horticulturae

MDPI

Article

Potential Role of the Yeast *Papiliotrema terrestris* Strain PT22AV in the Management of the Root-Knot Nematode *Meloidogyne incognita*

Trifone D'Addabbo [1,*], Silvia Landi [2], Davide Palmieri [3], Lea Piscitelli [4], Elena Caprio [5], Vincenzo Esposito [5] and Giada d'Errico [2]

1 Institute for Sustainable Plant Protection, National Council of Research, 70126 Bari, Italy
2 CREA Plant Protection and Certification, 50125 Florence, Italy; silvia.landi@crea.gov.it (S.L.); giada.derrico20@gmail.com (G.d.)
3 Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy; davide.palmieri@unimol.it
4 Mediterranean Agronomic Institute, International Centre for Advanced Mediterranean Agronomic Studies, 70010 Bari, Italy; piscitelli@iamb.it
5 BIOFARM S.R.L. Test Facility, 80056 Naples, Italy; e.caprio@biofarmsrl.com (E.C.); v.esposito@biofarmsrl.com (V.E.)
* Correspondence: trifone.daddabbo@ipsp.cnr.it

Abstract: The nematicidal potential of the yeast *Papiliotrema terrestris* strain PT22AV (YSY) was investigated against the root nematode (RKN) *Meloidogyne incognita* in in vitro bioassays on infective juveniles (J2) and experiments on tomatoes in pot and greenhouse conditions. The J2 nematodes were exposed to YSY solutions for 19 days, using abamectin (ABA), fosthiazate (FOS) and distilled water as controls. In the experiments on potted and greenhouse tomatoes, 0.5 and 1 kg ha^{-1} doses of YSY were tested in comparison to ABA, biocontrol agents *Purpureocillium lilacinus* strain 251 (PUL) and *Bacillus firmus* strain 1-1582 (BAF), a plant biostimulant/fertilizer (ERG) and the nematicide Fluopyram (FLU). J2's viability was affected by YSL after 7 days, decreasing to zero on the 15th exposure day, while ABA and FOS resulted in 83 and 100% J2 mortality within 24 h. Only the 1.0 kg ha^{-1} dose of YSY was able to significantly reduce the final nematode population in soil and gall formation on tomato roots, without significant differences from PUL and BAF. All treatments in comparison also resulted in a significant increase in tomato growth and crop yield, except for 0.5 kg ha^{-1} of YSY. Data indicated that YSY could represent an additional tool for organic and integrated RKN management.

Keywords: *Papiliotrema terrestris*; *Meloidogyne incognita*; sustainable management; biocontrol

Citation: D'Addabbo, T.; Landi, S.; Palmieri, D.; Piscitelli, L.; Caprio, E.; Esposito, V.; d'Errico, G. Potential Role of the Yeast *Papiliotrema terrestris* Strain PT22AV in the Management of the Root-Knot Nematode *Meloidogyne incognita*. *Horticulturae* **2024**, *10*, 472. https://doi.org/10.3390/horticulturae10050472

Academic Editors: Wenli Sun, Mohamad Hesam Shahrajabian and Yansu Li

Received: 10 April 2024
Revised: 29 April 2024
Accepted: 2 May 2024
Published: 5 May 2024

1. Introduction

Plant-parasitic nematodes (PPNs) are included among the most harmful crop pests, annually causing yield losses of over USD 100 billion worldwide [1]. The direct damages are often aggravated by a reduced yield quality and by synergistic effects raised by the contemporary presence of fungal and bacterial crop pathogens [2,3]. In particular, root-knot nematodes (RKNs) of the genus *Meloidogyne* are acknowledged as the most yield-limiting PPN species, widespread on a wide range of herbaceous and tree crops all over the world [4].

The harmful environmental impact of the synthetic nematicides, that effectively control PPNs and have been relied upon for many decades, has led to their removal from the market under a strict EU pesticide revision [5]. Following to the dramatic lack of chemical nematicides, there is a strong imperative to research more sustainable management strategies, including biological control, i.e., the soil application of microbes that are antagonistic to PPNs or the encouragement of antagonistic microbial species that are natively present in the soil [6,7]. The modes of action of the main PPN-antagonistic microorganisms rely on different mechanisms, such as a direct parasitism, the production of nematotoxic secondary metabolites or the induction of plant resistance [8–10].

Yeasts are unicellular fungi that play a role in essential soil ecological processes such as nitrogen and sulfur cycles, phosphate solubilization and organic matter mineralization [11]. Soil yeasts were also acknowledged as plant growth promoters due to their biostimulating effects on plant growth [11,12]. Various yeast genera were reported to have a biocontrol role in fungal postharvest diseases of fruits, vegetables and stored grains, as well as in the control of foliar and root diseases caused by *Sphaerotheca fuliginea* Schltdl. and *Rhizoctonia solani* (Cooke) Wint., respectively [13]. Adversely, minor attention has been paid to the potential role of yeasts in PPN management, mainly limited to the study of the suppressive effects of strains of *Saccharomyces cerevisiae* on the infestation of the RKN species *M. incognita* Kofoid et White and *M. javanica* Treub in fruit and vegetable crops [14–16].

The genus *Papiliotrema* (class Tremellomycetes, order Tremellales, family Rhynchogastremaceae) was described about twenty years ago according to morphological, ultrastructural, physiological and molecular data [17]. This genus includes more than twenty yeast species, among which is *P. terrestris*, a ubiquitous species commonly present in both forestry and agricultural soils [18,19]. The strain PT22AV of *P. terrestris* was isolated from the epiphytic microbial communities of olive carpospheres in Central Italy and characterized for its biocontrol activity against field and postharvest phytopathogenic fungi [20]. Strain PT22AV has been developed as the active component of a granular formulation already tested as a biological fungicide in about 150 field efficacy trials. In consideration of this biocontrol potential and of the positive nematicidal performance of other yeast species, this study aimed to assess the potential suppressiveness of the *P. terrestris* strain PT22AV against the RKN *M. incognita* through in vitro bioassays and pot and greenhouse trials on tomato crops, in comparison to formulations of abamectin, the biocontrol fungus *Purpureocillum lilacinus* Thomson (syn. *Paecilomyces lilacinus*, the bacterium *Bacillus firmus* Bredemann and Werner, and the synthetic nematicides Fosthiazate and Fluopyram.

