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Ecophysiological characteristics of the nematophagous fungus, *Plectosphaerella plurivora*, with biocontrol potential on *Nacobbus aberrans s.l.* in tomato

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Abstract The plant-parasitic nematode, *Nacobbus sp.,* is responsible for significant economic losses in horticultural production centers in Argentina and other countries in America, alone or in combination with other biotic and abiotic factors. Although the genus' distribution is restricted to the American continent, it has quarantine importance and is subject to international legislation to prevent its spread to other regions. The management of phytoparasitic nematodes using biological control strategies is a promising eco-compatible alternative, allowing for sustainability of the crop horticultural system. Firstly, this study ecophysiologically characterized *Plectosphaerella plurivora* SRA14, a strain with nematophagous activity on *N. aberrans s.l.* This fungal strain developed *in vitro* under a wide temperature range (20–30 °C), but the highest levels of water stress (Ψ: -7 and -10 Mpa; a_W: 0.95 and 0.93) inhibited its growth. While the production of extracellular enzymes by this strain was low, *P. plurivora* SRA14 was able to develop in the rhizosphere and endorhizosphere of the tomato and basil crops without affecting the plant vigor parameters or producing phytotoxicity signs. Secondly, this study evidenced the biocontrol activity of *P. plurivora* SRA14 on *N. aberrans s.l.* populations in tomato, implanted into both sterile (artificially inoculated) and naturally infested soils via greenhouse pot experiments. The results of this work revealed for the first time the potential of *P. plurivora* SRA14 as a biological control agent of the phytoparasitic nematode *N. aberrans s.l.* in horticultural crops.

Keywords Growth features · Enzymatic activity · Endorhizospheric colonization · Plant-parasitic nematode antagonism · Environmental factors

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Introduction

In the rhizospheric ecosystem, tomatoes develop in association with diverse microorganisms, many of which may be beneficial, while others may be pathogenic for this crop. The Argentine under-cover production model of fresh tomatoes, with a tendency

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towards monocultures, and intensification of the chemical fertilizer and pesticide applications, has triggered a higher incidence of pests and diseases. Among them, some plant-parasitic nematodes (PPN) can cause damage to many horticultural crops, with economic losses estimated at US$ 215.77 billion annually (Abd-Elgawad & Askary, 2015). *Nacobbus aberrans s.l.* (Thorne, 1935, Thorne and Allen, 1944) is considered one of the ten most relevant PPN worldwide (Jones et al., 2013). This phytopathogen pest induces the production of galls in the root system of susceptible hosts, and due to this it is commonly known as "the false knot nematode". Consequently, parasitized plants gradually lose their ability to absorb water and nutrients, reducing their growth and yield levels (Cristóbal et al., 2001; de Oliveira Bosco et al., 2009; Garita, 2019; Garita et al., 2021; Ploganou, 2018). *Nacobbus aberrans s.l.* is responsible for significant losses in numerous horticultural crops, negatively impacting 84 botanical species, including tomato among others of economic importance (Manzanilla-Lopez et al., 2002; Cabrera Hidalgo et al., 2014). This PPN is endemic to the American continent (Manzanilla-López, 2010) and has quarantine pest status, for which international phytosanitary standards attempt to prevent its spread to other regions (EPPO, 2022). On the other hand, this PPN can adapt to a wide range of soils and temperatures, hence in Argentina it is distributed in a great diversity of ecoregions, causing recurring problems in different centers of horticultural production of the country, such as Río Cuarto (Córdoba) (Doucet, 1989; Doucet & Lax, 2005; Lax et al., 2011; Sosa et al., 2018).

In an attempt to address this growing problem within an eco-compatible perspective, our research line has focused on finding an effective strategy using biocontrol, which preserves the sustainability of horticultural systems. A wide diversity of microorganisms have ecologically important roles, since they act as antagonists of nematodes, among which fungi stand out (Lax et al., 2022). Antagonistic fungi have great potential because they have developed various physical and biochemical mechanisms for PPN control (Stirling, 2014), are common inhabitants of the soil, have great diversity, are abundant, are capable of surviving as saprophytes, can be easily cultivated *in vitro*, and can be produced on a large scale for the management of nematodes in the field (Sosa et al., 2021). In previous *in vitro* studies, a native fungus isolated from the horticultural ecosystem demonstrated nematophagous activity against eggs (76.0%) and juveniles (J2s) (78.3%) of *N. aberrans s.l.*, using "adhesive conidia" as the adherence mechanism. Based on polyphasic studies, this nematophagous fungus (NF) was identified as *Plectosphaerella plurivora* (Sosa et al., 2018). Some studies have shown that another *Plectosphaerella* species, *P. cucumerina*, has been isolated from *Meloidogyne hapla* egg masses (Yu and Coosemans 1998), and has demonstrated its potential as a biological control agent (BCA) against the potato cyst nematodes (PCN) *Globodera rostochiensis* and *Globodera pallida* (Jacobs, 2000). However, up to now, there is no information available regarding the potential of this fungus for the control of *N. aberrans s.l.*

The establishment and effectiveness of BCA in the natural environment is a critical aspect to be considered. Tolerance to fluctuating abiotic factors, such as water potential and temperature, is a requirement for the successful development of ecologically competent BCA (Magan, 2001). In addition, to use a fungus as a BCA, it is necessary to know the pathogenicity mechanisms involved in the interaction with the host, such as virulence factors and infection mechanisms (López-Llorca et al., 2008). These aspects emphasize the importance of characterizing the *P. plurivora* SRA14 strain, whose nematophagous activity on *N. aberrans s.l.* was demonstrated in a preliminary study, evaluating the desirable characteristics of a potential BCA, that would allow guaranteeing results in field applications. Therefore, in the present work, *P. plurivora* SRA14 growth under different temperatures and hydric stress, enzymatic production capacity, the rhizospheric development characteristics, and the biocontrol capacity on *N. aberrans s.l.* in tomato plants were evaluated.

