

Control of root-knot nematodes by composted agro-industrial wastes in potting mixtures

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Abstract

The use of composted dry cork, dry-grape marc (fruit residue after pressing) and a 1:1 mixture of dry-olive marc + dry-rice husk as an amendment to potting mixtures was assessed for the management of *Meloidogyne* species. Amending the potting mixture with composted dry cork at rates of 0%, 25%, 50%, 75% and 100% v/v, reduced the root galling and final populations of *Meloidogyne incognita* race 1 and *M. javanica* in tomato, and final nematode population in olive plants, compared with the control. In tomato, increasing the rate of that amendment exponentially reduced the root galling caused by *Meloidogyne incognita* race 1 (40.8%) and the final nematode population (81.9%). Similarly, increasing rates of the amendment exponentially reduced the root galling of tomato caused by *M. javanica* (51.3%) and the final population (82.6%). Infection of olive roots by *M. incognita* race 1 did not cause visible galling; however, amendment with dry cork reduced the final nematode population by 87.9%. Amending the potting mixture with dry-grape marc also reduced the root galling and final populations of *M. incognita* race 1 and *M. javanica* in tomato, though the reductions in root galling (24.4% and 25.6%, respectively) and final nematode populations (34.2% and 34.7%, respectively) were not enough for effective nematode management. Root galling and final nematode population were not reduced in potting mixture amended with the 1:1 mixture of composted dry-olive marc and dry-rice husk.

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1. Introduction

Root-knot nematodes, *Meloidogyne* spp., are economically important plant pathogens that can be managed by cultural practices, chemical nematicides, and resistant cultivars. Use of nematicides for the management of root-knot nematodes is being restricted due to environmental and human health concerns. In addition, nematicides often do not provide long-term suppression of the pathogen. Therefore, there is a need to develop alternative, environmentally friendly management strategies for root-knot nematodes, including use of biocontrol agents and organic amendments (Noling and Becker, 1994). Soil amendments such as green manure, animal manure, and composted materials are effective in the management of several soilborne plant pathogens (Hoitink and Boehm, 1999). In

particular, several authors reported suppression of diseases caused by root-knot nematodes with composted agricultural wastes (McSorley and Gallaher, 1995; Oka and Yermiyahu, 2002). In addition, amending soil or potting mixtures with composts can improve soil fertility as well as quality and productivity of plant products (Boehm et al., 1993).

In Spain, agro-industrial processing in cork (*Quercus suber* L.), grape (*Vitis vinifera* L.), olive (*Olea europaea* L.) and rice (*Oryza sativa* L.) production give rise to large amounts of agro-industrial byproducts, such as olive and grape marc (fruit residues discarded after olive-oil and wine productions), rice husks, and cork wastes. The disposal of the large amounts of these waste materials is causing environmental concern. These large amounts of byproducts result from extensive olive (2.3×10^6 ha), grape (1.2×10^6 ha), and rice (100,000 ha) acreage, and cork production (90,000 t/year) (MAPA, 2000). Estimated yearly amounts of residues produced include 800,000 t of olive marc,

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550,000 t of grape marc, 220,000 t of rice husk, and 20,000 t of cork waste (MAPA, 2000). Therefore, finding appropriate methods for their disposal is important in Spain. During the last few years, composting was shown to be one of the most suitable methods for recycling these waste materials (Paredes et al., 2002).

Research at the Technical College of Agricultural Engineering, Seville University (Seville, Spain) developed protocols for production of composts from the agricultural wastes mentioned above, which provides stable composts with appropriate maturity parameters (Trillas et al., 2002). The objective of the present study was to determine the potential of composts from dry cork, dry-grape marc and a mixture of dry-olive marc + dry-rice husk for the management of *Meloidogyne* spp. in potting mixtures used for nursery production in Spain. We chose two experimental systems: (1) olive planting stocks, as they are susceptible to *Meloidogyne* spp. (Nico et al., 2002, 2003), and (2) tomato (*Lycopersicon esculentum* Mill.) seedlings, because *Meloidogyne* spp. are economically important pathogens of tomato worldwide (Jones et al., 1991).