2. Materials and Methods

2.1. Materials

Strain PT22AV of *P. terrestris* (YSY), used in this study, was formulated as wettable granules with a 3×10^9 CFU concentration of live cells per gram (patent pending, AgroVentures Srl/LLC, Latina, Italy/Southbury, CT, USA). The exact composition is confidential, but it roughly consists of 30% (*w*/*w*) active ingredient, 40% (*w*/*w*) mineral co-formulant, and 30% food additives. In all the experiments, YSY was used in comparison to other nematode biocontrol products and synthetic nematicides, selected according to their frequency of use by the farmers of the experimental areas. The comparative nematode biocontrol products were an abamectin formulate (Tervigo©, Syngenta, Italy, 1.67% a.i.) (ABA), a formulation of *P. lilacinus* strain 251 (Bioact Prime DC©, Bayer CropScience, Milano, Italy, 216 g L^{-1} a.i.) (PUR), a formulation of *B. firmus* strain 1-1582 (Flocter WP5 10©) (BAF) and a biostimulant/fertilizer formulate (Ergofert Nemacontrol©, B.E.A. S.r.l., Galazzano, San Marino) (ERG). This last product was described as a mixture of seaweed, yeast extracts and a microbial ensemble of rhizosphere bacteria (*Pseudomonas* spp., *Bacillus* spp., Actinomyces) and saprophytic fungi (*Trichoderma* spp.), in addition to humic, fulvic and polycarboxylic acids and enzymes (cellulase, protease, amylase, lipase). The comparative synthetic nematicidal products were commercial formulations of the nematicides Fosthiazate (Nemathorin 150 EC©, Syngenta, Italy 15% a.i.) (FOS) and Fluopyram (Velum Prime©, Bayer CropScience, Milano, Italy, 400 g L^{-1} a.i.) (FLU).

The population of *M. incognita* used in the in vitro assay and in the experiment on potted tomatoes was recovered from the infested roots of the invasive weed *Araujia sericifera* Brot; identified through morphological observations and molecular analysis [21]; and reared on tomato plants (*Solanum lycopersicum* L.) cv. Dolcetini in an unheated greenhouse located in Ercolano (province of Naples) until experimental use.

2.2. In Vitro Bioassay

Eggs of *M. incognita* were extracted by shaking the infested tomato roots for 3 min in a plastic bottle containing 500 mL of a 0.5% sodium hypochlorite solution [22]. The egg suspension was repeatedly washed with sterile distilled water on 100 and 20 µm sieves and the recovered eggs were incubated for 24 h in Petri dishes filled with sterile distilled water inside a growth chamber at 25 ± 1 °C. The emerged second-stage juveniles (J2) were recovered in water and immediately used for the experiment.

Batches of 30 nematode J2 were hand-picked with a needle and transferred to glass wells containing 2 mL of a 50 mg L^{-1} suspension of YSY. The same volumes of a 100 µL L^{-1} solution of ABA, a 500 µL L^{-1} solution of FOS and distilled water (DW) were used as controls. Concentrations of the test products were estimated according to label field doses of each product, hypothesizing their distribution in a 20,000 L Ha^{-1} volume of water. Four replicates were provided for each treatment. The glass wells were sealed with Parafilm and incubated in the dark at 25 ± 1 °C for a 19-day period [23]. The motility of J2 in each well was checked under a stereoscopical microscope at 24 h intervals, transferred in DW, with the specimens still immobile after a 10 s needle stimulation. The persistence of immobility after the 24 h permanence in DW was assumed as a confirmation of nematode death. Mortality rates were calculated by Abbott's formula, $m = 100 \times (1 - nt/nc)$, in which m = percent mortality; nt = number of J2 nematodes still viable after the treatment; nc = number of viable J2 nematodes in the water control [24]. The experiment was run twice with separate controls for each experimental run.

2.3. Experiment in Pot

Plastic pots with a 3 L volume were filled with steam-sterilized sandy soil and inoculated with 1800 *M. incognita* eggs (60 eggs 100 mL^{-1} soil) previously extracted with the Hussey and Barker method described above [22]. The initial nematode density was chosen according to the 0.55 eggs and J2 mL^{-1} soil damage threshold estimated for *M. incognita* on tomatoes [25]. Ten days after the inoculation, each pot was transplanted with a 4-leaf tomato seedling, cv. Dolcetini. Treatments with 25 and 50 mg L^{-1} concentrations of YSY were applied by a 30 min dipping of the tomato seedling roots before the transplant and by drenching each pot with 200 mL of the YSY suspensions at transplant and four more times at weekly intervals. Pots non-treated or treated with PUR or FLU were used as controls. PUR was applied at a dose corresponding to a 0.75 L ha^{-1} field rate both 7 days before tomato transplant and 5 weeks later. FLU was applied 3 days before and two weeks after tomato transplanting at a dose corresponding to a 0.625 L ha^{-1} field rate. For YSY, the comparative products were also applied by soil drenching with 200 mL suspensions of respective dosages. Non-treated soil (NT) received the same volume of water applied for the other treatments. The pots were arranged in a non-heated greenhouse located in the area of Ercolano (province of Naples, Southern Italy), according to a randomized block design with four replicates of each treatment. Tomato plants were reared over two months according to the agronomical practices (fertilization, irrigation, pesticidal treatments) currently applied by local farmers.

At the end of the experiment, the tomato plants were uprooted and the fresh weights of aerial parts and roots were recorded. Gall infestation on tomato roots was estimated according to a 0–10 scale [26], while the final soil population density of *M. incognita* was assessed by extracting the J2 nematodes from a 100 mL soil sample of each pot by the cotton wool filter method [27].

2.4. Experiments in Greenhouse

The experiments were carried out in two non-heated commercial plastic greenhouses located in Ragusa province, Southern Italy, in 2022 and 2023. The experimental areas were uniformly infested by *M. incognita* according to both the visual observation of root systems from the previous tomato crop and soil nematode population density assessed on soil samples collected before starting the experiments.

In the first experiment, a 290 m^2 area (7.2 × 40 m) was subdivided into twenty 14.5 m^2 (8 × 1.8 m) plots on 13 August 2022, while the 346 m^2 (7.2 × 48 m) experimental area of the second experiment was partitioned in twenty-four 14.4 m^2 (8.0 × 1.8 m) plots on 31 July 2023. In both experiments, the plots were arranged according to a randomized block design, providing four replicates of each treatment in comparison.

In the first experiment, four-leaf tomato seedlings of cv. Hummer were transplanted at a 40 plants/plot density in double rows (40 cm apart in the rows and 1.25 cm between rows) on 13 August 2022. In the second trial, the cv. Livanti tomatoes were transplanted at a similar density (40 cm apart in the rows and 1.40 cm between rows) on 4 August 2023.

In both experiments, soil treatments with YSY were provided at a rate of 0.5 and 1.0 kg ha^{-1} either on the same day of transplant (1st experiment) or four days before (2nd experiment) and in five following applications at weekly intervals. Soil treatments at transplant with the two doses of YSY were also preceded by a 30 min dipping of the seedling roots in 25 and 50 mg L^{-1} YSY suspensions of the product, respectively. In addition to NT, treatments with FLU were applied at 0.625 L ha^{-1} 3 days before tomato transplanting and two weeks later, and one or two non-chemical formulates were added as controls in both trials. In the experiment of the year 2022, the non-chemical control was ERG distributed at a rate of 6 L ha^{-1} at tomato transplant and in four following 3 L Ha^{-1} weekly applications. In the second greenhouse trial (2023) experiment, the non-chemical controls were PUR, applied at 0.75 L ha^{-1} 3 days before transplanting (7 August 2023) and 28 days later (4 September 2023), and BAF, distributed at a 40 kg ha^{-1} dose on the same dates. All soil treatments were carried out in fertigation by diluting the plot dose of each product in a volume of water equivalent to 6000 L ha^{-1}. No treatment was carried out in the non-treated plots, which received only the same volume of water provided to the treated plots. As in the pot experiment, both tomato crops were reared according to agronomical practices currently applied by local farmers.

