, flower, a	100.0*	100.0*
Liliaceae			
Asparagus officinalis L.	leaf, a	100.0*	13.8[z]
	root, a	100.0*	100.0*

Table 1. (Continued) Toxicity of aqueous extracts of selected plant species to *Xiphinema americanum sensu lato*.[v]

Plant family/species[w]	Plant part and type of extract[x]	Percentage of immobile nematodes	
		Concentration	
		Standard (S)	25% of S
Onagraceae			
Oenothera affinis Cabess.[w]	whole plant, a	100.0*	86.3*
Oxalidaceae			
Oxalis rosea Jacq.[w]	leaf, flower, a	100.0*	—
	whole plant, b	100.0*	—
Papilionaceae			
Galega officinalis L.	leaf, flower, a	100.0*	—
	root, a	100.0*	—
Plantaginaceae			
Plantago major L.	leaf, flower, a	100.0*	30.4[z]
	root, a	100.0*	85.4*
Portulacaceae			
Portulaca oleracea L.	whole plant, a	100.0*	—
Rosaceae			
Quillaja saponaria Mol.[w]	leaf, flower, a	100.0*	100.0*
Rutaceae			
Ruta graveolens L.	leaf, flower, a	100.0*	—
	root, a	100.0*	100.0*
Solanaceae			
Cestrum parqui L' Herit.[w]	leaf, flower, a	100.0*	35.0[z]
	bark, a	100.0*	53.6*[z]
Datura stramonium L.	leaf, flower, a	100.0*	—
	root, a	100.0*	—
Vestia lycioides Willd.[w]	leaf, b	100.0*	97.3*
Umbeliferae			
Coriandrum sativum L.	top, a	88.0*	—
	root, a	100.0*	—
Control (distilled water)	plant tops	6.3	5.9
	roots	6.6	5.9
Fisher's LSD ($P < 0.05$)	plant tops	27.5	44.4
	roots	36.4	47.2

[v]Time of exposure to the extracts: 24 h. Marked #: 48 h.
[w]Native plant species.
[x]Type of extract: a, solution from fresh plant material; b, infusion of air-dried plant material.
[y]*Significantly different from the control at $P < 0.05$.
[z]Nematostatic effect.

solution and plant material were processed in an electric blender for 2 minutes. The mixture was then squeezed through a cotton cloth and the liquid was filtered through filter paper. This filtrate (1:4 W/V or 1:20 W/V) was designated as standard (S), and it was prepared from all plants. Extracts from 21 of these plants were diluted in distilled water to provide extracts of 25% of S (Table 1). All extracts were kept at -20°C until used.

The bioassays used populations of *Xiphinema americanum sensu lato* extracted from soil collected in vineyards of the Region Metropolitana area, Central Chile (33° South). Nematodes were extracted by Cobb's sieving and decanting method (Southey, 1985). The nematodes caught on the 250 µm sieve were further freed from debris by a sieve with two layers of nylon gauze for *ca.* 24 h. Approximately 30 adults and juveniles of *X. americanum s.l.* were placed in glass tubes with 2 ml of the test solution and kept in the dark at 20 ± 1°C.

After 24 h, the nematodes were observed with a dissecting microscope. Nematodes were considered immobile if they failed to respond to stimulation with a needle. If nematodes continued to move, they were exposed to the extracts for an additional 24 h. Immobile nematodes were resuspended for 24 h in distilled water. In the case that immobile nematodes regained mobility after this time, the effect was considered as nematostatic. The treatments were replicated 3-5 times, and untreated controls using distilled water were included.

Nematode counts were transformed to log (x + 1) prior to analysis of variance, due to slight variations in the numbers of nematodes, and treatment means were compared by Fisher's LSD (least significant differences) pairwise procedure at $P < 0.05$. However, non-transformed data is presented in Table 1, as percentage of immobile nematodes.