2. Materials and methods

2.1. Nematode inoculum and plant material

The effect of compost amendments on root gallings and final nematode population was studied in artificial inoculation experiments of healthy plants with *Meloidogyne incognita* race 1 and *M. javanica*. These nematodes commonly infect tomato and olive planting stocks in Spain. *Meloidogyne incognita* race 1 and *M. javanica* were isolated from roots of infected olive planting stocks of cvs. Manzanilla and Picual, respectively, from nurseries in Córdoba province (Nico et al., 2002).

Inocula were increased on tomato plants (cv. Roma) starting from a single nematode egg mass for each species (Nico et al., 2002). Inoculum for experiments consisted of eggs and second-stage juveniles (J2s) extracted from tomato roots by the NaOCl procedure (Hussey and Barker, 1973). Briefly, inoculum was extracted from 5-g sub-samples of chopped tomato roots suspended in 1% NaOCl in sterile water for 4 min. Eggs and J2s released from the root pieces were poured through a 75- μ m sieve and collected onto a 5- μ m sieve; then, the 5- μ m mesh was submerged into a water bath to assure a quick dilution of NaOCl concentration. Afterwards, the extracted eggs and J2s were gently washed in a stream of tap water three times to remove NaOCl, and the nematode inoculum was separated from root residues by centrifugation (Coolen, 1979). The population density of eggs and J2s released was determined

from 10 replications of 1-ml aliquots of the inoculum suspension.

Plant material for experiments consisted of tomato seedlings cv. Roma and olive planting stocks cv. Picual. Tomato seedlings developed from seeds sown in 4 \times 4 cm plastic cell plug trays filled with vermiculite. Olive planting stocks, propagated by rooting of leafy stem cuttings in perlite under mist tunnels, were grown in an organic growing mixture (Max[®], Vehnemoor GmbH D-26683 Saterland Sedelsberg, Germany) in 5 \times 5 cm plastic cell plug trays for 3 months.

2.2. Growth chamber experiments

Two experiments were conducted in a growth chamber adjusted to 25 \pm 1 °C, 60–90% relative humidity, and a 14-h photoperiod of fluorescent light at 360 \pm 25 μ E m⁻²s⁻¹. These environmental conditions are favorable for development and reproduction of *Meloidogyne* spp. (Van Gundy, 1985).

Experiment I was conducted to determine whether amending a potting soil mixture with dry cork compost would suppress root gallings and final populations of *M. incognita* race 1 on olive and tomato, and of *M. javanica* on tomato. Dry cork compost was derived from non-valuable cork residues resulting from the first stages of manufacture, and provided by M. Avilés, Technical College of Agricultural Engineering, University of Seville (Trillas et al., 2002). Residues were chopped in approximately 5-mm-long pieces and subjected to a standard aerobic composting process (Trillas et al., 2002). The composted mixture was tested for phytotoxicity prior to use. Tomato seedlings and olive plants were transplanted into 15-cm-diameter clay pots (one plant per pot) filled with 0.5 l of an autoclaved (121 °C, 1 h, twice on two consecutive days) soil mixture (sand/clay loam, 2:1, v/v) amended with dry cork compost at rates of 0%, 25%, 50%, 75% and 100%, v/v. Plants in pots were watered as needed and fertilized with 100 ml of a 0.1%, 20–5–32 + micronutrients hydro-sol fertilizer (Haifa Chemicals Ltd., Haifa, Israel) solution every week. Tomato seedlings were inoculated by pouring 10,000 eggs + J2s of *M. incognita* race 1 or *M. javanica* in 10 ml of sterile distilled water around the root ball of a plant at transplanting. Olive plants were inoculated similarly with 5000 eggs + J2s of *M. incognita* race 1. The control treatment consisted of plants treated similarly to those inoculated but without nematodes. Plants were incubated in the growth chamber for 2 months. Treatments were replicated seven times in a randomized complete block design.

Experiment II was conducted to determine whether amending the potting mixture with either a dry-grape marc compost or a mixture of dry-olive marc + dry-rice husk (1:1 vol/vol) composts would suppress root gallings and final populations of *M. incognita* race 1 and

M. javanica on tomato. The source of the composts used in this experiment were described above. Inoculation, rates of composted amendments, and experimental treatment design and replications were as described.

