CONTROL OF *DITYLENCHUS DIPSACI* ON GARLIC (*ALLIUM SATIVUM*) WITH EXTRACTS OF MEDICINAL PLANTS FROM CHILE

V. Insunza B.[1] and A. Valenzuela A.[2]

Swedish University of Agricultural Sciences, Department of Plant Pathology, S-750 07 Uppsala, Sweden;[1] formerly[†] Departamento de Sanidad Vegetal, Facultad de Ciencias Agrarias y Forestales, Universidad de Chile, Santiago, Chile.[2]

ABSTRACT

Insunza B., V., and A. Valenzuela A. 1995. Control of *Ditylenchus dipsaci* on garlic (*Allium sativum*) with extracts of medicinal plants from Chile. Nematropica 25:35-41.

The potential nematicidal effects of 18 Chilean plants were evaluated on garlic (*Allium sativum*) seed cloves infected with *Ditylenchus dipsaci*. Cloves from infested fields were treated by immersion for 24 hours in plant extracts derived from fresh or air-dried plant parts. The garlic cloves were potted and allowed to grow for 14 weeks under outdoor conditions. At harvest, complete control of the nematode was achieved with fresh extracts from leaves of *Plantago major* and *Ruta graveolens* and resulted in the highest quality plants. Extracts of *Aristotelia chilensis*, *Chenopodium ambrosioides*, and *Ovidia pillopillo* reduced ($P \leq 0.05$) final nematode densities and disease symptoms compared to untreated control plants. None of the other plant extracts showed nematicidal activity against *D. dipsaci*.

Key words: Allium sativum, Aristotelia chilensis, bulb disinfection, *Chenopodium ambrosioides, Ditylenchus dipsaci,* garlic, nematicidal plants, *Ovidia pillopillo, Plantago major, Ruta graveolens.*

RESUMEN

Insunza B.,V. y A. Valenzuela A. 1995. Control de *Ditylenchus dipsaci* en ajo (*Allium sativum*) con extractos de plantas medicinales de Chile. Nematrópica 25:35-41.

El efecto nematicida potencial de 18 plantas de la flora chilena, se evaluó en dientes ("semilla") de ajo (*Allium sativum*) infectado con *Ditylenchus dipsaci*. Dientes de ajos provenientes de campos infestados, se trataron durante 24 horas por inmersión en los extractos de las plantas obtenidos de material vegetal fresco o secado al aire. Los dientes de ajo se plantaron en maceteros y crecieron durante 14 semanas en condiciones de campo. Al cosechar, se encontró que los extractos frescos de hojas de *Plantago major* y *Ruta graveolens* controlaron totalmente al nematodo y dieron los mejores resultados en cuanto a la calidad de las plantas. Extractos de *Aristotelia chilensis, Chenopodium ambrosioides* y *Ovidia pillopillo* redujeron ($P \leq 0.05$) las densidades finales del nematodo y los síntomas de la enfermedad comparados con las plantas del testigo sin tratar. Ninguno de los otros extractos vegetales mostró actividad nematicida contra *D. dipsaci*.

Palabras clave: Ajo, *Allium sativum, Aristotelia chilensis, Chenopodium ambrosioides,* desinfección de bulbos, *Ditylenchus dipsaci, Ovidia pillopillo, Plantago major,* plantas nematicidas, *Ruta graveolens.*

INTRODUCTION

The stem and bulb nematode (*Ditylenchus dipsaci* Kühn Filipjev) is one of the most destructive plant-parasitic nematodes, especially in temperate regions. In Chile, it is an economically important pest of garlic (*Allium sativum* L.), onion (*Allium cepa* L.), and alfalfa (*Medicago sativa* L.) crops. Losses of 30-80% have been reported in the main garlic production areas (8). *Ditylenchus dipsaci* causes necrosis

[†]Prof. Adelina Valenzuela Arce died in March 1992 to the great sadness of Nematologists.

or rotting of bulbs, swellings and distortion of aerial plant parts, leaf yellowing, and death of young plants.