In both experiments, the number and weight of tomato fruits and the total fruit yield were recorded on the 20 central plants of each plot on four harvest dates (20 September and 1, 10 and 17 October 2022, and 21 and 30 September and 9 and 16 October 2023, respectively). In experiment 2, SPAD index values were also determined by five readings on leaves from the central part of sampling plants by using an atLEAF® CHL PLUS chlorophyll meter combined with the atLEAFSoft 1.0 software (FT Green LLC, Wilmington, DE, USA). Root gall infestation was estimated on the same plants at the last harvest date of each experiment, according to the Zeck's 0–10 scale [26]. The initial and final population densities of *M. incognita* were determined on a 100 g subsample of composite soil samples collected in each plot before tomato transplant (11 August 2022 and 31 September 2023) and at the final harvest (17 October 2022 and 16 October 2023) by extracting nematode J2, either already present in soil or emerged from nematode eggs, with the nematode cotton wool filter method [27].

2.5. Statistical Analysis

Data from the two experimental runs of the in vitro bioassay were pooled in the absence of any significant experiment × treatment interaction. All data from the four experiments were subjected to a one-way analysis of variance, followed by a comparison of treatment means with Fisher's Least Significant Difference pairwise procedure ($p < 0.05$), using the statistical software PlotIT 3.2 (Scientific Programming Enterprises, Haslett, MI, USA).

3. Results

3.1. In Vitro Bioassay

After a 24 h exposure, the viability of *M. incognita* J2 was not affected by YSY, while about 83% and 100% mortality rates were recorded for the nematode specimens exposed to ABA and FOS solutions, respectively (Figure 1). The complete viability of J2 nematodes exposed to YSY persisted up to the seventh exposure day, after which the percentage of

mortality constantly increased up to 100% on the 15th day. Adversely, the J2 mortality was reached faster in the ABA solution, reaching 93 and 100% within 48 and 72 exposure hours, respectively. An almost complete correspondence of J2 immobility and mortality values was observed for both ABA and YSY, as only a few immobile specimens recovered their motility after the 24 h permanence in water following the treatments. A low mortality (7%) of specimens immersed in DW naturally occurred only after 16 exposure days.

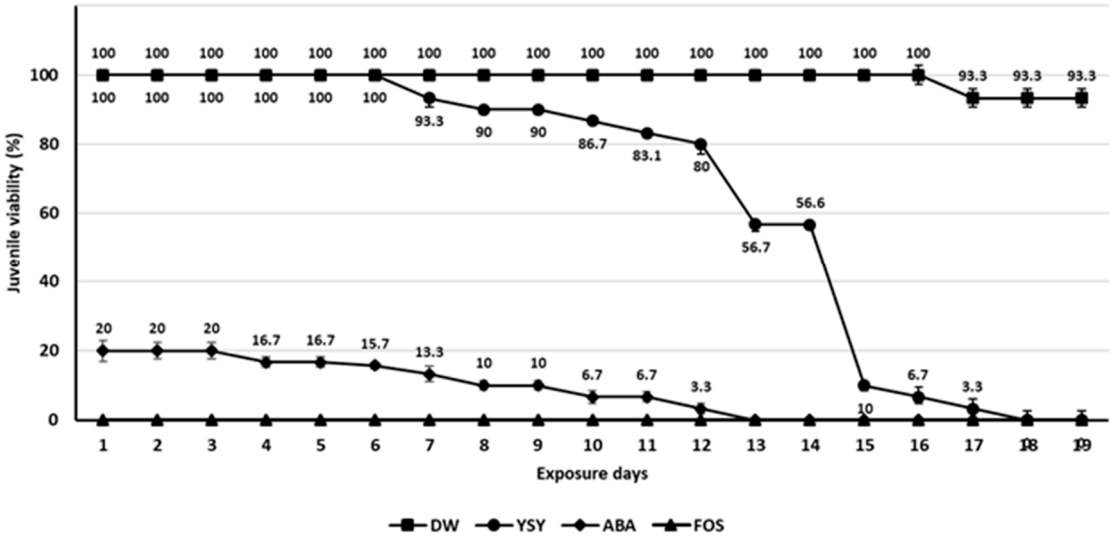

Figure 1. Effect of a 24 h exposure to *P. terrestris* PT22AV (YSY), abamectin (ABA), Fosthiazate and distilled water (DW) on percentage viability of *M. incognita* juveniles. Data are means of four replicates ± standard error.

3.2. Experiment in Pots

Only the 1 kg ha^{-1} dose of YSY was able to significantly reduce the soil population density of *M. incognita* and the gall formation on tomato roots compared to NT, without any statistical difference from PUR (Table 1). The lowest values of both nematode infestation parameters were recorded in the pots treated with FLU, and were statistically lower than both biocontrol agents.

Table 1. Effect of soil treatments with formulates of *P. terrestris* strain PT22AV (YSY), *P. lilacinum* strain 251 (PUR) and Fluopyram (FLU) on *M. incognita* infestation and growth of tomato plants in a greenhouse trial with artificially infested pots. Data are means of four replicates ± standard error.

Treatment	Dose	Eggs and J2 100 mL^{-1} Soil		Root Gall Infestation (0–10)		Plant Height (cm)		Plant Top Weight (g)		Plant Root Weight (g)	
YSY	0.5 [1]	137 ± 7.2	a	6.1 ± 0.5	a	69.2 ± 1.9	b	56.2 ± 3.3	a	8.1 ± 0.1	b
YSY	1 [1]	111 ± 8.4	b	4.8 ± 0.5	b	78.8 ± 2.0	c	77.7 ± 2.4	b	8.3 ± 0.2	b
PUR	0.75 [2]	109 ± 6.9	b	4.4 ± 0.4	b	75.5 ± 1.1	bc	75.4 ± 1.5	b	8.4 ± 0.1	b
FLU	0.625 [2]	72 ± 7.6	c	2.7 ± 0.1	c	76.9 ± 1.2	c	74.4 ± 3.9	b	8.5 ± 0.2	b
NT	-	153 ± 7.8	a	6.9 ± 0.4	a	61.3 ± 3.9	a	53.2 ± 2.8	a	7.3 ± 0.3	a

[1] kg ha^{-1}; [2] L ha^{-1}; means followed by the same letters on the same column are not significantly different according to the Least Significant Difference Test ($p \leq 0.05$).