RESULTS AND DISCUSSION

The nematicidal activity of the plant extracts tested was evaluated according to nematode immobility instead of mortality (Alphey *et al.*, 1988; Yamashita and Viglierchio, 1987). Nematodes remained inactive in the toxic environment, but they were not necessarily dead. Nematodes may regain mobility on transfer to water following exposure to plant extracts within some h, or even within days or weeks (Bunt, 1987; Jatala *et al.*, 1995) and we limited this time to 24 h.

Extracts of the tops and roots of *Tagetes erecta*, *T. patula nana*, *Zinnia elegans* were nematicidal or nematostatic to *X. americanum s. l.* after 24 h at the standard and 25% of S concentrations ($P < 0.05$) (Table 1). A similar effect was observed at both concentrations with extracts of the tops of *Aristotelia chilensis*, *Quillaja saponaria*, *Thymus serpyllum* and *Vestia lycioides*; of the roots of *Melissa officinalis* and *Ruta graveolens*; of the bark of *Cestrum parqui;* and the whole plant extracts of *Oenothera affinis*, *Oxalis rosea* and *Stachys albicaulis.*

Extracts of the tops and roots of *Chenopodium ambrosioides* and of the tops of *M. officinalis* were effective after 24 h at the standard concentration, and after 48 h at 25% of S. Root extracts of *Asparagus officinalis*, *Brassica campestris*, *Calendula officinalis*, and *Plantago major* were effective at the S and 25% of S concentrations after 24 h, but their top extracts were effective only at S.

Top and root extracts of *Cosmos bipinnatus, Gaillardia aristata* and tops of *C. parqui*, *Chamomilla recutita,* and *Mentha citrata* affected nematode mobility at the standard concentration, but not at 25%.

After 24 h at the standard concentration, the tops and roots of *Coriandrum sativum*, *Datura stramonium*, *Galega officinalis*, *Matricaria discoidea*, and *R. graveolens*, demonstrated high nematotoxic activity (88-

100% nematode immobility). In addition, strong nematicidal activity at the standard concentration was shown by tops of *Ocimun basilicum* and *Oxalis rosea*, the root extracts of *Mentha citrata* and *Raphanus sativus*, and whole plants extracts of *Centaurium cachanlahuen*, *O. rosea* and *Portulaca oleracea*.

In summary, at the standard concentration all the extracts showed nematicidal or nematostatic activity after 24 h. However, at dilute concentration, extracts from *ca.* 33% of the treatments showed reduced activity. Additional testing with solutions at concentrations lower than 25% of S may be required. Nevertheless, in bioassays with *Xiphinema index*, the concentration of 12.5% of S indicated loss of the nematicidal activity in most of the extracts (Insunza *et al.*, 1998).

These data support previous reports of nematicidal activity by some of these plants against other plant parasitic nematodes, i.e *Ditylenchus dipsaci*, *Heterodera schachtii* and *Pratylenchus penetrans* (Insunza, 1994; Insunza and Eriksson, 1989). Nematicidal activity of plants against *X. americanum s.s.* has only been reported in a few studies. Halbrendt and Jing (1994) reported toxicity to the nematode in bioassays of extracts derived from several *Brassica* cultivars (*B. napus*, *B. campestris*, *B. kaber*). Other plant species were reported to suppress populations of *X. americanum s.s.* in the field, including *Crotalaria spectabilis*, *Indigofera hirsuta* and *Tagetes minuta* (Good *et al.*, 1965); canola, *Brassica napus* (Halbrendt, 1991); and some species of *Tagetes* (*T. erecta*, *T. patula*, *T. patula nana*), *Asparagus officinalis*, *Melissa officinalis* and *Mirabilis jalapa* (Insunza and Aballay, 1995). Several of these plants were tested in our bioassays with a similar effect on *X. americanum s.l.*

The nematicidal activity shown by the plant extracts may be a consequence of various factors, as e.g. presence of water-soluble toxic compounds, microbial metabolites, high osmotic strength, alterations of pH, or simply removal of oxygen from the solutions. The plant compounds reported to have nematicidal activity include a wide variety of phytochemical structures including polythienyls, acetylenes, alkaloids, carboxylic acids, fatty acids and derivatives, phenolics and terpenoids (Chitwood, 1992).

