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ANTONIO COLOMBO (*) - SALVATORE CATALDI (*) - GIUSEPPE MARANO (**
GIACOMO GENNA (***)

CONTROL OF THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, IN ORGANIC PROTECTED CROPS IN SICILY

(*) *Regione Siciliana, Dip. Interventi Strutturali, Servizio IV, U.O. 21, Osservatorio per le Malattie delle Piante di Acireale, Sezione di Vittoria - C.da Fanello, 97019 Vittoria (RG), Italy;* e-mail: antoniocolombo@regione.sicilia.it
(**) *Regione Siciliana, Dip. Interventi Strutturali, Servizio IV, U.O. 21, Osservatorio per le Malattie delle Piante di Acireale - Via Sclafani n. 34, 95124 Acireale (CT), Italy.*
(***) *Regione Siciliana, Dip. Interventi Strutturali, Servizio IV - Viale Regione Siciliana n. 2675, 90145 Palermo, Italy.*

Colombo A., Cataldi S., Marano G., Genna G. – Control of the root-knot nematode, *Meloidogyne incognita,* in organic protected crops in Sicily.

The effectiveness of different methods for controlling the root-knot, *Meloidogyne incognita*, suitable in organic farming in protected crops, was assessed in tomato during the 2009-2010 crop cycle, on sandy soil in a coastal area of southern Sicily (Italy). The treatments tested were: 60 day soil solarization alone and combined with commercial formulations of plant extracts of *Quillaja saponaria* Molina, *Azadiractha indica* Juss., *Brassica carinata* A. Brown, *Tagetes* spp., mycorrhizal fungi (*Glomus* spp.), or pellets and extracts of *A. indica*. The formulations were applied three or four times at intervals of 30 days. Non treated plots served as a control.

The application of plant extracts or the mycorrhizal fungi combined with soil solarization significantly reduced the population of *M. incognita* in the soil and suppressed root galling on tomatoes compared to both non treated and solarized control plots. All plant derived formulations combined with soil solarization increased marketable yield. The greatest increases were obtained using *A. indica* or the mycorrhizal formulations in combination with soil solarization.

KEY WORDS: root-knot nematode, vegetable extracts, mycorrhizae, organic farming.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* species) are among the most widespread and damaging nematodes in vegetable crops. They cause average annual yield losses of 20.6% in tomato crops, 16.9% in eggplant, 13.8% in melon and 12.2% in pepper (SASSER & FRECKMAN, 1987). In the southern part of Sicily, these soil-borne pests represent the main biotic factor limiting the yield of many vegetable crops in plastic-houses. This area has experienced severe damage caused by the southern root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw., which often occurs along with *M. javanica* (Treub) Chitw. and *M. arenaria* (Neal) Chitw., causing production losses of about 25% in tomato crops in sandy soil, especially in plastic-houses (COLOMBO, 2002).

In conventional agriculture these nematodes are controlled by chemicals, which are not permitted in organic farming. Search for alternative control methods effective against nematodes and environmental friendly is an imperative in organic agriculture. The effectiveness of some sustainable strategies has been tested and demonstrated in recent years. Among these strategies are crop rotation (MCLEOD & STEEL, 1999; CURTO *et al.*, 2005), biofumigation (CURTO *et al.*, 2005; MATTHIESSEN & KIRKEGAARD, 2006) and application of natural substances (OKA *et al.*, 2000; CHITWOOD, 2002). Moreover, greenhouse tests have shown that plants inoculated with endotrophic mycorrhizal fungi are less susceptible to root-knot nematode attack than non-mycorrhized plants (SIKORA & SHRONBECK, 1975; SIKORA, 1979). Soil solarization combined with the application of either extracts of various plant species or mycorrhizae as a mean of biological control of nematodes can be seen as possible alternatives to the use of synthetic nematicides.

During the 2009-2010 crop cycle, a trial was conducted in unheated plastic-house in Sicily (Italy) to asses the efficacy of different strategies in managing the root-knot nematode *M. incognita* in organic tomatoes.

MATERIALS AND METHODS

The trial was conducted during the autumn-winter-spring cycle, on tomato crop (*Solanum lycopersicum* L.), hybrid Ikram, in an area of Sicily where protected vegetable crops are economically very important. Therefore, a sandy soil plastic-house, naturally infested by *M. incognita*, was selected in a coastal area at Marina di Ragusa (province of Ragusa) (36° 792' N. and 14°599' E., 60 m a.s.l.).

