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Effect of chestnut tannins on the root-knot nematode *Meloidogyne javanica*

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Summary

Among the natural products extracted from plants, tannins have been reported to possess antihelmintic properties especially for gastrointestinal nematodes in ruminants. Also, they are toxic to a wide range of fungi, bacteria and yeasts. Therefore, an *in vitro* and a glasshouse pot experiments were undertaken to evaluate the effect of chestnut tannins on the control of the root-knot nematode *Meloidogyne javanica*. In the *in vitro* experiment, different tannin concentrations in a geometric scale (from 0.32 to 20.48 g/l), were tested for their effect on hatching of the nematode, whereas in the pot experiment, 100, 250 and 450 g/m^2 of tannins in aqueous solutions, were used in pots at transplant or at transplant and two weeks later for their effect on nematode control. In both experiments treatments were compared to untreated and fenamiphos-treated controls. *In vitro* a nematostatic effect of tannins was observed, whereas in the pot experiment a significant reduction of eggs and juveniles/g root, total population density and reproduction rates of the nematode were recorded. The anatomical changes induced by *M. javanica* in tomato roots treated with tannins did not differ from those produced by this and other *Meloidogyne* species on various hosts reported earlier.

Keywords: *Meloidogyne javanica*; nematode control; chestnut tannins

Introduction

The recent European Legislations (Reg. CE 396/2005; 1095/2007; 33/2008, 299/2008 and 1107/2009) have deeply revised and restricted the use of pesticides on agricultural crops focusing the attention on environmental safety, human and animal health. Plant protection from phytoparasitic nematodes and soilborne pathogens should therefore rely on alternative control strategies that are both environmentally sound and economically sustainable.

During the last two decades, research on low environmental impact alternatives to chemicals has received a strong impulse. A wide range of options was considered, including agronomic strategies (green manures, amendments, crop rotations, biofumigations, mycorrhization, grafting, resistant cultivars) (Gamliel *et al*., 2000; Sasanelli *et al*., 2002; Nico *et al*., 2004; Castillo *et al*., 2006; Renčo *et al*., 2007; 2009), physical methods (soil solarization, steam, ozone treatments) (Sasanelli & Greco, 2000; Tamietti & Valentino, 2000; Tjamos *et al*., 2000; Ciccarese *et al*., 2008), the use of bio-pesticides such as biological control agents (mainly fungi and bacteria) (Vannacci & Gullino, 2000; Sasanelli *et al*., 2008); and biocidal plants belonging to different botanical families and/or their derived products (Gommers, 1981; Grainge & Ahmed, 1988; Sasanelli *et al*., 2007).

Focusing especially on plants, many products such as essences, essential oils and aqueous extracts, have been reported for their biocidal effect on fungi, weeds and bacteria (Kurita *et al*., 1981; Janssen *et al*., 1987; Müller-Riebau *et al*., 1995; Isman, 2000) as well as soil dwelling insects (McCaffrey *et al*., 1995; Elberson *et al*., 1996; Sharaby *et al*. 1997; Maistrello *et al*., 2001; 2003). Natural plant products could provide a potential alternative to synthetic chemicals also for the control of soilborne pathogens and parasites, such as phytoparasitic nematodes (Sasanelli, 1992; Sasanelli & D'Addabbo, 1993; Oka *et al*., 2000; Rodríguez-Kábana & Simmons, 2005).

Among the natural products extracted from plants, tannins have been reported in the literature to possess antihelmintic properties especially for gastrointestinal nematodes in ruminants and the role of condensed tannins in the antiparasitic activity seems to be strongly substantiated by results both *in vivo* and *in vitro* (Hukkeri *et al*., 1993; Lopez *et al*., 2005; Hoste *et al*., 2006). Tannins, with a high affinity to proteins and polysaccharides, are secondary plant polyphenols whose physical and chemical properties

can change according to the plants, parts of the plants and the season in which they are produced (Waterman, 1999; Waghorn & McNabb, 2003). Moreover, tannins protect several plants against herbivores (Feeny, 1976) and they are toxic to a wide range of fungi, bacteria and yeasts (Scalbert, 1991).

Very little information is available on the effect of tannins on free-living or phytoparasitic nematodes (Taylor & Murant, 1966; Saly, 1989; Hewlett *et al*., 1997), although the plant pests can cause severe damages to many agricultural crops (Seinhorst, 1965; 1979; Sasanelli, 1994).

The objective of the present work was to evaluate the effect of chestnut tannins on: i) the root-knot nematode *Meloidogyne javanica* (Treub) Chitw. both *in vitro* and in a pot experiment on tomato under controlled conditions and ii) the histopathological variations induced by the nematode attack.

Material and Methods

In vitro experiment

The tannins (SAVIOTAN®, Nuova Rivart, Radicofani, Siena Province, Italy) were extracted by vapour from chestnut wood, without chemical solvents, in powder form after dehydration. Technical data and chemical composition of the used product are reported in Table 1.