To prevent damage from *D. dipsaci*, the use of nematode-free planting material is advocated (3). This can be accomplished by producing garlic in nematode-free fields or by disinfecting seed cloves. A novel approach for disinfection would be the use of plant extracts having nematicidal properties. Such an approach should be ecologically sound and cheaper than chemical treatment. Some attempts to control *D. dipsaci* have been tried using garlic cloves macerated in water (8,26). In previous tests (10,11), 34 of 75 medicinal plant species from the Chilean flora appeared to possess nematicidal activity against *D. dipsaci*.

The objective of this work was to evaluate the nematicidal potential of extracts from some of these plants to disinfect garlic bulbs infected by *D. dipsaci*.

MATERIAL AND METHODS

An experiment was conducted in 1991-92 (September to January) on the El Canelo de Nos farm, 35 km South of Santiago, Chile. Eighteen plant species (11 native and 7 naturalized in Chile) possessing nematicidal activity (11,12) were evaluated in this experiment (Table 1).

Plant material was obtained from plants grown on farms, from wild vegetation, and from herbal shops. Plant extracts were prepared either from fresh sap obtained after processing plant parts in an electric blender, or from air-dried plant material by infusion (5-10% w/v) in tap water. In both cases, extracts were filtered through nylon gauze to remove debris and kept in a refrigerator at -5°C until used.

Field-grown, nematode-infected garlic cloves (var. Rosado), that had been harvested in 1990, were obtained from the Llay-Llay area (V Region, North of Santiago). The bulbs were of the same uniform size ("calibre" 3rd, 32-36 mm diam) used by local growers. A random sample revealed ca. 300 *D. dipsaci* per 10 g of garlic tissue. Prior to the treatment, all cloves were presoaked in tap water for 2 hours to activate the nematodes and increase permeability to the plant extracts (14,15). The infected garlic cloves were then immersed in the appropriate plant extract for 24 hours at room temperature (16°C). Two controls were used: one with nematode-infected garlic cloves and a second one with healthy cloves. Both were treated by immersion in tap water.

The treated cloves were planted individually in 250 cm^3 plastic pots filled with well-mixed compost derived from organic farm wastes. The compost was previously disinfested by solarization for 2 months under a black plastic tarpaulin. The pots, with seven replicates per treatment, were arranged in a randomized complete block design on a wooden table placed outdoors. To avoid contact and possible contamination between the different treatments, the pots were placed on a wire netting 10 cm above the table surface. When needed, shade was provided by a "totora" roof. The plants were maintained for 14 weeks at 10-32°C and watered as needed.

Observations on germination, plant development, and visual nematode symptoms were recorded weekly. After harvest, the garlic plants were evaluated for disease expression, top heights were measured, and the roots/bulbs were weighed.

Symptom expression of plants was indexed on an arbitrary scale from 0 to 5 as follows: 0 = plants without symptoms; 0.5 = no visual symptoms but *D. dipsaci* was detected; 1 = reduced growth or slight necrosis on the bulbs, small bulbs; 2 = symptoms on the top: distortion, swellings of false stem and leaves; leaf growth "fan-like"; bulbs apparently not infected; 3 =

Table 1. Plant species tested for nematicidal activity against *Ditylenchus dipsaci.*

Plant species[z]	Botanical family	Common name (Spanish, Chilean)	Common name (English)
Aristotelia chilensis (Mol.) Stuntz*	Elaeocarpaceae	maqui	—
Artemisia abrotanum L.	Compositae	éter	old man wormwood
A. absinthium L.	Compositae	ajenjo	common wormwood
Asparagus officinalis L.	Liliaceae	espárrago	asparagus
Cestrum parqui L'Her.*	Solanaceae	palqui	—
Centaurium cachanlahuen (Mol) Rob.*	Gentianaceae	cachanlagua	—
Chenopodium ambrosioides L.*	Chenopodiaceae	paico	American wormseed
Durvillea antarctica (Cham.) Hariot*	Phaeophyta	cochayuyo	—
Galega officinalis L.	Papilionaceae	galega	goats rue
Matricaria chamomilla L.	Compositae	manzanilla	German chamomile
Oenothera affinis Cabess.*	Onagraceae	Don Diego de la noche	—
Ovidia pillopillo (Gay) Meissn.*	Thymeliaceae	lloime	—
Oxalis rosea Jacq.*	Oxalidaceae	culle colorado	—
Plantago major L.	Plantaginaceae	llatén	broadleaf plantain
Ruta graveolens L.	Rutaceae	ruda	rue
Senna stipulacea (Ait.) Irw. et Barn.*	Caesalpiniaceae	sen	—
Stachys albicaulis Lindl.*	Labiatae	Yerba de Sta María	—
Vestia lycioides Willd.*	Solanaceae	huévil	—

[z]Plant species marked with an asterick * are native to Chile.

bulbs/roots rotted or necrotic, but no visual symptoms in the plant tops; 4 = death of the plant at the end of the experiment; stunting of stem base, being separated from the bulb; bulb base necrotic or rotted; 5 = death of young plants within 4-5 weeks after planting. A disease severity index was determined by the sum of the ratings for all plants divided by the number of plants in the treatment.

Nematodes were extracted separately from the plant tops and from bulbs plus roots by incubation on Baermann funnels for 18 hrs. The extracted *D. dipsaci* were counted under a dissecting microscope and expressed as numbers per 10 g of plant tissue. Dead plants were recorded but not included in nematode extractions since *D. dipsaci* does not develop in necrotic tissue. Nematode extraction and analysis was conducted in the laboratory of SAG (Servicio Agrícola y Ganadero, Ministry of Agriculture) in Santiago, Chile.

All data were subjected to analysis of variance and treatment means were compared by Fisher's LSD (least significant differences) pairwise procedure at $P \leq 0.05$ using Minitab Statistical software (Minitab Inc., State College, PA, U.S.A., 1991). Nematode counts and bulb/root weight data were transformed to $\log(x + 0.1)$ prior to analysis, however, non-transformed means are presented.

RESULTS AND DISCUSSION

Plant symptoms corresponded to those described by Bruna and Guíñez (1) and Tapia (25). Percentages of diseased plants and disease indices were the most descriptive parameters of disease severity. Disease assessment revealed 71.4% affected plants and a disease index of 2.3 in the infected control (Table 2). Compared to the infected control, all treatments reduced *D. dipsaci* damage to some degree. However, fresh leaf extracts of *Plantago major* and *Ruta graveolens* completely controlled *D. dipsaci* (no diseased garlic plants). On the other hand, infusion of dried leaf extracts of *R. graveolens* resulted in 42.9% diseased plants, suggesting that the active nematicidal principle might have been affected or destroyed during the infusion process or by the air-drying of the leaves. Low infection rates also were obtained in the treatments with fresh leaf extracts of *Aristotelia chilensis* and *Chenopodium ambrosioides* and with infusion (extract type "B") of the leaves and stems of *Ovidia pillopillo* (14.3% diseased garlic plants in all three cases, with disease indices of 0.2, 0.2, and 0.3, respectively).

Top heights and root/bulb weights in the treatments with *Durvillea antarctica* and *Oxalis rosea* were lower ($P \leq 0.05$) than those of the infected control and many other treatments (Table 2). Furthermore, in extracts of *O. rosea*, the percentage of germination was lower ($P \leq 0.05$) than that in several other treatments; only 57% of the bulbs germinated. Also, the disease index (2.5) corresponded to that of dead seedlings, although *D. dipsaci* was not found. Therefore, it is not clear if these results might be attributed to a toxic effect of the *O. rosea* extract on germination and growth rather than to nematode infection. *Oxalis rosea* contains oxalic, ascorbic, pyruvic and glioxalic acids (16). Oxalic acid seems to have an allelopathic effect and is known to inhibit the growth and germination of sugarcane (2).

At the end of the experiment, *D. dipsaci* was extracted from most of the diseased plants, and population counts ranged between 1 to 334 nematodes per 10 g of plant tissue, mainly in bulbs. The numbers of *D. dipsaci* found in the tissue samples were rather low, even in heavily affected plants, or the nematode was not recovered at all. This contrasts with observations in Chile of populations as high as 11,000 individuals per 10 g of bulb tissue during late spring (November - December) (25).

A possible explanation for the low nematode numbers may be that, because of the hot summer temperatures, nematodes migrated from the bulb into the potted soil (which was not analyzed) or had died by the end of the experiment (7). Another possibility may be that garlic bulbs possess nematicidal principles which may be liberated when the tissue is chopped for nematode extraction (19, 24). However, this explanation is less plausible because higher numbers were obtained at the beginning of the experiment, when the same methods were used.

Extracts from *Plantago major, Ruta graveolens*, and *Chenopodium ambrosioides* resulted in low or no diseased garlic plants and no phytotoxic effects. These three plant species were introduced into Chiles but have been naturalized and are now relatively abundant in Central and South Chile. *Plantago major* is a perennial herb which has found use as a medicinal plant, but there seems to be no earlier reports on nematicidal properties. However, Jermy (13) reported insecticidal activity. The active principles of *P. major* include alkaloids, glycosides, flavonoids, phenolic acids, tannins, and derivates of sulphur (16).

Table 2. Effects of extracts of 18 Chilean plants on *Ditylenchus dipsaci* and growth and disease expression in garlic (*Allium sativum*).[w]

Plant species	Plant part and type of extract[x]	Plants germinated (%)	Plants diseased (%)	Disease index[y]	Final number of *D. dipsaci* per 10 g tissue[z]	Plant top height (cm)	Root/bulb weight (g)
Aristotelia chilensis	L, A	100.0	14.3	0.2	1	35.1	4.2
Artemisia abrotanum	T, B	85.7	50.0	2.0	7	26.8	3.5
A. absinthium	T, A	100.0	42.9	1.4	30	32.0	4.9
Asparagus officinalis	R, B	85.7	33.3	1.5	1	25.0	3.4
Cestrum parqui	L, A	100.0	28.6	0.6	5	30.4	3.6
Centaurium cachanlahuen	W, B	85.7	33.3	1.3	20	31.8	4.4
Chenopodium ambrosioides	L, A	100.0	14.3	0.2	4	38.0	5.1
Durvillea antarctica	W, B	85.7	50.0	1.8	0	20.9	3.0
Galega officinalis	L, A	85.7	33.3	1.0	0	30.1	4.5
Matricaria chamomilla	T, B	85.7	28.6	1.7	0	32.4	4.8
Oenothera affinis	T, C	85.7	50.0	1.6	2	37.7	4.9
Ovidia pillopillo	L, B	100.0	14.3	0.3	2	39.1	5.4
Oxalis rosea	W, C	57.1	50.0	2.5	0	11.2	1.2
Plantago major	L, A	85.7	0.0	0.0	0	36.1	7.3
Ruta graveolens	L, A	85.7	0.0	0.0	0	38.1	5.4
	T, B	100.0	42.9	1.3	4	26.6	4.9
Senna stipulacea	L, B	100.0	28.6	0.9	1	29.0	4.7
Stachys albicaulis	T, C	85.7	28.6	0.6	7	37.2	4.7
Vestia lycioides	L, B	100.0	42.9	1.7	4	33.9	4.6
Infected control	tap water	100.0	71.4	2.3	112	32.4	4.9
Healthy control	tap water	100.0	14.2	0.3	5	30.2	4.1
Fisher's LSD ($P \leq 0.05$)		30.2	46.2	2.0	43	10.8	2.7

[w]Data are means of 4-7 replicates, depending on survival of garlic plants after 14 weeks of the experiment.
[x]Plant parts: L = leaves; R = roots; S = stems; T = plant top; W = whole plant. Type of extract: A = fresh sap; B = infusion of air-dried plant parts at 5% (w/v); C = infusion of air-dried plant parts at 10% (w/v).
[y]Disease index rated on scale 0 to 5 (0 = plants without symptoms; 5 = young plants dead).
[z]The initial population of *D. dipsaci* was ca. 300 individuals per 10 g of bulb tissue.

Ruta graveolens is a perennial shrub which has been used as an anthelminthic since colonial times in Chile (4). Nematicidal extracts of *R. graveolens* affected the hatching of eggs of *Heterodera schachtii* Schmidt (22), and *in vitro* nematicidal effects were also reported against several *Meloidogyne* spp. (23) and against *Xiphinema index* Thorne and Allen (21). The chemicals responsible for this nematicidal activity have not been identified, although insecticidal principles are known (6).

