Suppressiveness of soil amendments with pelleted plant materials on the root-knot nematode *Meloidogyne incognita*

T. D'ADDABBO[1]., V. D. MIGUNOVA[2], M. RENČO[3,*], N. SASANELLI[1]

[1]Institute for Sustainable Plant Protection, CNR, Via G. Amendola 122/D, 70126 Bari, Italy, E-mail: *trifone.daddabbo@ipsp.cnr.it*, *nicola.sasanelli@ipsp.cnr.it*; [2]A.N. Severtsov Institute of Ecology and Evolution (IEE RAS), Leninsky pr. 33, Moscow, 119071, Russian Federation, E-mail: *varvara.migunova@sevin.ru*; [3]Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, Košice, 04001 Slovakia, E-mail: *renco@saske.sk*

Article info

Summary

Soil treatments with formulated plant biomasses or waste materials can be an effective alternative to green manure crops for a sustainable management of root-knot nematode infestations. The suppressive performance of soil amendments with three commercial formulations of defatted seed meal from *Brassica carinata*, dry biomass of *Medicago sativa* and pressed pulp from *Beta vulgaris* was comparatively evaluated on the root knot nematode *Meloidogyne incognita* both on potted and field tomato (cv. Regina) trials. Products were applied at rates of 10, 20, 30 or 40 g/kg and 20 and 40 T/ha soil in pots and field, respectively. Soil non treated or treated with the nematicide Oxamyl were used as controls in both experiments. Amendments in potted soil significantly reduced *M. incognita* infestation on tomato roots compared to both the untreated control and treatment with Oxamyl, also increasing tomato plant growth up to the 30 g/kg soil rate. At the end of the field tomato crop, soil population density of *M. incognita* resulted significantly reduced by all the tested treatments, whereas tomato yield was significantly higher than the untreated control only at the lowest amendment rate. Soil amendments with the materials tested in this study demonstrated to be a potential additional tool for a satisfactory and safe management of root-knot nematodes.
Keywords: Nematode management; soil amendments; defatted seed meals; sugar beet pulp

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are included among the most dangerous crop pests due to the severe yield losses caused to a wide range of worldwide crops (Moens *et al*., 2009; Le *et al*., 2019). The severe restrictions to the use of synthetic nematicides, imposed by their negative environmental effects, have given a strong impulse to the research on more sustainable alternative control strategies (Tilman *et al*., 2002).
Soil amendments with plant-derived materials are traditionally applied for improving physico-chemical properties and water-holding capacity of soil as well for increasing soil content of nutrients and beneficial organisms (Sasanelli *et al*., 2002; Hu *et al*., 2018). Interestingly, soil amendments with a variety of plant materials have been documented as suppressive effect on a wide range of soil-borne plant pathogens and pests including fungi, bacteria, viruses and plant parasitic nematodes (Hoitink & Boehm, 1999; Abawi & Widmer, 2000).
Plant parasitic nematodes suppression by soil amendments with plant materials has been generally related to chemical and biological mechanisms, such as the release of nematotoxic compounds originally present in the incorporated material or generated during

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* – corresponding author

their degradation in the soil or the development of nematode-antagonistic microorganisms (Stirling, 1991; Akhtar & Malik, 2000; Thligene *et al.*, 2019). Plant materials from *Brassicaceae* species represent the most effective sources of plant-based nematicidal soil amendments, as releasing volatile compounds (isothiocyanates, organic cyanides and nitriles) toxic to a range of soilborne pests and pathogens, including nematodes and fungi by the myrosinase-catalyzed hydrolysis of glucosinolates present in their tissues (Matthiessen & Kirkegaard, 2006; Avato *et al.*, 2013). Biomasses from *Medicago* species have been also reported as highly suitable for nematicidal soil amendments, mainly due to their high content of saponins and flavonoids with a nematicidal activity (Argentieri *et al.*, 2008; Ibrahim & Srour, 2014), as well as to the nematotoxic nitrogen compounds released during their degradation in soil (Gilpatrick, 1969). Soil incorporation with dry plant biomass from *Medicago sativa* L. was proved to be a highly suppressiveness to the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. on tomato both in pot and field (D'Addabbo *et al.*, 2009), but did not negatively affect the beneficial soil nematophauna (Walker, 2007).