Materials and methods

Nematophagous fungus and phytonematode population

Plectosphaerella plurivora SRA14 (MF996823) was isolated from horticultural soil naturally infested with *N. aberrans s.l.* in Río Cuarto, Córdoba, Argentina (Sosa et al., 2018). This strain was stored in the Collection of the Department of Microbiology

and Immunology of the National University of Río Cuarto, Córdoba (Argentina). The fungal strain was cultured on Potato Dextrose Agar (PDA) (Britania®) for 7 days at 25 °C.

The population of *N. aberrans s.l.* (MH000315) isolated from a horticultural field in Río Cuarto, Córdoba, Argentina, and identified by Sosa et al. (2018) was maintained in tomato plants (*Solanum lycopersicum* L. *var. valouro*) under greenhouse conditions. To obtain the inoculum, egg masses were extracted by dilaceration under a stereoscopic microscope from infested plant galls and placed in conical tubes with sterile distilled water. The obtained solution was homogenized and observed under an optical microscope to reach the necessary concentration of eggs.

Fungal growth studies

This test was carried out following the methodology proposed by Girardi et al., (2022). PDA plates were inoculated in the center with a 0.5 cm diameter disc of the fungus. The water activity (a_W) of the culture medium was modified by adding glycerol (0.99, 0.98, 0.95, and 0.93) and the matric potential (Ψm) by PEG 8000 (-0.7, -3, -7 and -10 MPa) (Dallyn & Fox, 1980). Plates with 0.99 a_W were incubated at 20, 25, and 30 °C for 15 days to evaluate the effect of temperature. Each treatment was performed in triplicate. Fungal growth was examined daily, evaluating the diameter of the fungal colonies to calculate the growth rate (Passone et al., 2005).

Production of extracellular enzymes

Semiquantitative studies

Protease, amylase, lipase, and chitinase activities were evaluated by plate technique using the same methodology used by Girardi et al., (2022). The following culture media were used: media I (0.05 peptone, 0.03 meat extract, 0.05 NaCl, 15.0 agar–agar) (g l^{-1}) supplemented with gelatin (10.0 g l^{-1}), starch (10 g l^{-1}), sorbitan monolaurate 10 (g l^{-1}) (Barra et al., 2015) and media II (0.07 K_2HPO_4, 0.08 $MgSO_4$. $7H_2O$, 0.001 $FeSO_4$ $7H_2O$, 0.126 $MnSO_4$, 0.0001 $ZnSO_4$ $7H_2O$, 0.05 $ClNH_4$, 0.2 agar–agar) (g l^{-1}) supplemented with chitin (40 g l^{-1}) (Atlas, 2005). The a_W of the culture medium was modified by adding glycerol (0.98, 0.95, and 0.93) (Dallyn et al., 1980). Each plate was inoculated by central puncture with a suspension of fungal spores and incubated at 25 °C for 14 days. Enzymatic activity (EA) was estimated by recording the diameter of the substrate degradation halo. EA was expressed as an index: EA = D-d (D: diameter of the colony plus the halo of the degradation zone; d: diameter of the colony) (Price et al., 1982).

Quantitative studies

Erlenmeyer flasks containing 100 ml of culture medium (4.56 K_2HPO_4, 2.77 KH_2PO_4, 0.5 $MgSO_4$ $7H_20$, 0.5 KCl) (g l^{-1}) (Khan et al., 2003) supplemented with colloidal chitin (Sigma, St. Louis, USA) (Braga et al., 2013) (10 g l^{-1}) or milk (20 g l^{-1}) were inoculated with 1 ml of a conidia suspension (10^7 conidia ml^{-1}) and incubated on a rotary shaker (150 rpm) at 25 °C for 14 days. The uninoculated culture medium was used as a negative control. The study was performed in triplicate. Samples of the culture supernatant were taken at 7 and 14 days, which were centrifuged (7000 rpm, 15 min) and filtered (0.2 µm) before measuring the chitinolytic and proteolytic activities, following the methodologies described below.

Chitinolytic activity The production of chitinolytic enzymes was determined following the methodology proposed by Tikhonov et al., (2002). Five hundred µl of culture supernatant was mixed with 250 µl of colloidal chitin suspension (1% in 100 mM acetate buffer pH 5) and incubated at 40 °C for 20 h. After incubation, 250 µl dinitrosalicylic acid solution (1% in 0.7 M NaOH) and 100 µl 10 M NaOH were added and heated at 100 °C for 5 min. Finally, the mixture was allowed to cool, and the absorbance at 582 nm was measured (Miller, 1959). A calibration curve was constructed using N-acetyl-D-glucosamine (NAGA). The chitinolytic activity was expressed in NAGA units (NAGA-U). One unit of NAGA is equal to 1 µmol of NAGA released (1 unit of NAGA = 1 µmol of NAGA) per 1 ml of enzyme solution in 1 h under the conditions described ($U.h^{-1}$ ml^{-1}).