The total area of the plastic-house was arranged in eight parts of 160 m^2 (10x16 m), divided by a buffer zone two metres large. Into each block, the surface was subdivided in 4x10 m plots according to a randomised block design, with four replicates per each treatments. Every plot was planted with 100 tomato seedlings in four rows, spaced one metre apart. The treatments, rating and timing of application are described in Table 1. The eigth treatments tested were: 60 day soil solarization alone and 60 day soil solarization combined with commercial formulations of plant extracts of *Quillaja saponaria* Molina, *Azadiractha indica* Juss., *Brassica carinata* A. Brown, *Tagetes* spp., pellets and extracts of *A. indica*, mycorrhizal fungi (*Glomus* spp.). The formulations were applied three or

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Table 1 – Substances, doses and times used for the treatments in the trial.

Treatment/thesis	Before transplanting or at transplanting	After transplanting
1	Soil solarization for 60-days from July 19th 2009	
2	Soil solarization for 60-days + Formulation contains spores and mycelium of mycorrhizal fungi (*Glomus* spp.) distributed in the soil at transplanting around the plant (dose of 5 g/m^2)	
3	Soil solarization for 60-days + Formulation with watery extract of *Quillaja saponaria* and *Tagetes* spp. distributed at transplanting by drip irrigation (30 l/ha)	Formulation with watery extracts of *Quillaja saponaria* and *Tagetes* spp. distributed 30-60-90 days after transplanting by drip irrigation (30 l/ha)
4	Soil solarization for 60-days + Formulation with oily extract of *Azadirachta indica* distributed at transplanting by drip irrigation (7 l/ha	Formulation with oily extract of *Azadirachta indica* distributed 30-60-90 days after transplanting by drip irrigation (7 l/ha)
5	Soil solarization for 60-days + Formulation with oily extract of *Brassica carinata* distributed at transplanting (20 l/ha)	Formulation with oily extract of *Brassica carinata* distributed 30-60-90 days after transplanting by drip irrigation (20 l/ha)
6	Soil solarization for 60-days + Pellet based on defatted seeds meals of *Azadirachta indica* distributed 3 days before transplanting (100 g/m^2) + Formulation with oily extract of *Azadirachta indica* distributed at transplanting by drip irrigation (7 l/ha)	Formulation with oily extract of *Azadirachta indica* distributed 30-60 days after transplanting by drip irrigation (7 l/ha)
7	Soil solarization for 60-days + Formulation with watery extract of *Tagetes erecta* distributed at transplanting by drip irrigation (20 l/ha)	Formulation with watery extract of *Tagetes erecta* distributed 30-60-90 days after transplanting by drip irrigation (20 l/ha)
8	Untreated soil	

four times at intervals of 30 days. Non treated plots served as a control (Tables 1-3).

Soil solarization was performed in closed plastic-house by mulching the irrigated plots with 50 µm thick transparent polyethylene film for 60 days, from July 19th to September 19th, 2009. The natural nematicides were distributed after soil solarization, as granular formulation before transplanting or liquid formulation in split applications along the rows, using 800 ml of solution per plant. The control plots received only water.

Tomatoes were transplanted on October 9th, 2009 and harvested from February to May 2010.

During the soil solarization period, soil temperatures at 10 and 20 cm depth were recorded in both non-mulched and polyethylene mulched plots. Throughout the growing seasons standard organic practices were followed.

Soil samples, composite of 10 cores, were collected in the central area of each plot, corresponding at 4,0 m^2, from the top 20 cm soil. They were collected before soil solarization, at transplanting and after the last harvest of tomato fruits (May 9th, 2010). The nematode density in each plot was determined by extracting the second stage juveniles of the nematode from a 100 cm^3 soil sub-samples by sieves Cobb's method (THORNE, 1961). Then the reproduction rate of the nematode (Pf/Pi = final population/initial population) was determined. At the last harvest, the root galls index was evaluated on the root of 10 plants per plot, 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 per root (TAYLOR & SASSER, 1978). During the trial, in subsequent harvests, data on weight and number of fruits were collected from 10 plants in the central area of each plot.

Data were subjected to analysis of variance and, when significant differences were observed, the treatments were compared with the Student-Newman-Keuls test.