In intensive crop systems with strict crop cycle rotations, soil treatments with dry formulations (meals, granules, pellets) of plant green biomasses or waste agroindustrial materials can be an effective alternative to green manure crops specifically addressed to the incorporation of nematode-suppressive biomasses. An effective suppression of root-knot nematode infestation on horticultural crops was documented for defatted seed meals from different *Brassicaceae* species (Lazzeri *et al.*, 2009; Curto *et al.*, 2016), or from neem (*Azadirachta indica* Juss) (Abbasi *et al.*, 2005; Cavoski *et al.*, 2012). Soil treatments with pelleted *M. sativa* dry biomass were also documented for a strong reduction of the attacks of *M. incognita* and of the carrot cyst nematode *Heterodera carotae* Jones on field tomato and carrot, respectively (D'Addabbo *et al.*, 2010).

Pressed pulps are generated during industrial processing of sugar beet (*Beta vulgaris* L.) as a waste product generally marketed as compressed pellets destined to livestock fodder. Soil amendments with sugar beet pulp were demonstrated for an enhancement of soil physical and biological properties (Tejada *et al.*, 2007; Schaffers, 2000), as well as for a significant reduction of some soilborne fungal pathogens (Santos *et al.*, 2008; Domınguez *et al.* 2014). At the best of our knowledge, there is no information on a potential suppressiveness of sugar beet pulp to phytoparasitic nematodes, though the availability of a pelleted formulation easily applicable to soil makes this product a potential candidate for nematicidal soil amendments. This study was addressed to a comparative evaluation of the suppressive performance of three industrial formulations of *B. vulgaris* pressed pulp, *M. sativa* dry biomass and seed meal from *Brassica carinata* Braun on the root knot nematode *M. incognita* in two experiments on potted and field tomato (*Solanum esculentum* L.).

Materials and Methods

Experiment in pot

A sandy soil artificially infested with *M. incognita* (7 eggs and juveniles/mL soil) was added with 10, 20, 30 or 40 g/kg soil rates of three commercial formulations of *B. carinata* defatted seed meal (BSM), *M. sativa* dry biomass (MSB) and *B. vulgaris* pulp (SBP). The amended soil was poured into 1.2 L clay pots with five replicates per treatment, that were arranged in a randomised block design on benches in a greenhouse at $25 \pm 2°C$. Soil treated with a liquid formulation of the nematicide Oxamyl (10 % a.i.), applied at an amount corresponding to a 20 L/ha field rate at transplanting and 15 and 30 days later, and non-amended soil, either non infested or infested by *M. incognita*, were used as controls. Three weeks after the amendment incorporation, a one month-old seedling of tomato cv. Regina was transplanted in each pot.

During the experiment, plants were maintained in the glasshouse randomizing the position of the blocks and at the same time re-positioning 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.).

After two months, at the end of the pot experiment, tomato plants were uprooted. Fresh top and root weight were recorded on each replicate, as well as gall formation was evaluated on each tomato root according to a 0 – 5 scale, in which 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). In each pot, soil nematode population density was assessed by processing 500 mL soil by the Coolen's method (Coolen, 1979). Numbers of *M. incognita* eggs and second stage juveniles (J2) in roots were determined by cutting up each root system into small pieces and further comminuting them in a blender, containing 1 % aqueous solution of sodium hypochlorite for 20 sec (Marull & Pinochet, 1991). The water suspension was then sieved through a 250 μm pore sieve put over a 22 μm pore sieve. Nematodes and root debris gathered on the 22 μm pore sieve were further processed by centrifuging at 2,000 rpm for five min in 400 ml of a magnesium sulphate (MgSO$_4$) solution of 1.16 specific gravity. Eggs and J2 in the water suspension were sieved through the 22 μm pore sieve, sprayed with tap water to wash away the MgSO$_4$ solution and collected in about 50 ml water. Recovered eggs and J2 were microscopically counted and final nematode population density (*Pf*) in each pot was determined by summing counts from roots and soil. The nematode reproduction rate *r* was expressed as ratio between final and initial *M. incognita* population density (*Pf/Pi*). The experiment was performed twice.

