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Integrated management strategies of *Meloidogyne incognita* and *Pseudopyrenochaeta lycopersici* on tomato using a *Bacillus firmus*-based product and two synthetic nematicides in two consecutive crop cycles in greenhouse

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ABSTRACT

Because of the restrictions on chemical pesticide use and their negative effects on the environment, as well as on human and animal health, alternative strategies for plant pest and pathogen managements are highly desirable. The objective of this work was to evaluate the suitability of a commercial formulation of *Bacillus firmus* strain 1-1582, applied either alone or in combination with oxamyl or fosthiazate, to control the southern root-knot nematode *Meloidogyne incognita* and the fungal plant pathogen *Pseudopyrenochaeta lycopersici* under greenhouse conditions during two tomato crop cycles. Application of *B. firmus* suppressed nematode population levels during the second crop cycle and when the treatments were repeated on soil previously amended with organic matter. In contrast, fungal infection was reduced during both crop cycles regardless of the application of organic matter. The combinations of the bioformulation and chemicals induced the lowest Root Galling Index compared to all other treatments in both crop cycles. The suppression of nematode populations levels and infection rate of the fungus induced by *B. firmus* alone or in combination with the chemicals was more pronounced during the second tomato crop cycle than the first crop cycle, also because the temperatures during the second crop cycle were unfavorable to the nematode development. The greatest increase in tomato yield induced by the combined treatments was observed during the second crop cycle, and it was up 50% compared to the untreated control. The applications of the bionematocide and two chemicals used in this study did not result in dramatic suppression of nematode and fungal populations. However, the application of these products alone or in combination, supplemented by organic amendment increases the yield of tomato plants compared to that of the untreated control, although the plants were infected by the pest and the pathogen. These results indicate that early spring/early fall application of *B. firmus* is an effective biopesticide treatment for management of the southern root-knot nematode and *P. lycopersici* on tomato crops growing in the integrated pest management system of this experiment and in the environmental conditions of southern Italy.

1. Introduction

Tomato root disorders due to soil exhaustion, accumulation of fungal pathogens and plant-parasitic nematodes are a consequence of agronomic cropping systems based on monoculture. The interaction between fungal pathogens and plant-parasitic nematodes may synergistically increase damage to the host plants (Ragozzino and d'Errico, 2011). In particular, severe corky root rot and root-knot disease complex symptoms caused by *Pseudopyrenochaeta lycopersici* (Schneider *et* Gerlach) Valenz-Lopez, Crous, Stchigel, Guarro & Cano and *Meloidogyne* spp., respectively, have commonly been reported in tomato-growing areas both under field and greenhouse conditions. The soil-borne pathogen

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Table 1
Schedule of treatments applied 7 days before (BT), during (AT) or 14 days after transplanting (DAT) of tomato seedlings into *Meloidogyne incognita* infested soil. Treatments were tested during the first (unamended-soil) and second (amended-soil) tomato growing cycles. Water-treated plants served as controls. Time and number of applications are indicated by the "x".

Treatments	Commercial product	Dose	Time of application		
			BT	AT	DAT
Bacillus firmus	Flocter (Bayer CropScience)	80 kg ha^{-1}	x		
oxamyl	Vydate (DuPont)	10 L ha^{-1}		x	xxx
fosthiazate	Nemathorin (Syngenta)	10 L ha^{-1}		x	
B. firmus + oxamyl	Flocter +	80 kg ha^{-1}	x		
	Vydate	10 L ha^{-1}		x	xxx
B. firmus + fosthiazate	Flocter +	80 kg ha^{-1}	x		
	Nemathorin	10 L ha^{-1}		x	
control	water				

may affected the root system with varying degrees of severity depending upon inoculum density, plant cultivar, soil type, and environment. The soil temperature (up to 15–20 °C) play a key role in the *P. lycopersici* infection and in the development of the corky root disease.

