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Nematicidal potential of materials from *Medicago* spp.

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Abstract The nematicidal effect of soil amendments with dry top and root material from *Medicago sativa* and/or *Medicago arborea* was evaluated on the root-knot nematode *Meloidogyne incognita* and on the cyst nematode *Globodera rostochiensis* in potting mixes. All amendments suppressed root and soil population densities of both nematode species compared to non-treated and chemical controls. The suppressiveness of *M. sativa* differed between top and root material and among the amendment rates. In field conditions soil amendments with 20 or 40 t ha^{-1} of a pelleted *M. sativa* meal increased tomato crop yield and reduced soil population densities and root galling by *M. incognita*. It is suggested that saponins were at least partly responsible for the nematicidal activity.

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Introduction

Root-knot nematodes (*Meloidogyne* spp.) occur worldwide and are responsible for a large part of the annual yield losses attributed to nematodes (Trudgill and Blok 2001). Potato cyst nematode, *Globodera rostochiensis*, is also a serious crop pathogen in the major potato growing areas of the world (Mai 1977). Environmental and health concerns are imposing the withdrawal of most nematicides and soil fumigants for the control of these nematodes leading to a search for alternative strategies, including the use of organic amendments (Ghorbani et al. 2008).

Organic amendments can be effective for the management of several soil-borne plant pathogens (Hoitink and Boehm 1999), and have a suppressive effect on phytoparasitic nematodes (Abawi and Widmer 2000). Biological and chemical mechanisms, either alone or in combination, were reported to play a role in nematode suppression by organic amendments (Akhtar and Malik 2000). Allelochemicals are often produced in large amounts in plant material or in agricultural wastes; the use of organic amendments is an effective means for the release of such compounds (Kokalis-Burelle and Rodriguez-Kabana 2006). A direct relationship between nitrogen of organic amendments and nematicidal activity through the release of toxic ammoniacal compounds was reported by Bailey and Lazarovits (2003).

Soil amendments with green or dry biomass from *Medicago* spp. may have some nematicidal potential since they contain high amounts of saponins which have antimicrobial, fungicidal and nematicidal activity (Argentieri et al. 2008; Avato et al. 2006). In addition, the high nitrogen content of *Medicago* plants may ensure the release of toxic nitrogen compounds and the development of a nematode-competitive, antagonistic or parasitic microflora (Gilpatrick 1969). In previous studies, soil incorporation of pelleted meal of *Medicago sativa* suppressed phytoparasitic nematode populations without affecting the free-living soil nematophauna (Walker 2007), and the dried plant material from *M. sativa* inhibited mycelial growth and reduced severity of symptoms of *Phytophthora* spp. (Demirci and Dolar 2006; Gilpatrick 1969). The nematicidal potential of tissues from *Medicago* spp. was demonstrated in *in vitro* experiments (Argentieri et al. 2008), but no comparative information is available on their suppression of plant parasitic nematode populations in soil.

Three glasshouse experiments were undertaken to evaluate the suppressive effect of soil amendments with plant material from *M. sativa* or *M. arborea* on the root-knot nematode *Meloidogyne incognita* and the potato cyst nematode *G. rostochiensis* in potting mixes. The aim of this investigation was to develop a feasible control technique, in order to evaluate these materials under field conditions.

Materials and methods

Plant material

Top and root material of *M. sativa* and top material of *M. arborea* for potting mixes was derived from plants previously grown at C.R.A.−FLC Centro di Ricerca per le Produzioni Foraggere e Lattiero Casearie, Lodi, Italy. Plant samples were collected, dried at 50°C and mechanically pulverised (1 mm). Pelleted *M. sativa* meal for the field experiment was obtained by processing dried and pulverised plant top material from a conventional alfalfa crop in a pellet mill.

Chemical analysis

All materials were characterised for their content of total nitrogen, carbon and phenolics (Table 1). Total nitrogen and carbon were measured according to the Dumas' method (Kristen 1983), whereas total phenolics content was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi 1965). The amount of saponins present in each plant sample was determined as reported in Tava et al. (1993). Saponins were also characterised by evaluating their aglycone composition by gas chromatography (GC) and GC/mass spectrometry (MS) of derivatised sapogenins as obtained after acid hydrolysis (Tava et al. 2005).