2.3. Nematode population analysis

After the 2-month incubation period, individual plants were cut at the soil level and the roots washed free of soil. Severity of root galling in the *Meloidogyne*-infected tomato plants was assessed on a 0–5 rating scale according to the percentage of galled tissue, in which 0=0–10% of galled roots; 1=11–20%; 2=21–50%; 3=51–80%; 4=81–90%; and 5=91–100% (Barker, 1985). Severity of nematode galling in olive roots was not assessed because galls did not develop in infected roots. Nematodes from 100-cm³ samples of infested potting mixtures and from 5-g samples of roots were extracted by centrifugation (Coolen, 1979), as described for inoculum preparation. Extracted nematodes were used to estimate final nematode population densities.

2.4. Statistical analysis

All experiments were performed twice. Similarity among experiments was tested by preliminary analyses of variance using experimental runs as a factor, so that experiment \times treatment interaction could be determined. Such an interaction was not significant and allowed combining data for analyses of variance and nonlinear regression. Data of root-gall severity (RGS) and final nematode population were normalized before analysis by transforming them to $\log_{10}(X + 1)$ (Gomez and Gomez, 1984). Analyses of variance were carried out using Statistix 7.0 (NH Analytical Software, Roseville, MN). Mean values of RGS and final nematode population were compared using Fisher's protected least significant difference test (LSD) at $P = 0.05$.

Suppression of root-knot nematode development by treatments was described by the expanded negative exponential model: $Y = C \exp[-r \log(AR)] + K$. In this model, Y is RGS or final nematode population, C is a constant, AR is the amendment rate, r is a rate of decrease of RGS and final nematode population, and K is the asymptote (Campbell and Madden, 1990). Regression analyses were conducted by the least-squares program for nonlinear models of the Statistical Analysis System 6.08 (SAS Institute Inc., Cary, NC). The coefficient of determination (R^2), the mean square error, the asymptotic standard error associated with the estimated parameter, and the pattern of the standardized residuals plotted against either predicted values or the independent variable, were used to evaluate the goodness-of-fit of data to the model (Campbell and Madden, 1990).

3. Results

3.1. Amendment with dry-cork compost

In the first growth chamber experiment, amending the potting mixture with dry cork compost reduced ($P < 0.001$) the severity of root galling and final nematode population in tomato, irrespective of *Meloidogyne* species or amendment rate (Table 1, Fig. 1A and C). In the non-amended potting mixture, both *M. incognita* race 1 and *M. javanica* caused root galling on more than 60% of the tomato root system and the final nematode populations increased 11.5- and 7.4-fold, respectively, compared with initial nematode populations (Table 1). In contrast, root galling did not occur in inoculated olive planting stocks grown in the non-amended potting mixture, and the final population of *M. incognita* race 1- was 1.7-fold that of the initial nematode population (Table 1). In the potting mixture amended with dry cork compost, tomato root galling caused by *M. incognita* race 1 was reduced ($P < 0.001$) by 27.3–54.5% and that caused by *M. javanica* by 27.5–75.0%, compared with the non-amended control (Table 1). For both nematode species, root galling decreased exponentially with an increase in the rate of dry cork compost (Fig. 1A and C). Similarly, the final nematode population in tomato roots was reduced by 65.3–98.5% for *M. incognita* race 1 and by 65.5–99.7% for *M. javanica*, compared with the non-amended control (Table 1). For both *Meloidogyne* spp., the final nematode population decreased exponentially with an increase in the rate of dry cork compost (Fig. 1B and D). Suppression of tomato galling and final populations of *M. incognita* race 1 or *M. javanica* was not influenced by increasing the dry cork compost amendment rate from 50% to 100% or 75% to 100%, respectively (Table 1). The dry cork compost amendment reduced ($P < 0.001$) the final population of *M. incognita* race 1 in olive roots, with that decrease following a negative exponential pattern with the increase in the rate of compost (Table 1, Fig. 2). Compared with the nonamended control, the percentage reduction in the final nematode population ranged from 81.3% to 98.4% (Fig. 2).