Root-knot nematodes (*Meloidogyne* spp.) are pests of great economic importance for agriculture and cause dramatic yield losses (Greco and Di Vito, 2009; d'Errico et al., 2016) especially in rather sandy soils and under warm conditions (up to 20 °C). An effective strategy to control pests and pathogens is given by the use of nematicides (d'Errico et al., 2011, 2017a; Giacometti et al., 2010). However, environmental pollution, phasing-out of some active ingredients and restricted registration procedures for new agrochemicals have prompted research to find novel eco-friendly control strategies. The use of microbial pesticides (i.e. based on bacteria, fungi, viruses or protozoans) for the management of pests and pathogens has potential for reducing yield losses (Arora et al., 2000). Rhizobacteria have been reported to release compounds which have nematicidal effects (Zeng et al., 2015; Luo et al., 2018), induce plant systemic resistance (Hasky-Günther et al., 1998), alter nematode behaviour (Sikora and Hoffmann-Hergarter, 1993), affect plant recognition (Oostendorp and Sikora, 1990), and promote plant growth (El-Nagdi and Youssef, 2004). Numerous studies demonstrated that *Bacillus firmus* effectively controls different nematode genera on various crops (Crow, 2014; Xiong et al., 2015). Nevertheless, worldwide researches suggest that better nematode control can be obtained using integrated measures (Zasada et al., 2010; Soppelsa et al., 2011; d'Errico et al., 2017b; Landi et al., 2018). The objective of this study was to evaluate the efficacy of a commercial bioproduct based on *B. firmus* strain I-1582, when applied alone or in combination with two synthetic nematicides and organic amendment for the management of the southern root-knot nematode, *M. incognita* (Kofoid & White) Chitwood, and *P. lycopersici* on greenhouse grown tomato plants.

2. Materials and methods

2.1. Commercial products

In this study, conducted on two consecutive tomato (*Solanum lycopersicum* L.) crops, a commercial bio-product containing *B. firmus* strain I-1582 (5% w/v) and two synthetic nematicides were used singly and in combination, as described in Table 1. The commercial products were used at doses and application times as recommended by the manufacturers. Products were applied through the drip irrigation system at a depth of about 10 cm.

2.2. Greenhouse trials

The trials were conducted for two consecutive crop cycles in a greenhouse located in the nocerino-sarnese industrial district (Salerno, Italy) and previously used for up to 60 years for the production of conventional tomato cv. San Marzano. Soil at the experimental site was sandy loam (40% sand, 54% silt and 6% clay), with a low organic matter content (17 g kg^{-1} soil), pH 7.9 and with a residual nematode population of second stage juveniles (J2) of *M. incognita* (45 J2s per 100 ml soil) associated with spores of *P. lycopersici* from the previous tomato crops. Tomato seedlings cv. Grinta, susceptible to *M. incognita* and certified to be free from nematodes, were used in the experiments. Seedlings were grown in polystyrene alveolate planting trays and when at four true-leaf stage, 28 seedlings were transplanted in 2 rows per plot for a total of 24 plots (2.5 × 5 m each) inside the greenhouse covered with plastic film. Then, plants were staked up and lightly tied to supports. The experiments were arranged according to a randomized block design with four replicates per treatment and repeated twice during each crop cycle. During the first crop cycle (from March 30th to August 26th, 2015) the treatments were as follows: (1) *B. firmus*; (2) oxamyl; (3) fosthiazate; (4) *B. firmus* plus oxamyl; (5) *B. firmus* plus fosthiazate; and (6) untreated control (receiving only the same amount of water). The second crop cycle trial (from September 7th, 2015 to January 29th, 2016) was established on the same experimental plots as the first cycle. The above mentioned treatments were applied following the incorporation of the organic amendment into differently treated soils (Table 1). The amendment (Stalfert, Organazoto fertilizzanti) consisting of 60% organic matter, 5% organic Nitrogen and 30% organic Carbon, was incorporated into *M. incognita*-infested soil of each plot at the recommended dose (18 q ha^{-1}), 7 days before transplanting (BT) using an harrow (Table 1). During both crop cycles, tomato plants were watered periodically, treated for insect control, exposed to beneficial pollinators (bumblebees) and monitored for symptoms of phytotoxicity. Plants were also staked and tied as needed during the seasons.