Experiment in field

A sandy soil uniformly infested by *M. incognita* (1.6 eggs and juveniles/mL soil), located at Zapponeta (province of Foggia, Apulia region) (41° 44' 93" N, 15° 96' 13" E), was divided in 10 m^2 (5 x 2 m) plots, spaced 1 m apart. The same formulations used in

Table 1. Effect of soil amendments with formulated *M. sativa* biomass, *Beta vulgaris* pressed pulp and *B. carinata* seed meal on the infestation of *M. incognita* and growth of potted tomato (cv Regina).

Rate (g/kg soil)	Tomato plant weight (g)		Root gall index (0 – 5)	Nematode population (Eggs and J2/)		Reproduction rate (Pf/Pi)
	aerial part	roots		g root	mL soil	
B. carinata seed meal						
10	73.0 ± 1.5* i**	37.5 ± 1.2 f	2.5 ± 0.3 cd	98 ± 5.3 hi	2.8 ± 0.3 cd	0.8 ± 0 g
20	60.5 ± 0.9 f	28.0 ± 0.4 e	2.0 ± 0 de	37 ± 0.6 ij	2.0 ± 0.1 ef	0.4 ± 0 h
30	52.7 ± 1.1 de	27.5 ± 1.0 de	1.0 ± 0 f	23 ± 0.4 j	1.8 ± 0.3 f	0.3 ± 0 h
40	50.7 ± 0.8 cd	25.3 ± 0.8 cd	1.0 ± 0 f	19 ± 1.0 j	1.0 ± 0 g	0.2 ± 0 h
B. vulgaris pressed pulp						
10	30.2 ± 0.8 b	28.2 ± 0.9 e	1.0 ± 0 f	906 ± 6.3 d	2.8 ± 0.3 cd	3.4 ± 0.1 d
20	55.0 ± 1.2 e	38.0 ± 0.9 f	1.3 ± 0.3 f	781 ± 6.5 e	2.0 ± 0 ef	3.8 ± 0.1 c
30	61.0 ± 0.4 fg	45.5 ± 1.0 h	1.0 ± 0 f	405 ± 3.6 g	2.0 ± 0.1 eg	2.5 ± 0.1 f
40	47.7 ± 1.5 c	19.0 ± 0.9 b	1.5 ± 0.3 ef	53 ± 2.3 hij	1.0 ± 0 g	0.3 ± 0 h
M. sativa biomass						
10	64.0 ± 2.0 gh	29.8 ± 1.0 e	2.5 ± 0.3 cd	968 ± 28.0 cd	3.0 ± 0.2 cd	3.9 ± 0.2 c
20	89.7 ± 0.9 k	51.8 ± 0.6 i	1.5 ± 0.3 ef	601 ± 5.6 f	2.5 ± 0.3 de	4.1 ± 0.2 c
30	83.0 ± 1.1 j	52.8 ± 0.6 i	1.3 ± 0.3 f	436 ± 13.8 g	1.0 ± 0 g	2.9 ± 0.1 e
40	26.0 ± 0.4 a	3.8 ± 0.3 a	1.0 ± 0 f	114 ± 1.7 h	0.8 ± 0.3 g	0.2 ± 0 h
Oxamyl	54.7 ± 1.5 e	40.7 ± 1.4 g	2.8 ± 0.3 bc	1,007 ± 6.6 bc	3.2 ± 0.3 bc	5.3 ± 0.1 b
Non treated	47.7 ± 0.6 c	24.0 ± 0.4 c	4.0 ± 0 a	4,710 ± 81.1 a	10.2 ± 0.6 a	14.9 ± 0.3 a
Non infested	64.7 ± 1.5 h	45.0 ± 0.7 h	-	-	-	-

*Each value is an average ± SE of five replications from two independent experiments;
**Data flanked in each column by the same letter are not statistically different according to Fisher's Least Significant Difference pairwise procedure (P ≤ 0.05).

the pot experiment were uniformly distributed on plot surface at the rate of 20 and 40 T/ha and then incorporated into the soil by rotavation. Each treatment was replicated in four plots according to a randomized block design. Plots non treated or treated with Oxamyl at the same rate of the experiment in pot were used as controls. The three amendments were incorporated into the soil four weeks before tomato transplanting whereas Oxamyl was applied in fertirrigation at 20 L/ha, by PVC drip lines (Ø 1.6 cm) with water emitters (flow rate 4 L/h), three days before and 15 and 30 days after transplant.

