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DOI 10.2478/s11687-012-0048-4

VERSITA

HELMINTHOLOGIA, 49, 4: 259 – 269, 2012

Nematicidal and fertilizing effects of chicken manure, fresh and composted olive mill wastes on organic melon

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Summary

The fertilizing and nematicidal effects of three organic amendments were evaluated in a pot experiment on melon plants infested by the root-knot nematode *Meloidogyne incognita*. A soil artificially infested with 4 eggs and juveniles/ml soil of the nematode was amended with: a) virgin olive pomace (VOP); b) composted olive pomace (COP); c) chicken manure based fertilizer (CM) and d) chicken manure based fertilizer combined with the biological control agent *Paecilomyces lilacinus* strain 251, brand name BioAct WG (CMB). VOP was applied at doses of 11 (VOP-A), 22 (VOP-B) and 44 t/ha (VOP-C); COP at 4.5 (COP-A), 9 (COP-B) and 18 t/ha (COP-C); CM at 3 t/ha and CMB at 3 t/ha combined with 4 kg/ha of BioAct WG. Untreated soil was used as control. The treatments CM, CMB, VOP-B and COP-B were established on the basis of N requirement of melon plants (120 kg/ha) taking into account soil and amendments N availability. Two weeks later amendment application and nematode inoculation, the soil was poured in 4.8 l clay pots which were arranged in a greenhouse according to a randomized block design with ten replications for each treatment. A one-month old melon seedling (cv. Galia) was transplanted in each pot and organic farming management practices were used during the growing period. At the end of the experiment, 60 days after transplant, plants were uprooted and height, fresh and dry shoot and root weights were recorded. Root gall index, on the roots, caused by the nematode attack, was estimated according to a 0-5 scale. Final nematode population density and reproduction rate were also calculated for each pot. All data were subjected to statistical analysis of variance (ANOVA) and means compared according to Least Significant Difference's Test. Nematode population and root infestation were significantly suppressed by the addition of all amendments, compared to untreated control. However, CM and CMB resulted in a total more suppressive effect and in a significantly higher plant growth in comparison to all the other treatments. A significant correlation was found between root gall index and eggs and juveniles/g root and final nematode population density. No signifycant correlations were found between nematological parameters or plant growth parameters and amendment doses.

Keywords: *Meloidogyne incognita*; nematode control; composts; N requirement

Introduction

Root-knot nematodes *Meloidogyne* spp. are important plant parasites that can cause severe economic yield losses to numerous agricultural crops including vegetables (Sasanelli, 1994). The impact of these species is enhanced by their wide host ranges of more than 5,000 plant species belonging to different botanical families (Trudgill & Blok, 2001). Generally, in conventional farming systems, these plant parasitic nematodes can be managed by the use of chemical nematicides or cultural practices (resistant cultivars and crop rotation) (Mai, 1985). However, root-knot nematode resistant varieties are available only for a limited number of vegetable crops and crop rotation is often difficult because of the wide host range of *Meloidogyne* spp. (Taylor & Sasser, 1978). Moreover, the range of effective chemical products is limited, they are expensive, and their use on agricultural crops has been deeply restricted and revised by the recent European Legislation because of they have generally a negative impact on the environment and on the general public health (Reg. EC 396/2005, 1095/2007, 33/2008, 299/2008 and 1107/2009).

Several studies have been carried out to evaluate the effectiveness of control strategies at low environmental impact alternative to chemicals. A wide range of options was considered: amendments, biofumigations, crop rotation, grafting, green manures, mycorrhization, resistant cultivars

(Gamliel *et al*., 2000; Sasanelli *et al*., 2002; Nico *et al*., 2004; Castillo *et al*., 2006; D'Addabbo *et al*., 2011; Renčo *et al*., 2007; 2009; 2011), soil solarization, ozone and steam applications (Sasanelli & Greco, 2000; Tamietti & Valentino, 2000; Tjamos *et al*., 2000; Ciccarese *et al*., 2008), biocidal plants or natural byproducts (Gommers, 1981; Grainge & Ahmed, 1988; Sasanelli & D'Addabbo, 1993; Sasanelli *et al*., 2007, 2009; Maistrello *et al*., 2010; Renčo *et al*., 2012) and biological control agents especially fungi and bacteria (Vannacci & Gullino, 2000; Sasanelli *et al*., 2008).

