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NEMATICIDAL ACTIVITY OF AQUEOUS PLANT EXTRACTS ON *XIPHINEMA INDEX*

by
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Summary. Nematicidal activity of aqueous extracts prepared from 30 plant species was screened on a Chilean population of *Xiphinema index*. The extracts were tested at a standard concentration of 1:4 W/V in fresh plant material, or 1:20 W/V in air-dried plant parts and at 25% and 12.5% of standard. Nematicidal activity was evaluated by nematode immobility after 24-48 hours immersion in the extracts, followed by 24 hrs in distilled water for eventual recovery of movement. Most of the plant extracts showed nematicidal or nematostatic activity, but this effect decreased in the lowest concentration. Nematode immobility was observed at 12.5% of standard with extracts from *Chenopodium ambrosioides* (tops and roots); *Cosmos bipinnatus* (tops); *Chamomilla recutita* (flowers); *Oxalis rosea* (whole plant); *Vestia lycioides* (leaves); and *Zinnia elegans* (roots).

Nematodes are among the most serious problems in grapevine (*Vitis spp.*), an economically important crop in Chile, both as table and as wine grape. The most damaging pathogens are dagger nematodes, such as *Xiphinema index*, and several species of the *Xiphinema americanum* - group, which act as root pathogens as well as vectors of viruses.

Nematode control is still heavily dependent on chemical nematicides, with all their adverse side effects and risks to humans and the environment. Consequently, in order to find more acceptable and ecocompatible control methods, the possibilities of using nematode antagonistic plants in *Xiphinema* control was investigated.

Some information about *in vitro* nematicidal activity of plant extracts on *X. index* were given by Dechet (1991), Sasanelli and Catalano (1991) and Sasanelli (1992). In previous tests in Chile, using other plant parasitic nematodes, 34 of 75 plants of the Chilean flora appeared to possess nematicidal activity (Insunza and Eriksson, 1989; Insunza, 1994).

The present work started with screening of some selected plant species already known for their nematicidal properties. The objective was to evaluate the *in vitro* nematicidal activity of some selected plants against Chilean populations of *X. index*.

Materials and methods

The experiments were conducted in the laboratory. The tests included 30 selected plant species (9 native and 21 naturalized in Chile), belonging to 14 botanical families (Table I). Plant material was obtained from plants grown

TABLE I - *Toxicity of aqueous extracts of some plant species on* Xiphinema index *after 24 and 48 hours of exposure.*

Plant family/species	Plant part(s) (a)	Percentage of immobile nematodes		
		Time of exposure		
		24 hrs	24 hrs	48 hrs
		Concentration		
		Standard solution (S)	25% of S	12.5% of S
Asteraceae				
Calendula officinalis L.	leaf, flower	97.6*	64.1*	0.0
	root	94.9* (c)	88.2*	0.0
Cosmos bipinnatus Cav.	leaf	100.0*	100.0*	34.7*
	root	16.2 (c)	24.0	0.0
Chamomilla recutita (L.) Rausch.	flower (a)	100.0*	100.0*	21.3*
Gaillardia aristata Pursh.	leaf, flower	100.0*	11.9	6.2
	root	17.5 (c)	22.2	3.4
Matricaria discoidea DC.	leaf, flower	100.0*	–	–
	root	100.0*	–	–
Tagetes erecta L.	leaf	100.0*	84.7*	2.4
	root	46.9 (c)	91.7*	1.2
Tagetes patula nana L.	leaf, flower	72.5* (c)	66.9*	0.0
	root	41.7	61.6*	0.0
Zinnia elegans Jacq.	leaf	78.2*	100.0*	–
	root	100.0*	90.6*	71.1*
Brassicaceae				
Brassica campestris L.	leaf	100.0*	30.0	2.4
	flower	95.4*	–	–
	root	94.2*	25.9	0.0
Brassica juncea (L.) Czern.	leaf, flower	100.0*	–	–
	root	100.0*	–	–
Brassica napus L.	leaf, flower	100.0*	–	–
	root	100.0*	–	–
Raphanus sativus L.	leaf, flower	100.0*	–	–
	root	45.0 (c)	–	-
Raphanus raphanistrum L.	leaf	21.8	–	–
	root	61.6* (c)	–	–
Chenopodiaceae				
Chenopodium ambrosioides L. (b)	top	76.6* (c)	82.3*	25.1*
	root	71.7* (c)	83.3*	10.3*
Elaeocarpaceae				
Aristotelia chilensis (Mol.) Stuntz. (b)	leaf	100.0*	93.3*	6.2