All treatments significantly increased the tomato plant root weight and height compared to NT, while the lower YSY rate failed to increase the weight of green biomass. The plant growth increase in pots treated with 1 kg ha^{-1} YSY was always statistically similar to that which occurred for both ABA and FLU treatments.

3.3. Greenhouse Trials

At the start of the first experiment, the soil was uniformly infested by *M. incognita*, without statistical differences among plots assigned to the different treatments (Table 2).

Table 2. Effect of soil treatments with *P. terrestris* strain PT22AV (YSY), biofertilizer (ERG) and Fluopyram (FLU) on the infestation of *M. incognita* on tomatoes in a trial of natural infestation in 2022. Data are means of four replicates ± standard error.

Treatment	Dose	Eggs and J2 100 mL^{-1} Soil				Reproduction Rate (Pf/Pi)		Root Gall Infestation (0–10)	
		Initial (Pi) (11/08/22)		Final (Pf) (17/10/22)					
YSY	0.5 [1]	87 ± 2.5	a	204 ± 4.9	c	2.3 ± 0.1	cd	2.5 ± 0.3	cd
YSY	1 [1]	86 ± 1.4	a	164 ± 6.2	d	1.9 ± 0.1	e	1.9 ± 0.2	d
ERG	6 + 3 [2]	89 ± 4.3	a	230 ± 18.7	bc	2.6 ± 0.1	bc	3.2 ± 0.5	bc
FLU	0.625 [2]	84 ± 1.8	a	162 ± 6.8	d	1.9 ± 0.1	de	1.9 ± 0.1	d
NT	-	87 ± 4.8	a	368 ± 20.0	a	4.2 ± 0.3	a	5.2 ± 0.3	a

[1] kg ha^{-1}; [2] L ha^{-1}; means followed by the same letters on the same column are not significantly different according to the Least Significant Difference Test ($p \leq 0.05$).

At the crop end, all the treatments in comparison significantly reduced the final soil population density and the reproduction rate of *M. incognita* and gall infestation on tomato roots. Nematode suppression in soil treated with 1 kg ha^{-1} of YSY was significantly higher compared to both the 0.5 kg ha^{-1} dose and soil application with ERG, but was not different from FLU.

All treatments resulted in a significant increase in the tomato yield compared to NT (Figure 2). The tomato yield did not statistically differ among the 1 kg ha^{-1} dose of YSY and ERG or FLU treatments, with an average 38–40% increase compared to NT, though this was significantly lower in plots treated with 0.5 kg ha^{-1} of YSY than the other treatments, mainly due to a lower mean fruit weight.

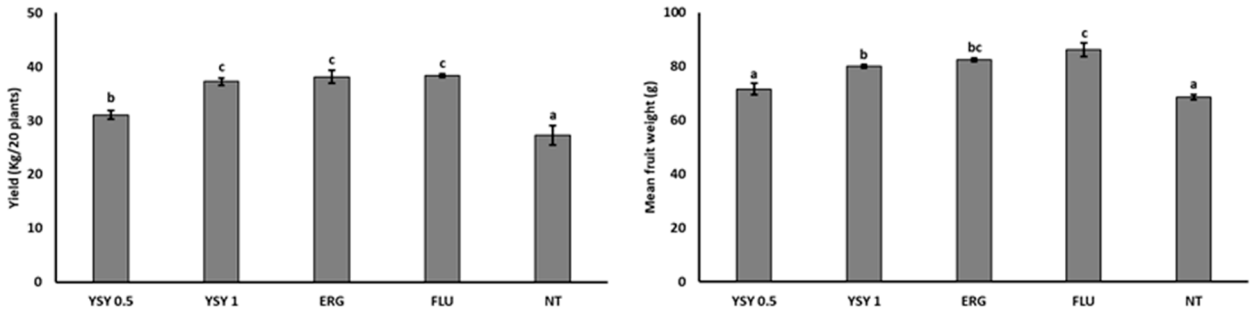

Figure 2. Effect of soil treatments with *P. terrestris* strain PT22AV (YSY), biofertilizer (ERG) and Fluopyram (FLU), in comparison to non-treated soil (NT), on tomato yield and mean fruit weight in a trial in a greenhouse naturally infested by *M. incognita* in 2022. Data are means of four replicates ± standard error. Bars marked by the same letters are not significantly different according to the Least Significant Difference Test ($p \leq 0.05$).

The initial soil population density of *M. incognita* also did not differ among the plots at the start of the experiment in 2023, while both the final nematode population and root gall infestation were significantly lower in all treated plots than in NT (Figure 3). The 1 kg Ha^{-1} dose of YSY and both PUR and BAF treatments resulted in the lowest values of nematode infestation parameters, while a significantly higher nematode population and root gall infestation occurred for both the 0.5 kg Ha^{-1} YSY and FLU treatments, resulting in nematode reproduction rates that were not significantly different from NT.

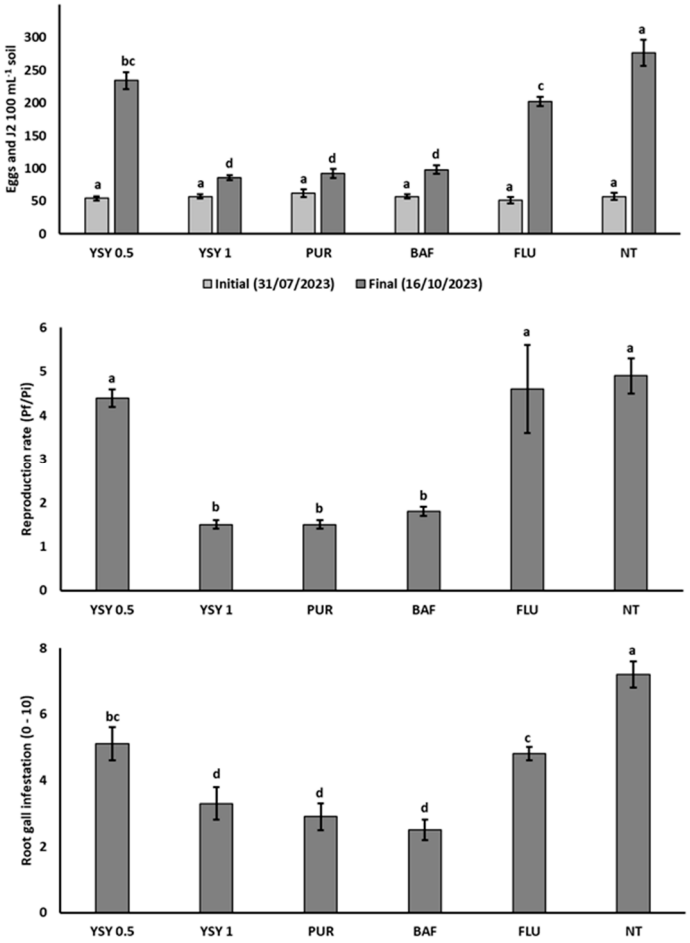

Figure 3. Effect of soil treatments with formulations of *P. terrestris* strain PT22AV (YSY), *Purpureocillum lilacinus* (PUR), *Bacillus firmus* (BAF) and Fluopyram (FLU) on the infestation of *M. incognita* on tomato plants in a trial in a naturally infested greenhouse in 2023. Bars marked by the same letters are not significantly different according to the Least Significant Difference Test ($p \leq 0.05$).