2.3. Soil sampling and trial evaluations

Soil samples (5 sub-samples/plot), consisting of 15 cores removed with a sampling tool from 15 plants, were collected from the central area of each plot to the soil depth of 0–20 cm for the evaluation of initial (IP) and final (FP) nematode population densities. During the first crop cycle, soil samples were collected eight days BT, whereas in the second crop cycle sampling was conducted 1 day before the incorporation of the amendment into the soil. *M. incognita* second stage juveniles were extracted from 100 ml of each soil sample by combining the Cobb's sieving and decanting method with the funnel method for 48 h at room temperature (~25 °C). The nematode reproduction factor was calculated as FP/IP. Root galling index (RGI) was evaluated on 15 plants per plot using a 0–10 scale (Bridge and Page, 1980).

The severity of corky root development was estimated on 10 plants per plot at 50 DAT according to a 0–5 scale (0 = root healthy; 1 = 1–10% affected root surface (a.r.s.); 2 = 11–25% a.r.s.; 3 = 26–50% a.r.s.; 4 = 51–75% a.r.s. and 5 ≥ 76% a.r.s.). Diagnosis of *P. lycopersici* infection was confirmed by extraction of the fungus. Therefore, symptomatic tissues of tomato plants (1 mm^2) were sterilized, rinsed in sterile distilled water and placed in Petri dishes containing acidified potato dextrose agar medium (PDA), amended with streptomycin sulfate (0.1 g L^{-1}). Plates were incubated at 24 °C under dark conditions. Briefly, genomic DNA was extracted from the mycelia using DNeasy Plant Mini Kit (QIAGEN, USA). An aliquot (50 ng) of the extracted DNA was used as template for PCR reactions with primers ITS4 and ITS5, specific for the ITS regions and the 5.8S gene in the cluster of ribosomal genes of fungi. PCR products of the expected size (~500 bp) were purified from agarose gel using the Nucleospin Extract Kit (Macherey-Nagel, Germany) and sequenced. Then, sequences were analyzed using the BLASTn program with default parameters (http://blast.ncbi.nlm.nih

Table 2
Effect of treatments of *Bacillus firmus*, oxamyl and fosthiazate alone or in combination and without (first crop cycle, in March–August) or with soil amendments (second crop cycle, in September–January) on population densities (100 cm^3 of soil), reproduction factor and on root galling index (RGI) of *Meloidogyne incognita* infesting tomato cv Grinta plants growing in a greenhouse. Nematode reproduction factor was calculated as a ratio FP/IP, where IP and FP are the final and initial nematode population density before transplanting, respectively. RGI was evaluated at the end of each crop cycle (in August and January, respectively) using a scale ranged from 0 to 10, where 0 = no galls and 10 = 100% of the root system galled. Means followed by the same letters in a column are not significantly different ($P < 0.05$) according to Student-Newman-Keuls test.

Treatments	First crop cycle				Second crop cycle			
	IP	FP	FP/IP	RGI	IP	FP	FP/IP	RGI
Bacillus firmus	5 a	846 a	169 a	7.9 a	615 ab	269 b	0.4 a	5.9 b
oxamyl	7 a	595 b	85 b	6.6 ab	499 b	237 b	0.5 a	5.5 b
fosthiazate	6 a	612 b	102 b	6.7 ab	613 ab	245 b	0.4 a	5.8 b
B. firmus + oxamyl	4 a	424 c	106 b	5.0 b	361 c	142 c	0.4 a	4.3 c
B. firmus + fosthiazate	6 a	453 c	75 b	5.2 b	354 c	154 c	0.4 a	4.0 c
control	5 a	912 a	182 a	8.1 a	702 a	323 a	0.5 a	7.7 a

.gov/Blast.cgi).