Table 1. Chemical composition and characteristics of Saviotan®

Characteristic	Unit measure	% content	Analytic Method (UNI 4632)
Density Baumè	°Bè / 15 °C	27	NR LAB 006
Tannin	% w/w	75	NR LAB 001-002
Non tannin	% w/w	18	NR LAB 001-002
Non soluble	% w/w	1	NR LAB 003
Water	% w/w	6	NR LAB 001-002
Dry content	% w/w	94	NR LAB 007
Tannin/Non tannin rate	---	4.1	Calculation
pH (solutions 6.9 °Bè)	---	3.5	NR LAB 004
Setting materials (solutions 6.9 °Bè)	% v/v	1	NR LAB 008

Different concentrations from 0.32 to 20.48 g/l, in a geometric series, were obtained by dissolving the largest rate of tannin in distilled water (Table 2).

An Italian population of *M. javanica* (Treub) Chitwood, from Torchiarolo (Brindisi Province), southern Italy, was reared for two months on tomato (*Solanum lycopersicum* L.) cv. Rutgers in a glasshouse at 25 ± 2 °C. Batches with fifty egg masses of *M. javanica* (averaging 10,000 eggs per batch) were prepared by collecting egg masses from infected tomato roots. The batches were placed on 2 cm diameter sieves (215 µm aperture) in a 3.5 cm diameter Petri dish. Three ml of each test solution, sufficient to cover egg masses, were then added to four batches of egg masses. Distilled water and an aqueous solution containing 5 µg/ml fenamiphos were used as controls (Greco & Thomason, 1980). The dishes were arranged in a completely randomised design with four replicates per treatment and incubated in a growth cabinet at 20 ± 2 °C (Ekanayake & Di Vito, 1984).

Every week, emerged juveniles were counted and removed, and the hatching solutions renewed. Overall, the test lasted twelve weeks. After the first two weeks, the egg masses were removed from the tannin and fenamiphos solutions and the incubation continued for ten more weeks in distilled water only, according to an already described methodology (Sasanelli & Di Vito, 1991; Sasanelli & D'Addabbo, 1992).

At the end of the experiment, the egg masses were shaken for 3 min in a 1 % sodium hypochlorite aqueous solution (Hussey & Barker, 1973) and the unhatched eggs were counted. Numbers of juveniles emerging weekly were expressed as cumulative percent of the total initial population (hatched + unhatched eggs).

Data were subjected to analysis of variance (ANOVA), after arcsin square root transformation (Bliss' Tables), and means were compared by Least Significant Difference's Test. All statistical analysis were performed using the PlotIT program.

Glasshouse experiment

The same population of *M. javanica* used in the *in vitro* experiment was reared for two months on tomato cv. Rutgers in a glasshouse at 25 ± 2 °C. When large mature egg masses were formed, tomato roots were finely chopped and eggs and juveniles were quantified by processing 10 root samples of 5 g each with 1 % aqueous solution of NaOCl (Hussey & Barker, 1973). The roots were then thoroughly mixed with 3 kg of steam sterilised sandy soil (pH 7.2; sand > 99 %; silt < 1 %; clay < 1 % and organic matter = 0.75 %) and used as inoculum. Appropriate amounts of this inoculum were thoroughly mixed with the steam sterilised sandy soil in each clay pot containing 1,000 ml soil to give

Table 2. Effect of different aqueous concentrations of tannins on hatching of the root-knot nematode *Meloidogyne javanica*

Treatment	Cumulative percentage of juveniles emerging weekly																					
	Incubation period (weeks)																					
	In test solutions				In distilled water																	
	1		2		3		4		5		6		7		8		9		10		11		12	
Tannin 0.32 g/l	8.3*	c**	11.2	c	23.8	cd	36.3	ef	46.1	cde	51.3	de	53.6	c	54.1	bc	54.4	bc	54.4	bc	54.4	bc	54.4	b
Tannin 0.64 g/l	3.1	b	12.2	c	25.4	cde	36.8	ef	48.2	de	51.1	de	52.6	bc	53.6	bc	54.3	bc	54.7	bc	54.8	bc	54.8	b
Tannin 1.28 g/l	0.7	a	2.4	b	17.4	c	28.9	de	41.7	cd	50.4	de	55.0	c	56.5	bc	57.1	bc	57.3	bc	57.4	bc	57.4	b
Tannin 2.56 g/l	0.2	a	2.0	b	26.7	de	45.9	f	57.2	e	64.9	e	72.5	d	75.3	d	76.7	d	77.3	d	77.4	dc	77.4	c
Tannin 5.12 g/l	0.1	a	0.1	ab	6.4	b	21.0	cd	33.5	c	43.0	cd	55.4	c	61.6	cd	64.9	cd	67.0	cd	67.2	cd	67.3	bc
Tannin 10.24 g/l	0.1	a	0.1	ab	3.7	ab	14.3	bc	19.8	b	26.9	b	37.7	b	42.3	b	45.5	b	50.2	b	51.9	b	52.1	b
Tannin 20.48 g/l	0.1	a	0.1	a	3.1	ab	9.6	b	18.7	b	32.5	bc	46.5	bc	53.0	bc	57.0	bc	61.8	bc	63.6	bc	63.8	b
Fenamiphos 5 mg/l	0.4	a	0.5	ab	1.0	a	2.0	a	3.1	a	4.6	a	5.2	a	5.4	a	5.9	a	6.4	a	6.5	a	6.5	a
Control	7.8	bc	21.2	d	36.9	e	49.8	f	55.1	de	56.1	de	56.6	c	56.8	bc	56.8	bc	56.9	bc	56.9	bc	56.9	b

* Each value is an average of 4 replications;

** Data followed by the same letters in each column are not statistically different according to Least Significant Difference's Test (P=0.05).

a nematode population density of 5 eggs and juveniles/cm^3 soil (*Pi*).

The pots were arranged on benches in a glasshouse at 25 ± 2 °C in a randomized block design with 10 replicates for each treatment. Three tannin concentrations were considered: i) 100 g/m^2; ii) 250 g/m^2 and iii) 450 g/m^2, applied only at transplanting or at transplanting and two weeks later, for a total of six tannin treatments (Table 3). Nematode-infested untreated soil and fenamiphos treated soil were used as controls. In addition, an un-infested and untreated soil was considered. Fenamiphos was applied one day before transplanting at the rate of 62.5 l/ha. Tannins were applied as aqueous solutions (400 ml/pot), calibrated on the water holding capacity of the soil.

After all treatments had been performed (for treatments 2, 4, 6 after the first session of treatments), in each pot a one-month-old tomato seedling cv. S. Marzano was transplanted.

During the experiment tomato plants were maintained in the glasshouse randomizing the position of the blocks and at the same time repositioning each plant within a block every week, to avoid a block position effect and at the same time the factor position of the plant within the block. Plants received all the necessary maintenance (irrigation, fertilization, etc.).

At the end of the experiment, two months later, plants were uprooted and height, fresh and dry top and root weight were recorded. Root gall index (GI) was estimated according to a 0 – 5 scale, where 0 = no galls; 1 = 1 – 2 galls; 2 = 3 – 10 galls; 3 = 11 – 30 galls; 4 = 31 – 100 galls and 5 > 100 galls (Taylor & Sasser, 1978).

Soil nematode population density in each pot was determined by processing 500 cm^3 soil by the Coolen's method (Coolen, 1979). Numbers of *M. javanica* eggs and second stage juveniles in roots were assessed by cutting up each root system into small pieces and further comminuting them in a blender, containing 1 % aqueous solution of NaOCl for 20 sec (Marull & Pinochet, 1991). The water suspension was then sieved through a 250 μm pore sieve put over a 5 μm pore sieve. Nematodes and root debris gathered on the 5 μm pore sieve were further processed by centrifuging at 2,000 rpm for five min in 500 ml of a magnesium sulphate solution of 1.16 specific gravity. Then eggs and juveniles in the water suspension were sieved through the 5 μm pore sieve, sprayed with tap water to wash away the magnesium sulphate solution and collected in about 30 – 40 ml water. Then they were counted and final nematode population density (*Pf*) in each pot was determined by summing nematodes recovered from soil and roots. The nematode reproduction factor *r* was expressed as ratio between final and initial population density (*Pf*/*Pi*) of *M. javanica*.

Data from the experiment were subjected to analysis of variance (ANOVA) and means compared by Least Significant Difference's Test. All statistical analysis were performed using the PlotIT program.

Histopathology

Galled roots from *M. javanica* infected tomato plants were used for histopathological studies. Roots were gently washed free of adhering soil and debris, and individual galls were selected together with root segments of un-infected plants. Galled and healthy root tissues were fixed in formaldehyde chromo-acetic solution for 48 h, dehydrated in a tertiary butyl alcohol series (40 – 70 – 85 – 90 – 100 %) and embedded in 58 °C melting-point paraffin. Embedded tissues were sectioned with a rotary microtome. Sections 10 – 12 μm thick were mounted on glass slides, stained with safranin and fast-green, mounted in dammar xylene and examined microscopically (Johansen, 1940).

Results

In vitro experiment

During the first two weeks, emergence of juveniles from egg masses was suppressed in all the aqueous solutions of tannin and fenamiphos (Table 2). All treatments with tannin in the range 1.28 – 20.48 g/l did not significantly differ from fenamiphos. Only at the concentrations of 0.32 and 0.64 g/l tannin the emergence of juveniles was significantly larger ($P = 0.05$) than that in fenamiphos, but less than that in distilled water (control) (Table 2).

When aqueous extracts of tannin and fenamiphos were removed and the incubation continued in distilled water, egg hatch of egg masses previously incubated in tannin solutions resumed, however, during the following three weeks for the egg masses which had been incubated in tannin at 5.12 – 20.48 g/l, hatching remained still lower (18.7 – 33.5 %) than the untreated control (Table 2), where more than 50 % of the egg masses hatched. Emergence of juveniles in tannin at 10.24 g/l was significantly lower than that in the control until the seventh week (Table 2). At the end of the incubation period (twelve weeks), the final cumulative hatch percentages in all tannin solutions did not differ from that in distilled water, except in aqueous solution at 2.56 g/l, in which it resulted significantly higher.