Soil amendments, commonly used in organic farming, are effective in the management of several soil borne plant pathogens and phytoparasitic nematodes (Nico *et al*., 2004) and at the same time can improve soil fertility as well as productivity and quality of plant products (Bulluck *et al*., 2002; Rivera & Aballay, 2008). In particular, in the Mediterranean countries, in the areas of cultivation of olive, the disposal of olive mill wastes represents a serious environmental problem as large amounts of these materials are produced in a short period every year. Incorporation of these materials into the soil may represent a possible solution to the problem of their disposal and in addition could result as an important resource of nitrogen, phosphorus, calcium and other elements as zinc, copper, magnesium essential to plant growth (Tester, 1990).

Various mechanisms are involved in the suppressive action among which the development of nematode natural enemies and the release of toxic compounds were found to be the most relevant (Stirling, 1991; Abawi & Thurston, 1994; Oka *et al*., 2000). For plant-parasitic nematodes, the effectiveness of suppression varies depending from nematode species, type of amendment and amendment dose (Nico *et al*., 2004). According to Kaplan and Noe (1993) the use of the optimal amendment rate seems to be of primary importance indicating that the nematicidal activity is regulated by dose-response functions.

Although various organic amendments can have diverse effects on soil properties and nematode communities (Nahar *et al*., 2006; Hu & Qi, 2010), all amendments tend to increase availability of plant nutrients, microbial biomass and bacterivore and fungivore nematodes (Briar *et al*., 2007). Chicken manure is also known to be effective in the control of root knot nematodes (Akhtar, 1997; Lopez-Pérez *et al*., 2005; Oka *et al*., 2000; D'Addabbo *et al*., 2003) and the mode of action is thought to be based on the release of toxic levels of ammonium, although alterations in soil structure, the stimulation of antagonistic organisms, and the increase of plant tolerance also may play a role (Oka *et al*., 2000; Lopez-Pérez *et al*., 2005).

Therefore, a pot experiment was carried out to compare the nematicidal and fertilizing effects of different amendments on organic melon. In particular virgin olive pomace and composted olive pomace were used at 3 different doses and compared to a chicken manure based commercial fertilizer in the presence and absence of the biological control agent *Paecilomices lilacinus* strain 251.

The purpose was to compare the effectiveness of different amendments and to establish for each of them the most suitable dose for nematode control and plant growth.

Materials and methods

The pot experiment was carried out at the Mediterranean Agronomic Institute of Bari (MAIB) (41º.0539 North; 16º.8773 East Greenwich) from May to July 2009. Treatments with soil amendments were established on the basis of N requirement of melon plants (120 kg/ha) taking into account soil and amendments N availability (Rodriguez *et al*., 2005).

Preparation of nematode inoculum

An Italian population of *Meloidogyne incognita* (Kofoid *et* White) Chitw. was reared for two months on tomato (*Solanum lycopersicum* L.) cv. Rutgers in a glasshouse at 25 ± 2 ºC. When large mature egg masses were formed, tomato roots were finely chopped and eggs and juveniles were quantified by processing 6 root samples of 10 g each with 1% aqueous solution of NaOCl (Hussey & Barker, 1973). The roots were then throughly mixed with 3 Kg of steam sterilised sandy soil (pH 7.1; sand > 99 %; silt < 1 %; clay < 1 % and organic matter = 0.73 %) and used as inoculum.

Soil sampling and soil analysis

Soil samples were collected in a naturally *M. incognita* infested field. Soil sampling followed a W scheme and 20 single samples were mixed to obtain a final composite sample for physical, chemical and nematological analysis. Each single sample was collected at 30 cm depth after the removal of weeds and the first 5 cm topsoil. Before amendment application the soil sample and amendments were analyzed for physico-chemical parameters to establish the optimal amendment application doses.

The dose of each organic amendment to apply was calculated taking into account i) soil nitrogen content; ii) the mineralization rate of soil organic matter (Stanford & Smith, 1972); iii) N melon requirement according to the Italian good agricultural practice (Min. D. n. 86, 1999); iv) amendment N content; v) the mineralization rate of each amendment in order to calculate the amount of N released by organic fertilizers during the growing cycle (Cabrera *et al*., 2005; Whitmore, 2007). The purpose was to calculate the amount of available N released by soil and amendment during the growing period to support melon growth. Considering the N potential rate of mineralization and length of the melon crop cycle, doses of amendments able to satisfy completely the melon N requirement were: 22 t/ha for VOP; 9 t/ha for COP and 3 t/ha for CM and CMB (on a dry weight base).