Proteolytic activity The culture supernatant (1 ml) was mixed with 5 ml of a casein solution (0.65% in 50 mM potassium buffered phosphate, pH 7.5) (37 °C) and incubated at 37 °C for 10 min. After incubation, 5 ml trichloroacetic acid (110 mM) was

added and incubated at 37 °C for 30 min. The mixture was then filtered (0.45 µm) and 2 ml was placed in a vial containing 5 ml sodium carbonate (500 mM) and 1 ml Follin-Ciocalteu reagent and incubated at 37 °C for 30 min. The mixture was then cooled, filtered (0.45 µm), and the absorbance at 660 nm was measured. A calibration curve was constructed using tyrosine. Proteolytic activity was expressed as the enzyme unit (U) needed to hydrolyze an amount of casein equivalent to 1 µmol of tyrosine per minute, at pH 7.5 and 37 °C (U. min^{-1} ml^{-1}).

Micro-rhizosphere assay

Surface disinfected tomato seeds (*Solanum lycopersicum* cv. "Perita Roma vf") were germinated in water-agar in the dark at 25 °C ± 2 °C. Two-week-old tomato seedlings were transplanted into micro-ROC chambers and kept in a growth chamber, under controlled environmental conditions (25 ± 2 °C; 80% RH; 12:12 h photoperiod) for the development of roots. Sterile vermiculite and a solution of macro and micronutrients (Acuagarden ®) were used for the growth medium. After 2 weeks, the root seedlings were inoculated with 1 ml of *P. plurivora* SRA14 suspension (1×10^8 conidia ml^{-1}) that were stained with 1 ml of FITC/$NaHCO_3$ 0.1 mg ml^{-1} (FITC, Sigma Aldrich, USA) en $NaHCO_3$ 0.1 M, pH 9) (Ayliffe et al., 2013). Also, a 100 µl solution containing 3 J2s $µl^{-1}$ of *N. aberrans s.l.* staining with PKH26 (4×10^{-6} M, as per manufacturer's protocol, Sigma Aldrich, USA) was inoculated (Kooliyottil et al., 2016). Root samples were taken 5 days after inoculation, and then subjected to fluorescent stereomicroscopy (Olympus Optical Co. Ltd., BX50F4) under blue-light and green-light excitation to observe the infection process of the fungus and nematode, respectively.

Rhizospheric colonization of tomato and basil plants

Tomato and basil plants (3 weeks) were transplanted into pots containing a sterile soil mixture (peat, soil, and sand (1:1:1)) (100 g). Seven days after transplanting, the soil was inoculated with 5 ml of an aqueous suspension of *P. plurivora* SRA14 (1×10^6 conidia ml^{-1}) with four replicates per treatment and uninoculated plants were used as control. The plants were kept in a greenhouse in a completely randomized design with natural light and daily watering for 35 days. After the incubation period, the plant growth parameters and the fungal colonization of soil and roots, and the presence of browning symptoms were evaluated.

Plant vigor parameters

After removing each plant from its pot, the following characteristics were evaluated: stem length, aerial weight, and root length and weight.

Plant pathogenicity test

After evaluating the vigor parameters, the radicle and neck of the basil plants were carefully washed, and the browning of the roots and neck were examined according to Raimondo and Carlucci (2018).

Fungal saprophytic development

Fungal counts were determined following the methodology proposed by Girardi et al. (2022). Dilutions of the soil samples were made, and an aliquot (0.1 ml) of each dilution was spread on PDA supplemented with antibiotics (0.05 streptomycin sulfate, 0.05 chloramphenicol, and 0.075 rose Bengal) (g l^{-1}) and were incubated at 25 °C for 7 days. The identification of *P. plurivora* isolates was based on morphological characteristics (Zhang et al., 2019).

Fungal endophytic development

The fungal colonization of the roots was determined using the methodology proposed by Girardi et al., (2022). The superficial disinfection of the roots was performed with NaClO (0.5%) for 5 min, followed by three successive washings (distilled water, 5 min). The roots were macerated with PBS (10^{-1} dilution), and an aliquot (0.1 ml) was spread on PDA with antibiotics. In addition, samples (100 µl) from the last washing step were processed to verify the effectiveness of the disinfection process. The Petri plates were incubated at 25 °C for 7 days.

In plant antagonism assay

Tomato plants (3 weeks) were transplanted, at the rate of one seedling per pot containing 5 kg of a mixture of sterile substrate (soil: peat: sand: compost 1:1:1:1)

or naturally infested soil, with *N. aberrans s.l.* The pots with sterile soil were inoculated with a suspension of 600 eggs of *N. aberrans s.l.* (initial population; Pi) at the time of transplanting, while naturally infested soil contained 2.8 J2s g^{-1}. In addition, the pots were inoculated with 5 ml of an aqueous suspension of *P. plurivora* SRA14 (1×10^6 conidia ml^{-1}).

The treatments evaluated were the following:

- Control 1 (C1): tomato seedling in sterile soil
- Fungal control (FC): tomato seedling in sterile soil + *P. plurivora* SRA14
- Nematode control (NC): tomato seedling in sterile soil + *N. aberrans s.l.*
- Treatment 1 (T1): tomato seedling in sterile soil + *N. aberrans s.l.* + *P. plurivora* SRA14
- Control 2 (C2): tomato seedling in naturally infested soil
- Treatment 2 (T2): tomato seedling in naturally infested soil + *P. plurivora* SRA14

All the treatments were irrigated daily (Trebo ® watering duration: 1 min; irrigation frequency: 12 h) and kept under greenhouse conditions for 96 days. Five replicates of each treatment were performed. The following determinations were made at the end of the trial.