For both trials tomato shoot fresh weight and yield plant^{-1}, fruit number plant^{-1} and fruit weight of 15 plants per treatment were recorded. Tomato plant fresh weight was determined at the end of each crop cycle (August and January, respectively), while tomato fruits were harvested, as they matured, once in June and July, and twice in August (I and II harvest) for the first trial, and once in November and December and twice in January (I and II harvest) for the second trial. Fifty days after transplanting and at the end of both trials, the chlorophyll content from basal, median and apical leaves of 10 plants per treatment was determined by a Minolta SPAD-502 apparatus (Minolta camera Co., Ltd., Osaka, Japan). Soil temperatures (min and max) were recorded monthly (the 1st day of each month) at the soil depth of 10 cm using a data logger (Lutron).

2.4. Data analysis

Statistical analysis was performed using SPSS 15.0 software (SPSS for Windows). As the results from the repeated experiments were similar, data were pooled for the analysis of variance (ANOVA). Means were compared using Student Newman Keuls multiple comparison test at $P < 0.05$.

3. Results

3.1. Effects of treatments on nematode infestation and fungal infection

At the beginning of the first crop cycle the nematode soil population density was evenly distributed across all treatments while it was not at the beginning of the second crop cycle as a consequence of the differences observed at the end of the first crop cycle (Table 2).

In the first crop cycle, the nematode soil densities and reproduction rates were significantly reduced only in the plots treated with the two synthetic nematicides both alone and in combination with application of *B. firmus*, with the greatest reduction of the nematode population occurring in plots treated with either one of the chemicals combined with *B. firmus*. In the second crop cycle the final densities and reproduction of the nematode declined drastically due to drop of the temperature. However, both the nematicides and the bioagent significantly controlled the nematode and, again, the greatest reduction occurred in the plots in which the two synthetic nematicides were combined with the bioagent (Table 2).

The severity of the nematode attack (RGI) was reduced only in the plots treated with either chemicals combined with *B. firmus* in the first cycle and by all treatments during the second crop cycle, when the greatest reduction was achieved combining *B. firmus* with the two nematicides (Table 2). During the second crop cycle, the effect of *B. firmus* alone on RGI was comparable to that of both nematicides applied singly.

PCR analysis confirmed that the corky root was due to *P. lycopersici*

Table 3
Effect of treatments of *Bacillus firmus*, oxamyl and fosthiazate alone or in combination and without (first crop cycle) or with soil amendments (second crop cycle) on root disease index induced by *Pseudopyrenochaeta lycopersici* at the middle and end of each crop cycle (in May and August and October and January, respectively) on tomato cv. Grinta growing in a greenhouse. The scale used for root disease index ranged from 0 to 5 (0 = root healthy and 5 ≥ 76% affected root surface). Means followed by the same letters in a column are not significantly different ($P < 0.05$) according to Student-Newman-Keuls test.

Treatments	*P. lycopersici*			
	First growing cycle		Second growing cycle	
	May	Aug.	Oct.	Jan.
Bacillus firmus	2.3 b	-*	2.1 b	2.0 b
oxamyl	3.2 ab	–	3.1 a	2.8 a
fosthiazate	3.0 ab	–	3.0 a	2.9 a
B. firmus + oxamyl	2.1 b	2.7 a	1.9 b	2.0 b
B. firmus + fosthiazate	2.2 b	2.9 a	1.8 b	2.1 b
control	3.9 a	–	3.3 a	–

* Infection was not assessable because roots were severely galled and damaged by nematode.