To evaluate the effect of applied dose on nematode suppression and on plant growth, the corresponding double and half doses for VOP and COP were used.

Soil sample was air dried, weighted and sieved 2 mm. Stones and gravels were collected and weighted after washing and the fine earth fraction was used for chemical and physical analyses. Soil texture was determined by using the pipette method (Indorante *et al*., 1990). The textural class of the soil was calculated using the USDA soil textural classification system. Soil pH was measured on a soil suspension, 1:2.5 soil to water ratio (w/v) and 1:2.5 soil to $CaCl_2$ 1M solution ratio (w/v) using a pH meter Crison model Basic 20 and a glass electrode Crison 52-00 (Thomas, 1996). The soil salinity was assessed by the measurement of the electrical conductivity on an water extract 1:2 (w/v) soil to water ratio using a Conductivity meter XS cond 510. The electrical conductivity (EC) was expressed in dS/m at 25 ºC (Rhoades, 1996). Organic carbon was determined by the Walkley-Black method and Organic Matter was calculated by multiplying Soil Organic Carbon by the factor 1.72 (Nelson & Sommers, 1996). Total nitrogen was determined by the Kjeldahl method (Bremner, 1996). Inorganic nitrogen was extracted from soil by a KCl 2 M solution using a soil to extractant ratio of 1:10 (w/v). Nitrates and ammonium were then analyzed on 50 ml of the extract by steam distillation in the presence and absence of 0.2 g of Devarda alloy respectively (Bremner & Keeney, 1965). Available phosphorus was determined colorimetrically by using the Ascorbic Acid Method (Olsen & Sommers, 1982). Absorbance was measured at 650 nm by a spectrophotometer model Megatech SP 930. Total carbonate content was determined by the volumetric calcimeter method (Loeppert & Suarez, 1996) and active calcium according to the Drouineau method (Drouineau, 1942). Exchangeable bases (Ca, Mg, K, Na) were extracted from soil by using a solution of Barium Chloride (100 g/l) and Triethanolamine (22.5 ml/l) at pH to 8.2. Ca, K, Mg and Na concentrations were estimated by mean of Inductively Couple Plasma Optical Emission Spectrometry (ICP-OES) using a Thermo Electron ICAP 6000 Series spectrometer (Gessa & Ciavatta, 2000).

Organic amendments analysis

Olive compost (COP) was produced at MAIB using virgin olive pomace (VOP) derived from a two phase olive oil industry. Chicken manure (CM and CMB) was the main raw material of a commercial fertilizer. Representative samples of each amendment were analyzed for physical and chemical parameters before their use as soil amendments. pH was determined on a sample suspension (3:50 w/v compost to water ratio) using a pH meter Crison model Basic 20 and a glass electrode Crison 52-00. Salinity was assessed by the measurement of electrical conductivity on an aqueous extract (1:10 w/v compost to water ratio) (Italian Official Methods for Fertilizer Analysis, 2002).

Organic matter was determined by difference between dry sample and ash content obtained by ignition at 550 ºC till constant weight (US. TMECC, 1997). Organic Carbon was calculated according to the Italian Official Methods for Fertilizer Analysis (2002). Total nitrogen was analyzed by using the Kjeldahl method (US. TMECC, 1997).

Total phosphorus content was determined by using the Ascorbic Acid Method (Olsen & Sommers, 1982) on the digested sample. Acid digestion was carried out on 0.5 g of dry and grinded sample using a microwave system (US. TMECC, 1997). Absorbance was measured at 650 nm by a spectrophotometer model Megatech SP 930. Concentration was reported on a sample dry weight basis determined at 105 ºC.

The relative secondary, micro-nutrient and heavy metals content of the digestate were determined by mean of Inductively Couple Plasma Optical Emission Spectrometry (ICP-OES) using a Thermo Electron ICAP 6000 Series spectrometer. Concentration was reported on a sample dry weight basis determined at 105 ºC (US. TMECC, 1997).