Pot experiments

Soil from the field experimental area was steam-sterilised (8 h at 100°C) and mixed with finely chopped tomato roots infested with *M. incognita* or with cysts of *G. rostochiensis* to provide an initial population density of 20 eggs and juveniles ml^{-1} soil of both species. The tomato roots were derived from plants artificially infested with the root-knot nematode and previously grown in the greenhouse, whereas cysts of *G. rostochiensis* were extracted from a naturally infested soil. Soil infested with *M. incognita*

Table 1 Total saponins, total phenolics, total nitrogen and total carbon of *Medicago* plant samples

Plant material	Total saponins* (% dw)	Total phenolics[1] (mg g^{-1} dw)	Total N (% dw)	Total C (% dw)
M. sativa tops	2.1±0.3	8.5±0.3	29.2	42.5
M. sativa roots	1.9±0.2	2.9±0.1	12.7	43.2
M. sativa pellet	0.8±0.3	7.3±0.4	15.2	45.6
M. arborea tops	2.2±0.4	5.1±0.5	19.5	40.4

*Each value was obtained by calculating the average of three determinations± standard deviation. [1]Expressed as gallic acid equivalent.

was added with dry top material of *M. sativa* or *M. arborea* in the first experiment, or with dry top or root material of *M. sativa* in the second experiment, at rates of 5 g kg^{-1}, 10 g kg^{-1}, 20 g kg^{-1}, and 40 g kg^{-1} soil. In the third experiment, soil infested with *M. incognita* or *G. rostochiensis* was mixed with dry top material of *M. sativa* at the same rates plus an additional 30 g kg^{-1} soil treatment. Mixtures were poured into 1.2 l clay pots arranged in a randomised block design, five replicates per treatment, on a greenhouse bench. Soil treated with granular fenamiphos, broadcast on the soil surface at amounts corresponding to a 15 kg a.i. ha^{-1} field rate 3 days before transplanting, and non-amended soil, either non-infested or infested by the nematode species, were used as controls. Three weeks after amendment incorporation, a 1 month-old tomato (*Solanum lycopersicum*) cv. San Marzano seedling was transplanted in to soil infested with *M. incognita*, whereas a potato (cv. Spunta) tuber was placed in soil infested with *G. rostochiensis*. Plants were maintained in the greenhouse at a constant temperature of 25±2°C and 20±2°C, for *M. incognita* and *G. rostochiensis* infested pots, respectively. After 2 months, plants were uprooted and plant height and fresh top and root weight recorded in the first two experiments. In all experiments galling caused by *M. incognita* was evaluated on each tomato root according to a 0–5 scale (0=no galls, 1=1–2 galls, 2=3–10 galls, 3=11–30 galls, 4=31–100 galls and 5>100 galls) (Taylor and Sasser 1978). Final population density of *M. incognita* in each pot was determined by processing each tomato root using the sodium hypochlorite method (Hussey and Barker 1973) and extracting nematodes from 500 cm^3 soil by the Coolen's method (Coolen 1979) and then counting eggs and juveniles. Due to the large growth effects of amendments, data of eggs and juveniles on tomato roots were presented on a per plant basis rather than per gram of roots. A 100 g soil sample was taken from the pots infested with *G. rostochiensis* and nematode cysts were extracted by the Fenwick can procedure and then crushed to count the number of viable eggs and juveniles. In the third experiment, percent mortality of *M. incognita* and *G. rostochiensis* was also calculated according to the Abbott's formula (Finney 1978), where $m=100\times(1-Pt/Pc)$, in which: m = % nematode mortality; Pt = final nematode population in treated soil; Pc = final nematode population in non-treated soil.