In the present work, the native plants were found to be nematicidal or nematostatic to *X. americanum*, confirming previous reports (Insunza, 1994). These included the perennials *Aristotelia chilensis*, *Cestrum parqui*, *Quillaja saponaria* and *Vestia lycioides*, and the herbs *Centaurium cachanlahuen*, *Chenopodium ambrosioides*, *Oenothera affinis*, *Oxalis rosea* and *Stachys albicaulis*. All of these plants have been used in traditional medicine in Chile as anthelminthics (Montes and Wilkomirsky, 1985; Niemeyer, 1995). Therefore, the present work appears to confirm indigenous knowledge.

Aristotelia chilensis, *Cestrum parqui*, and *Quillaja saponaria* are relatively common in Central Chile. Several indolic alkaloids with antitumoral and antimicrobial activity, such as aristotelina, aristotelone, aristotelinine, aristona have been isolated from leaves of *A. chilensis* (Montes and Wilkomirsky, 1985).

Cestrum parqui is poisonous to animals, although its leaves and cortex have been safely used as medicinal treatments. Its toxicity is probably due to kaurene glycosides, carboxyparquin and parquin (Pearce *et al.*, 1992). This plant also contains alkaloids such as parquina, solanine, solasodine, digitogenine, and steroid sapogenins.

Quillaja saponaria is an endemic tree rich in saponines, which are found mainly in the cortex but also in its leaves and flowers. The tree also contains glycosides, polyphenols and tannins. It has been traditionally used as an insect repellent and detergent. Extracts of *Q. saponaria* have recently been

investigated as nematicides, with promising results, against species of *Xiphinema*, *Meloidogyn*e and others (Saínz, 1999).

Vestia lycioides is an endemic Solanaceae from South Chile, but not very common. The species contains triterpenes, coumarin, steroidal-sapogenins, flavonoids, glycosides, and an indolic alkaloid (guevillina), similar to solanine (Montes and Wilkomirsky, 1985). Solanine and other steroid-glycoalkaloids isolated from Solanaceae have shown nematicidal and fungicidal activity (Allen and Feldmesser, 1971).

Chenopodium ambrosioides is an annual or perennial herb, which is known for its antihelminthic properties. It also contains substances which are antiviral, antibiotic and insecticidal. The most active component is an oil, consisting of ascaridol, a terpenoid peroxide (Quarles, 1992).

Centaurium cachanlahuen and *Oenothera affinis* are not very abundant herbs. Compounds extracted from *C. cachanlahuen* include several xanthones; erytroncentaurine, a glycoside; diverse acids, tanins, and mucilages (Montes and Wilkomirsky, 1985).

Oxalis rosea, is a herb rich in oxalic, ascorbic, pyruvic and glioxalic acids (Montes and Wilkomirsky, 1985). Oxalic acid is reported to be nematoxic to the mycophagous nematode *Aphelenchus avenae* (Mankau, 1969).

The nematicidal activity of the plant extracts used in this study may serve as leads for development of plant-based agrochemicals. Nevertheless, it is not known whether the nematicidal activity was due to a single compound or to a complex of compounds, or other mechanisms and/or interactions. In the case of native plant species, future research should include preservation of germplasm. However, the main objective of this study was to find plants with nematicidal properties that could be used in nematode management, as cover crops or green manures in vineyards. For example, a crop grown to be incorporated into the soil as green manure may correspond to *ca.* 25 t/ha of biomass, a rate which may be comparable to the 25% of the standard solution used in our tests, if the green manure is mixed in the upper 5 cm of soil to favor decomposition. Ongoing research is evaluating methods to use some of these materials as alternative methods for *Xiphinema* control.

ACKNOWLEDGEMENTS

This study was supported by the Fondo Nacional de Desarrollo Cientifico y Tecnológico (FONDECYT), Chile.

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Received: *3.VII.2000*

Recibido:

Accepted for publication: *8.XII.2000*

Aceptado para publicación: