IN VITRO SENSITIVITY OF *MELOIDOGYNE INCOGNITA* TO EXTRACTS FROM NATIVE YUCATECAN PLANTS

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ABSTRACT

Cristóbal-Alejo, J., J. M. Tun-Suárez, S. Moguel-Catzín, N. Marbán-Mendoza, L. Medina-Baizabal, P. Simá-Polanco, S. R. Peraza-Sánchez, and M. M. Gamboa-Angulo. 2006. *In vitro* sensitivity of *Meloidogyne incognita* to extracts from native Yucatecan plants. Nematropica 36:89-97.

Screening of 55 plant extracts against second stage juveniles of *Meloidogyne incognita* was conducted. These extracts were obtained from leaves, stems, and roots of 20 native Yucatecan plants, of which 13 species were characterized as endemic. These were *Acalypha gaumeri*, *Ageratum gaumeri*, *Ambrosia hispida*, *Bidens alba*, *Blechum piramidatum*, *Caesalpinia yucatanensis*, *Calea urticifolia*, *Carlowrightia myriantha*, *Croton chichenensis*, *Eugenia yucatanensis*, *Eugenia winzerlingii*, *Furcraea cahum*, *Stenandrium nanum*, *Tephrosia cinerea*, *Trichilia arborea*, *Trichilia minutiflora*, *Randia longiloba*, *Randia obcordata*, *Randia standleyana*, and *Vitex gaumeri*. An *in vitro* nematicidal assay, carried out at 250 and 500 ppm showed that extracts from *Calea urticifolia* leaves and roots, *Eugenia winzerlingii* leaves, and *Tephrosia cinerea* stems were the most active against *M. incognita*. The plant extracts were evaluated at 0, 50, 100, 200, 300, 400, and 500 ppm to obtain their median effective dose. Results showed that *Eugenia winzerlingii* leaf extract induced at 300 ppm mortalities of 77% and 84% after 48 and 72 hr, respectively. The activity demonstrated by *Eugenia winzerlingii* was good enough to propose this plant for further studies at greenhouse and field stages to determine its efficacy in soil.

Key words: in vitro assays, *Meloidogyne incognita*, native Yucatecan plants, nematodes, screening.

RESUMEN

Cristóbal-Alejo, J., J. M. Tun-Suárez, S. Moguel-Catzín, N. Marbán-Mendoza, L. Medina-Baizabal, P. Simá-Polanco, S. R. Peraza-Sánchez, and M. M. Gamboa-Angulo. 2006. Sensibilidad *in vitro* de *Meloidogyne incognita* a extractos de plantas nativas de Yucatán. Nematropica 36:89-97.

Se evaluaron 55 extractos vegetales para el control de juveniles del segundo estadio de *Meloidogyne incognita*. Estos extractos fueron obtenidos de hojas, tallos y raíces de 20 plantas nativas de Yucatán, de las cuales 13 especies son catalogadas como endémicas. Las plantas fueron *Acalypha gaumeri*, *Ageratum gaumeri*, *Ambrosia hispida*, *Bidens alba*, *Blechum piramidatum*, *Caesalpinia yucatanensis*, *Calea urticifolia*, *Carlowrightia myriantha*, *Croton chichenensis*, *Eugenia yucatanensis*, *Eugenia winzerlingii*, *Furcraea cahum*, *Stenandrium nanum*, *Tephrosia cinerea*, *Trichilia arborea*, *Trichilia minutiflora*, *Randia longiloba*, *Randia obcordata*, *Randia standleyana* y *Vitex gaumeri*. El bioensayo nematicida *in vitro* a 250 y 500 ppm indicó que los extractos de hojas y raíz de *Calea urticifolia*, hojas de *Eugenia winzerlingii* y tallo de *Tephrosia cinerea* presentaron la mayor actividad contra *M. incognita*. Las plantas seleccionadas fueron evaluadas a 0, 50, 100, 200, 300, 400 y 500 ppm para determinar su dosis efectiva media. Los resultados indican que el extracto de las hojas de *Eugenia winzerlingii* a 300 ppm induce mortalidades de 77% y 84% después de 48 y 72 hr, respectivamente. La actividad demostrada por *Eugenia winzerlingii* permite proponer continuar con otros estudios en invernadero y campo para conocer su efectividad en el suelo.

Palabras clave: ensayos *in vitro*, evaluación, *Meloidogyne incognita*, nematodos, plantas nativas de Yucatán.

INTRODUCTION

Meloidogyne spp. are recognized as the most destructive plant parasites in the world. These polyphagous nematodes have world-wide distribution, and are found in important economic crops such as coffee, cotton, tomato, citrus fruits, and olive (Whitehead, 1998). Infections by *Meloidogyne* spp. have a negative impact in crop production and, therefore, affect economies in the region where damage is extensive (Carrillo *et al.*, 2000; Bauske *et al.*, 1994). In Mexico, the species *M. incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood, *M. javanica* Chitwood, and *M. hapla* Chitwood are the most commonly found, with *M. incognita* being the most pathogenic and evolved species (Cid del Prado-Vera *et al.*, 2001).Traditionally, synthetic nematicides are used to control *Meloidogyne* spp. As a result, production costs are increased as well as impacts on the environment and non-target organisms. Because of these inconveniences, it is necessary to look for other less harmful management options to control *Meloidogyne* spp. as well as other pests (Agrios, 2001).

A viable strategy is the use of natural metabolites produced by plants that possess nematode-antagonist properties. To move forward in the discovery of natural plant metabolites, plant extracts are submitted to screening programs looking for potential candidates with nematicidal activity (Chitwood, 2002; Lorimer *et al.*, 1996; Inzunsa *et al.*, 2001). Some of these studies have reported interesting metabolites against *M. incognita*, such as lantanoside and lantanone from *Lantana camara* (Verbenaceae) (Begum *et al.*, 2000; Qamar *et al.*, 2005).

The biodiversity of Mexican plant life may lend itself to the discovery of unique plant metabolites. In particular, the Yucatan peninsula possesses almost 800 different plant species, of which 117 are classified as endemic (Duran *et al.*, 1998). However, very little is known about the chemical and pesticidal properties of this natural resource. The overall goal of this research is to identify native Yucatecan plants that produce antagonist compounds to nematodes, and eventually screen these compounds against nematodes in soil. The main objective of this study was to evaluate *in vitro* 55 ethanolic extracts obtained from 20 selected native Yucatecan plants against second-stage juveniles of *M. incognita*.

MATERIAL AND METHODS

Plant Material

Plants were collected from different localities of the Yucatan peninsula, Mexico, under the supervision of botanists on September and November, 2001. Voucher samples of each plant were deposited in the herbarium at Unidad de Recursos Naturales of Centro de Investigación Científica de Yucatán. After collection, all plants were separated as leaves (L), stems (S), and roots (R), except *Ambrosia hispida* (L and S+R) and *Stenandrium nanum* (L+S, and R). The plant material was dried under artificial light (50-60°C) for three days, and afterwards stems and roots were ground in a Brabender mill (mesh No. 2, Duisburg, Germany); leaves were manually triturated (Table 1).

Extraction

Dried and ground plant material (20 g) was macerated with ethanol (300 ml) three times at room temperature for 48 h, each time. The solvent was separated by filtration, and the material was vacuum-concentrated in a rotary evaporator (Büchi, model RE-111, Flawil, Switzerland) at 40°C, obtaining the corresponding organic crude extracts (Table 1).

Table 1. Yucatecan plants selected for evaluation of ethanolic crude extract against *Meloidogyne incognita*, and percentage of *Meloidogyne incognita* J_2 mortality after 72 hr evaluated at 500 and 250 ppm.

Family	Scientific name	Voucher No.	Part used[v]	% Mortality 72 h	
				500 ppm[w]	250 ppm[x]
Acanthaceae	*Blechum piramidatum* (Lam.) Urb.	PS 1988	L	58 cd	NE[y]
			S	58 cd	NE
			R	80 ab	NE
	Carlowrightia myriantha (Standl.) Standl.[z]	PS 2589	L	100 a	23 cdf
	Stenandrium nanum (Standl.) T.F. Daniel[z]	PS 2597	L	100 a	13 df
			S+R	100 a	14 df
Agavaceae	*Furcraea cahum* Trel[w]	PS 2583	L	45 cd	NE
			S	45 cd	NE
			R	86 ab	NE
Asteraceae	*Ageratum gaumeri* B.L. Rob. for *gaumeri*[w]	FM 1990	L	100 a	22 cdf
			S	94 ab	15 df
			R	86 ab	14 df
	Ambrosia hispida Pursh	PS 2579	L+S	63 bc	12 df
			S	63 bc	11 df
	Bidens alba (L.) DC. var. *alba*	FM 1989	L	100 a	24 cdf
			S	100 a	11 df
			R	100 a	19 cdf
	Calea urticifolia (Mill.) DC.[w]	FM 1721	L	90 ab	97 a
			S	100 a	30 cd
			R	88 ab	80 cdf
Euphorbiaceae	*Acalypha gaumeri* Pax & K. Hoffm.[w]	PS 2584	L	95 ab	13 df
			S	95 ab	22 cdf
			R	100a	14 df
	Croton chichenensis Lundell[w]	PS 2571	L	58 bc	NE
			S	72 b	NE
			R	32 cd	NE
Fabaceae	*Caesalpinia yucatanensis* Greenm.[w]	PS 2569	L	66 abcd	NE
			S	80 abc	16 df
			R	93 ab	15 df
	Tephrosia cinerea (L.) Pers.	PS 2580	L	95 ab	30 cd
			S	96 ab	85 ab
			R	100 ab	56 cd

[v]L = leaves, S = stem, R = root.
[w]SD^2 (500 ppm) = 0.092.
[x]SD^2 (250 ppm) = 0.1207.
[y]Not evaluated.
[z]Endemic plant

Table 1. (Continued) Yucatecan plants selected for evaluation of ethanolic crude extract against *Meloidogyne incognita*, and percentage of *Meloidogyne incognita* J_2 mortality after 72 hr evaluated at 500 and 250 ppm.

Family	Scientific name	Voucher No.	Part used[y]	% Mortality 72 h	
				500 ppm[w]	250 ppm[x]
Meliaceae	*Trichilia arborea* C. DC. (*Trichilia glabra* L.)	PS 2596	L	85 ab	13 df
			S	100 a	18 cdf
	Trichilia minutiflora Standl.[w]	PS 2586	L	34 cd	NE
			S	44 cd	NE
			R	80 ab	9 df
Myrtaceae	*Eugenia winzerlingii* Standl.[w]	PS 2595	L	100 a	60 ab
			S	100 a	22 cdf
			R	100 a	28 cdf
	Eugenia yucatanensis Standl.[w]	PS 2585	L	25 cd	NE
			S	24 cd	NE
			R	64 cd	NE
Rubiaceae	*Randia longiloba* Hemsl.[w]	PS 2578	L	95 ab	25 cdf
			S	75 bc	NE
			R	66 bcd	NE
	Randia obcordata S. Watson	PS 2582	L	60 cd	NE
			S	63 cd	NE
			R	61 cd	NE
	Randia standleyana (L.) Williams[w]	PS 2581	L	95 ab	8 df
			S	75 bc	NE
			R	66 bcd	NE
Verbenaceae	*Vitex gaumeri* Greenm.	PS 2598	L	75 b	NE
			S	43 cd	NE
			R	46 cd	NE
	Furadan, 3 µl/ml			100 a	100 a
	Control (distilled water)			6	6 d
	DMSO-water			15 d	15 d

[y]L = leaves, S = stem, R = root.
[w]SD^2 (500 ppm) = 0.092.
[x]SD^2 (250 ppm) = 0.1207.
[y]Not evaluated.
[z]Endemic plant

Preparation of Nematode Inoculum

Meloidogyne incognita egg masses were obtained from the roots of greenhouse-grown tomato plants (*Lycopersicon esculentum* Mill.) cv. Maya. Eggs masses were collected from knotted-roots, and incubated at 28 ± 1°C for three days to obtain second stage juveniles (J_2) (Cristóbal *et al.*, 2001). A stock solution of nematodes was prepared with a final concentration of 100 ± 5 J_2 per ml.

Nematicidal Assay

All crude extracts (500 ppm) were dissolved in dimethyl sulfoxide (DMSO ≤0.5%, 5 µl), and diluted with water (995 µl). Twenty freshly hatched J_2 were placed in suspension, and incubated at room temperature in special dishes for J_2 mortality studies. Furadan (Carbofuran 750 CE) was used as positive control (3, 6, and 9 µl/ml), and negative controls included solvent mixtures (DMSO-water, 0.5%) and sterile distilled water (100%). Each extract was replicated four times (Table 1).

Extracts of *Acalypha gaumeri* (L, S, R), *Ageratum gaumeri* (L, S, R), *Bidens alba* (L, S, R), *Caesalpinia yucatanensis* (S, R), *Calea urticifolia* (L, S, R), *Carlowrightia myriantha* (L), *Eugenia winzerlingii* (L, S, R), *Stenandrium nanum* (R), *Randia longiloba* (L), *Randia standleyana* (L), *Tephrosia cinerea* (L, S, R), *Trichilia arborea* (L, S), and *Trichilia minutiflora* (R) were evaluated again at 250 ppm, using four replicates of each one and the same controls.