Field experiment

The trial was carried out on a sandy-silty soil (64.4% sand, 18.7% silt, 16.9% clay, 0.8% organic matter, pH7.5) almost uniformly infested by *M. incognita* (1.6 eggs and juveniles ml^{-1} soil) at Monteroni (Lecce province, southern Italy). The field was divided in 10 m^2 (5×2 m) plots, spaced 1 m apart and arranged in a randomised block design with five replicates per treatment (Table 3). Experimental treatments were the pelleted *M. sativa* meal at 20 or 40 t ha^{-1}rates, 30 l ha^{-1} of an industrial formulate of quillay (*Quillaja saponaria*) aqueous extracts recommended for nematicidal treatments in organic agriculture, and fenamiphos emulsifiable concentrate at 7 kg a.i. ha^{-1}, i.e. the field rate normally used by local farmers. Non-treated plots were used as the control. Pelleted *M. sativa* meal was uniformly distributed on the plot surface and then incorporated into the soil by rotavation 2 weeks before transplanting, whereas the quillay formulation and fenamiphos were both applied by fertirrigation in a 10 l m^{-2} water volume 1 day and 1 week before transplanting, respectively. On 18 July 1 month-old tomato seedlings cv. Faino were transplanted in the plots at a distance of 0.60 m in the row and 1 m between rows (1.7 plants m^{-2}). All plots received standard maintenance. Tomatoes were harvested weekly, from 28 September to 23 October 2006, and plot yields recorded. On 28 October the galling index was estimated on all plants in each plot, according to the same 0–5 scale applied in pot experiments. A composite 40-core soil sample was collected with a soil probe (1.5 cm diam and 30 cm long) in the central m^2 of each plot, before transplanting (30 June 2006) and after crop harvest (7 November 2006). Eggs and juveniles were extracted from 500 cm^3 aliquots by Coolen's method and counted.

Statistical analysis

Nematode data from the first and second experiment in pots and from the field trial were L*n* (x+1) transformed and mortality data from the third pot experiment arcsin-transformed before statistical analysis, due to homogenisation of error variances. All data were subjected to a one-way or factorial analysis of variance or to Student's *t* test and

treatment means were compared using Fisher's Least Significant Difference pairwise procedure at $P \leq 0.05$.

Results

Chemical analysis

High amounts of phenolics and total nitrogen were detected in all plant materials but *M. sativa* roots (Table 1). Total content of saponins averaged 2% dw in plant materials for potting mixes and 0.8% dw in the pelleted *M. sativa* meal. Relative content of saponins, considered as their aglycones after acid hydrolyses of the corresponding glycosides, differed among the different plant materials (Table 2). Saponins from *M. sativa* tops were characterised by a high amount of medicagenic acid and zanhic acid in a 2:1 ratio (38.7 vs 20.6, respectively). The same trend was also observed for the pelleted *M. sativa* meal, in which medicagenic and zanhic acids accounted for 40.2 and 19.8%, respectively. In contrast the saponins in the *M. sativa* roots were almost exclusively medicagenic acid glycosides (medicagenic acid 65.9%), with a low content of zanhic acid saponins (5.1%) but a relatively higher amount of hederagenin (7.9%) glycosides if compared to the other plant material (Table 2). Medicagenic acid and zanhic acid were also the main aglycones from saponins of *M. arborea* tops, 31.7% and 28.9%, respectively, although bayogenin was also found in higher amounts (14.1%) compared to the other materials.

Pot experiments

In the first trial tomato plant growth was significantly increased by both *M. sativa* and *M. arborea* amendments compared to the infested control, and the highest rate of *M. arborea* top material resulted in significantly heavier tomato plants in comparison with the non-infested and the fenamiphos-treated soil (Table 3). Root systems of the plants in soil amended with both materials at rates up to 20 g kg^{-1} soil developed normally and had only small galls on the lateral roots, whereas the roots from the infested control soil were severely deformed (Fig. 1). The highest rate of the amendment caused some phytotoxicity on tomato roots and the weight was not statistically different from the infested control. Numbers of eggs and juveniles either on tomato roots or in soil and formation of galls on plant roots were significantly reduced by *M. sativa* and *M. arborea* amendments in comparison with the non-amended control, resulting in no differences from fenamiphos at the highest rates of both species. Main effects of *Medicago* species and amendment rate and their interactions were always highly significant for root and soil nematode densities and root gall index, whereas the plant growth parameters were significantly affected only by the amendment rate (Table 3).