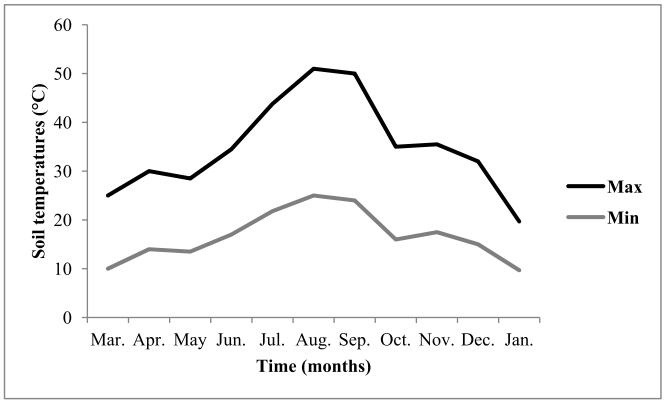

Fig. 1. Maximum and minimum average soil temperatures from March 1st to August 1st, 2015 (first crop cycle) and from September 1st to January 1st, 2016 (second crop cycle).

infection. Root disease incidence caused by *P. lycopersici* was evaluated only once during the first crop cycle because in August the roots were severely galled by the nematode, except for the combined treatments (Table 3) making difficult the rating of the corky root. During the second crop cycle, significant reduction of disease incidence by *P. lycopersici* was observed in all treated plants compared to control (Table 3). This diminished root disease incidence reflected the reduction in root galling observed during the second tomato crop. *M. incognita* is a species

Table 4
Effects of treatments of *Bacillus firmus*, oxamyl and fosthiazate alone or in combination and without (first crop cycle in March–August) or with soil amendments (second crop cycle in September–January) on shoot weight and on average chlorophyll content of leaves of tomato cv Grinta plants infested/infected by *Meloidogyne incognita* and *Pseudopyrenochaeta lycopersici* and growing in a greenhouse. Shoot fresh weights were recorded at the end of each crop cycle. Chlorophyll content was measured using a SPAD apparatus; data were collected twice in the middle and at the end of each crop cycle (in May and August and in October and January, respectively). Means followed by the same letters in a column are not significantly different ($P < 0.05$) according to Student-Newman-Keuls test.

Treatments	First crop cycle			Second crop cycle		
	Fresh weight (g plant^{-1})	SPAD		Fresh weight (g plant^{-1})	SPAD	
		May	Aug.		Oct.	Jan.
Bacillus firmus	21.4 b	27.3 b	24.2 c	18.9 ab	26.4 ab	22.8 a
oxamyl	26.3 ab	28.4 ab	27.9 b	17.5 ab	26.7 ab	23.4 a
fosthiazate	25.9 ab	28.1 ab	28.0 b	18.1 ab	25.9 ab	22.7 a
B. firmus + oxamyl	30.2 a	30.4 a	33.3 a	22.2 a	29.9 a	24.1 a
B. firmus + fosthiazate	29.7 a	30.0 a	32.8 a	22.6 a	28.7 a	24.2 a
control	19.7 b	26.9 b	23.8 c	12.7 b	24.9 b	21.7 a

belonging to the thermophile group of root-knot nematodes that thrive at temperature above 20 °C (Karssen et al., 2013). The final population densities attained by the nematode at the end of the two tomato crop cycles reflected the average temperature occurred during the final months of tomato growth during the two cycles. The average temperature above 20 °C was more favorable to the nematode development and reproduction than those below 20 °C recorded at the end of the second crop cycle (Fig. 1 and Table 2). Soil temperatures were also suitable for the reproduction of *P. lycopersici* and for the development of the corky root disease.

3.2. Effects of treatments on plant growth, leaf chlorophyll content and yield

No phytotoxic effect of the treatments was observed. In both trials, the greatest shoot weights were obtained by tomato plants treated with *B. firmus* in combination with oxamyl or fosthiazate compared to those treated with the biopesticide and chemicals alone and the untreated control, which all were of the same size (Table 4).