Dose-inhibitory response curves using a dilution series (0, 50, 100, 200, 300, 400, and 500 ppm) were prepared for extracts of *Calea urticifolia* (L, R), *Eugenia winzerlengii* (L), and *Tephrosia cinerea* (S). One hundred J_2 were placed in 10 ml of the extract solutions of each concentration in Petri dishes. The same positive and negative controls were used, with four replicate samples at each concentration (Table 2).

In each assay, the J_2 were counted for mortality and non-mortality under a stereoscopic microscope after 24, 48, and 72 hr. Nematodes were considered dead if still immobile after touching them with a needle. After 72 hr, the nematodes were transferred to water, counted and confirmed dead. All experiments were carried out under laboratory conditions, at 26 ± 2°C, using a completely randomized design.

The percentage of J_2 mortality was obtained by analysis of variance, previous transformation with arcsin [y = arcsin (sqrt y/100)] followed by multiple means comparison (Tukey $P = 0.05$) (Steel and Torrie, 1986). Effective concentrations (ED_{50} and ED_{95}) were obtained by transforming to "Probit" and ten-base logarithms the calculated percent mortality of the second nematicidal assay (Thorne *et al.*, 1995).

RESULTS AND DISCUSION

Results of the initial crude extract screening showed induction of mortality in the extracts at 500 ppm with statistical differences ($P = 0.01$) after 24, 48, and 72 hr. Exposure of *M. incognita* to *Acalypha gaumeri* (R), *Ageratum gaumeri* (L), *Bidens alba* (L, S, R), *Calea urticifolia* (S), *Carlowrightia myriantha* (L), *Eugenia winzerlingii* (L, S, R), *Stenandrium nanum* (L+S, R), *Tephrosia cinerea* (R), and *Trichilia arborea* (S) resulted in 100% mortality.

Extracts from *Acalypha gaumeri* (L, S), *Ageratum gaumeri* (S, R), *Caesalpinia yucatanensis* (S, R), *Calea urticifolia* (L, R), *Randia longiloba* (L), *Randia standleyana* (L), *Tephrosia cinerea* (L, S), *Trichilia arborea* (L), and *Trichilia minutiflora* (R) resulted in mortalities higher than 80%. These results were compared with those obtained with Furadan (100% mortality evaluated at three concentrations), a nematicide used as standard.

Among the 20 species (9 families) of plants tested, 11 species had some nematicidal activity. Three species belonging to Asteraceae family (*Ageratum gaumeri*, *Bidens alba*, and *Calea urticifolia*) showed active properties. On the other hand, only *Bidens alba* and *Eugenia winzerlingii* were able to induce activity by all three extract plant parts tested.

All active extracts were evaluated *in vitro* against *M. incognita* at 250 ppm. The results

Table 2. Median effective dose of ethanolic extract from *Calea urticifolia* leaf, *Eugenia winzerlingii* leaf, and *Tephrosia cinerea* stem against *Meloidogyne incognita* J_2 after 24, 48 and 72 hr.

		% Mortality		
Extracts	ppm	24 h[w]	48 h[x]	72 h[y]
Eugenia winzerlingii leaf	0	6 f[z]	6 f	6 f
	50	8 f	25 def	36 dc
	100	8 f	26 def	29 dce
	200	15 cefd	47 dc	47 c
	300	48 b	77 bc	84 b
	400	82 a	87 ab	87 ba
	500	93 a	99 a	99 a
Tephrosia cinerea stem	0	6 f	6 f	6 f
	50	7 f	11 ef	13 dfe
	100	7 f	16 ef	18 def
	200	5 f	12 ef	28 dfce
	300	11 ef	29 def	29 dce
	400	7 f	24 def	28 dfce
	500	8 f	33 de	37 dc
Calea urticifolia leaf	0	6 f	6 f	6 f
	50	4 f	8 ef	9 fe
	100	4 f	10 ef	12 fe
	200	7 f	16 ef	16 dfe
	300	11 efd	11 ef	12 dfe
	400	13 cefd	17 ef	20 dfe
	500	10 e	16 ef	19 dfe
Calea urticifolia root	0	6 f	6 f	6 f
	50	10 ef	11 ef	12 dfe
	100	11 efd	11 ef	15 dfe
	200	13 cefd	13 ef	17 dfe
	300	30 cebd	30 def	30 dce
	400	31 cb	31 de	33 dce
	500	30 cbd	30 def	35 dc
Furadan		100	100	100
Water		3	3	4