In the second experiment both top and root material from *M. sativa* always resulted in a significant increase in tomato plant growth in comparison with the infested control, with no statistical difference among the amendment rates (Table 4). Top and root weights of almost all the tomato plants in amended soil were significantly greater than in fenamiphos-treated or non-infested soil, although the highest amendment rate generally resulted, as in the previous experiment, in a reduced root growth compared to the lower dosages. All *M. sativa* amendments significantly suppressed root and soil population densities of *M. incognita* and tomato root galling in comparison

Table 2 Sapogenin content of *Medicago* plant materials

	% of total sapogenins						
Plant material	Medicagenic acid	Zanhic acid	Hederagenin	Bayogenin	Soyasaponin A	Soyasaponin B	Soyasaponin E
M. sativa tops	38.7	20.6	2.0	2.1	3.1	18.0	2.6
M. sativa roots	65.9	5.1	7.9	4.7	1.3	4.1	0.6
M. sativa pellet	40.2	19.8	3.1	2.6	3.3	15.8	2.1
M. arborea tops	31.7	28.9	1.5	14.1	1.0	12.7	1.6

Table 3 Effect of soil amendments with dry leaf biomass of *Medicago sativa* and *Medicago arborea* tops on the growth of tomato (cv. San Marzano) plant and the infestation of the root-knot nematode *Meloidogyne incognita*

Treatment	Amendment rate (g kg^{-1} soil)	Plant growth parameters				Final nematode population (eggs and juveniles)				Root gall index[2]	
		Height (cm)		Top weight (g)		per plant (x 1,000)		ml^{-1} soil			
M. arborea	5	51.0	b[1]	32.8	b	185.5	b	25.3	c	4.0	bc
M. arborea	10	58.0	bc	34.4	b	182.0	b	22.7	c	2.8	d
M. arborea	20	61.5	bc	33.7	b	52.2	cd	23.0	c	1.7	e
M. arborea	40	50.2	b	47.9	cd	9.7	ef	11.3	d	1.0	f
M. sativa	5	50.0	b	37.0	bc	181.9	b	42.0	b	3.8	c
M. sativa	10	55.6	b	56.9	d	69.9	c	7.3	d	1.8	e
M. sativa	20	59.0	bc	55.9	d	18.3	def	3.0	d	1.0	f
M. sativa	40	51.0	b	26.2	b	5.8	f	10.0	d	1.0	f
Fenamiphos	-	51.6	b	33.2	b	48.1	cde	3.3	d	1.6	ef
Non-treated soil	-	23.0	a	6.9	a	222.9	a	95.7	a	5.0	a
Non-infested soil	-	67.2	c	32.9	b	-	-	-	-	-	-
ANOVA F values[3]											
Species		0.23		3.3		10.7 **		24.2 **		8.4 **	
Rate		38.3 **		21.9 **		78.1 **		73.4 **		126.8 **	
Species x rate		0.01		7.4 **		5.1 **		17.9 **		2.3	

[1] Data followed by the same letters in each column are not significantly different ($P \leq 0.05$) according to Least Significant Difference's Test; [2] According to a 0–5 scale (0 = no galls, 1=1–2 galls, 2=3–10 galls, 3=11–30 galls, 4=31–100 galls and 5>100 galls); [3] Statistically significant at $P \leq 0.01$ (**).

with the infested soil and were generally not different from the fenamiphos treatment. Amendment suppressivity was significantly higher at 40 g kg^{-1} soil than at the lower rates, and the incorporation of *M. sativa* top material always resulted in a significantly lower soil nematode population than the corresponding rate of root material. Factorial analysis of variance showed highly significant effects of amendment rate both on nematode infestation parameters and on plant growth.