All treatments except 0.5 kg ha^{-1} of YSY also provided a significant increase in tomato yield, with peak yield values recorded in plots treated with PUR and BAF, due to a significantly larger mean fruit weight and a higher number of fruits per plant (Table 3).

Table 3. Effect of soil treatments with formulations of *P. terrestris* strain PT22AV (YSY), *P. lilacinus* strain 251 (PUR), *B. firmus* strain 1-1582 and Fluopyram (FLU) on tomato yield parameters in a trial in a greenhouse naturally infested by *M. incognita* in 2023. Data are means of four replicates ± standard error.

Treatment	Dose	Yield of 20 Sample Plants				Mean Fruit Weight (g)	
		Weight (kg)		No of Fruits			
YSY	0.5 [1]	15.8 ± 0.8	ab	69.0 ± 2.5	b	21.1 ± 0.5	ab
YSY	1 [1]	18.0 ± 0.7	cd	71.8 ± 2.7	bc	22.9 ± 0.6	c
PUR	0.75 [2]	19.1 ± 0.8	d	76.5 ± 3.1	c	24.1 ± 0.4	cd
BAF	40 [1]	19.3 ± 0.3	d	77.5 ± 1.3	c	26.4 ± 0.1	e
FLU	0.625 [2]	16.5 ± 0.4	bc	66.0 ± 1.6	b	21.4 ± 0.6	b
NT	-	14.6 ± 0.6	a	58.5 ± 2.2	a	20.0 ± 0.3	a

[1] Kg ha^{-1}; [2] L ha^{-1}; means followed by the same letters on the same column are not significantly different according to the Least Significant Difference Test ($p \leq 0.05$).

In addition, treatments with the three biocontrol agents also resulted in a higher plant photosynthetic efficiency, as indicated by SPAD values significantly higher than NT and the FLU treatment (Figure 4).

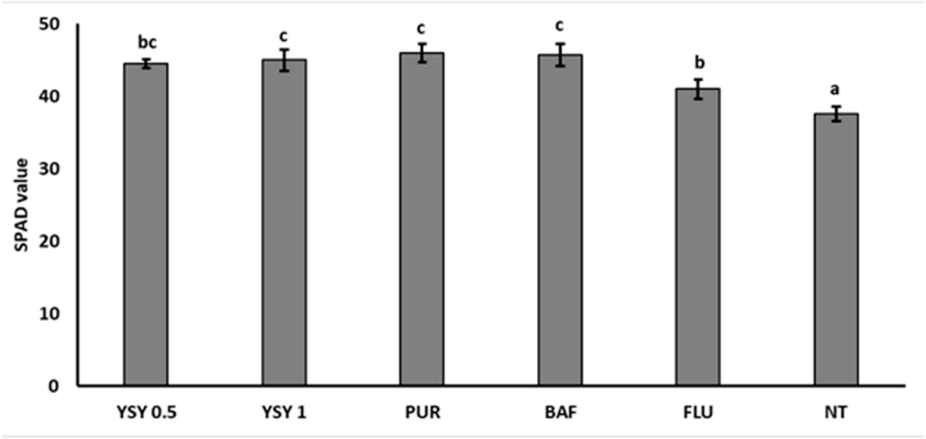

Figure 4. Effect of soil treatments with formulations of *P. terrestris* strain PT22AV (YSY), *P. lilacinus* strain 251 (PUR), *B. firmus* strain 1-1582 and Fluopyram (FLU) on plant SPAD values in a trial in a greenhouse naturally infested by *M. incognita* in 2023. Data are means of four replicates ± standard error. Bars marked by the same letters are not significantly different according to the Least Significant Difference Test ($p \leq 0.05$).

4. Discussion

This study is the first report of the nematicidal activity of strain PT22AV of *P. terrestris*, which had been previously documented only for the control of brown rot disease caused by the fungal pathogen *Monilinia fructigena* Aderhold and Ruhland [20]. The delayed effect of YSY on the viability of *M. incognita* J2 observed in the in vitro assay suggests an earlier start of soil treatments with this product, i.e., at least one week before crop transplanting or even earlier. The experiment on potted tomatoes and the two trials in a greenhouse indicated that the suppressive performance of *P. terrestris* PT22AV on *M. incognita* can be comparable to that of other commercial biocontrol agents, though only at sufficiently high treatment doses. In all three experiments in soil, the initial RKN soil population density was higher than the tomato damage threshold but consistently lower than the infestation levels occurring in soils of many horticultural areas of Southern Italy. In these soils, single treatments with biocontrol products are not able to provide an effective control of RKN infestation on long-cycle crops as tomato, which require an initial application of a synthetic nematicide for reducing RKN population at levels manageable with the YSY product.

Adversely to *P. terrestris*, nematicidal activity has been proven for a wide range of other yeast species. A significant reduction in RKN J2 viability and egg hatchability and a delayed J2 penetration in plant roots were caused by culture filtrates from selected strains of several yeast species, such as *Candida oleophila*, *C. albicans*, *Cryptococcus albidus*, *Pichia guilliermondii*, *S. cerevisiae*, *Sporobolomyces roseus P. manshurica*, *P. caribbica*, *Lachancea thermotolerans*, *Hanseniaspora opuntiae* and *Kodamaea ohmeri* [28–31]. Isolates of *C. albicans*, *Geotichum terrestre*, *P. guilliermondii* and *Pachytrichospora transvaalensis* were documented to have strong suppression of RKN infestation on greenhouse and field grapevines [32], while the suppressiveness of *S. cerevisiae* against RKNs was also repeatedly documented for banana plants [14,15]. Adversely, Egyptian isolates of *Saccharomyces* species were less effective than the antagonistic bacteria *B. subtilis* and *B. thuringiensis* and fungi *T. harzianum*, *Gliocladium vimes* and *P. lilacinus* for controlling *M. javanica* infestation on greenhouse tomatoes [33]. Contrasting effects on RKN infestation were also documented for the combined application of yeasts and other biocontrol agents, as suppressiveness against RKNs was improved by the combination of *S. cerevisiae* with *P. fluorescens* or *P. lilacinus*,

whereas this was reduced by the combination of *P. guilliermondii* with the cyanobacterium *Calothrix parietina* [14,34].

Yeast suppressiveness against PPNs is generally attributed to the release of nematotoxic metabolites such as alcohols, esters and organic acids or enzymes such as proteases and chitinases [32,35,36]. The increased content of compounds like phenolics or the increased activity of enzymes such as catalase and pectin methyl esterase in plants treated with yeasts also suggest the induction of plant resistance to PPN attack [15,16]. Yeast's competition for nutrients and the creation of soil physical and chemical conditions that are unsuitable for PPN activities have also been hypothesized, though are still not confirmed by specific studies [37].