Plant vigor parameters

Root and aerial length and fresh weight, aerial dry weight, and the number and weight of fruits were evaluated.

Population of N. aberrans s.l.

The number of galls, density of egg masses in the roots, number of eggs and the number of J2s per gram of soil were determined according to Regaieg et al. (2011). The final population (Pf) of *N. aberrans s.l.* was estimated for each treatment by adding the number of eggs and larval stages extracted from the soil. The Pf value was used to calculate the Reproduction Factor (RF = Pf/Pi).

Fungal rhizospheric development

This methodology was performed according to that previously described in 2.4.3

Data analyses

Statistical analyses were performed using the program InfoStat version 2017. InfoStat Group, FCA, National University of Córdoba, Argentina. http://www.infostat.com.ar URL. Means data on fungal growth, EA, and fungal colonization were determined by analyses of variance (ANOVA). To establish significant differences, a posteriori DGC test (Di Rienzo, Guzmán and Casanoves test) ($p < 0.05$) was performed. MLGyM (General and Mixed Linear Models) ($p < 0.05$) was performed to establish differences in plant growth parameters. In the case of the number of galls, egg masses, and reproduction factor of *N. aberrans s.l.* population (Poisson distribution) were analyzed through Mixed Generalized Linear Models (MGLM). The best model that adjusts heterogeneous variances was selected. LSD Fisher a posteriori test was used ($p < 0.05$) for the comparison of means. The analysis of the images in jpg format was carried out with the image processor, Image J, according to the specifications of González (2018).

Results

Fungal growth

The growth performance of *P. plurivora* SRA14 was determined at different temperature levels, a_W and Ψm. The incubation temperature significantly affected ($F = 16.08$; $p = 0.0039$) this parameter. The growth rate of *P. plurivora* was reduced by 9% ($p < 0.05$) at the highest temperature tested (30 °C), compared to the values obtained at 20 and 25 °C (0.60 ± 0.02 cm day^{-1}). The a_W of the culture medium also significantly affected fungal growth ($F = 89.67$; $p < 0.0001$). The growth rate reached significantly different values ($p < 0.05$) at a_W of 0.98 (0.27 ± 0.10 cm day^{-1}) and 0.99 (0.59 ± 0.02 cm day^{-1}); while at low levels of a_W (0.93 and 0.95) fungal development was not observed. Similarly, the growth rate was significantly affected ($F = 197.87$; $p < 0.0001$) when the fungus was grown on PDA conditioned at different matric potentials (Ψm). Statistically significant differences ($p < 0.05$) were observed in the growth rates observed at -3.0 (0.35 ± 0.03 cm day^{-1}) and -0.7 MPa (0.53 ± 0.06 cm day^{-1}), and fungus development was

not observed in the conditions of greatest stress Ψm (-10.0 and -7.0 MPa) (Fig. 1).

Enzymatic activity

Semiquantitative analyses (protease, amylase, lipase and chitinase) only showed the production of chitinolytic enzymes by *P. plurivora* SRA14 at the highest a_W tested (0.98) with a mean enzymatic activity value of 1.3 ± 1.12 U. h^{-1} ml^{-1}. The quantitative analysis showed low levels of chitinases (7 days: 0.05 ± 0.02; 14 days: 0.07 ± 0.03 U. h^{-1} ml^{-1}) and proteases (7 and 14 days: 0.01 ± 0.01 U.min^{-1} ml^{-1}) when they were incubated under inductive conditions, regardless of the incubation time.

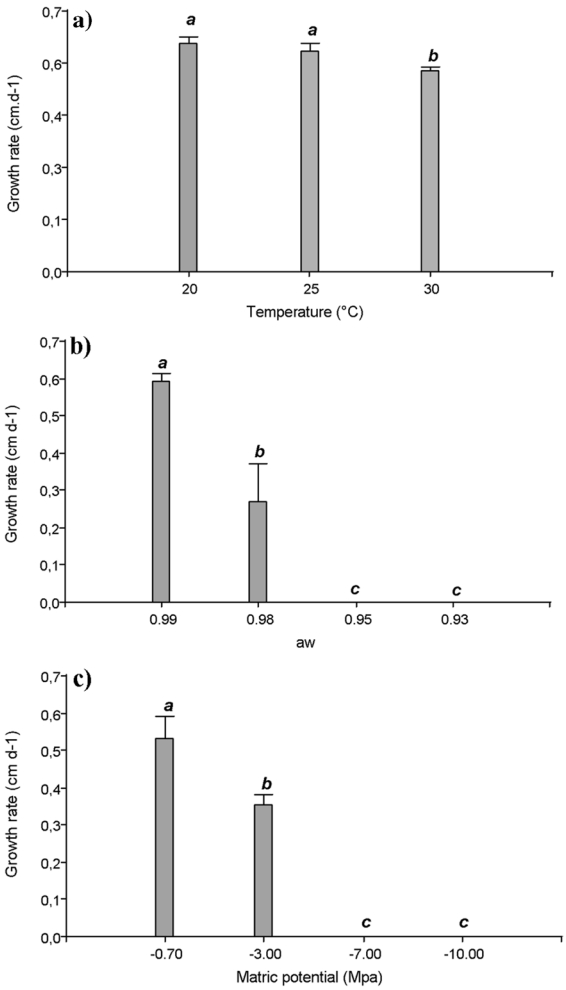

Fig. 1 Effect of (**a**) temperature, (**b**) water activity (a_W) and (**c**) matric potential (Ψm), on the growth rate of *P. plurivora* SRA14. Mean values based on three data and standard deviation. Data with the same letter are not significantly different according to DGC Test ($p < 0.05$)

Endoparasitism and endophytism studies

Both fluorochromes (FITC and PKH26) combined with micro-ROC allowed *in situ* observation of *P. plurivora* SR14 and *N. aberrans s.l.* J2 migration through root tissue in its host (Fig. 2).