Seed germination and root elongation tests were applied to ensure the absence of any toxic effect for plant growth. The test was performed using the modified Zucconi's method (Zucconi *et al.*, 1981). Briefly, organic amendment samples were kept to 85 % of humidity to obtain a water extract. The suspension was shaken for 2 hours, centrifuged, filtered on 0.45 µm sieve and dilutions 1:1 (v/v) and 3:1 (v/v) extract to water ratios were prepared. One ml of each diluted extract was used to inoculate 9 cm diameter Petri dishes in which 10 seeds of *Lepidium sativum* L. were then placed. Five replicates for each treatment and a control with distilled water were used. Petri dishes were incubated at 23 ºC for 48 h and the germination index (G.I.) was calculated according to the formula:

$$GI(\%) = \frac{Gc \times Lc}{Gt \times Lt} \times 100$$

in which Gc: average No. of germinated seeds for sample; Lc: average of root length for sample; Gt: average No. of germinated seeds in the control and Lt: average of root length in the control.

Pot experiment

The soil collected and analysed as previously described was throughly mixed and amended with: a) virgin olive pomace (VOP); b) composted olive pomace (COP); c) chicken manure based fertilizer (CM) and d) chicken manure based fertilizer combined with the biological control agent *P. lilacinus* strain 251 (commercial name BioAct WG, CMB). VOP was applied at doses of 11 (VOP-A), 22 (VOP-B) and 44 t/ha (VOP-C); COP at 4.5 (COP-A), 9 (COP-B) and 18 t/ha (COP-C); CM at 3 t/ha and CMB at 3 t/ha combined with 4 kg/ha of BioAct WG. Untreated soil was used as control. Each amended soil was then throughly mixed with appropriate amounts of the inoculum to give a population density of 4 eggs and juveniles/ml soil and stored in big plastic containers for two weeks to allow juveniles emergence and to start mineralization phase of the applied composts. The same soil, before transplant, was subjected to nematological analysis to verify the true initial nematode population density at the time of transplantation (*Pi* = 1 eggs and juveniles/ml soil). The treated and infested soils were then used to fill 4.8 l pots. One-month old melon (*Cucumis melo* L., subsp. *melo*, var. *cantalupensis* Nardin) seedling, cv. Galia, was transplanted in each pot. Pots were arranged on banches in a greenhouse according to a randomized block design with 10 replications per each treatment. During the experiment melon plants were maintained in the greenhouse 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.

Moreover, during the growing period chlorophyll content of melon plants was recorded 3 times using a chlorophyll meter (SPAD). Each value resulted from the average of four readings on two leaves. Soil temperature was recorded at 1 hour intervals from 28th May to 28th July 2009 using a special temperature recorded (Temp 1000 by Madge Tech).

Two months later, at the end of the experiment, plants were uprooted from the soil and height, fresh and dry top and root weight, number of leaves and chlorophyll content were recorded. Root gall index (RGI) 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 eas determined by processing 500 ml soil by the Coolen's method (Coolen, 1979). Numbers of *M. incognita* 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 400 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 40 – 50 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 at time of transplant (*Pf*/*Pi*) of *M. incognita*.

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. Table Curve program was used to analyze the relationships between different compost doses and nematological parameters of the root-knot nematode.

Results and discussion

Soil physical and chemical parameters

Physical and chemical soil parameters are shown in Table 1. Soil used in the pot trial was characterized by a silt-clay-loam texture according to the USDA soil textural classification system and modest resulted the amount of stones and gravels.

Soil reaction was slightly alkaline mainly due to the con-

Table 1. Physical and chemical characteristics of soil

Parameter	Unit	Value
Stones and Gravels (ø>2mm)	g/kg	22*
Sand (ø<0.5mm)	g/kg	156
Silt (0.002mm<ø<0.5mm)	g/kg	563
Clay (ø<0.002mm)	g/kg	281
Textural Class (USDA)	/	Silty Clay Loam
pH (1:2,5 H2O)	/	8.0
pH (1:2,5 $CaCl_2$)	/	7.3
Electrical conductivity (1:2 H_2O at 25 °C)	dS/m	0.2
Total Carbonate	g/kg	29.0
Active Calcium	g/kg	27.2
Organic Carbon	g/kg	12.7
Total Nitrogen	g/kg	1.3
C/N	/	10.1
Available Phosphorus	g/kg	23.8
Exchangeable Calcium	g/kg	5556
Exchangeable Potassium	g/kg	346
Exchangeable Magnesium	g/kg	244
Exchangeable Sodium	g/kg	90
Exchangeable K_2O	g/kg	419
Exchangeable MgO	g/kg	402
Ca/Mg	/	13.8
K/Mg	/	0.9

* Each data is an average of four replications

tent of total carbonate and active calcium. According to electrical conductivity (0.2 dS/m) the soil can be defined non saline (salinity class = 0). Organic carbon and total N content indicated a medium level of fertility. C/N ratio indicated a good level of humification of organic matter (USDA, 1995; Ryan *et al*., 2001). Available phosphorus (P) content resulted low probably because the high calcium content that is responsible of the removal of phosphorus as insoluble calcium phosphates salts.