Chenopodium ambrosioides, an annual or perennial herb, has long been recognized for its anthelminthic properties in traditional American cultures. This and other *Chenopodium* species contain substances that are either antiviral, antifungal, antibiotic, nematicidal, molluscicidal, insecticidal, or allelopathic. The most active principle is an oil, consisting of up to 86% ascaridol, a terpenoid peroxide (16, 20). *In vitro* nematicidal effects of *C. ambrosioides* have been reported against *Hoplolaimus indicus* Sher, *Tylenchorhynchus brassicae* Siddiqi, and *Rotylenchulus reniformis* Linford and Oliveira (9); and in potted soil against *Meloidogyne* spp. (5,17). However, the nematicidal component(s) is unknown.

The native shrubs *Ovidia pillopillo* and *Aristotelia chilensis* contain several active principles (16) and have been used traditionally as anthelminthics. Another plant species, *Daphne odorata* Thun., which is closely related to the genus *Ovidia*, has shown nematicidal activity against *Aphelenchoides besseyi* Christie (18).

In conclusion, our results indicate five plant species have potential to control *D. dipsaci* in garlic bulbs: *Plantago major*, *Ruta graveolens*, *Chenopodium ambrosioides*, *Ovidia pillopillo*, and *Aristotelia chilensis*. In previous screening work (12), *A. chilensis*, *C. ambrosioides*, *O. pillopillo*, *P. major*, and *R. bracteosa* DC. (closely related to *R. graveolens*) showed nematoxic or nematostatic activity *in vitro* against *D. dipsaci*, *Heterodera schachtii*, and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven. It is not known whether the nematicidal activity demonstrated by these plants is due to a single compound or to a complex of compounds. Additional work is needed to clarify the mechanisms involved.

The use of plant extracts for bulb disinfection to control *D. dipsaci* needs further study before being recommended as an alternative to chemical control. Main factors to be considered are plant availability in the area, possible phytotoxicity of the candidate plant extract, and alternative economic value of the plant to agriculture.

ACKNOWLEDGEMENTS

Sincere thanks to Agr. Eng. E. Aballay, Dr. B. Eriksson and Dr. N. Greco for comments and critical reading of the manuscript, and to the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for financial support.

LITERATURE CITED

1. BRUNA, A., and A. GUIÑEZ. 1980. Identificación del nematodo del tallo y de los bulbos *Ditylenchus dipsaci* (Kühn) Filipjev y porcentaje de infestación en ajo (*Allium sativum* L.) y cebolla (*Allium cepa* L.). Agricultura Técnica (Chile) 40:137-142.
2. CHOU, C. H. 1986. The role of allelopathy in subtropical agroecosystems in Taiwan. Pp. 57-73 *in* A. R. Putnam and C. S. Tang, eds. The Science of Allelopathy. Wiley and Sons, New York, New York, U.S.A.
3. EPPO/CABI. 1992. *Ditylenchus dipsaci*. Pp. 371-374 *in* I. M. Smith, D. G. McNamara, P. R. Scott, and K. M. Harris, eds. Quarantine pests for Europe. CAB International, Wallingford, U.K.
4. FARGA, C. y J. LASTRA. 1988. Ruda. Pp. 74-78 *in* Plantas medicinales de uso común en Chile. SOPRAMI Ltda., Santiago de Chile.
5. GARCIA ESPINOZA, R. 1980. *Chenopodium ambrosioides* L., planta con uso potencial en el