TABLE I - *(cont.)*

Plant family / species	Plant part(s) (a)	Percentage of immobile nematodes		
		Time of exposure		
		24 hrs	24 hrs	48 hrs
		Concentration		
		Standard solution (S)	25% of S	12.5% of S
Gentianaceae				
Centaurium cachanlahuen (Mol.) Rob. (b)	whole plant (a)	100.0*	–	–
Lamiaceae				
Melissa officinalis L.	leaf	100.0*	43.8	5.2
	root	11.0		0.0
Mentha citrata Ehrh.	leaf, flower	100.0*	–	–
	root	73.3*	–	–
Stachys albicaulis Lindl. (b)	whole plant	57.6* (c)	74.1*	2.5
Thymus serpyllum L.	leaf, flower	90.2* (c)	100.0*	0.0
	root	100.0*	–	–
Liliaceae				
Asparagus officinalis L.	leaf	92.2* (c)	59.0*	1.2
	root	98.3* (c)	97.5*	0.0
Onagraceae				
Oenothera affinis Cabess. (b)	whole plant	100.0*	76.8*	0.0
Oxalidaceae				
Oxalis rosea Jacq. (b)	leaf, flower	100.0*	100.0*	2.5
	whole plant (a)	100.0*	86.7*	100.0*
Papilionaceae				
Galega officinalis L.	leaf, flower	100.0*	–	–
	root	91.7*	–	–
Plantaginaceae				
Plantago major L.	leaf, flower	100.0*	33.1	2.2
	root	87.5*	88.2*	1.3
Rosaceae				
Quillaja saponaria Mol. (b)	leaf, flower	100.0*	92.9*	–
Rutaceae				
Ruta graveolens L.	leaf, flower	100.0*	84.2*	0.0
	root	100.0*	97.5*	0.0
Solanaceae				
Cestrum parqui L'Herit. (b)	leaf, flower	100.0*	100.0*	11.5
	bark	100.0*	100.0*	0.0

TABLE I - *(cont.)*

Plant family / species	Plant part(s) (a)	Percentage of immobile nematodes		
		Time of exposure		
		24 hrs	24 hrs	48 hrs
		Concentration		
		Standard solution (S)	25% of S	12.5% of S
Datura stramonium L.	leaf, flower	100.0*	–	–
	root	100.0*	–	–
Vestia lycioides Willd. (b)	leaf (a)	100.0*	97.4*	38.5*
Control (distilled water)**	top and whole plant	6.7	9.1	1.2
	root	4.0	4.5	1.2

(a): most plant parts were used as solution (i.e. aqueous extracts of fresh plant parts), except those marked (a) which were used as infusion (i.e. aqueous extracts of air-dried plant parts); (b): native plant species; (c): nematostasis; * significantly different from the control determined by Fisher's LSD ($P<0.05$); ** control in the ANOVA.

in farms, glasshouse, wild vegetation, and from herbal shops (dried plant material).

Aqueous extracts were prepared either as solutions from fresh plant material, or as infusions from air-dried plant parts, aerial parts and roots kept apart. Plant material, finely chopped, was soaked in cold distilled water, at the rates of 1:4 W/V, or of 1:20 W/V, by infusion of air-dried plant parts, and placed in open containers at room temperature (*ca.* 20 °C). After 24 hrs, time considered sufficient to solubilize the active principles, the plant material was homogenised in an electric blender and filtered through nylon gauze to remove débris. The filtrate (1:4 W/V or 1:20 W/V) was designated as standard (S), and it was prepared from all the plants. Besides, extracts from 22 plants were diluted in distilled water to provide extracts of 25% of S and 12.5% of S, respectively. All extracts were kept at -20 °C until used.