[w]SD^2 (24 h) = 0.8364.
[x]SD^2 (48 h) = 0.1223.
[y]SD^2 (72 h).
[z]Median between columns is statistically equal (Tukey p = 0.05).

showed statistical differences ($P = 0.01$). In general, at this concentration some extracts induced less than 30% mortality at 24 hr. After 48 hr, mortality increased to 50%, and 100% mortality could be detected at 72 hr. In this experiment, the best results were obtained with extracts from leaves and roots of *Calea urticifolia* (97 and 80%, respectively), leaves of *Eugenia winzerlingii* (60%), and stems of *Tephrosia cinerea* (85%). The effects observed at 250 ppm suggested the presence of nematicidal metabolites produced by these four plant extracts. Therefore, these species were evaluated at different concentrations (0, 50 100, 200, 400, and 500 ppm) to determine the median effective dose (ED_{50}).

The plant with the best activity detected was *Eugenia winzerlingii* leaf at 300, 400, and 500 ppm, which led to mortality percentages of 48, 82, and 93%, respectively, in 24 hr. After 48 hr of exposure, *Eugenia winzerlingii* leaf extract continued showing the same tendency, including mortalities of 77, 87, and 99% at 300, 400, and 500 ppm, respectively. Finally, after 72 hr, *Eugenia winzerlingii* leaf extract increased its effectiveness to 84% at 300 ppm (Table 2). The rest of the extracts did not show nematicidal effects over 50%. On the other hand, the negative control showed 3% mortality, while Furadan, 100%. These results were not quantitatively reproducible compared with the initial assay at 500 and 250 ppm, probably due to the presence of volatile or unstable metabolites in the extracts.

As expected, when the effective doses were calculated, *Eugenia winzerlingii* leaf extract showed lower ED_{50} and ED_{95}, with 133.4 and 645.5 ppm, respectively, compared to *Tephrosia cinerea* stem and *Calea urticifolia* leaf. These results indicate that *Eugenia winzerlingii* leaf extract has high potential to be used in future greenhouse tests to control *M. incognita*.

Some species belonging to the genus *Eugenia* are widely recognized for their medicinal properties as antimicrobial (Locher *et al.*, 1995; Nuñez *et al.*, 2001; Li *et al.*, 2005), antihypertensive (Consolini *et al.,* 2002), anticonvulsant (Pourgholami *et al.*, 1999), and trypanocidal (Adewunmi *et al.*, 2001), or used against pests due to its insecticidal properties (Yang *et al.*, 2003). However, only one study about its nematicidal action has been done with *Eugenia caryophyllata* (Sangwan *et al.*, 1990; Chitwood, 2002). In general, several metabolites have been identified from *Eugenia* species, mainly sesquiterpene lactones, polyoxygenated flavonoids, anthocyanins, and essential oils (NAPRALERT Database). Specifically, eugenol has been isolated as an active compound from the flowers of *Eugenia caryophyllata* (Sangwan *et al.*, 1990). So far, there is no information reported on the biological and chemical properties of *Eugenia winzerlengii* (NAPRALERT Database).

In conclusion, in view of the nematicidal properties exhibited by the ethanolic extract of *Eugenia winzerlingii* leaf, it is advisable to further study this plant. These studies should be performed at greenhouse and field stages to evaluate its effectiveness against nematodes in soil, and based on the results obtained, the plant could be recommended for use in agriculture in the future. Therefore, phytochemical studies to isolate and purify the active principles of *Eugenia winzerlingii* leaf are in progress. The present work constitutes the first report on the nematicidal properties of plant species from the Yucatecan flora.

ACKNOWLEDGMENTS

We thank Filogonio May-Pat (Centro de Investigación Científica de Yucatán) for plant material collection and identifica-

tion; the technical assistance of Rita Arzápalo-Marín, Laura Pérez-Marrufo, and William Mena-Sierra (Instituto Tecnógico de Conkal) is greatly appreciated. This research was supported by Fundación Produce Yucatán (project No. 0704). We are also thankful to NAPRALERT database (University of Illinois at Chicago, Chicago, USA) for providing information on the plant material used in this study.

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Received: *Accepted for Publication:*
6/II/2006 *10/III/2006*
Recibido: *Aceptado para Publicación:*

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