One month-old seedlings of tomato cv. Regina 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), on 23/06/2018. All plots received standard maintenance (irrigation, weed control, mineral nutrition, etc.) during the growing season.

Tomato crop was harvested at weekly intervals from 31 August to 15 September 2018, recording the yield of each plot at the same time. At the end of tomato harvest, root gall index was estimated on all the tomato roots from each plot, according to 0 – 5 scale (0 no galls and 5 root system completely deformed by large and numerous galls) (Lamberti, 1971). A composite 40-core soil sample was also collected from the central square meter of each plot, either before soil amendments and after crop harvest. Eggs and juveniles were extracted from 500 mL soil aliquots by the Coolen's method (Coolen, 1979) and microscopically counted using a stereo microscope at 20x magnification.

Statistical analysis
Data from the two experimental runs of the experiment in pot were pooled, as a preliminary analysis of variance showed no significant interaction of experiment × treatment (Finney, 1979). All data were subjected to analysis of variance and means compared by Fisher's Least Significant Difference pairwise procedure at P ≤ 0.05 using PlotIT 3.2 (Scientific Programming Enterprises, Haslett, MI).

Ethical Approval and/or Informed Consent

This article does not contain any studies with human participants or animals by any of the authors, so formal consent is not required.

Results

Experiment in pot
All the soil amendments significantly reduced the number of *M. incognita* eggs and J2 on tomato roots compared to the non treated control, according to a dose effect relationship and even in comparison to the nematicide Oxamyl (Table 1). The relationship between the number of eggs and J2 per gram of tomato roots and the rates of the three amendments was calculated by using different interpolation formulae. The best fit to the experimental data was provided by the logarithmic equation ln $y = a + bx^{0.5}$, as showing the highest correlation indices (r^2 > 0.99) (Fig. 1). The

final soil nematode population was always significantly lower in all the amended pots than in soil non treated or treated with the synthetic nematicide. Root gall formation was also statistically lower in soil amended with the three pelleted plant materials than in the two controls.

Weight of aerial part and roots of tomato plants was significantly increased by treatments up to 30 g/kg soil rate, but not statistically different from the non treated control at the maximum rate of SBP and BSM or even statistically lower for the 40 g/kg soil rate of MSB (Table 1). Finally, most of treatments with MSB and BSM resulted also in a tomato growth significantly higher, or at least not different, compared to the chemical and the non infested controls.
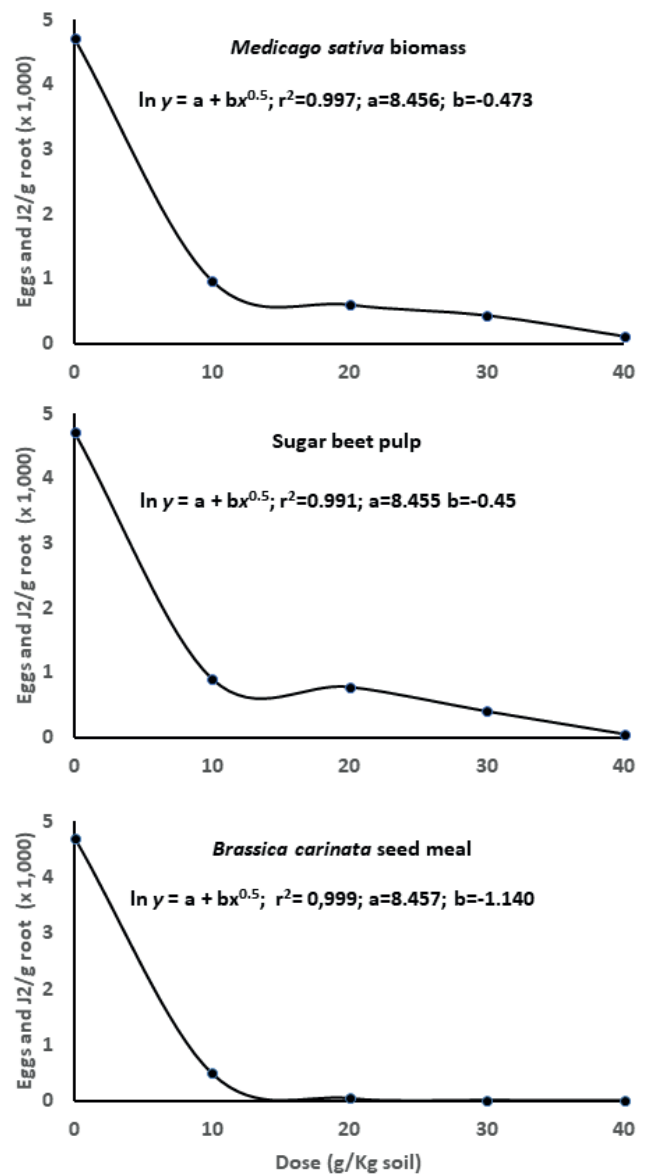