3.2. Amendment with dry-grape marc, dry-olive marc+dry-rice husk

In the second growth chamber experiment, amending the potting mixture with dry-grape marc compost reduced ($P < 0.001$) the severity of root galling and final nematode populations in tomato, irrespective of *Meloidogyne* species. (Table 1). Similar to experiment I, in the non-amended potting mixtures *M. incognita* race 1 and *M. javanica* caused root galling on more than 80% of the tomato root system, and the final nematode population increased by 6-fold, compared with the

Table 1

Effects of composted amendments of potting mixtures on the root galling and final population of *Meloidogyne incognita* race 1 and *M. javanica* on tomato and olive planting stocks^a

Composted amendment		Tomato				Olive
		<i>M. incognita</i> race 1		<i>M. javanica</i>		<i>M. incognita</i> race 1
Material	Rate (%)	RGS ^b	Final population ^c	RGS	Final population	Final population
Dry cork						
		Experiment I				
	0	3.3 ^d a	114,612 a	4.0 a	73,777 a	8379 a
	25	2.4 b	39,752 b	2.9 b	25,470 b	1565 b
	50	1.8 c	17,396 bc	2.1 c	16,138 c	573 c
	75	1.5 c	8435 c	1.1 d	3483 d	248 c
	100	1.5 c	1737 c	1.0 d	215 d	132 c
Dry-grape marc						
		Experiment II				
	0	4.1 a	59,480 a	4.3 a	61,490 a	
	25	3.1 b	42,926 b	3.2 b	43,946 b	
	50	3.2 b	39,144 b	3.3 b	40,164 b	
	75	3.3 b	41,144 b	3.3 b	42,164 b	
	100	3.2 b	44,296 b	3.3 b	45,316 b	
Dry-olive marc + dry-rice husk (1:1)						
	0	3.7 a	69,499 a	3.8 a	65,609 a	
	25	3.4 a	64,914 a	3.5 a	60,750 a	
	50	3.4 a	72,931 a	3.5 a	68,249 a	
	75	3.6 a	71,208 a	3.4 a	68,654 a	
	100	3.4 a	64,078 a	3.4 a	63,561 a	

^aPlants were inoculated with 10,000 eggs + second-stage juveniles (J2s) (tomato) or 5000 eggs + J2s (olive) at the time of transplanting into the amended potting mixture. Plants were incubated in a growth chamber under conditions favorable for nematode development for 2 months.

^bSeverity of root galling (RGS) was rated on a 0–5 scale according to the percentage of galled tissue, in which 0 = 0–10% of galled roots; 1 = 11–20%; 2 = 21–50%; 3 = 51–80%; 4 = 81–90%; and 5 = 91–100%.

^cFinal nematode population determined by extracting nematodes from 100-cm³ samples of infested soil mixtures and from 5-g root samples of each plant 2 months after inoculation.

^dData are the average of two trials each with seven replicated plants per treatment combination. Means followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's protected LSD test.

initial nematode populations (Table 1). In the amended potting mixtures, tomato root galling caused by *M. incognita* race 1 and *M. javanica* was reduced by 19.5–24.4% and 23.3–25.6%, respectively, compared with that in the control treatment (Table 1). Amendment with dry-grape marc compost also reduced ($P = 0.036$) the final nematode population by 25.5–34.2% for *M. incognita* race 1 and by 26.3–34.7% for *M. javanica*, compared with the control. However, for both nematode species, neither root galling nor the final nematode populations on tomato were influenced by the rate of amendment (Table 1). Amendment of the potting mixture with the mixture of dry-olive marc + dry-rice husk (1:1 v/v) composts did not influence root galling or the final nematode population, irrespective *Meloidogyne* species or rate of amendment (Table 1).

4. Discussion

The effectiveness of compost amendments in the suppression of plant diseases is well documented, particularly when used in containerized systems (Hoitink

and Fahy, 1986). For plant-parasitic nematodes, effectiveness of suppression varies depending upon nematode species and type of compost (Akhtar and Alam, 1993). The present study was designed to determine the potential of amendment with three kinds of composts: dry cork, dry-grape marc and a mixture of dry-olive marc + dry-rice husk composts, in the suppression of root-knot nematodes in potting mixtures. Our results demonstrated that amending potting mixtures with dry cork compost suppressed root-knot diseases of tomato caused by *M. incognita* race 1 and *M. javanica*, and of olive planting stocks caused by *M. incognita* race 1. Such suppression was expressed both by reducing both the amount of root galling and the amount of secondary nematode inoculum in the infected plants. The suppression of root galling and nematode populations by dry-grape marc was too low for efficient nematode management in potting mixtures because secondary inoculum in infected plants was larger than damage thresholds for *Meloidogyne* spp. (Van Gundy, 1985). No suppression of root galling or final nematode population was shown by the composted 1:1 mixture of dry-olive marc and dry-rice husk.