RESULTS

In the solarized plots daily mean soil temperatures at 10 cm depth reached 45 °C between the end of July and the first days of August 2009. During the same period, at 20 cm depth, soil temperature reached 46 °C. Temperatures of 50 °C were recorded for three or four hours per day at both 10 and 20 cm depths. At the same time, in the control plots the soil temperature on average increased of only four degrees at 10 and 20 cm depth in comparison to the air temperature inside the plastic-house.

For each developmental stage of *Meloidogyne* there is a temperature-dependent death rate that increase with the increase of the temperature (FERRIS & VAN GUNDY, 1979). If a level of temperature lethal to nematode lasts for several hours at a given soil depth, high mortality of nematodes is likely to occur.

In our trial, only 10-20% of *M. incognita* juveniles survived in the top 20 cm of the soil solarized for 60-days compared with a reduction of 50% in non-solarized plots (Table 2). The treatments with plant extracts in association with soil solarization significantly suppressed nematode infestation. A general reduction in the number of juveniles was recorded at end of the tomato growing period (Table 2). The number of *M. incognita* juveniles in the soil was significantly decreased by the combination of all the products tested with soil solarization.

The plants showing the lowest rates of nematode attack were in the plots solarized and treated with plant extract formulations. At the end of the growing season higher gall

Table 2 – Effect of treatments on *Meloidogyne incognita* soil population.

Treatment	Time of application	Nematode population (N. J2/100 cm^3 of soil)				Galls index
		Before soil solarization	Initial (Pi)	Final (Pf)	Pf/Pi	
Soil solarization (60 days)	Before transplanting	1.007 a	253 b	1.031 b	4.0 c	1.7 b
Soil solarization + mycorrhizal fungi (5 g/m^2)	At transplanting	988 a	75 a	288 a	3.8 c	0.1 a
Soil solarization + Watery extract of *Quillaja saponaria* and *Tagetes* spp. (30 l/ha)	At transplanting + 30-60-90 days after transplanting	1.054 a	56 a	167 a	3.0 b	0.4 a
Soil solarization + Oily extract of *Azadirachta indica* (7 l/ha)	At transplanting + 30-60-90 days after transplanting	922 a	84 a	269 a	3.2 b	0.7 a
Soil solarization + Oily extract of *Brassica carinata* (20 l/ha)	At transplanting + 30-60-90 days after transplanting	978 a	215 a	202 a	1.0 a	0.2 a
Soil solarization + Pellet of *Azaditachta+ indica* (100 g/m^2) + Oily extract of *Azadirachta indica* (7 l/ha)	At transplanting 30-60 days after transplanting	1.109 a	375 ab	1.727 c	4.6 c	0.2 a
Soil solarization + Watery extract of *Tagetes erecta* (20 l/ha)	At transplanting + 30-60-90 days after transplanting	1.087 a	235 a	207 a	1.0 a	0.6 a
Untreated soil	–	1.072 a	938 c	1.881 c	2.0 b	3.5 c

Means followed by the same letters on the same column are not significantly (P = 0,05) different according to Student-Newman-Keuls test.

index values were recorded in the control plots, along with a very large juvenile population of *M. incognita* in the soil (Table 2).

All treatments increased the marketable yield of tomatoes (Table 3). The greatest yield increase was obtained in the plots solarised and integrated with *A. indica* extracts. Yield increases of 79% and 74% were obtained when these extracts were applied with either dual (meal and oily) or single (only oily) formulation, respectively. Significant (P = 0.05) yield increases of 78% were also obtained with mycorrhizal fungi (Table 3). Soil solarization alone was less satisfactory than the other treatments (Table 3).

In all treated plots, the number of fruits per plant was larger than in the control (Table 3). The greatest average weight of a single fruit was attained in the treated plots and the least in the control (Table 3).

DISCUSSION AND CONCLUSIONS

The results of this study demonstrate that an effective control of *M. incognita* and satisfactory tomato yield is possible using organic farming techniques. The experiment has confirmed that in Sicily soil solarization alone reduces the population of *M. incognita* in the soil, but nevertheless nematode control may not be sufficient. Instead, soil solarization combined with split applications of plant derived formulations, before and after transplanting, is more effective than soil solarization alone in preventing the invasion of tomato roots by *M. incognita*. Satisfactory results were also obtained combining soil solarization with the endotrophic mycorrizal fungi. The fungal symbiont may suppress the nematode by altering root attractiveness, impeding juvenile penetration into the roots and retarding giant cell formation (SIKORA, 1979), probably as a result of complex physiological changes associated with mycorrhizal infection (TULLIO *et al.*, 2002).