Fungal colonization of rhizosphere

Studies in target plants (tomato)

The results of the rhizosphere colonization assays showed that *P. plurivora* SRA14 remained viable in the rhizospheric tomato soils 30 days after the inoculation, with count levels of 1.5×10^3 CFU g^{-1} soil. However, not all plants showed fungal colonization of the rhizosphere. Furthermore, tomato root analyses revealed that *P. plurivora* SRA14 was able to endophytically colonize this plant organ (3.5×10^4 CFU g^{-1} of the root). Fungal development was not observed in the water samples from the last root washing step of all the plants evaluated. Data analysis by LGM models showed that inoculation with *P. plurivora* SRA14 did not significantly affect plant vigor parameters (stem length ($F = 1.30$, $p = 0.2867$), root length ($F = 0.00$, $p > 0.9999$), aerial part weight ($F = 0.10$, $p = 0.7555$), or root weight ($F = 0.12$, $p = 0.7432$) (Fig. 3). Table 1 shows the data corresponding to the vigor parameters of tomato plants treated with *P. plurivora* SRA14 and control (untreated).

Studies in non-target plants (basil)

The results observed 30 d after basil soil inoculation showed that *P. plurivora* SRA14 developed saprophytically in the rhizosphere with mean counts of 1.0×10^3 CFU g^{-1} soil 30 days after inoculation. Furthermore, basil root analyses revealed that *P. plurivora* SRA14 was able to endophytically colonize this plant organ (2.5×10^4 CFU g^{-1} of root). Fungal development was not observed in the water samples from the last root washing step of all the plants evaluated. Plants inoculated with *P. plurivora* SRA14 did not

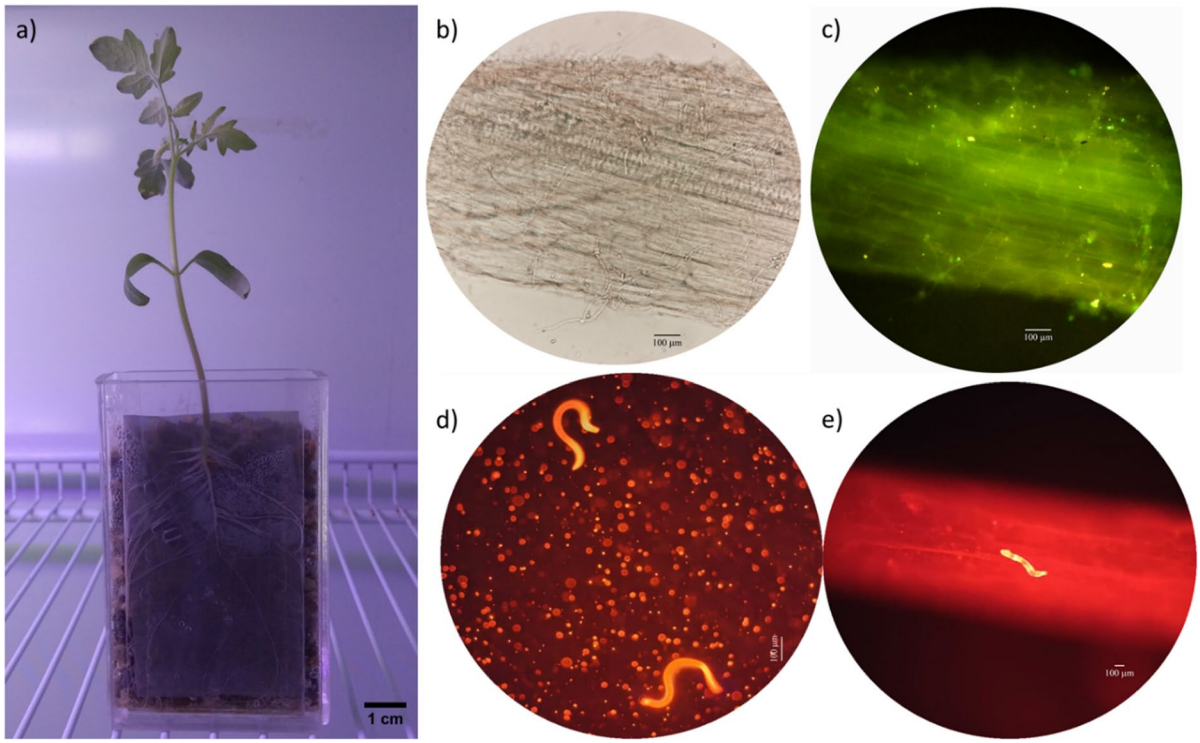

Fig. 2 Tomato seedlings (**a**) growing in microscopy rhizosphere chambers (micro-ROC); endorizospheric colonization of *P. plurivora* SRA14 (**b**); FITC endorizospheric colonization of stained *P. plurivora* SRA14 (**c**); PKH26 stained *N. aberrans s.l.* J2s before (**d**) and after inoculation (**e**). Scale bars: a = 1 cm; b-e = 100 µm

show symptoms of root and collar browning (Fig. 4). Data analysis by LGM models showed that inoculation with *P. plurivora* SRA14 did not significantly affect plant vigor parameters (stem length (F = 0.13, $p = 0.7272$), root length (F = 3.57, $p = 0.0955$), aerial part weight (F: 5.76, $p = 0.0432$), root weight (F: 0.44, $p = 0.5236$)). Table 2 shows the data corresponding to the vigor parameters of tomato plants treated with *P. plurivora* SRA14 and control (untreated).