In good agreement with the literature data, a significant suppressiveness against *M. incognita* was also found for all other biocontrol agents comparatively included in our study, i.e., abamectin, *P. lilacinum* and *B. firmus*. Abamectin is a mixture of avermectins, macrocyclic lactones derived as fermentation products from *Streptomyces avermitilis* and known for insecticidal, acaricidal and nematicidal properties [38]. This product has been extendedly documented to have nematicidal activity against various PPNs, also including a suppressive efficacy against RKN infestation ranging from 49 to 62% and from 55 to 97% on field tomatoes and cucumbers, respectively [39–41]. *P. lilacinum* is a multitrophic fungal species known as a biofertilizer and a biocontrol agent against pathogenic fungi and PPNs, and is also documented to provide up to about a 60% reduction in gall formation on tomato roots and even a 80% reduction in RKN population density in the soil [42–44]. Formulations of *B. firmus* strain I-1582 were also proven to reduce RKN egg hatching and J2 viability in in vitro conditions and to suppress their populations in soil, either by producing toxic metabolites or by inducing plant systemic resistance [45–47]. However, the field nematicidal efficacy of this biocontrol agent was reported as generally lower compared to abamectin and *P. lilacinus*, with a 13–24% reduction in root gall formation and a 15–60% suppression of soil nematode populations in trials on cucumbers and tomatoes [46,47].

The suppressiveness against *M. incognita* of the biostimulant/fertilizer applied in the first trial in the greenhouse can be reasonably ascribed to its compositional profile, which is based on compounds (humic, fulvic and polycarboxylic acids, enzymes) and a microbial pool (*Pseudomonas* spp., *Bacillus* spp., *Actinomycetes* spp. and *Trichoderma* spp.) that are already proven to directly suppress RKNs and/or trigger plant systemic resistance and increase the biodiversity of antagonistic microflora [48–51]. However, it must be noted that ERG's positive suppressive performance against *M. incognita* could have been much more limited in the presence of a higher initial nematode infestation level as well as a 30–40-day-longer tomato cycle.

The low nematicidal efficacy of FLU in the second trial in the greenhouse could be due to the unfavorable physical and/or chemical conditions of the experimental soil, while the other two experiments on tomatoes confirmed the strong suppressive activity of this nematicide on RKNs, documented by studies in the literature [52,53].

In addition to the suppressive activity on *M. incognita* on tomatoes, YSY was also able to enhance the growth of the potted tomato plants as well as increase the tomato yield in both greenhouse crops, though only when applied at the highest dosage. These positive performances of YSY can be attributed to both the reduced nematode infestation and a plant growth stimulation effect, as also indicated by the higher values of SPAD indices in soil treated with YSY. In agreement, a dose-dependent stimulation of plant root and shoot growth and a higher seed germination rate were observed following the treatment of maize (*Zea mays* L.) seeds with a strain of *P. terrestris* [54]. Plant-growth-promoting potential has been acknowledged for yeasts in general, providing plants with considerable amounts of amino acids, mineral elements, carbohydrates, enzymes, vitamins and cytokinins during their fermentation processes [11,55]. The positive effects on tomato growth and yield found for the other biocontrol products in comparison are also in full agreement with the literature data, which reported the enhancement of plant growth and crop yield as a side effect of

soil treatments with abamectin, *P. lilacinus*, *B. firmus* and other biocontrol agents against RKNs [56].

5. Conclusions

The data from this study suggest that YSY exhibits promising nematicidal properties against *M. incognita*. While it may not be as rapid-acting as some conventional nematicides like ABA and FOS, YSY demonstrates effectiveness in reducing nematode populations and mitigating root gall formation, particularly at higher application rates. Furthermore, the positive impact on tomato growth and yield suggests that YSY could serve as a valuable tool in organic and integrated RKN management strategies.

Further research is warranted to optimize the application methods and dosage rates of YSY for maximum efficacy. Additionally, field trials under varied environmental conditions would provide valuable insights into its practicality and reliability as a nematicidal agent in agricultural settings. Furthermore, investigations into its mode of action and potential impacts on non-target organisms would contribute to a comprehensive understanding of its utility and safety in sustainable agriculture practices.

Author Contributions: Conceptualization, G.d., S.L., D.P. and T.D.; methodology, G.d., S.L. and D.P.; software, E.C. and T.D.; validation, G.d., S.L. and D.P.; formal analysis, G.d. and T.D.; investigation, D.P., L.P., E.C. and V.E.; resources, D.P., L.P., E.C. and V.E.; data curation, E.C., D.P. and V.E.; writing—original draft preparation, T.D.; writing—review and editing, G.d., S.L. and T.D.; visualization, T.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author due to privacy reasons.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Perry, R.N.; Moens, M. Introduction to plant-parasitic nematodes; modes of parasitism. In *Genomics and Molecular Genetics of Plant-Nematode Interactions*; Jones, J., Gheysen, G., Fenoll, C., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 3–20. [CrossRef]
2. Palomares-Rius, J.E.; Escobar, C.; Cabrera, J.; Vovlas, A.; Castillo, P. Anatomical alterations in plant tissues induced by plant-parasitic nematodes. *Front. Plant Sci.* **2017**, *8*, 288614. [CrossRef] [PubMed]
3. Ragozzino, A.; d'Errico, G. Interactions between nematodes and fungi: A concise review. *Redia* **2011**, *94*, 123–125.
4. Jones, J.T.; Haegeman, A.; Danchin, E.G.J.; Gaur, H.S.; Helder, J.; Jones, M.G.K.; Kikuchi, T.; Manzanilla-Lopez, R.; Palomares-Rius, S.J.E.; Wesemael, W.M.L.; et al. Top ten plant-parasitic nematodes in molecular plant pathology. *Mol. Plant Pathol.* **2013**, *14*, 946–961. [CrossRef] [PubMed]
5. Regulation (EC) of 25 May 2011 implementing Regulation (EC) No1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. No. 540/2011. *Off. J. Eur. Union* **2011**, *L153*, 1–186.
6. Stirling, G.R. Biological control of plant-parasitic nematodes. In *Diseases of Nematodes*; Poinar, G.O., Jansson, H.B., Eds.; CRC Press: Boca Raton, FL, USA, 2018; pp. 103–150. [CrossRef]
7. Timper, P. Utilization of biological control for managing plant-parasitic nematodes. In *Biological Control of Plant-Parasitic Nematodes: Building Coherence between Microbial Ecology and Molecular Mechanisms*; Davies, K., Spiegel, Y., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 259–289. [CrossRef]
8. Poveda, J.; Abril-Urias, P.; Escobar, C. Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: Trichoderma, mycorrhizal and endophytic fungi. *Front. Microbiol.* **2020**, *11*, 992. [CrossRef]
9. Xiang, N.; Lawrence, K.S.; Donald, P.A. Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: A review. *J. Phytopathol.* **2018**, *166*, 449–458. [CrossRef]
10. Schouten, A. Mechanisms involved in nematode control by endophytic fungi. *Annu. Rev. Phytopathol.* **2016**, *4*, 121–142. [CrossRef]
11. Botha, A. The importance and ecology of yeasts in soil. *Soil Biol. Biochem.* **2011**, *43*, 1–8. [CrossRef]
12. Cloete, K.J.; Valentine, A.J.; Stander, M.A.; Blomerus, L.M.; Botha, A. Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub, *Agathosma betulina* (Berg.) Pillans. *Microb. Ecol.* **2009**, *57*, 624–632. [CrossRef]
13. El-Tarabily, K.A. Suppression of *Rhizoctonia solani* diseases of sugar beet by antagonistic and plant growth-promoting yeasts. *J. Appl. Microbiol.* **2004**, *96*, 69–75. [CrossRef]
14. Abokorah, M.S.; Fathalla, A.M. The nematicidal efficacy of fulvic acid, yeast fungus (*Saccharomyces cerevisiae*) and L-tryptophan on plant parasitic nematodes, growth, and yield of banana plants. *Egypt. J. Crop Prot.* **2022**, *17*, 27–37. [CrossRef]