Emergence of juveniles in the fenamiphos solution did not resume after transferring the egg masses in water and a final cumulative emergence of only 6.5 % was recorded.

Glasshouse experiment

All treatments with tannin did not significantly ($P = 0.01$) increase tomato plants growth variables compared with the untreated control (Table 3). However, treatments with the highest doses of tannin (450 and 250 g/m^2) applied at transplant and again 2 weeks later, significantly reduced root gall index in comparison to inoculated and untreated control, similarly to the treatment with fenamiphos (Table 4).

The remaining treatments with aqueous solutions of tannin (100 and 250 g/m^2) applied only at transplant, did not suppress nematode GI on the roots compared to untreated control, which resulted also significantly higher than that recorded in fenamiphos treated plants (Table 4).

Table 3. Effect of different concentrations of aqueous solutions of tannins on the growth of tomato plants (cv. S. Marzano) in soil inoculated with 5 eggs and juveniles/cm^3 of *Meloidogyne javanica*

Treatment	Dose g/m^2	Application time	Top weight (g)						Height (cm)			Root weight (g)		
			fresh			dry								
Tannin	100	At transplant	91.5*	a**	A	8.9	a	A	94.2	a	A	22.6	ab	A
Tannin	100	At transplant and 2 weeks later	127.8	a	A	13.0	a	A	101.0	a	A	30.5	b	A
Tannin	250	At transplant	96.3	a	A	9.4	a	A	93.5	a	A	24.2	ab	A
Tannin	250	At transplant and 2 weeks later	92.8	a	A	9.7	a	A	90.7	a	A	23.3	ab	A
Tannin	450	At transplant	111.4	a	A	11.1	a	A	91.0	a	A	29.0	ab	A
Tannin	450	At transplant and 2 weeks later	98.7	a	A	9.7	a	A	91.8	a	A	19.2	ab	A
Fenamiphos in liquid formulation 240 EC	1.2 ml/m^2	At transplant	92.5	a	A	9.0	a	A	96.7	a	A	16.3	a	A
Control (inoculated and untreated)	--		89.6	a	A	9.4	a	A	86.8	a	A	26.0	ab	A
Control (non inoculated and untreated)	--		153.9	--	--	17.2	--	--	86.5	--	--	44.3	--	--

* Each value is an average of 10 replications;

** Data followed by the same letters in each column are not statistically different according to Least Significant Difference's Test (small letters for P=0.05; capital letters for P=0.01).

Table 4. Effects of different concentrations of aqueous solutions of tannins on the root-knot nematode *Meloidogyne javanica*

Treatment	Dose (g/m^2)	Application time	Root gall index (0-5)			Eggs and juveniles /g root			Final population/cm^3 soil (from roots and soil)			Reproduction rate *r = Pf/Pi*		
Tannin	100	At transplant	3.8[1]	cd[2]	CD	10.098	c	D	290	bc	BC	58	bc	BC
Tannin	100	At transplant and 2 weeks later	3.0	bc	ABCD	9.866	c	CD	381	c	C	76	c	C
Tannin	250	At transplant	3.7	cd	BCD	7.715	bc	BCD	248	bc	ABC	50	bc	ABC
Tannin	250	At transplant and 2 weeks later	2.7	ab	ABC	4.896	ab	ABC	151	ab	AB	30	ab	AB
Tannin	450	At transplant	2.7	ab	ABC	4.469	ab	AB	179	ab	ABC	36	ab	ABC
Tannin	450	At transplant and 2 weeks later	2.5	ab	AB	5.271	ab	ABCD	149	ab	AB	30	ab	AB
Fenamiphos in liquid formulation 240 EC	1.2 ml/m^2	At transplant	1.8	a	A	2.482	a	A	55	a	A	11	a	A
Control (inoculated and untreated)		--	4.0	d	D	16.196	d	E	594	d	D	119	d	D
Control (non inoculated and untreated)		--	0.0	--	--	0	--	--	0	--	--	--	--	--

* Each value is an average of 10 replications;

** Data followed by the same letters in each column are not statistically different according to Least Significant Difference's Test (small letters for P=0.05; capital letters for P=0.01).

The number of eggs and juveniles of the nematode per gram of root in the inoculated untreated plants was significantly higher than that observed in all other treatments (P = 0.01) (Table 4).
In plants treated with the highest doses of tannins, the number of eggs and juveniles per gram of root was not significantly different from that recorded in the fenamiphos treatment, whereas significant differences (P = 0.05) were observed in comparison to treatments with the lowest dose (100 g/m^2) applied at transplant and two weeks later (Table 4).
The treatments with medium and highest doses of tannins and fenamiphos significantly (P = 0.01) reduced *M. javanica* final population density (*Pf*) in the soil (eggs and juveniles/cm^3 soil) in comparison to untreated control. For this parameter, a significant difference was observed between fenamiphos and the lowest dose of tannin.
In the untreated control, nematode reproduction rate (r = *Pf*/*Pi*) was significantly larger than that of all other treatments (P = 0.01) (Table 4). With the exception of the lowest dose (100 g/m^2) (P = 0.01), tannin treatments, applied at transplant or at transplant and two weeks later, were not significantly different from fenamiphos (Table 4).