The chlorophyll content, as determined by measuring the SPAD index, was not increased by *B. firmus* (Table 4). Nevertheless, the combination of *B. firmus* with oxamyl or fosthiazate resulted in the greatest accumulation (P 0.05) of chlorophyll in tomato leaves (Table 4) from May throughout October. Oxamyl and fosthiazate alone increased leaf chlorophyll content only in August of the first crop cycle.

In the first trial, the number of tomato fruit was not affected by the treatments, while the weight of a single fruit increased only in August in the plots in which *B. firmus* was combined with either nematicides. The cumulative fruit yield did not significantly vary between single treatments and controls during the first three months of the experiment (Table 5). However, both combined treatments resulted in significant yield increase by the second August harvest and total yield of the entire crop cycle ($P < 0.05$), with a slightly increase (~15%) *vs.* control (Table 5). In the second growing cycle, the effects of the treatments on production variables were much more marked compared to the first crop cycle. In the second cycle, the numbers of tomato fruits were not affected by the treatments, but significant increases in yield and fruit weights were observed since the early harvest in November and December. Thereafter, tomato yield was still increased throughout January, while increases in tomato fruit weights were significant only at the last harvest (Table 6). However, the cumulative fruit yield was significantly

Table 5
Effects of treatments of *Bacillus firmus*, oxamyl and fosthiazate alone or in combination and without soil amendment (first crop cycle in March–August) on yield, number of fruits, and weight of each fruit of tomato cv. Grinta plants infested/infected by *Meloidogyne incognita* and *Pseudopyrenochaeta lycopersici* growing in a greenhouse. Data were collected in June, July and twice in August (I and II harvest). Means followed by the same letters in a column are not significantly different ($P < 0.05$) according to Student-Newman-Keuls test.

Treatments	Time (month)										Total yield (kg)	Increase *vs.* control (%)
	Jun.			Jul.			Aug. (I harvest)			Aug. (II harvest)				
	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)		
Bacillus firmus	5.6a	42a	134a	6.5a	52a	126a	6.8a	56a	122b	7.1b	61a	117b	26.0b	1.2
oxamyl	5.8a	44a	132a	6.4a	50a	129a	7.2a	56a	128 ab	7.6 ab	63a	121b	27.0 ab	5.1
fosthiazate	5.6a	43a	131a	6.4a	50a	128a	7.2a	57a	127 ab	7.8 ab	64a	122b	27.0 ab	5.1
B. firmus + oxamyl	6.1a	45a	135a	7.1a	55a	130a	7.9a	55a	144a	8.8a	64a	137a	29.9a	16.3
B. firmus + fosthiazate	6.0a	45a	134a	7.1a	56a	127a	7.8a	54a	145a	8.5a	59a	144a	29.4a	14.4
control	5.6a	44a	128a	6.5a	53a	122a	6.7a	58a	115b	6.9b	60a	115b	25.7b	–

Table 6
Effects of treatments of *Bacillus firmus*, oxamyl and fosthiazate alone or in combination and with soil amendment (second crop cycle in September–January) on yield, number of fruits, and weight of each fruit of tomato cv. Grinta plants infested/infected by *Meloidogyne incognita* and *Pseudopyrenochaeta lycopersici* growing in a greenhouse. Data were collected in November, December and twice in January (I and II harvest). Means followed by the same letters in a column are not significantly different ($P < 0.05$) according to Student-Newman-Keuls test.