The better performance of the natural products and mycorrhizal fungi, in combination with soil solarization, in controlling the root knot nematode resulted also in greater yield increase compared with soil solarization alone.

Most probably, soil solarization would have little effect on nematodes present at more than 25 cm depth. Therefore, to improve nematode control the combination of natural products with soil solarization may represent a satisfactory option to control *M. incognita* in protected organic crops. Also, inoculation of tomato seedling at transplant with entotrophic mycorrhizal fungi could be another useful option for the integrated control of root-knot nematodes.

Table 3 – Effect of treatments on yield and its components in the experiment.

Treatment (l/ha or g/m^2)	Time of application	Yield components			Increased compared with control (%)
		Weight (g/plant)	Fruits (N./plant)	Fruits weight average (g)	
Soil solarization (60 days)	Before transplanting	1.824 ab	21 b	88 a	37
Soil solarization + mycorrhizal fungi (5 g/m^2)	At transplanting	2.370 b	25 b	93 b	78
Soil solarization + Watery extract of *Quillaja saponaria* and *Tagetes* spp. (30 l/ha)	At transplanting + 30-60-90 days after transplanting	2.220 b	24 b	93 b	67
Soil solarization + Oily extract of *Azadirachta indica* (7 l/ha)	At transplanting + 30-60-90 days after transplanting	2.315 b	24 b	99 b	74
Soil solarization + Oily extract of *Brassica carinata* (20 l/ha)	At transplanting + 30-60-90 days after transplanting	2.118 b	22 b	99 b	59
Soil solarization + Pellet of *Azaditachta indica* (100 g/m^2) + Oily extract of *Azadirachta indica* (7 l/ha)	At transplanting + 30-60 days after transplanting	2.388 b	24 b	100 b	79
Soil solarization + Watery extract of *Tagetes erecta* (20 l/ha)	At transplanting + 30-60-90 days after transplanting	2.112 b	20 b	98 b	59
Untreated soil	–	1.331 a	16 a	85 a	–

Means followed by the same letters on the same column are not significantly (P = 0,05) different according to Student-Newman-Keuls test.

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RIASSUNTO

IL CONTENIMENTO DEI NEMATODI GALLIGENI NELLE COLTURE BIOLOGICHE SICILIANE IN AMBIENTE PROTETTO

L'efficacia di diverse sostanze di origine naturale, impiegate in strategie di contenimento dei nematodi galligeni in regime di agricoltura biologica, è stata accertata attraverso una verifica sperimentale condotta nella fascia costiera della Sicilia sud-orientale (provincia di Ragusa) su un terreno sabbioso naturalmente infestato da *Meloidogyne incognita*. La prova è stata condotta su pomodoro cv Ikran F_1 in ambiente protetto, a ciclo autunno-primaverile, con trapianto effettuato nel mese di ottobre 2009 e raccolta da febbraio ad maggio 2010.

Le tesi a confronto prevedevano l'impiego della solarizzazione del terreno per 60 giorni, durante il periodo di luglio-agosto 2009, da sola o combinata con applicazioni di estratti vegetali (*Quillaja saponaria* Molina, *Azadiractha indica* Juss., *Brassica carinata* A.Brown, *Tagetes* spp.) o di funghi micorrizici (*Glomus* spp.), in formulazioni e combinazioni diverse di intervento, a confronto con un testimone non trattato.

Tutti i trattamenti hanno ridotto significativamente le infestazioni del nematode galligeno. Comunque, la solarizzazione del terreno, da sola, ha dimostrato una efficacia parziale, mentre l'integrazione della solarizzazione con tutte le sostanze impiegate è risultata efficace nel contenimento del nematode galligeno.

Tutti i trattamenti hanno migliorato la qualità e la quantità della produzione di pomodoro. Gli incrementi produttivi più alti sono stati ottenuti dall'integrazione della solarizzazione con estratti di *Azadiractha indica* e dall'associazione della solarizzazione con i funghi micorizzici. Risultati intermedi sono stati rilevati dalla combinazione della solarizzazione con gli estratti di *Quillaja saponaria* e di *Brassica carinata*.

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