combate de nematodos fitoparásitos. Agricultura Tropical 2:92-97.
6. GRAINGE, M., and S. AHMED. 1988. P. 238 *in* Handbook of plants with pest-control properties. Wiley and Sons, New York, New York, U.S.A.
7. GRECO, N. 1993. Epidemiology and management of *Ditylenchus dipsaci* on vegetable crops in southern Italy. Nematropica 23:247-251.
8. GUIÑEZ, A. 1986. Control del nematodo del tallo y de los bulbos en ajo. IPA, La Platina, Chile 34:17-20.
9. HASSEB, A., B. SINGH, A. M. KHAN, and S. K. SAXENA. 1978. Evaluation of nematicidal property in certain alkaloid bearing plants. Geobios 5:116-118.
10. INSUNZA, V. 1988. Nematicidal activity of Chilean plants: *in vitro* tests on *Ditylenchus dipsaci.* International Nematology Network Newsletter 7:44-46.
11. INSUNZA, V. 1994. Propiedades nematicidas de plantas Chilenas. I. Evaluación en bioensayos con tres nematodos fitoparásitos. Resumenes de los trabajos presentados en el VII Congreso Latinoamericano de Fitopatología, Fac. Agronomía, Universidad Católica, Santiago, Chile:53-54 (resumen).
12. INSUNZA, V., and B. ERIKSSON. 1989. Nematicidal properties of Chilean plants and their potential in nematode control: evaluating tests on three plant-parasitic nematodes. Papers of Nordisk Plantevaernskonference 1989:269-274.
13. JERMY, T. 1966. Feeding inhibitors and food preference in chewing phytophagous insects. Entomology Experimentalis et Applicata 9:1-12.
14. LEAR, B., and D. E. JOHNSON. 1962. Treatments for eradication of *Ditylenchus dipsaci* in cloves of garlic. Plant Disease Reporter 46:635-639.
15. MEREDITH, J. A. 1977. Control of *Ditylenchus dipsaci* in garlic "seed" in Venezuela. Nematropica 7:3.
16. MONTES, M., and T. WILKOMIRSKY. 1985. Medicina tradicional Chilena. Editorial Univ. de Concepción Chile, 205 pp.
17. MORISHITA, T., T. TAGAMI, and T. MATSUZAKA. 1978. Soil nematode control with *Chenopodium ambrosioides.* Chemical Abstracts 88:100366.
18. MUNAKATA, K. 1979. Nematicidal substances from plants. Advances in Pesticide Sciences. Symposia Papers, 4th International Congress Pesticide Chemistry, Zurich, Switzerland, Pp. 295-302.
19. NATH, A., N. K. SHARMA, S. BHARDWA, and C. D. THAPA. 1982. Nematicidal properties of garlic. Nematologica 28:253-255.
20. QUARLES, W. 1992. Botanical pesticides from *Chenopodium*? IPM Practitioner 14:1-11.
21. SASANELLI, N. 1992. Nematicidal activity of aqueous extracts from leaves of *Ruta graveolens* on *Xiphinema index.* Nematologia Mediterranea 20:53-55.
22. SASANELLI, N., and T. D'ADDABBO. 1992. The effect of *Cineraria maritima, Ruta graveolens* and *Tagetes erecta* extracts on the hatching of *Heterodera schachtii.* Nematologia Mediterranea 20:49-51.
23. SASANELLI, N., and T. D'ADDABBO. 1993. The effect of *Cineraria maritima, Ruta graveolens* and *Tagetes erecta* leaf and root extracts on Italian populations of *Meloidogyne* species. Nematologia Mediterranea 21:21-22.
24. SUKUL, N. C., P. K. DAS, and G. C. DE. 1974. Nematicidal action of some edible crops. Nematologica 20:187-191.
25. TAPIA, E. M. 1984. Aporte al estudio de la incidencia del "nematodo del bulbo y tallo" (*Ditylenchus dipsaci* (Kühn) Filipjev en el cultivo del ajo (*Allium sativum* L.), tipo rosado, en sectores de la V región. Thesis, Ingeniero Agrónomo, Escuela de Agronomía, Dpto de Horticultura, Universidad Católica de Valparaíso, Valparaíso, Chile.
26. TORO, M. S. del, J. A. GARAY, and M. A. COLOMBINO. 1988. Experiencias para el control de *Ditylenchus dipsaci* (Kühn) Filipjev, en ajo c.v. Colorado, en San Luis, Argentina. Revista de la Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo 24 (1-2):35-47.

Received:
Recibido: *4.XI.1994*

Accepted for publication:
Aceptado para publicación: *17.III.1995*