In the comparative experiment on *M. incognita* and *G. rostochiensis*, number of eggs and juveniles of *M. incognita* on tomato plants and the soil population of the cyst nematode were always significantly lower in pots amended with top material of *M. sativa* than in non-treated soil and for *G. rostochiensis*, than in fenamiphos-treated pots (Table 5). Differences among amendment rates were always significant for the cyst nematode populations and less consistent for plant and soil populations of the root-knot nematode. A direct relationship between nematode mortality and amendment rate was found for both nematode species, although amendment suppressivity significantly differed between the two species at rates > 5 g kg^{-1} soil. Percentage mortality of *G. rostochiensis* was slightly but significantly higher than in *M. incognita* at 20 g kg^{-1} soil, whereas root-knot nematode mortality was consistently higher at the other amendment rates and in the fenamiphos-treated pots. An analytical relationship between amendment rate and nematode mortality was also fitted to the experimental data (Fig. 2). The best fit was provided for both nematode species by the power equation $y = a + bx^c$, in which: y = percent nematode mortality; x = amendment rate (% w/w); a, b, c = coefficients. High values of coefficients for determination of r^2, indicate that almost all the total variation in nematode mortality can be explained by the above relationship.

Field experiment

In the field experiment tomato yield was significantly higher in the plots amended with both rates of pelleted *M. sativa* meal than in soil treated with

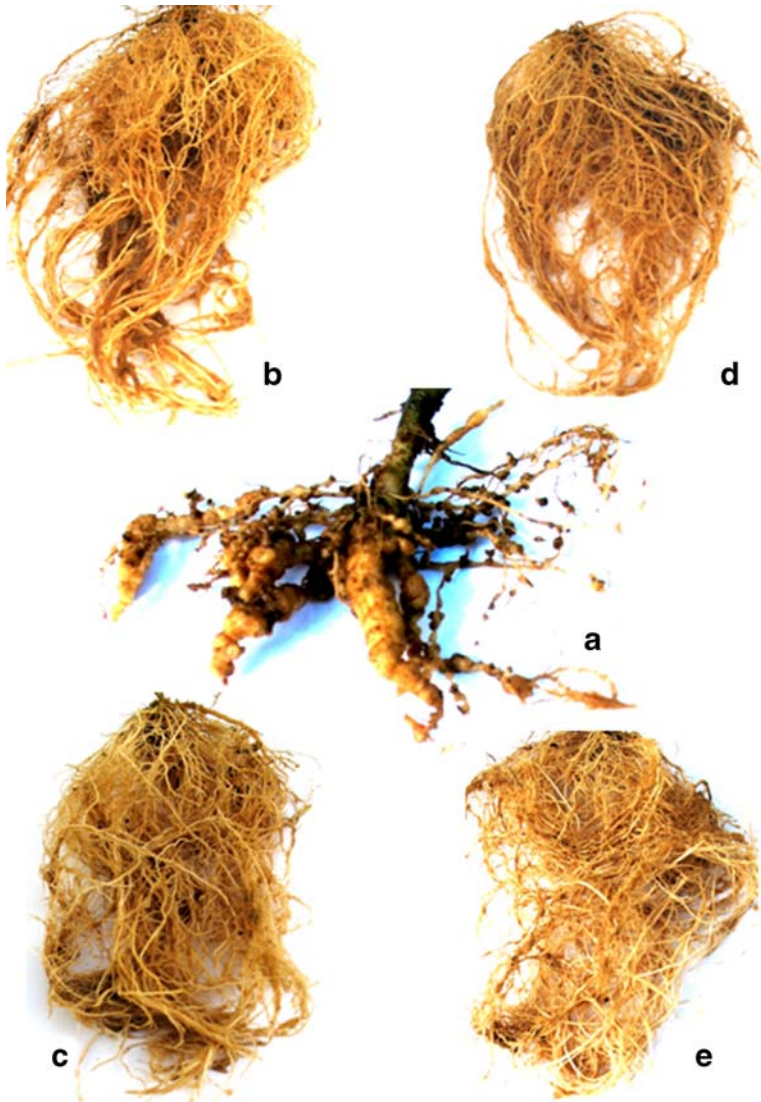

Fig. 1 Roots of tomato plants from non-treated soil **a**, soil amended with *M. sativa* **b** or *M. arborea* **c** top material, soil treated with fenamiphos **d** and non-infested soil **e**

fenamiphos or the quillay formulation, although all treatments significantly increased the crop yield compared to the non-treated control (Table 6). Moreover, both amendment rates strongly suppressed soil population density of *M. incognita* and gall formation on tomato roots, as both parameters were significantly lower in the plots amended with pelleted *M. sativa* meal than in non-treated soil. The 40 t ha^{-1} amendment rate provided values of nematode infestation parameters significantly < 20 t ha^{-1} and not statistically different from the chemical control, resulting also in the lowest nematode reproduction rate. However, *M. incognita* multiplication in the plots amended with 20 t ha^{-1} pelletted meal was significantly lower than in soil non-treated or treated with the quillay formulation, but higher than in fenamiphos-treated soil.