15. Hamouda, R.; Al-Saman, M.; El-Ansary, M. Effect of *Saccharomyces cerevisiae* and *Spirulina platensis* on suppressing root-knot nematode, *Meloidogyne incognita* infecting banana plants under greenhouse conditions. *Egypt. J. Agronematol.* **2019**, *18*, 90–102. [CrossRef]
16. Karajeh, M.R. Efficacy of *Saccharomyces cerevisiae* on controlling the root-knot nematode (*Meloidogyne javanica*) infection and promoting cucumber growth and yield under laboratory and field conditions. *Arch. Phytopathol. Plant Prot.* **2013**, *46*, 2492–2500. [CrossRef]
17. Sampaio, J.P.; Weiß, M.; Gadanho, M.; Bauer, R. New taxa in the Tremellales: *Bulleribasidium oberjochense* gen. Et sp. Nov., *Papiliotrema bandonii* gen. Et sp. Nov. And *Fibulobasidium murrhardtense* sp. Nov. *Mycologia* **2002**, *94*, 873–887. [CrossRef]
18. Vadkertiova, R.; Dudasova, H.; Stratilova, H.; Balaskakova, M. Diversity of yeasts in the soil adjacent to fruit trees of the Rosaceae family. *Yeast* **2019**, *36*, 617–631. [CrossRef] [PubMed]
19. Crestani, J.; Fontes Landell, M.; Faganello, J.; Henning Vainstein, M.; Simpson Vishniac, H.; Valente, P. *Cryptococcus terrestris* sp. Nov., a tremellaceous, anamorphic yeast phylogenetically related to *Cryptococcus flavescens*. *Int. J. Syst. Evol. Micr.* **2009**, *59*, 631–636. [CrossRef] [PubMed]
20. Palmieri, D.; Ianiri, G.; Conte, T.; Castoria, R.; Lima, G.; De Curtis, F. Influence of biocontrol and integrated strategies and treatment timing on plum brown rot incidence and fungicide residues in fruits. *Agriculture* **2022**, *12*, 1656. [CrossRef]
21. d'Errico, G.; Crescenzi, A.; Landi, S. First report of the southern root-knot nematode *Meloidogyne incognita* on the invasive weed *Araujia sericifera* in Italy. *Plant Dis.* **2014**, *98*, 1593. [CrossRef]
22. Hussey, R.S.; Barker, K.R. A comparison of methods of collecting inocula of *Meloidogyne* spp. Including a new technique. *Plant Dis. Rep.* **1973**, *57*, 1025–1028.
23. d'Errico, G.; Giacometti, R.; Roversi, P.F.; d'Errico, F.P.; Woo, S.L. Mode of action and efficacy of iprodione against the root-knot nematode *Meloidogyne incognita*. *Ann. Appl. Biol.* **2017**, *171*, 506–510. [CrossRef]
24. Finney, D.J. *Statistical Method in Biological Assay*, 3rd ed.; Charles Griffin & Company Ltd.: High Wycombe, UK, 1978; pp. 1–508.
25. Sasanelli, N. Tables of nematode-pathogenicity. *Nematol. Medit.* **1994**, *22*, 153–157.
26. Zeck, W.M. A rating scheme for field evaluation of root-knot infestations. *Pflanzenschutz-Nachrichten* **1971**, *24*, 141–144.
27. van Bezooijen, J. *Methods and Techniques for Nematology*; Wageningen University: Wageningen, The Netherlands, 2006; pp. 1–112.
28. El-Sagheer, A.; El-Mesalamy, A.; Anany, A.M.; Mahmoud, N. Nematicidal properties of some yeast culture filtrates against *Meloidogyne javanica* infecting squash plants (in vitro and in vivo). *Pak. J. Nematol.* **2021**, *39*, 111–121. [CrossRef]
29. Mioranza, T.M.; Schwan-Estrada, K.R.F.; Zubek, L.; Miamoto, A.; Hernandes, I.; Rissato, B.B.; Mizuno, M.S.; Schwan, R.F.; Dias-Arieira, C.R. Effects of yeast fermentation broths on the *Meloidogyne incognita* population in soybean. *Trop. Plant Pathol.* **2020**, *45*, 112–121. [CrossRef]
30. Jothi, G.; Poornima, K.; Prasad, G. In vitro studies of antagonistic yeast against *Meloidogyne incognita*. *Ann. Plant Prot. Sci* **2019**, *27*, 146–151. [CrossRef]
31. Vyshali, G.; Jothi, G.; Poornima, K. Effect of antigonistic yeast on penetration and biology of *Meloidogyne incognita* in tomato. *Ann. Plant Prot. Sci.* **2019**, *27*, 390–393. [CrossRef]
32. Hashem, M.; Omran, Y.A.; Sallam, N.M. Efficacy of yeasts in the management of root-knot nematode *Meloidogyne incognita*, in flame seedless grape vines and the consequent effect on the productivity of the vines. *Biocontr. Sci. Technol.* **2008**, *18*, 357–375. [CrossRef]
33. Shawky, S.M.; El-Shennawy, R.Z.; Shady, A.M. Biological control of Meloidogyne javanica on tomato plants with some isolated bioagents in Egypt. *J. Plant Prot. Pathol.* **2006**, *31*, 6049–6063. [CrossRef]
34. Hashem, M.; Abo-Elyousr, K.A. Management of the root-knot nematode *Meloidogyne incognita* on tomato with combinations of different biocontrol organisms. *Crop Prot.* **2011**, *30*, 285–292. [CrossRef]
35. Fialho, M.B.; Bessi, R.; Inomoto, M.M.; Pascholati, S.F. Nematicidal effect of volatile organic compounds (VOCs) on the plant-parasitic nematode *Meloidogyne javanica*. *Summa Phytopathol.* **2012**, *38*, 152–154. [CrossRef]
36. Osman, H.A.; Ameen, H.H.; Mohamed, M.; Elkelany, U.S. Efficacy of integrated microorganisms in controlling root-knot nematode *Meloidogyne javanica* infecting peanut plants under field conditions. *Bull. Nat. Res. Cent.* **2020**, *44*, 134. [CrossRef]
37. El-Nuby, A.S.M. Effect of some amino acids and yeast on root-knot disease on tomato plants. *Egypt. J. Agronematol.* **2021**, *20*, 17–33. [CrossRef]
38. Cabrera, J.A.; Menjivar, R.D.; Dababat, A.E.F.A.; Sikora, R.A. Properties and nematicide performance of avermectins. *J. Phytopathol.* **2013**, *161*, 65–69. [CrossRef]
39. Liu, Y.; Liu, Y.; Yang, L.; Zhang, T.; Jin, Y.; Liu, L.; Du, J.; Zhang, D.; Li, B.; Gao, C.; et al. The effect of abamectin application in combination with agronomic measures on the control efficacy of cucumber root-knot nematodes and the cucumber yield. *Pest Manag. Sci.* **2023**, *79*, 3190–3199. [CrossRef] [PubMed]
40. d'Errico, G.; Marra, R.; Vinale, F.; Landi, S.; Roversi, P.F.; Woo, S.L. Nematicidal efficacy of new abamectin-based products used alone and in combination with indolebutyric acid against the root-knot nematode *Meloidogyne incognita*. *Redia* **2017**, *100*, 95–101. [CrossRef]
41. Qiao, K.; Liu, X.; Wang, H.; Xia, X.; Ji, X.; Wang, K. Effect of abamectin on root-knot nematodes and tomato yield. *Pest Manag. Sci.* **2012**, *68*, 853–857. [CrossRef] [PubMed]
42. Saeed, M.; Mukhtar, T.; Ahmed, R.; Ahmad, T.; Iqbal, M.A. Suppression of *Meloidogyne javanica* infection in peach (*Prunus persica* (L.) Batsch) using fungal biocontrol agents. *Sustainability* **2023**, *15*, 13833. [CrossRef]