Fig. 1. Analytical relationship between number of *M. incognita* eggs and J2 per gram of tomato roots and rates of the three amendments.
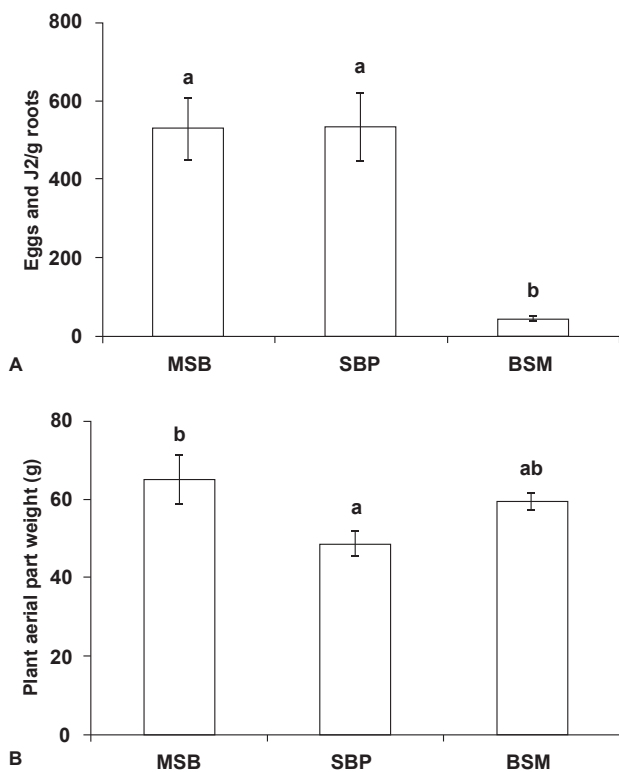
Fig. 2. Comparison of cumulative effect of soil amendments with *M. sativa* biomass (MSB), *B. vulgaris* pressed pulp (SBP) and *B. carinata* seed meal (BSM) on number of *M. incognita* eggs and J2 on tomato roots (A) and weight of plant aerial parts (B).

The two-way ANOVA comparison of cumulative effects of the three amendments showed significant differences only for two parameters, i.e. the number of *M. incognita* eggs and J2 per gram of tomato roots and the weight of plant aerial parts (Fig. 2). In particular, BSM was significantly more suppressive on *M. incognita* multiplication than MSB and SBP, whereas SBP resulted in a significantly lower growth of tomato green biomass than the other two products.

Experiment in field
At the end of tomato crop, the soil population density of *M. incognita* resulted significantly reduced by all the tested amendments compared to the non-treated control (Fig. 3). Moreover, nematode population in soil treated with 40 T/ha BSM was also significantly lower than in soil treated with Oxamyl. No significant difference was found between the two rates of MSB, whereas SBP and BSM were significantly more suppressive at the higher amendment rate. Gall formation on tomato roots was significantly lower than the control only for the higher rate of MSB and both dosages of BSM, which resulted also statistically not different from the chemical treatment.