Among the exchangeable basis, the amount of Ca^{++} resulted very high (90 % of the sum of all the exchangeable basis), while the Na^{+} content was low (1.29 %) and the concentration of Mg^{++} and K^{+} intermediate (USDA, 1995; Ryan *et al*., 2001). Finally the ratio Ca/Mg and K/Mg indicated the presence of competition in the uptake of Mg^{++} due to the high content of Ca^{++} and K^{+} (Ryan *et al*., 2001).

Amendment physical and chemical parameters

Physical and chemical properties of organic amendments used in the experiment are shown in Table 2. COP was characterized by a high organic C and N content while C/N ratio (19.8) indicated an adequate level of stability and humification of the organic matter, being 20 the highest threshold for this parameter in stabilized organic amendments (Bernal *et al*., 2009; Min. D. n. 75/2010). Heavy metals content was lower than the limits established by the Italian Regulation (Min. D. n. 75/2010) and the germination index (84.1 %) indicated the absence of phytotoxicity (GI > 50 %) (Bernal *et al*., 1998; 2009) and a high level of maturity (GI > 80 %) (US. TMECC, 1997; CCQC, 2001). Analytical values indicated that compost organic matter mineralization in soil, and as consequence the availability of nutrients for plant growth, will not be altered neither by NH_3 production that occurs in case of low C/N ratio nor by deficiency of N that occurs in case of high C availability for microbial metabolism and low N supply (Senesi, 1989; Bernal *et al*., 2009).

Physical and chemical properties of VOP resulted in line with data reported by Roig *et al.* (2006). VOP showed a slightly acidic pH, a high organic matter (OM) content but a low N one. As a consequence, C/N ratio resulted very high (C/N = 53) as reported by other authors (Saviozzi *et al*., 2001; Roig *et al*., 2006; López-Piñeiro *et al*., 2008). These authors suggested the addition of nitrogen fertilisers together with VOP to avoid immobilization of soil mineral N and P and a general nutritional imbalance for plant growth. The same authors indicated VOP as a K rich fertilizer, but in our case the amount of K as well as of P resulted lower than most of data already published. The second problem often related to the use of VOP as soil amendment is the high polyphenols content that is associated with the phytotoxicity and antimicrobial properties of the residue (Cayuela *et al*., 2008). In our study, the germination index resulted higher than the limit of toxicity (GI > 50 %) (Bernal *et al*., 2009) but we cannot define the VOP used in the experiment as a mature or completely not phytotoxic amendment since a high level of maturity is assured by GI values greater than 80 % (Iglesias-Jimenez & Perez-Garcıa, 1992; CCQC, 2001; Bernal *et al*., 2009; Saidi *et al*., 2009). In the present study no nitrogen fertilizers were

Table 2. Physical and chemical characteristic of different organic amendments (virgin olive pomace, composted olive pomace and chicken manure)

Parameter	**Unit**	**VOP**	**COP**	**CM**
Humidity	%	53.5	61.4	9.0
pH (3:50 H_2O)		5.6	9.4	6.9
Organic Matter	g/kg	924.5	864.1	691.5
Organic Carbon	g/kg	481.5	450.0	360.2
Total Nitrogen	g/kg	9.1	22.8	63.9
C/N		53.0	19.8	5.6
Total P	g/kg	0.6	3.0	65.5
Total Cd	mg/kg	< 0.05	0.06	3.4
Total Cu	mg/kg	11.2	104.8	46.4
Total Mn	mg/kg	70.9	73.8	275.8
Total Ni	mg/kg	10.1	8.3	14.1
Total Pb	mg/kg	4.7	2.5	0.5
Total Zn	mg/kg	16.6	41.6	314.6
Total Ca	g/kg	5.3	23.5	68.6
Total K	g/kg	6.3	13.5	33.7
Total Mg	g/kg	0.9	2.1	12.5
Total Na	g/kg	0.4	0.8	5.0
Total Fe	g/kg	2.8	2.1	2.7
Total phenols	mg GA/g	3.8	/	1.2
Germination index 50 – 75 %	%	62.3	84.1	/