Histopathology
Root galls induced by *M. javanica* on tannin treated tomato roots varied in size and shape and occurred on both the main and lateral roots. Usually, galls contained more than one female. Observation of stained root sections revealed tissue hypertrophy and hyperplasia as well as disorganization and disruption of xylem elements and primary phloem cells. The nematode induced permanent feeding sites adjacent to vascular tissues consisting of groups of two to five large, multinucleate giant cells. Active multinucleated giant cells showed characteristic dense cytoplasm and numerous hypertrophied nuclei and nucleoli. Additionally, hyperplasia of tissues adjacent to the giant cells caused the root tissue expansion, leading to the formation of the observed root galling. The anatomical changes induced by *M. javanica* in tomato roots treated with tannins did not differ from those produced by this and other *Meloidogyne* species on various hosts reported earlier (Huang, 1985) (Fig. 1).

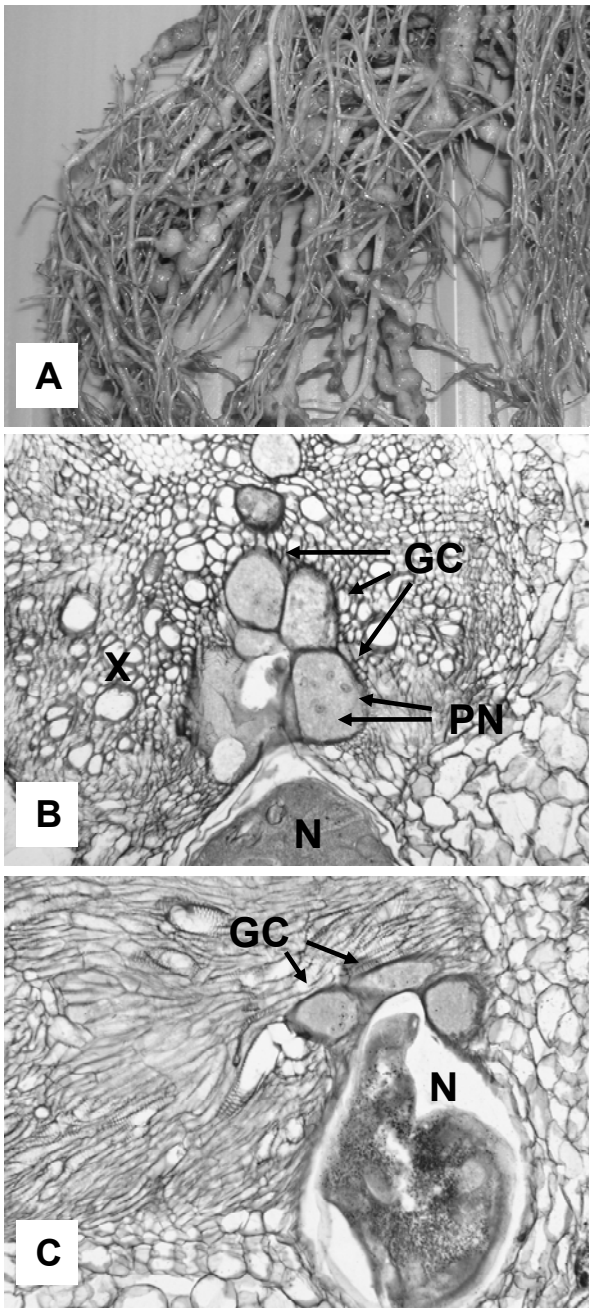

Fig. 1. Host-parasite relationship between *Meloidogyne javanica* and tomato plants treated with tannin solutions. Nematode infected tomato root (A). Transverve (B) and longitudinal sections (C) of root infested by *M.javanica*. GC = giant cells; PN = hypertrophic nucleous; N = nematode; and X = compressed xylem.

Discussion and conclusions

The *in vitro* experiment showed that tannin solutions greatly reduced nematode egg hatch. Between the third and the seventh week, the cumulative egg hatch percentage was gradually reduced as the applied concentrations increased. Probably a longer exposure to the tannin solutions (for more than 2 weeks) could have shown a nematicidal effect of these solutions. Hewlett *et al*. (1997) showed that tannic acid is an attractant for the root-knot nematodes *M. arenaria* (Neal) Chitw. and *M. incognita* (Kofoid & White) Chitw., whereas it is repellent for *Radophulus similis* (Cobb) Thorne and without any effect on *Heterodera glycines* Ichinohe. Few attractants or repellents for *M. javanica* have been identified (Bird, 1959; 1960; Oteifa & Elgindi, 1961). Application of attractants or repellents at planting or before transplanting may serve to disorientate phytoparasitic nematodes causing them difficulties in locating the root systems and potentially reducing plant damage. Therefore, the observed nematostatic effect of aqueous solutions of tannins together with the combined characteristics of attractants might represent a possible control tactics for plant-parasitic nematodes, alternative to the repeated use of chemicals.
Results from the glasshouse experiment seem to confirm this hypothesis. In the pot experiment all doses of tannin