Antagonism assay in tomato plants

Fungal count

The counts of total fungi and of *Plectosphaerella* spp. were determined from samples of sterile soil inoculated with *P. plurivora* SRA14 (FC and T), and soil from farms in the Río Cuarto horticultural belt (with natural infestation of *N. aberrans s.l.*) (C2 y T2) (Fig. 5). Significant differences were observed in the total fungal counts when the soil samples of different origins were compared at the end of the trial (F = 5.51; $p = 0.0046$), with significantly lower counts registered in T2. However, no statistically significant differences (F = 3.26; $p = 0.0595$) were observed between *Plectosphaerella* spp. counts 96 days after inoculation in sterile soil (FC and T) (mean count: 5.27×10^3 CFU g^{-1}) and in naturally infested soil (T2) (mean count: 1.96×10^3 CFU g^{-1}). Moreover, it was possible to determine that in T2, the levels of *Plectosphaerella* spp. represented a lower percentage of the total isolated fungi (49.33%) than in the treatments in which the fungal inoculum was applied on sterile soil (CF and T) (72.78%).

Population of N. aberrans s.l.

The tomato plants were parasitized with *N. aberrans s.l.*, regardless of the treatment (control and treatment) and soil type (sterile and naturally

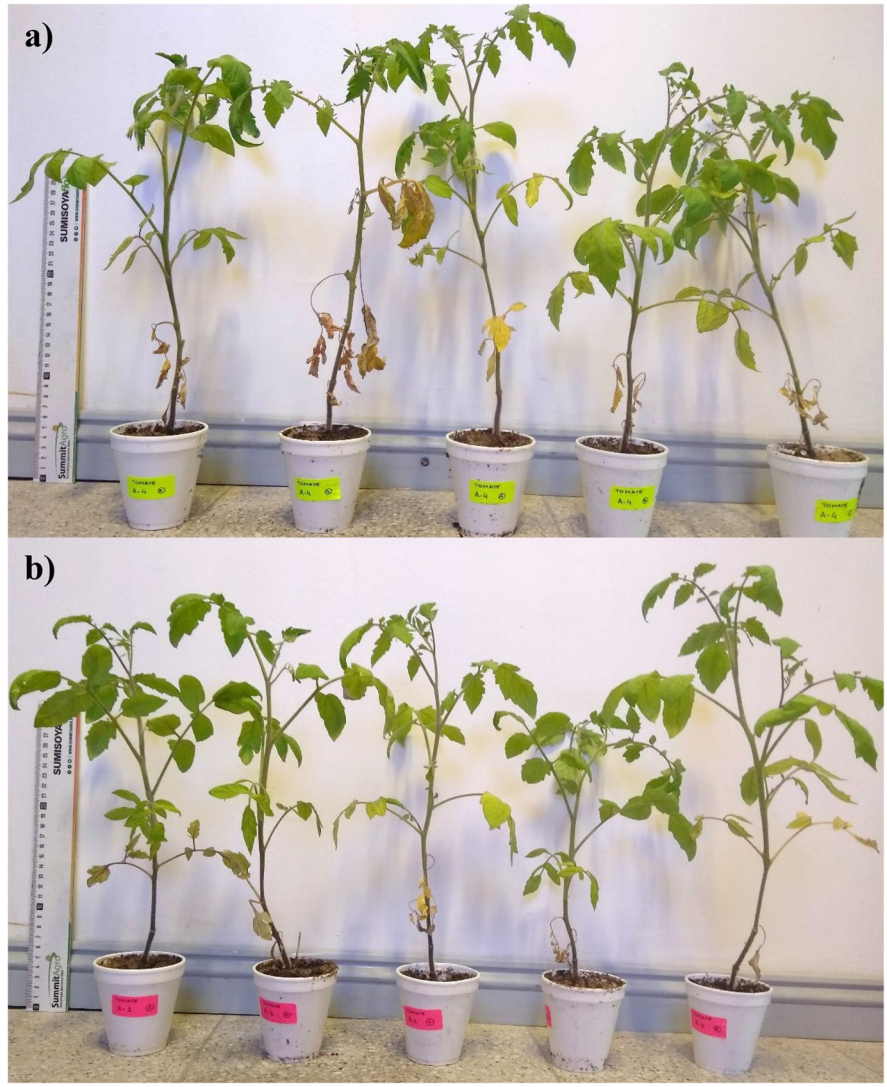

Fig. 3 Tomatoes plants (**a**) inoculated with *P. plurivora* SRA14 and (**b**) control

infected). Significant differences were observed for the number of galls ($F = 31.53$; $p < 0.0001$) and number of egg masses ($F = 12.02$; $p = 0.0006$). Plants grown in sterile soil inoculated with *N. aberrans s.l.* had a significantly higher number of galls (mean: 27.62) and egg masses (mean: 9.75) than those developed in naturally infested soil (mean of gall numbers: 6.12; mean of egg masses numbers: 1.75). However, as shown in Fig. 6, no significant differences ($F = 0.85$; $p < 0.4935$) were observed