43. Santos, C.H.B.; De Andrade, L.A.; Frezarin, E.T.; Sales, L.R.; Rigobelo, E.C.C. *Purpureocillium lilacinum* for biocontrol, bioremediation and biofertilization. *Preprints* **2023**, 2023051926. [CrossRef]
44. Singh, S.; Pandey, R.K.; Goswami, B.K. Bio-control activity of *Purpureocillium lilacinum* strains in managing root-knot disease of tomato caused by *Meloidogyne incognita*. *Biocontr. Sci. Technol.* **2013**, *23*, 1469–1489. [CrossRef]
45. Ghahremani, Z.; Escudero, N.; Beltrán-Anadón, D.; Saus, E.; Cunquero, M.; Andilla, J.; Loza-Alvarez, P.; Gabaldón, T.; Javier Sorribas, F. *Bacillus firmus* Strain I-1582, a nematode antagonist by itself and through the plant. *Front. Plant Sci.* **2020**, *11*, 796. [CrossRef]
46. d'Errico, G.; Marra, R.; Crescenzi, A.; Davino, S.W.; Fanigliulo, A.; Woo, S.L.; Lorito, M. 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. *Crop Prot.* **2019**, *122*, 159–164. [CrossRef]
47. Giannakou, I.O.; Karpouzas, D.G.; Prophetou-Athanasiadou, D. A novel non-chemical nematicide for the control of root-knot nematodes. *Appl. Soil. Ecol.* **2004**, *26*, 69–79. [CrossRef]
48. Giffard, B.; Winter, S.; Guidoni, S.; Nicolai, A.; Castaldini, M.; Cluzeau, D.; Coll, P.; Cortet, J.; LeCadre, E.; d'Errico, G.; et al. Vineyard management and its impacts on soil biodiversity, functions, and ecosystem services. *Front. Ecol. Evol.* **2022**, *10*, 850272. [CrossRef]
49. Lombardi, N.; Salzano, A.M.; Troise, A.D.; Scaloni, A.; Vitaglione, P.; Vinale, F.; Marra, R.; Caira, S.; Lorito, M.; d'Errico, G.; et al. Effect of Trichoderma bioactive metabolite treatments on the production, quality, and protein profile of strawberry fruits. *J. Agric. Food Chem.* **2020**, *68*, 7246–7258. [CrossRef] [PubMed]
50. Marra, R.; Coppola, M.; Pironti, A.; Grasso, F.; Lombardi, N.; d'Errico, G.; Sicari, A.; Bolletti Censi, S.; Woo, S.L.; Rao, R.; et al. The application of *Trichoderma* strains or metabolites alters the olive leaf metabolome and the expression of defense-related genes. *J. Fungi* **2020**, *6*, 369. [CrossRef]
51. D'Addabbo, T.; Laquale, S.; Perniola, M.; Candido, V. Biostimulants for plant growth promotion and sustainable management of phytoparasitic nematodes in vegetable crops. *Agronomy* **2019**, *9*, 616. [CrossRef]
52. Jones, J.G.; Kleczewski, N.M.; Desaeger, J.; Meyer, S.L.; Johnson, G.C. Evaluation of nematicides for southern root-knot nematode management in lima bean. *Crop Prot.* **2017**, *96*, 151–157. [CrossRef]
53. Faske, T.R.; Hurd, K. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to Fluopyram. *J. Nematol.* **2015**, *47*, 316–321. [PubMed]
54. Labancová, E.; Šípošová, K.; Kučerová, D.; Horváthová, Á.; Schusterová, H.; Vivodová, Z.; Vadkertiová, R.; Kollárová, K. The Tremellaceous Yeast: *Papiliotrema* terrestris–As the Growth Stimulant of Maize Plants. *J. Plant Growth Regul.* **2023**, *42*, 3835–3850. [CrossRef]
55. Ignatova, L.V.; Brazhnikova, Y.V.; Berzhanova, R.Z.; Mukasheva, T.D. Plant growth-promoting and antifungal activity of yeasts from dark chestnut soil. *Microbiol. Res.* **2015**, *175*, 78–83. [CrossRef]
56. Kozacki, D.; Soika, G.; Skwiercz, A.; Malusà, E. Microbial-based products and soil management practices to control nematodes in organic horticultural crops. In *Sustainable Management of Nematodes in Agriculture, Vol. 2: Role of Microbes-Assisted Strategies*; Chaudhary, K.K., Meghvansi, M.K., Siddiqui, S., Eds.; Springer International Publishing: Cham, Switzerland, 2024; Volume 19, pp. 3–31. [CrossRef]