(100, 250 and 450 g/m^2) reduced the number of eggs and juveniles/g root, the final population/cm^3 soil and the reproduction rate in comparison to infested untreated control. In particular, a significant reduction of the nematode gall index on the roots was obtained by the use of tannins at 250 g/m^2 applied two times, at transplant and two weeks later, demonstrating the protective and positive effect of repeated treatments. However, the lowest dose (100 g/m^2) of tannin was not sufficient to contain the initial nematode attack on the roots. Also in this case, there might be a close association of the level of this compound with nematode invasion. Tannin deposition occurred in parenchyma and endodermis cells of alfalfa roots invaded by *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven (Townsend *et al*., 1989; Zunke, 1990) and in banana roots infected by *R. similis* (Valette *et al*., 1997). The phenols content in roots is associated with the defence strategy of plants to nematode infection. Tannins are compounds that could be involved in the passive defence of the plant as chemical barriers to the invasion of the parasite in the roots and they might increase host resistance to nematode infection (Taylor & Murant, 1966). It is necessary to consider that the nematicidal effect of various organic amendments and in particular of grape pomace against *Meloidogyne* spp. may be related also to the release of compounds such as polyphenols and tannins from the berry epidermis in which the tannin content can vary from 0.4 to 3 % (D'Addabbo *et al*., 2000; Flanzy, 2000).

In conclusion, the use of tannins appears promising for the control of plant-parasitic nematodes in sustainable agriculture. However, further studies are suggested to investigate the effect of tannins in different types of soils and on different nematode species.

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References

BIRD, A. F. (1959): The attractiveness of roots to the plant-parasitic nematodes *Meloidogyne javanica* and *M. hapla*. *Nematologica*, 4: 322 – 335

BIRD, A. F. (1960): Additional notes on the attractiveness of roots to plant-parasitic nematodes. *Nematologica*, 5: 217

CASTILLO, P., NICO, A. I., AZCON-AGUILAR, C., DEL RIO RINCON, C., CALVET, C., JIMENEZ-DIAZ, R. M. (2006): Protection of olive planting stocks against parasitism of root-knot nematodes by arbuscular mycorrhizal fungi. *Plant Pathol.*, 55: 705 – 713. DOI**:** 10.1111/j.1365-3059. 2006.01400.x

CICCARESE, F., PACIOLLA, C., SASANELLI, N., CICCARESE, A., DE LEONARDIS, S., SCIACOVELLI, M. (2008): Can the ozone be used in the control of plant diseases? In *Abstracts of 10th Annual Congress FISV (Federazione Italiana Scienza della Vita), September 24 – 27, 2008*. Riva del Garda, Italy, pp. 18

COOLEN, W. A. (1979): Methods for the extraction of *Meloidogyne* spp. and other nematodes from roots and soil. In: LAMBERTI, F., TAYLOR, C. E. (Eds.) *Root-knot nematodes* (Meloidogyne *species*), *Systematics, Biology and Control.* London, UK: Academic Press, pp. 317 – 329

D'ADDABBO, T., SASANELLI, N., LAMBERTI, F., CARELLA, A. (2000): Control of root-knot nematodes by olive and grape pomace soil amendments. *Acta Hortic.*, 532: 53 – 57

ELBERSON, L. R., BOREK, V., MCCAFFREY, J. P., MORRA, M. J. (1996): Toxicity of rapeseed meal-amended soil to wireworms, *Limonius californicus* (Coleoptera: Elateridae). *J. Agr. Entomol.,* 13: 323 – 330

EKANAYAKE, H. R. M. K., DI VITO, M. (1984): Influence of root leachates and temperatures on egg hatch of *Meloidogyne* species. *Nematol. Mediterr.*, 12: 119 – 127

FEENY, P. (1976): Plant apparency and chemical defence. *Recent Adv. Phytochem.*, 10: 1 – 40

FLANZY, C. (2000): *Enología: Fundamentos científicos y tecnológicos*. Madrid, España, Mundi-Prensa (Eds), 389 pp.

GAMLIEL, A., AUSTERWEIL, M., KRITZMAN, G. (2000): Non-chemical approach to soilborne pest management-organic amendments. *Crop Prot.*, 19, 847 – 853. DOI: 10.1016/S0261-2194(00)00112-5

GOMMERS, F. J. (1981): Biochemical interactions between nematodes and plants and their relevance to control. *Helminthol. Abst. Series B, Plant Nematol.*, 50: 9-24

GRAINGE, M., AHMED, S. (1988): *Handbook of Plants with Pest-Control Properties*. 1th Edition, New York, WILEY J. & SONS (Eds.). 470 pp.