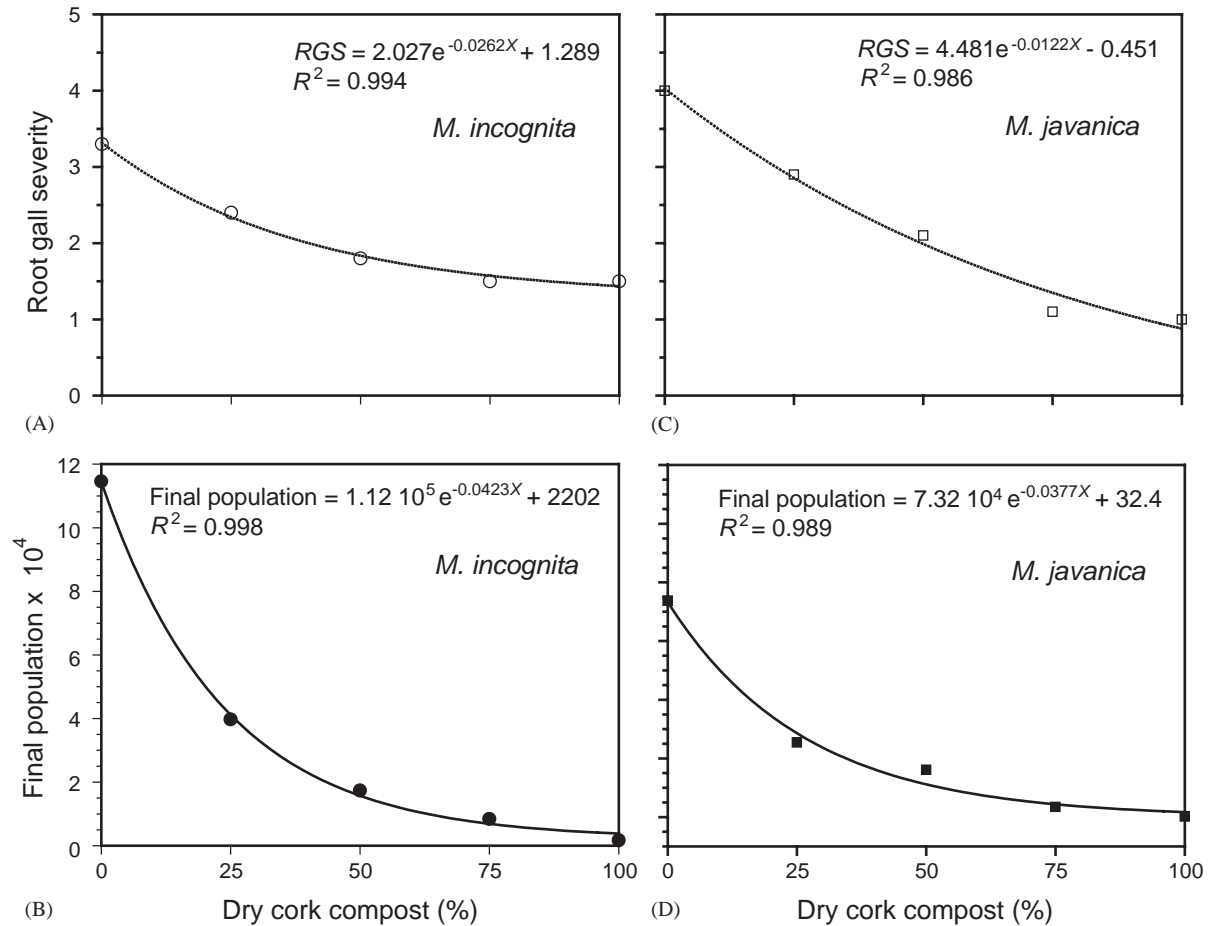


Fig. 1. Relationship between severity of root gall (RGS) (A, C) and final nematode population (B, D) of *Meloidogyne incognita* (A, B) and *M. javanica* (C, D) on tomato cv. Roma plants, and the rate of dry cork compost amending the potting mixture (0%, 25%, 50%, 75%, 100%). Each point represents the average of two experiments with seven replicated plants. Lines represent the predicted function calculated by fitting the expanded negative exponential model to data.