* Each data is an average of four replications

Table 3. Effect of different amendments and doses on the growth of melon plants (cv. Galia) in soil infested by the root-knot nematode *Meloidogyne incognita*

Treatment	Dose (dw) (t/ha)	Shoot weight (g) fresh	Shoot weight (g) dry	Shoot length (cm)	N° of leaves	Root weight (g)
VOP-A	11	48.7[*] b[**]	6.8 b	60 a	23 b	15.0 a
VOP-B	22	31.7 ab	4.3 ab	61 a	16 a	12.4 a
VOP-C	44	20.2 a	2.6 a	54 a	14 a	6.2 a
COP-A	4.5	90.6 c	15.0 c	85 b	30 c	15.7 a
COP-B	9	95.7 cd	15.6 cd	125 c	39 d	36.3 b
COP-C	18	89.7 c	14.6 c	84 b	33 c	20.6 ab
CM	3	370.6 e	52.7 e	124 c	56 e	96.4 c
CMB	3	398.6 f	57.4 f	133 c	58 e	88.5 c
Control (untreated)	---	118.6 d	18.9 d	96 b	34 cd	33.2 b

[*] Each value is an average of ten replications;

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

used in addition to VOP to avoid interference in the nematicide effect of the amendment. Our goals were to evaluate the nematode suppression capacity of VOP and COP as well as the ability of these amendments to support plant growth. Different doses were used to find the best solution for nematode control and plant growth.

CM could be considered an organic fertilizer rather than an amendment. It was characterised by a neutral pH, a high N content and a low C/N ratio. Also high resulted the content of P, Ca, K, Mg that probably were quickly mineralized in soil considering the low C/N ratio (C/N = 5.6) of this fertilizer.

The nematicidal activity of ammonia released during its mineralization greatly depends on environmental conditions, such as pH, temperature and humidity. The slightly alkaline pH of the soil used, the high temperature and the frequent irrigations probably favoured the mineralization of CM organic matter and the release of NH_3 in soil (Oka *et al*., 2000).

Plant growth parameters and chlorophyll content

Type and rates of amendments significantly affected plant growth and biomass compared to untreated control (Table 3). Plants amended with CM and CMB showed the highest values of plant growth and biomass in comparison to the other treatments (*P* = 0.05). However, CM and CMB treatments were not significantly different each other (*P* = 0.05) for root weight, number of leaves and wine length indicating that *P. lilacinus* did not have any influence in plant nutrients uptake. VOP resulted the worst treatment for plant growth and among the different doses 11 t/ha showed the higher values for shoot and root weight, shoot length and number of leaves. As expected, data obtained confirm the phytotoxic effect of the amendment although seed germination and root elongation tests did not indicate a high level of toxicity (Table 3).

All COP treatments showed significantly lower values of shoot and root weights in comparison to untreated control (*P* = 0.05). No statistical differences were found among COP treatments and untreated control in the number of leaves and shoot length (Table 3). Only the treatment COP-B (9 t /ha) produced a significantly higher number of leaves and shoot length in comparison to COP-A and COP-C tretaments (Table 3).

As observed for plant growth parameters, CM and CMB treatments showed significantly higher chlorophyll content during the whole life cycle of melon plants with respect to untreated control, VOP and COP treatments *(P* = 0.05) (Table 4). Significantly lower values were recorded for VOP at all doses applied in comparison to all other treatments (COP, CM and CMB). No differences in chlorophyll content were observed between COP treatments and untreated control indicating that the release of nutrients was probably lower than that was expected *(P* = 0.05) (Table 4).

Nematological parameters

All treatments with soil amendments significantly reduced root gall index (RGI) of the nematode on roots in comparison to untreated control (*P* = 0.05) (Table 5). The lowest gall index (1.2) was recorded on the roots of the plants treated with CMB, although no statistical difference was observed in comparison to CM treatment. Probably, *P. lilacinus* was not effective to improve significantly the reduction of RGI because of the fungus required a longer time to colonize the rhizosphere and/or the high amount of ammonium released by CM had a toxic effect not only on *M. incognita* but also on the antagonistic fungus. CM application, alone or in combination with *P. lilacinus*, resulted in a nematicidal action on the root-knot nematode population at rate of 3 t/ha lower than those previously reported in the literature (Rodriguez-Kabana, 1986; Kaplan & Noe, 1993). No differences were observed in RGI among the different applied rates of VOP and COP treatments (Table 5).