GRECO, N., THOMASON, I. J. (1980): Effect of fenamiphos on *Heterodera schachtii* and *Meloidogyne javanica*. *J. Nematol.*, 12: 91 – 96

HEWLETT, T. E., HEWLETT, E. M., DICKSON, D. W. (1999): Response of *Meloidogyne* spp., *Heterodera glycines* and *Radopholus similis* to Tannic Acid. *J. Nematol*, 29: 737 – 741

HOSTE, H., JACKSON, F., ATHANASIADOU, S., THAMSBORG, S. M., HOSKIN, O. S. (2006): The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol.*, 22: 253 – 261. DOI: 10.1016/j.pt.2006.04.004

HUANG, C. S. (1985): Formation, anatomy and physiology of giant cells induced by root-knot nematodes. In: SASSER J. N. & CARTER C. C. (Eds) *An advanced treatise on Meloidogyne*, *Biology and Control*, Vol. I. Raleigh, U.S.A.: North Carolina State University Graphics, pp. 155 – 164

HUKKERI, V. I., KALYANI, G. A., HATPAKI, B. C., MARVI, V. F. (1993): *In vitro* antihelmintic activity of aqueous extracts of fruit rind of *Punica granatum*. *Fitoterapia*, 64: 69 – 70

HUSSEY, R.S., BARKER, K.S. (1973): A comparison of

methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Reptr*., 57: 1025 – 1028

ISMAN, M. B. (2000): Plant essential oils for pest and disease management. *Crop Prot.*, 19: 603 – 608

JANSSEN, A. M., SHEFFER, J. J. C., BAERHEIM SVENDSEN, A. (1987): Antimicrobial activity of essential oils: a 1976-1986 literature review: Aspects of the test methods. *Planta Med*., 53: 395 – 398

JOHANSEN, D. A. (1940): Plant Microtechnique. McGraw-Hill Book Co., New York, NY (U.S.A.), 523 pp.

KURITA, N., MIYAJI, M., KURANE, R., TAKAHARA, Y. (1981): Antifungal activity of components of essential oils. *Agr. Biol. Chem.*, 45: 945 – 952

LOPEZ, J., IBARRA, O. F., CANTO', G. J., VASQUEZ, C. G., TEJADA, Z. I., SHIMADA, A. (2005): *In vitro* effect of condensed tannins from tropical fodder crops against eggs and larvae of the nematode *Haemonchus contortus*. *Int. J. Food, Agric. & Environ.*, 3(2): 191 – 194

MAISTRELLO, L., HENDERSON, G., LAINE, R. A. (2001): Efficacy of vetiver oil and nootkatone as soil barriers against *Formosan subterranean* termite (Isoptera: *Rhinotermitidae*). *J. Econ. Entomol.*, 94: 1532 – 1537

MAISTRELLO, L., HENDERSON, G., LAINE, R.A. (2003): Comparative effects of vetiver oil, nootkatone and disodium octaborate tetrahydrate on *Coptotermes formosanus* and its symbiotic fauna. *Pest Manag. Sci.,* 59: 58 – 68. DOI: 10.1002/ps.601

MARULL, J., PINOCHET, J. (1991): Host suitability of *Prunus* rootstock to four *Meloidogyne* species and *Pratylenchus vulnus* in Spain. *Nematropica*, 21:185 – 195

MCCAFFREY, J. P., WILLIAMS, L., BOREK, V., BROWN P. D., MORRA, M. J. (1995): Toxicity of ionic thiocyanate-amended soil to the wireworm *Limonius californicus* (Coleoptera, *Elateridae*). *J. Econ. Entomol,* 88: 793 – 797

MÜLLER-RIEBAU, F., BERGER, B., YEGEN, O. (1995): Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *J. Agr. Food Chem*., 43(8): 2262 – 2266

NICO, A. I., JIMÉNEZ-DÌAZ, R. M., CASTILLO, P. (2004): Control of root-knot by composted agro-industrial wastes in potting mixtures. *Crop Prot.*, 23: 581 – 587. DOI: 10.1016/j.cropro.2003.11.005

OKA, Y., NACAR, S., PUTIEVSKY, E., RAVID, U., YANIV, Z., SPIEGEL, Y. (2000): Nematicidal activity of essential oils and their components against the root-knot nematodes. *Phytopathol.*, 90(7): 710 – 715

OTEIFA, B. A., ELGINDI, D. M. (1961): Physiological studies on host-parasite relationship on the root-knot nematode, *Meloidogyne javanica*. *Plant Dis. Reptr.*, 45: 928 – 929

RENČO, M., D'ADDABBO, T., SASANELLI, N., PAPAJOVA, I. (2007): The effect of five composts of different origin on the survival and reproduction of *Globodera rostochiensis*. *Nematology*, 9(4): 537 – 543. DOI: 10.1163/15685410778 1487260

RENČO, M., SASANELLI, N., SALAMUN, P. (2009): The effect of two compost soil amendments, based on municipal green and penicillin production wastes, on plant parasitic nematodes. *Helminthologia*, 46: 190 – 197. DOI: 10.2478/ s11687-009-0035-6

RODRÍGUEZ-KÁBANA, R., SIMMONS, L. (2005): Fungicidal, herbicidal, and nematicidal activities of essential oils in slow-release formulations. In: *Abstracts XXVII Annual Meeting of ONTA, October 17 – 21, 2005*. Viña del Mar, Chile, pp. 96

SALY, A. (1989): Seasonal dynamics of free-living nematodes in the rhizosphere of *Fagus sylvatica* in the Little Carpathians (Czechoslovakia). *Helminthologia,* 26: 73 – 83