Treatments	Time (month)										Total yield (kg)	Increase *vs.* control (%)
	Nov.			Dec.			Jan. (I harvest)			Jan. (II harvest)				
	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)	Yield (kg)	Number of fruits (No plant^{-1})	Fruit weight (g)		
Bacillus firmus	3.9b	32a	121 ab	5.5a	43a	127a	3.2 ab	31a	104a	2.1 ab	21a	102a	14.7b	38.7
oxamyl	4.1b	33a	123 ab	5.2a	41a	126a	3.2 ab	30a	106a	2.2 ab	22a	100a	14.7b	38.7
fosthiazate	4.0b	33a	120 ab	5.1a	40a	128a	3.0 ab	28a	109a	1.9 ab	20a	97a	14.0b	32.1
B. firmus + oxamyl	4.5a	34a	133a	5.7a	42a	135a	3.9a	34a	108a	2.7a	24a	113a	16.8a	58.5
B.firmus + fosthiazate	4.4a	34a	130a	5.5a	41a	133a	3.7a	34a	110a	2.5a	23a	109a	16.1a	51.9
control	2.9c	29a	102b	3.6b	37a	97b	2.6b	27a	99a	1.5b	21a	74b	10.6c	–

($P < 0.05$) higher in all treated plots, including those treated with *B. firmus* alone, with the combination of *B. firmus* plus chemical nematicides giving the greatest yield with an increase of 51.9–58.5% over the control (Table 6), ensuring an economical return.

4. Discussion

Application of *B. firmus* alone suppressed population densities and reproduction rate of *M. incognita* only during the second tomato crop cycle when an organic amendment was applied to the soil and probably because of residual *B. firmus* from the previous crop cycle. In contrast, the treatment of this pesticide suppressed *P. lycopersici* infection in both tomato crop cycles regardless of the application of the organic amendment.

Damages caused by nematode infestation seems to have increased the severity of *P. lycopersici* infection, probably because the trophic activity of *M. incognita* J2s on the root tissue favored the fungal penetration. Cultural practices such as the addition of organic amendments have been reported to enhance decline in pathogen and pest populations, and improve plant growth and yield (Weller et al., 2002; Marra et al., 2018). The suggested mechanism is probably related to changes in the composition of the soil microbial populations, which are strongly involved in disease suppressiveness (Mazzola, 2002). This approach can be implemented through the establishment of beneficial microbial communities (e.g. rhizobacteria) or fungal microorganisms (e.g. *Trichoderma* spp.) into the soil, thus promoting the ecosystem quality leading to containment of plant pathogens and pests (Burkett-Cadena et al., 2008; Lombardi et al., 2018).

The combined use of *B. firmus* and nematicides was the most effective in suppressing nematode and fungus populations and increasing the yield of the treated tomato plants. *B. firmus* enhanced the knock down effect on the two pathogens caused by chemicals and may have favored the suppressive activity of beneficial microorganisms, which can be more effective in presence of low nematode infestation levels. The suppression of nematode populations levels and infection rate of the fungus induced by *B. firmus* alone or in combination with the chemicals was more pronounced during the second tomato crop cycle than the first crop cycle. The economic advantage in adopting these management strategies was justified in the improved tomato yield income.

Abiotic factors, such as the soil temperatures played a key role in the suppression of the populations of the two pathogens in the second crop cycle. During the last two months of the second crop cycle (December and January), temperatures were unfavorable to the development of *M. incognita* and this resulted in decline of the nematode reproduction. Reproduction rates of this thermophil species (*sensu* Karssen et al., 2013) are low at temperatures $< 20\,^{\circ}C$. The infective J2 of the southern root-knot nematode are mostly active in warm soils rather than in soil with temperatures $\leq 18\,^{\circ}C$.

The lower temperatures during the second crop cycle induced, lower total yield and growth of the tomato plants compared to those of the summer crop cycle. The beneficial effect of the organic amendment in improving the performance of the bionematocide was shown by the results of this study. The organic amendment may have induced increasing microbial activity resulting in a positive effect on the soil characteristics. As concluding remarks, we would like to point out that the applications of the bionematocide and chemical used in this study did not result in dramatic suppression of nematode and fungal populations. However, the application of these products alone or in combination and supplemented by organic amendment increases the yield of tomato plants compared to that of untreated control, although the plants were infected by the two pathogens. These compounds should be taken into considerations by the tomato growers as components of an integrated pest management system of tomatoes grown in a greenhouse in the environmental conditions of southern Italy.

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