Discussion

Amendments with *Medicago* spp. plant materials in potting mixes were demonstrated as strongly suppressive, both on the root-knot nematode *M.*

Table 4 Effect of soil amendments with dry tops or root biomass of *Medicago sativa* on the growth of tomato (cv. San Marzano) plant and the infestation of the root-knot nematode *Meloidogyne incognita*

Treatment	Amendment rate (g kg^{-1} soil)	Plant growth parameters				Final nematode population (eggs and juveniles)				Root gall index[2]	
		Height (cm)		Top weight (g)		per plant (x 1,000)		ml^{-1} soil			
Tops	5	71.2	bcd[1]	40.8	cd	166.7	cd	12.1	e	3.0	cd
Tops	10	71.5	bcd	54.7	e	142.8	cd	8.3	f	3.0	cd
Tops	20	74.0	bcd	62.3	e	86.2	de	4.6	g	2.5	d
Tops	40	65.0	bc	43.2	d	8.8	e	2.5	h	1.5	e
Roots	5	70.7	bcd	44.5	d	198.0	bc	22.1	c	3.0	cd
Roots	10	77.0	cd	55.0	e	184.0	cd	17.1	cd	3.2	bc
Roots	20	68.7	bcd	54.0	e	127.3	cd	15.4	de	2.5	d
Roots	40	79.7	d	53.6	e	5.4	e	5.0	g	1.0	e
Fenamiphos	-	63.2	bc	27.7	b	190.3	c	22.5	bc	3.0	cd
Non-treated soil	-	28.0	a	6.7	a	328.8	a	57.5	a	4.7	a
Non-infested soil	-	59.7	b	33.1	bc	-	-	-	-	-	-
ANOVA F values[3]											
Tissue		1.1		0.4		0.6		57.5 **		0.1	
Rate		42.9 **		95.9 **		14.4 **		83.2 **		69.1 **	
Tissue x rate		1.6		2.6		0.1		5.4 **		0.8	

[1] Data followed by the same letters in each column are not significantly different ($P \leq 0.05$) according to Least Significant Difference's Test; [2] According to a 0 – 5 scale (0 = no galls, 1=1–2 galls, 2=3–10 galls, 3=11–30 galls, 4=31–100 galls and 5>100 galls); [3] Statistically significant at $P \leq 0.01$ (**).

incognita and on the potato cyst nematode *G. rostochiensis*, in the presence of high initial nematode population densities. Moreover, effectiveness and feasibility of this technique for the control of root-knot nematodes were confirmed under field conditions.

Powdered aerial parts of different *Medicago s*pp. have been shown to have an antifungal activity on soil-borne pathogens (Demirci and Dolar 2006; Jurzysta and Waller 1996), whereas almost no information is available on the nematicidal activity of *Medicago* spp. amendments in soil. The suppressive effect of a commercial *M. sativa* pellet, applied at rates slightly lower than those used in our field experiment, was reported by Walker (2007) on some phytoparasitic species, without any effect on free-living nematophauna. To the best of our knowledge, the present article is the first report on the suppressivity of *M. sativa* soil amendments on the cyst nematode *G. rostochiensis*, which is sensitive to other soil amendments (Renco et al. 2007).