Bioassays were undertaken with populations of *Xiphinema index* Thorne *et* Allen extracted from field soils collected in vineyards from Copiapó (III Region) and Region Metropolitana area, in North and Central Chile respectively. Nematodes were extracted from soil by the Cobb's sieving and decanting method. The suspension of nematodes caught on the 250 µm sieve was further freed from débris by a sieve with a layer of nylon gauze (of 80 meshes) for *ca.* 24 hrs. In the tests, batches of about 30 adults and juveniles of *X. index*, were placed in glass tubes with 2 ml of the test solution. The glass tubes were covered with aluminium foil in order to eliminate light, and kept in the dark at 20 °C ± 1 °C.

After 24 hrs exposure, the nematodes were observed with a stereoscopic microscope. Nematodes were considered immobile if they failed to respond to stimulation with a needle. When nematodes continued to move, they were exposed to the extracts for an additional 24 hrs. Immobile nematodes were resuspended for 24 hrs in distilled water. In the case that paralyzed 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.

The data presented in percentage of immobile nematodes were normalized using arcsin transformation, and subjected to analisis of variance (ANOVA). Treatment means were compared by Fisher's LSD (least significant differences) pairwise procedure at $P<0.05$ (Table I).

Results and discussion

Extracts of either top or root of *Chenopodium ambrosioides*, and whole plant extract of *Oxalis rosea* have clearly shown nematicidal or nematostatic effect on *X. index* at all three concentrations, demonstrated by nematode immobility at $P<0.05$ (Table I).

Asparagus officinalis, *Calendula officinalis* and *Ruta graveolens* top and root extracts were effective only at the S and 25% of S concentrations. *Brassica juncea*, *B. napus*, *Datura stramonium*, *Galega officinalis*, *Matricaria discoidea*, *Mentha citrata* top and root extracts affected nematode mobility at the standard concentration, but no other concentrations were tested with these plants. *Brassica campestris* top and root extracts were effective only at the standard concentration.

Top extracts of *Chamomilla recutita*, *Cosmos bipinnatus*, *Vestia lycioides* and *Zinnia elegans* demonstrated significant nematicidal or nematostatic effect at all concentrations tested. Tops of *Aristotelia chilensis*, *Cestrum parqui*, *Oxalis rosea* (fresh plant top), *Quillaja saponaria*, *Stachys albicaulis*, *Tagetes erecta*, *Tagetes patula nana*, *Thymus serpyllum* and *Oenothera affinis* (whole plant), were effective only at the S and 25% of S concentrations. At the standard concentration, top extracts of *Gaillardia aristata*, *Plantago major* and *Raphanus sativus* were effective.

The root extract of *Z. elegans* was effective at all three concentrations. *P. major* root extract was effective at the S and 25% of S concentrations and *Raphanus raphanistrum* and *T. serpyllum* root extracts, tested only at the standard concentration, showed significant nematicidal activity.

Finally, root extracts of *C. bipinnatus*, *G. aristata*, *M. officinalis* at all concentrations, and of *R. sativus*, *T. erecta* and *T. patula nana* at the standard concentration, were without effect. However, at 25% of S, root extracts of the *Tagetes* species tested have shown nematicidal activity. These results appear as controversial, and a possible explanation is that photoactivation of the *Tagetes* extracts occurred in the tests at 25% of S, but not at the standard, and probably by an experimental error. Nematicidal compounds found in roots of *Tagetes*, as alfaterthienyl and analogues needs photoactivation, which is necessary for nematicidal activity (Gommers and Bakker, 1988).