Almost all the amendments also resulted in a significantly higher tomato yield compared to the non-treated control (Fig. 3). How-
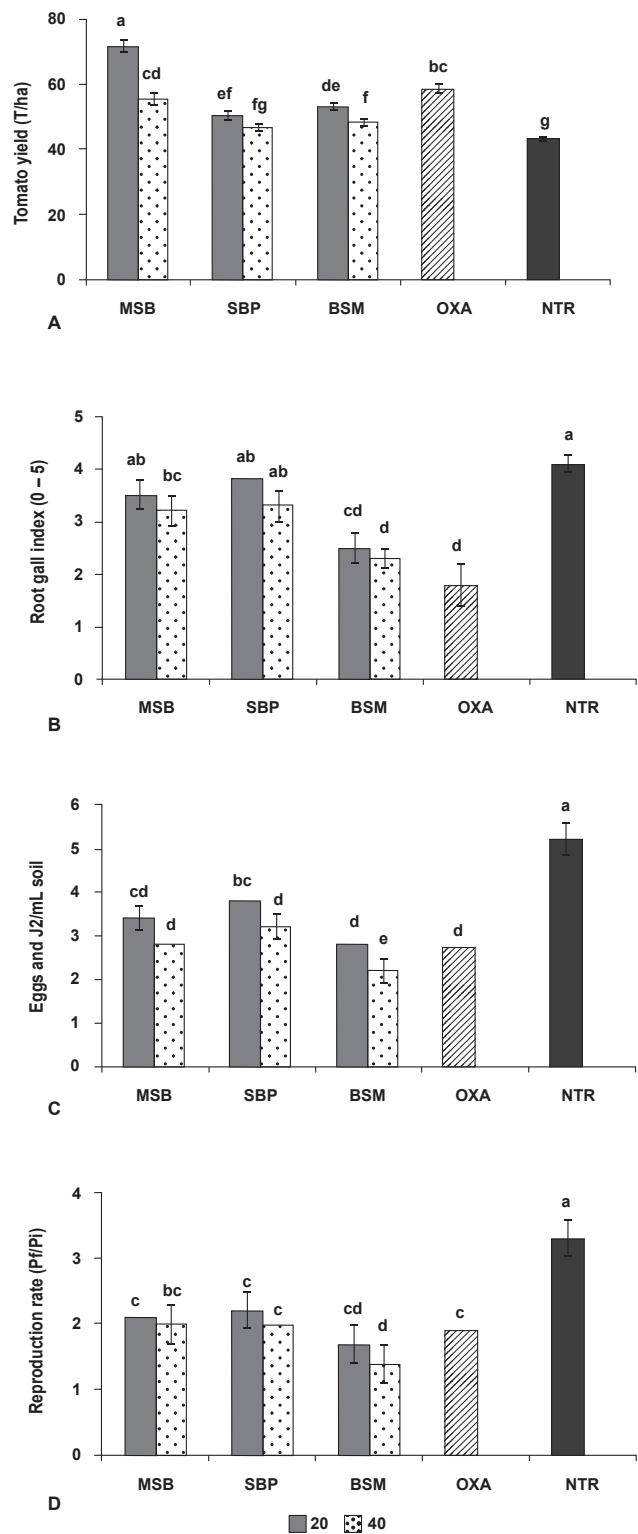

Fig. 3. Effect of soil amendments with formulated *M. sativa* biomass (MSB), *B. vulgaris* pressed pulp (SBP) and *B. carinata* seed meal (BSM) in comparison to Oxamyl (OXA) and untreated control (NTR) on yield of tomato cv Regina (A), gall formation on tomato roots (B), final soil population density (C) and reproduction rate (D) of *M. incognita* in field.

ever, only the 40 T/ha rate of MSB resulted in a crop yield not statistically lower than the chemical treatment.

Discussion and Conclusions

Soil amendments with MSB, BSM and SBP demonstrated to be suppressive to the root-knot nematode *M. incognita* either in pot and field conditions.