Table 4. Effect of different amendments (VOP, COP and CM) and their doses on chlorophyll content of leaves of melon plants (cv. Galia), infested with *M. incognita*, during the growing period

Treatment	Dose (dw) t/ha	Chlorophyll content (Spad unit) Date 13/06/2009	01/07/2009	19/07/2009
VOP-A	11	25.3* b**	27.5 a	26.2 a
VOP-B	22	18.2 a	27.4 a	26.9 a
VOP-C	44	19.8 a	23.5 a	25.6 a
COP-A	4.5	37.9 c	31.9 b	35.0 b
COP-B	9	35.4 c	32.3 b	32.4 b
COP-C	18	34.4 c	34.8 b	34.8 b
CM	3	46.4 d	46.2 c	45.7 c
CMB	3	49.8 d	49.5 c	47.4 c
Control (untreated)	---	37.7 c	34.6 b	32.3 b

* Each value is an average of 4 replications;

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

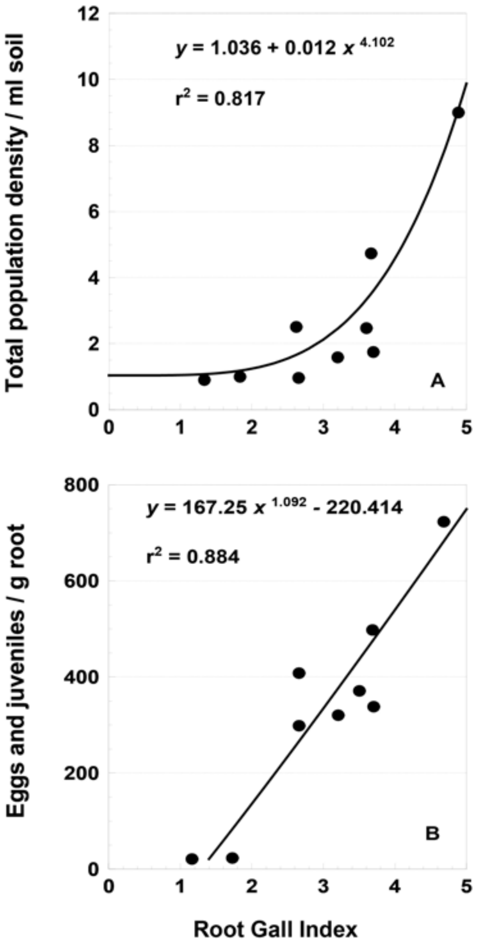

Fig. 1. Relationship between root gall index (RGI) observed on roots of melon plants infested with the root-knot nematode *Meloidogyne incognita* and nematode population density/ml soil (A) and eggs and juveniles/g root (B)

Number of eggs and second stage juveniles of the nematode and females per g of root ($P = 0.05$) were significantly reduced by all treatments with soil amendments in comparison to the untreated control (Table 5). The lowest numbers of eggs and juveniles per g of root were observed in CM and CMB treatments with no significant difference between them.

In VOP and COP treatments, the number of eggs and juveniels per g root was significantly higher than that calculated in CM and CMB treatments and significantly lower than that evaluated in the untreated control. No statistical differences were observed among the different doses applied of VOP and COP amendments. The highest number of eggs and juveniles per g of root was observed in the untreated control (751 eggs and juveniles/g root) (Table 5). Moreover, no significant differences were found in number of females/g root among the different amendments and the applied doses (Table 5).

These data confirm that all amendments incorporated into the soil were effective in nematode suppression and among them CM and CMB were the most effective. With regards to application doses, no differences in nematode control were observed indicating that, in the case of VOP application, the lowest dose can be used to minimize its phytotoxicity while, in the case of COP or CM/CMB amendments, the dose can be calculated on the basis of the nutrient content and their mineralization rate.