The high number of plants with nematicidal activity against *X. index* confirms results obtained with some of these plants on other plant parasitic nematodes, i.e. *Ditylenchus dipsaci*, *Heterodera schachtii* and *Pratylenchus penetrans* (Insunza and Eriksson, 1989; Insunza, 1994). Nematicidal activity of plants on *X. index* has been reported in a few studies only. Dechet (1991) reported that, in screening tests, several plants showed toxicity to *X. index*, between them e.g. *Asparagus officinalis*, *Calendula officinalis*, *Chenopodium ambrosioides*, *Raphanus sativus* var *oleiferus* cv Pegletta, and *Thymus serpyllum*. All these plant species have shown similar response to *X. index* in our bioassays. However, Dechet tested crude extracts in methanol of these plants and we tested crude extracts in water.

Another plant tested in our work, *Ruta graveolens*, has been reported by Sasanelli (1992) as highly nematicidal against *X. index*, *in vitro*, when used as leaf aqueous extracts and with LD-50 values until 1.79% of a standard solution at 1:4 W/V, after 48 hrs exposure. In our tests, root and top extracts of *R. graveolens* did not show effect at 12.5% of S at 48 hrs exposure. These differences may be attributed to the different experimental conditions of the tests.

In our study, the native plant species tested have been found to vary in their nematicidal or nematostatic effect on *X. index*. Some of these plants are trees or shrubs, as *Aristotelia chilensis*, *Cestrum parqui*, *Quillaja saponaria* and *Vestia lycioides*; other are weeds, as *Centaurium cachanlahuen*, *Oenothera affinis*, *Oxalis rosea* and *Stachys albicaulis*. Most of these

plant species have been used in traditional medicine, also as anthelmintics, in Chile (Montes and Wilkomirsky, 1985). Therefore, the present work refers to and exploits indigenous knowledge.

The compounds occurring in the plants with nematicidal activity, among them the plants included in our study, comprise a wide variety of phytochemical structures, e.g. polythienyls, acetylenes, alkaloids, fatty acids and derivatives, phenolics, terpenoids (Chitwood, 1992). It is not known, though, whether the nematicidal activity of these plants is due to a single compound or to a complex of compounds, or other mechanisms. However, our main concern is to select plants with nematicidal properties to be used in agriculture, e.g. as cover crops or green manures, for the management of *X. index* in grapevine.

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Literature cited

CHITWOOD D. J., 1992. Nematicidal compounds from plants. Pp. 185-204. *In*: *Phytochemical resources for medicine and agriculture* (H. N. Nigg and D. Seigler, eds). Plenum Press, New York, USA.

DECHET F., 1991. Untersuchungen zur Wirkung von Pflanzen und Pflanzeninhaltsstoffen auf *Xiphinema index* Thorne et Allen, 1950 (Nematoda: Dorylaimida). Doctoral Dissertation, Universität Kaiserslautern, Deutschland, 118 pp.

GOMMERS F. J. and BAKKER J., 1988. Physiological diseases induced by plant responses or products. Pp. 11-16. *In*: Diseases of nematodes. Vol. 1. (G. O. and H. B. Jansson, eds). CRC Press. Boca Ratoon, Florida, U.S.A.

INSUNZA V., 1994. Propiedades nematicidas de plantas chilenas. I. Evaluación en bioensayos con tres nematodos fitoparásitos. *Fitopatología, 29*: 44-45.

INSUNZA V. and ERIKSSON B., 1989. Nematicidal properties of Chilean plants and their potential in nematode control: evaluating tests on three plant parasitic nematodes. Pp. 269-274. *In*: Proc., Nordic Plant Protection Conference, Helsingoer (Denmark), Dec. 5-6, 1989.

MONTES M. and WILKOMIRSKY T., 1985. *Medicina popular chilena.* Univ. Concepción, Chile. 205 pp.

SASANELLI N., 1992. Nematicidal activity of aqueous extracts from leaves of *Ruta graveolens* on *Xiphinema index*. *Nematologia Mediterranea, 20*: 53-55.

SASANELLI N. and CATALANO L., 1991. Attività nematocida *in vitro* di *Capsicum annuum* su *Xiphinema index*. *Informatore fitopatologico, 41* (10): 55-56.

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