SASANELLI, N., DI VITO, M. (1991): The effect of *Tagetes* spp. on the hatching of an Italian population of *Globodera rostochiensis*. *Nematol. Mediterr.*, 19: 135 – 137

SASANELLI, N. (1992): Nematicidal activity of aqueous extracts from leaves of *Ruta graveolens* on *Xiphinema index*. *Nematol. Mediterr.*, 20: 53 – 55

SASANELLI, N., D'ADDABBO, T. (1992): The effect of *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* extracts on the hatching of *Heterodera schachtii*. *Nematol. Mediterr.*, 20: 49 – 51

SASANELLI, N., D'ADDABBO, T. (1993): Effect of *Cineraria maritima*, *Ruta graveolens* and *Tagetes erecta* leaf and root extracts on Italian populations of *Meloidogyne* species. *Nematol. Mediterr.,* 21: 21 – 25

SASANELLI, N. (1994): Tables of nematode-pathogenicity. *Nematol. Mediterr.*, 22: 153 – 157

SASANELLI, N., GRECO, N. (2000): Formulation of a model to relate nematode populations with exposure times to a range of temperatures. *Acta Hortic.*, 532: 131 – 135

SASANELLI, N., D'ADDABBO, T., CONVRTINI, G., FERRI, D. (2002): Soil Phytoparasitic Nematodes Suppression and Changes of Chemical Properties Determined by Waste Residues from Olive Oil Extraction. In: *Proceedings of 12th ISCO Conference*, May 26 – 31, 2002. Beijing, China. Vol. III: 588 – 592

SASANELLI, N., ATTILA, A., D'ADDABBO, T., TAKACS, T. (2007): Nematicidal properties of leaf extracts of *Ruta graveolens* inoculated with arbuscular mycorrhizal fungi. *Russ. J. Nematol.*, 15: 65 – 73

SASANELLI N., CICCARESE F., PAPAJOVA I. (2008): *Aphanocladium album* by via sub-irrigation in the control of *Pyrenochaeta lycopersici* and *Meloidogyne incognita* on tomato in a plastic-house. *Helminthologia*, 45: 137 – 142. DOI: 10.2478/s11687-008-0027-y

SCALBERT, A. (1991): Antimicrobial properties of tannins. *Phytochem.*, 30: 3875 – 3883

SEINHORST, J. W. (1965): The relationship between nematode density and damage to plants. *Nematologica*, 11: 137 – 154

SEINHORST, J. W. (1979): Nematodes and growth of plants: formulation of the nematode-plant system. In: LAMBERTI F. & TAYLOR C.E. (Eds) *Root-knot nematodes* (Meloidogyne *species*) *Systematics, Biology and Control*. London, Academic Press, pp. 231 – 256

SHARABY, A., AMMAR, N. (1997): Biological activity of extracts of *Tephrosia nubica* (Boiss) Baker against *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufn.).

Tropenlandwirt, 98: 143 – 150
TAMIETTI, G., VALENTINO, G. (2000): Effectiveness of soil solarization against soil borne plant pathogens and weeds in Piedmont (Northern Italy). *Acta Hortic.*, 532: 151 – 156
TAYLOR, C. E., MURANT, A. F. (1966): Nematicidal activity of aqueous extracts from raspberry canes and roots. *Nematologica*, 12: 488 – 494
TAYLOR, A. L., SASSER, J. N. (1978): *Biology, Identification and Control of Root-knot Nematodes* (Meloidogyne *spp*.). Raleigh, North Carolina State University Graphic, N.C. (U.S.A.), 111 pp.
TJAMOS, E. C., POLYMNIA, P. A., TJAMOS, S. E. (2000): Implementation of soil solarization in Greece: Conclusions and suggestions. *Crop Protection*, 19: 843 – 846. DOI: 10.1016/S0261-2194(00)00132-0
TOWNSEND, J. L., STOBBS, L., CARTER, R. (1989): Ultrastructural pathology of cells affected by *Pratylenchus penetrans* in alfalfa roots. *J. Nematol.*, 21: 530 – 539
VALETTE, C., NICOLE, M., SARAH, J. L., BOISSEAU, M., BOHER, B., FARGETTE, M., GEIGER, J. P. (1997): Ultrastructure and cytochemistry of interactions between banana and the nematode *Radopholus similis*. *Fund. Appl. Nematol*., 21: 65 – 77
VANNACCI, G., GULLINO, M. L. (2000): Use of biological agents against soil borne pathogens: Results and limitations. *Acta Hortic.*, 532: 79 – 88
WAGHORN, G. C., MCNABB, W. C. (2003): Consequences of plant phenolic compounds for productivity and health of ruminants. *Proc. Nutr. Soc*., 62: 383 – 392
WATERMAN, P. G. (1999): The tannins – an overview. In: BROOKER J. D. (Eds) *Tannins in livestock and human nutrition*. Adelaide, Australian Centre for International Agricultural Research, Australia, pp. 10 – 13
ZUNKE, U. (1990): Observations on the invasion and endoparasitic behaviour of the root lesion nematode *Pratylenchus penetrans*. *J. Nematol*, 22: 309 – 320

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