All soil amendments strongly and significantly reduced soil nematode population density in comparison to untreated control ($P = 0.05$) (Table 5). No statistical differences were observed among the doses applied of VOP and COP with the exception of COP-B treatment. Moreover, these treatments were not significantly different from CM and CMB. The addition of *P. lilacinus* did not significantly reduce soil nematode population density in comparison to CM. The reproduction rate (*Pf/Pi*) showed the same trend of final soil nematode population density and it was rather low, especially in the untreated control, according to the high temperatures recorded during the experiment. Indeed, soil temperature ranged from 15.6 ºC to 42.8 ºC. Organic matter mineralization was probably influenced by soil

Table 5. Effect of different amendments (VOP, COP and CM) and their doses on root gall index, eggs and juveniles and females/g root, final nematode population density/ml soil and reproduction rate of melon plants (cv. Galia) infested with *M. incognita*

Treatment	**Dose (dw) (t/ha)**	**Root Gall Index (RGI)**	**Nematodes/g root**		**Final nematode population density**	**Reproduction rate (*Pf*/*Pi*)**
		Scale (0-5)	**Eggs and J_2**	**Females**	**(Eggs and J_2/10 ml soil)**	
VOP-A	11	3.1* c**	326 b	0.94 a	15 a	1.5 a
VOP-B	22	2.7 bc	315 b	0.41 a	30 a	3.0 a
VOP-C	44	2.7 bc	409 b	1.43 a	13 a	1.3 a
COP-A	4.5	3.5 c	385 b	1.76 a	22 a	2.2 a
COP-B	9	3.6 c	523 bc	1.20 a	54 b	5.4 b
COP-C	18	3.6 c	344 b	0.46 a	18 a	1.8 a
CM	3	1.8 ab	6 a	0.12 a	11 a	1.1 a
CMB	3	1.2 a	7 a	0.03 a	9 a	0.9 a
Untreated control	---	4.9 d	751 c	4.00 b	87 c	8.7 c

* Each value is an average of ten replications;
** Data followed in each column by the same letters are not statistically different according to Least Significant Difference's Test ($P = 0.05$)

temperature being nitrification severely restricted and eventually stopped by temperature above 37 ºC while the optimum range of temperature for C-mineralization is supposed to be between 25 and 45 ºC (De Neve *et al.*, 1996).

Based on this results, indipendently from the amendment, significant positive correlations were found in the relationship between root gall index and total nematode density into the soil (Fig. 1A) and eggs and juveniles/g root (Fig. 1B) of *M. incognita*. The equations reasonably explain the above relationships, as indicated by the high values of the correlation coefficients (*r*).

Conclusions

According to the obtained biometric and nematological data (Table 4 and 5) CM and CMB resulted the most effective treatments in the root-knot nematode control and melon plant growth. The reason could be due to the higher availability of nitrogen and phosphours in CM and CMB treatments in comparison to VOP and COP treatments. The presence of a higher content of available N might have affected and improved plant growth with a greater plant resistance to nematode attacks. So, plant growth enhanced by CM and CMB treatments could be due to the combination of the suppressive effect on the root-knot nematode with a direct fertilizing effect on melon plants, as observed in previous trials on tomato (D'Addabbo *et al.*, 2003).

Results from this experiment confirm the suppressivity of VOP, COP and CM on *M. incognita*, previously reported in other trials (D'Addabbo & Sasanelli, 1996; D'Addabbo *et al*., 2000) in which the suppressive action was related to soil amendments rates.

Phytotoxic effect observed in VOP amended pots, as previously reported (Rodriguez-Kabana *et al.,* 1992; 1995), could possibly be explained by the short interval between VOP incorporation and transplanting (only 2 weeks), which probably did not allow the degradation of phytotoxic compounds.

The suppressive effect on nematode population was not significantly increased in CM by the addition of *P. lilacinus*, showing a greater and direct suppressive effect of chicken manure in comparison to the biological control agent, which require time to colonize the rhizosphere.

Also soil temperature recorded, higher than 40 °C, might have influenced the suppressive nematode effect of the different amendments tested, as previously reported by Lopez-Perez *et al.* (2005) for the nematicidal efficacy on *M. incognita* of three plant residues (broccoli, melon and tomato) at three different temperatures (20, 25 and 30 ºC). A strong significant reduction of the root-knot nematode was observed at the highest temperature, probably due to the increase of ammonia concentration.

Since the composting process could lead to the decomposition of nematicide compounds resulting in a non suppressive effect of mature compost (Cayuela *et al.*, 2008) the combination of two amendments, COP and CM, or VOP and CM could result in a higher nematode suppression and a higher crop yield compared to single application, as previously observed on tomato by Marull *et al.* (1997). Therefore the mixtures of these materials could be suggested to optimize the beneficial effects of each amendment either for nematode control and plant growth.

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RECEIVED JUNE 23, 2011 ACCEPTED DECEMBER 16, 2011