There is some scepticism among nematologists about the nematicidal effects of organic amendments because of the difficulty in providing adequate controls (Bailey and Lazarovits 2003). The evident and constantly repeated suppressive effect of soil amendments with the tested materials from *Medicago* spp., also supported by previous data from in vitro experiments (Argentieri et al. 2008), could indicate that saponins are likely to be partly responsible for the lower nematode densities in amended pots/plots. *Medicago* species are a particularly rich source of saponins, previously found to be responsible for many different biological properties (Tava et al. 2005; Tava and Avato 2006), though few data are available on saponin activity and mechanisms of action against phytonematodes. Argentieri et al. (2008) recently documented the nematicidal properties of saponins from different *Medicago* spp. and related prosapogenins and sapogenins on the virus-vector nematode *Xiphinema index*, and reported that saponins from *M. arborea*

Table 5 Suppressivity of soil amendments with dry leaf biomass of *Medicago sativa* tops on the root-knot nematode *Meloidogyne incognita* on tomato cv. San Marzano and the cyst nematode *Globodera rostochiensis* on potato cv. Spunta

Treatment	Amendment rate (g kg^{-1} soil)	Final nematode population								% mortality				
		M. incognita (eggs and juveniles)				*G. rostochiensis*								
		per plant (x 1,000)		ml^{-1} soil		Cysts g^{-1} soil		Eggs g^{-1} soil		*M. incognita*		*G. rostochiensis*		t^2
M. sativa	5	23.9	bc[1]	6.1	bc	2.2	c	503	c	58.7	b	56.5	c	-
M. sativa	10	18.7	cd	5.1	cd	1.9	d	413	d	67.7	c	64.3	d	*
M. sativa	20	15.9	d	4.1	de	1.8	d	281	e	72.5	d	75.7	e	**
M. sativa	30	4.8	e	2.9	de	1.5	e	230	f	91.7	e	80.1	f	**
M. sativa	40	3.1	e	2.5	e	1.5	e	201	g	94.6	f	82.6	g	**
Fenamiphos	-	2.1	cd	2.3	e	2.2	bc	550	b	64.1	c	52.5	b	**
Non-treated soil	-	57.9	a	14.5	a	3.3	a	1,157	a	0.0	a	0.0	a	-

[1] Data followed by the same letters in each column are not significantly different ($P \leq 0.05$) according to Least Significant Difference's Test. [2] Student's t values significant at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).

were less nematotoxic than those from *M. sativa*. In previous *in vitro* and pot experiments, saponins were found to reduce total populations, number of eggs and juvenile motility and viability of root-knot nematodes (Meher et al. 1988; Omar et al. 1994). San Martin and Magunacelaya (2005) found that the saponin fraction from an aqueous extract of *Q. saponaria* controlled phytoparasitic nematodes only when combined with a non-saponin fraction containing polyphenols. Nematicidal effect of saponins may be related to their interaction with cell membrane constituents, such as sterols, proteins and phospholipids, resulting in their destruction and in the increase of ion permeability, and it has been shown to be also influenced by the side sugar chains attached to the sapogenins, as monodesmoside saponins are more active than bidesmosides (Tava and Avato 2006).

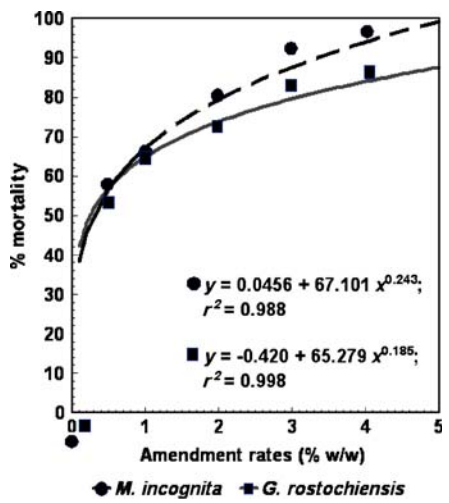

Fig. 2 Relationship between rates (x) of amendments with *Medicago sativa* plant material and percent mortality (y) of *Meloidogyne incognita* (●) and *Globodera rostochiensis* (■)

Saponin mixtures from the *Medicago* plant materials used in our previous *in vitro* experiments (Argentieri et al. 2008) differed in their composition, but medicagenic acid was the main aglycone of both *M. sativa* and *M. arborea* saponins and was associated with various rates (22 to <1%) of zanhic acid. As the above *in vitro* nematicidal activity of *M. sativa* was related to the content of medicagenic acid, the bioactivity of *Medicago* plant materials used in the present *in vivo* investigations could also be ascribed to the high content of medicagenic acid. However, as *M. sativa* roots showed a lower nematicidal activity compared to *M. sativa* tops in the presence of a high content of medicagenic acid and a low content of zanhic acid, 65.9% and 5.1%, respectively, it might be inferred that zanhic acid also contributes to the nematotoxic effect of *Medicago* spp. plant material.