Table 1 Vigor parameters of tomato plants

Treatment	Plant vigor parameters (mean ± SE)[a]			
	Stem length (cm)	Aerial part weight (g)	Root length (cm)	Root weigth (g)
Control	26.90 ± 3.42 a	8.70 ± 1.22 a	13.00 ± 0.94 a	7.60 ± 1.63 a
P. plurivora SRA14	28.90 ± 1.92 a	9.10 ± 2.49 a	13.00 ± 3.14 a	7.90 ± 1.12 a

[a]Mean values based on five data. SE: standard error. Values in the same column with different letters are significantly different according to LSD test ($p < 0.05$)

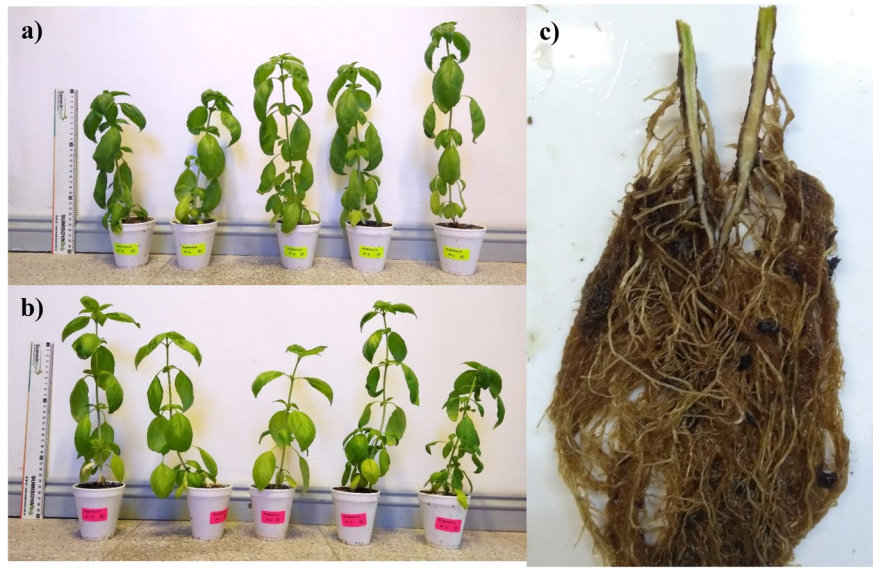

Fig. 4 Basil plants (**a**) inoculated with *P. plurivora* SRA14; (**b**) control and (**c**) basil roots inoculated with *P. plurivora* SRA14

in the RF of the *N. aberrans s.l.* populations, the effectiveness of the NF was evident in both types of substrates assayed. Reductions of this parameter were evidenced when each treatment was compared with its respective control, estimated at 65 and 42% for the sterile and natural-infested soils, respectively.

Plant vigor parameters

The applied treatments did not significantly affect the vigor characteristics of the tomato plants: aerial part length ($F=0.77$; $p=0.5860$), root length ($F=1.18$; $p=0.3293$), number of fruits per plant ($F=2.57$, $p=0.0685$) and total fruit weight ($F=2.58$, $p=0.0681$); meanwhile the fresh ($F=11.37$; $p=0.001$) and dry ($F=10.55$; $p=0.001$) weight of the aerial part of the plants and the fresh weight of the root ($F=3.12$; $p=0.0374$) were significantly affected. The mean values of the vigor parameters are shown in Table 3. The plants grown in the naturally infested soil (C2 and T2) presented aerial fresh and dry weight and root fresh weight significantly lower ($p<0.05$) than those grown on the sterile soil.

Discussion

Current knowledge of integrated pest and disease management shows that there is more than one efficient and sustainable tool for controlling the impact of phytonematodes on horticultural crops. However, in Argentina there are few registered biological control products, especially bio-agents, against PPN. In the present study, an isolate of *P. plurivora* SRA14 with potential biocontrol capacity on *N. aberrans s.l.* was ecophysiologically characterized, evaluating desirable features of a potential BCA. Consequently,

Table 2 Vigor parameters of basil plants

Treatment	Plant vigor parameters (mean ± SE)[a]			
	Stem length (cm)	Aerial part weight (g)	Root length (cm)	Root weigth (g)
Control	24.30 ± 1.43 a	7.20 ± 0.71 a	11.20 ± 0.41 a	5.16 ± 0.71 a
P. plurivora SRA14	25.20 ± 2.04 a	9.24 ± 0.47 a	12.70 ± 0.68 a	5.74 ± 0.50 a

[a] Mean values based on five data. *SE* standard error. Values in the same column with different letters are significantly different according to LSD test ($p<0.05$)