A reduction of soil population density of phytoparasitic nematode, without any detrimental effect on beneficial nematophauna, was already documented following to the field incorporation of a *M. sativa* hay (Walker, 2007). Recently, soil treatments with MSB resulted in a strong suppression of the infestation of both *M. incognita* and the cyst nematode *H. carotae* on field tomato and carrot, respectively (D'Addabbo *et al.*, 2009; 2011). The high saponin content of *Medicago* plant materials may be the main responsible of the reduced nematode multiplication in the amended soil, as the nematicidal activity of saponins from different *Medicago* species were reported on different phytoparasitic nematodes (Argentieri *et al.*, 2008; D'Addabbo *et al.*, 2010). Besides saponins, a role in phytoparasitic nematode suppression may also be played by the ammoniacal nitrogen released by MSB decomposition in soil, as a suppressiveness to phytonematode populations was often documented for soil amendments with a low C/N ratio, such as MSB (Janzen & McGinn, 1991; Bailey & Lazarovits, 2003). Moreover, amendments with organic materials as MSB were also found to increase soil nematode antagonistic or parasitic microflora (Stirling, 1991; Jaffee, 2006).

Suppressiveness to root-knot nematodes of BSM was also documented by literature data, as Curto *et al.* (2008) reported a significant reduction of *M. incognita* infestation on a field melon crop following to soil amendment with the same formulation used in this study. Analogously, soil treatment with BSM from *B. carinata* or other *Brassica* species were found to significantly suppress *M. incognita* infestation on zucchini or tomato also in field conditions (Curto *et al.*, 2016; Lazzeri *et al.*, 2009). It is largely acknowledged that the suppressive effect of *Brassica* seed meals on phytoparasitic nematodes, as well as on soilborne phytopathogens, should be attributed to the biofumigant activity of the products, mainly isothiocyanates, released during the myrosinase-catalyzed hydrolysis of glucosinolates such as sinigrin, the main glucosinolate component of BSM (Avato *et al.*, 2013).

Soil amendments with SBP in combination with *Brassica juncea* pellets were reported for a significant reduction of charcoal rot caused by *Macrophomina phaseolina* in field (Domínguez *et al.*, 2014), as well as for an up to 100 % inhibition of other phytopathogenic fungi, such as *Fusarium*, *Phytophthora, Pythium* and *Sclerotinia* f. spp. (Santos *et al.*, 2008).

The positive effects of the tested products on plant growth and yield of tomato also agree with literature studies. Soil addition with MSB was significantly found to increase tomato growth both in pots and field and also carrot yield (D'Addabbo *et al.*, 2009, 2010).

In addition, an increased tomato plant growth and yield response was also documented in soil amended with *M. sativa* hay (Walker, 2007). Positive side effects on plant vigour and crop yield were also proved in soil amended with BSM (Lazzeri *et al.*, 2009; Curto *et al.*, 2008; 2016). Aditionally to the reduced nematode infestation, the general improvement of soil physical, chemical and microbiological properties should also be considered as a concurrent cause of plant growth and yield increase by amendments with both MPB and BSM (Bulluck *et al.*, 2002). Adversely to our data, SBP application reduced the yield of the first-year wheat crop and did not significantly affected the second-year sugar beet yield in a field study of Kumar *et al.* (2009), mainly due to a reduced availability of mineralized nitrogen in the amended soil.

In conclusion, soil amendments with the materials tested in this study demonstrated to be a potential tool for an effective and environmentally safe management of root-knot nematodes. Use of these products can particularly be suitable for organic agriculture where the available control strategies are quite limited. Positive effects on soil fertility represent an added value of these products, as improving crop yield performances and reducing inputs of inorganic fertilizers. These additional effects should be taken into account in a cost-benefit analysis, as to highlight the convenience of tested amendments beyond the simple cost of the products. However, a reduction of amendment rates and consequently of their cost can be achieved by a combination with other nonchemical techniques, such as soil solarization, or with reduced doses of nematicides.

At the best of our knowledge, this is the first report of a suppressive activity of SBP on phytoparasitic nematodes, previous studies stated only its suppressiveness to some fungal soilborne pathogens.

Conflict of Interest

Authors have no potential conflict of interest pertaining to this submission to Helminthologia.

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