Table 6 Effect of soil amendments with commercial pellets of *M. sativa* in a field infested by the root-knot nematode *Meloidogyne incognita* on tomato cv. Faino

Treatment	Dose	Application time	Tomato yield (t ha^{-1})		Nematode population (Eggs and juveniles ml^{-1} soil)						Root gall index***	
					Initial (30/06/06)		Final (07/11/06)		Reproduction rate			
M. sativa	20 tha^{-1}	2 weeks b.t.*	68.6	c**	1.4	a	3.2	c	2.3	b	1.9	c
M. sativa	40 tha^{-1}	2 weeks b.t.*	71.6	c	1.9	b	1.5	d	0.8	d	1.3	d
Quillay formulate	30 lha^{-1}	at transplanting	55.5	b	1.3	a	3.8	bc	3.0	a	3.1	b
Fenamiphos	7 kg a.i. ha^{-1}	1 week b.t.*	58.3	b	1.7	ab	2.7	cd	1.6	c	1.8	cd
Non-treated soil	-	-	46.0	a	1.6	ab	5.2	a	3.3	a	4.0	a

*Before transplanting. **Data followed by the same letters in each column are not significantly different ($P \leq 0.05$) according to Least Significant Difference's Test. ***Root gall index estimated according to a 0–5 scale (0=no galls, 1=1–2 galls, 2=3–10 galls, 3=11–30 galls, 4=31–100 galls and 5>100 galls).

Metabolites other than saponins, such as phenolics and canavanine, may also be involved in the nematicidal effect of *Medicago* amendments (Anaya 2006; Natelson 1985). Moreover, as Jansen and McGinn (1991) reported the release of ammonia during the decomposition of legume crop green manures, ammoniacal nitrogen could also be hypothesised as contributing to nematode suppression in soil (Rodríguez-Kábana 1986). Therefore, lower amounts of total phenolics and total nitrogen in *M. sativa* root material could reasonably account for their lower nematicidal activity.

Incorporation of both *M. sativa* and *M. arborea* plant materials in potting mixes stimulated tomato plant growth; soil amendments with pelleted *M. sativa* meal increased tomato crop yield in the field. An increased plant growth and crop yield response in soil amended with pelleted *M. sativa* meal was also reported by Walker (2007), but at amendment rates much higher than those used in our field experiment. Beneficial effects of soil amendments with biomasses from *Medicago* spp. on plant growth and crop yield could be only partially attributed to their suppressivity on phytoparasitic nematode populations, as an improvement of physical, chemical and microbiological soil properties after the incorporation of organic amendments was also previously documented (Bulluck et al. 2002). An allelopathic potential was previously reported among biological activities of *Medicago* spp. (Miller 1996; Tava and Avato 2006). This allelopathic activity was experimentally found to be related to plant saponin content and resulted in detrimental effects on seed germination and plant growth (Waller et al. 1995). In our experiment only reduced root growth at the highest amendment rate in potting mixes may be ascribed to some allelopathic effect, whereas nematotoxic effect and improvement of soil properties prevailed in all the other treatments, resulting in plant growth and crop yield increase.

Soil amendments with plant material from *Medicago* spp. or their pelleted formulation seem to be a valuable option for an environmentally safe control of root-knot and cyst nematodes. Application of these amendments could be particularly suitable for vegetable cropping systems in organic agriculture, where the range of available control tools is particularly restricted, and can be easily extended to conventional farms, especially if combined with other non-chemical methods (i.e. soil solarisation or post-plant treatments with plant-derived liquid formulations). Beneficial potential of these amendments is further strengthened by their collateral positive effects on soil fertility, technical feasibility and economic convenience, due to a lower cost than chemicals and to the reduced input of inorganic fertiliser. Finally, cost-benefit analysis should also take into consideration the environmental benefits related to the withdrawal of chemical treatments.

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