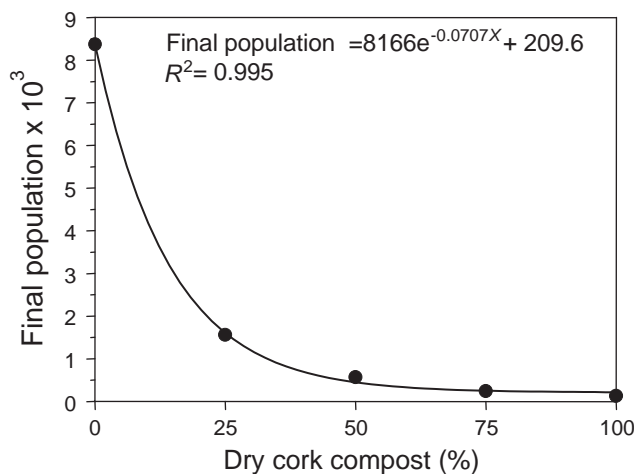


Fig. 2. Relationship between final nematode population of *Meloidogyne incognita* race 1 on olive planting stocks cv. Picual and the rate of dry cork compost amending the potting mixture (0%, 25%, 50%, 75%, 100%). Each point represents the average of two experiments with seven replicated plants. Lines represent the predicted function calculated by fitting the expanded negative exponential model to data.

Our results confirm the potential of some composted agro-industrial wastes as a management option for suppression of plant-parasitic nematodes (Akhtar and Alam, 1993; Akhtar and Malik, 2000). Such suppression was also demonstrated for organic amendments derived from forestry industry residues (Miller et al., 1973). The effect of organic amendments on suppression of plant-parasitic nematodes may be due to enhancement of the indigenous soil microflora (Rodríguez-Kabana et al., 1987). However, results of this present study are not likely associated with enhancement of microbial activity in the potting mixture because the composted agro-industrial wastes were added to sterilized soil mixture. On the other hand, reports of biological control of plant-parasitic nematodes by antagonists located in the amending material are scarce and refer mainly to materials very different from those used in this study, such as manures (Aryantha et al., 2000).

We suggest that nematode suppression in our work may rely on nematotoxic compounds released from the composted material. For example, ammonia produced

in the dry cork compost might be involved in nematode suppression since the C:N ratio of cork compost (C:N<20, Ordovás, *pers. comm.*) are sufficient for nematode management (Rodríguez-Kabana et al., 1987). Other high-molecular-weight substances, such as tannins or phenolic compounds, might also be associated with nematode suppression by the composted dry cork and dry-grape marc residues. Such substances are constituents of cork (Varea et al., 2001) and were regarded as responsible for nematode suppression by other organic amendments (Mian and Rodríguez-Kabana, 1982). The exact mechanism(s) of action of the released compounds is not known at this time. However, they could interfere with viability or pre-penetration activities of the egg and J2 stages, because once juveniles penetrate roots to complete their life cycle they are protected from chemical compounds unless those compounds are systemic.

Dose–response functions obtained in other studies (Kaplan and Noe, 1993) show that selection of the optimal amendment rates for nematode management is of primary importance to use this approach. In the present study, the reduction of root galling and nematode population was described by a negative exponential function with increasing rates of dry cork compost in the potting mixture. This dose–response activity was similar to reduction of *Meloidogyne artiellia* egg hatch and J2 survival by essential oils of garland chrysanthemum (*Chrysanthemum coronarium* L.) observed by Pérez et al. (2003). From our results, amendment with 50% dry cork compost seems the most suitable for *Meloidogyne* spp. management in potting mixtures for nursery production, because it provided a reasonable level of nematode control even though it did not result in the highest nematode suppression, since the additional nematode suppression of higher compost rates do not justify their cost. Conversely, the limited reduction of root galling and nematode populations by amendment with composted dry-grape marc and 1:1 mixture of dry-olive marc + dry-rice husk suggests that those composts have limited nematicidal activity, and therefore are not suitable for the management of root-knot nematodes on potting mixtures. In conclusion, composted agro-industrial wastes derived from andalusian agro industries byproducts may be a viable approach for the management of root-knot nematodes on potting mixtures. Moreover, this approach may be suitable to integrate with other management strategies to minimize yield losses in vegetable and woody crops caused by *Meloidogyne* spp.

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