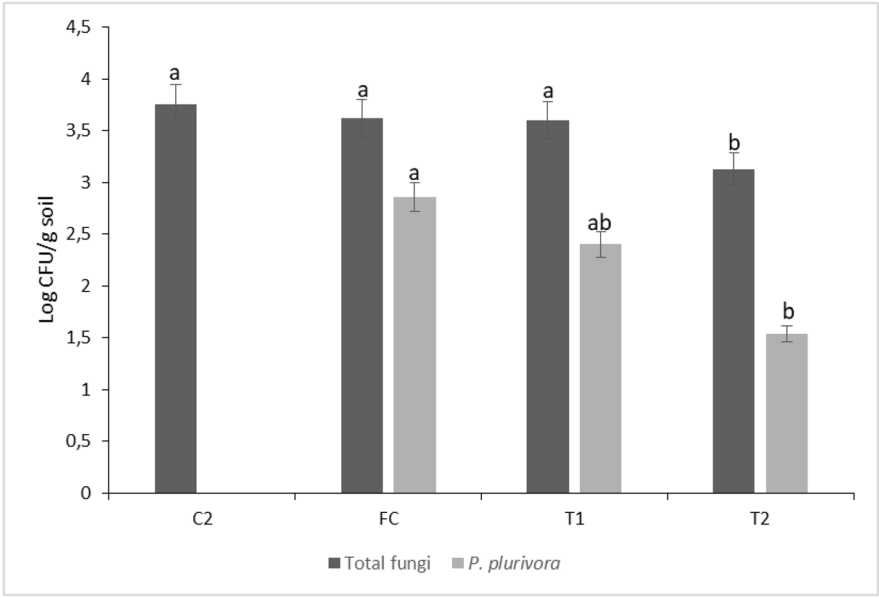

Fig. 5 Fungal count in rhizospheric soil tomato samples at the end of the greenhouse antagonism test. Mean values based on eight data and standard error. Data with the same letter are not significantly different according to LSD Test ($p < 0.05$). C2: Control 2 (tomato seedling in naturally infested soil + *P. plurivora* SRA14); FC: Fungal control (tomato seedling in sterile soil + *P. plurivora* SRA14); T1: Treatment 1 (tomato seedling in sterile soil + *N. aberrans s.l.* + *P. plurivora* SRA14); T2: Treatment 2 (tomato seedling in naturally infested soil + *P. plurivora* SRA14)

the growth parameters in variable environmental conditions, the enzymatic production of the fungus, as well as the capacity to develop as saprophyte and endophyte of the target (tomato) and non-target (basil) plants for *N. aberrans s.l.* were evaluated.

On the one hand, *in vitro* growth assays allowed the determination of the capacity of *P. plurivora* SRA14 to develop under a wide range of temperatures (20–30 °C). However, reductions in the fungal growth rate (9.0%) were observed at the highest temperature evaluated (30 °C). In addition, the highest levels of water stress tested (Ψm: -7 and -10 MPa; a_W: 0.95 and 0.93) inhibited the growth of this fungus. Therefore, this assay reflects how environmental factors can affect the survival of the fungal strain, which provides a projection of the survival capacity of the antagonist

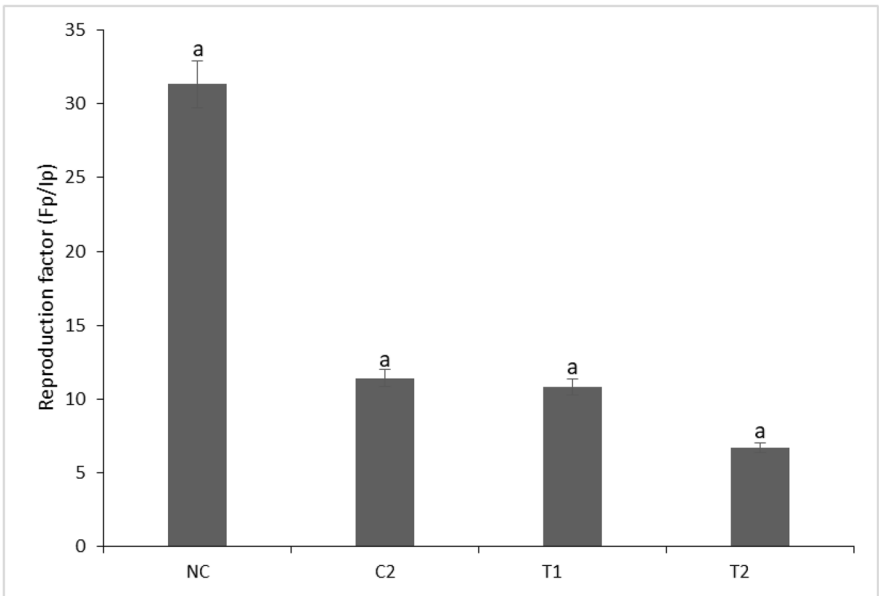

Fig. 6 Reproduction factor of *N. aberrans s.l* in soil at the end of the greenhouse antagonism test. Mean values based on four data and standard error. Data with the same letter are not significantly different according to LSD Test ($p < 0.05$). NC: Nematode control (tomato seedling in sterile soil + *N. aberrans s.l.*); C2: Control 2 (tomato seedling in naturally infested soil); T1: Treatment 1 (tomato seedling in sterile soil + *N. aberrans s.l.* + *P. plurivora* SRA14); T2: Treatment 2 (tomato seedling in naturally infested soil + *P. plurivora* SRA14)

Table 3 Vigor parameters of tomato plants at the end of the greenhouse antagonism assay

	Plant vigor parameters (mean ± SE)[a]						
	Aerial part			Root part		Fruits	
	Wet weight (g)	Dry weight (g)	Length (cm)	Wet weight (g)	Length (cm)	N°/plant	Total weight (g)
C1	401.50 ± 20.51 a	63.50 ± 4.95 a	1.72 ± 0.19 a	68.85 ± 23.83 ab	35.50 ± 3.54 a	3.00 ± 1.41 a	46.35 ± 49.14 a
FC	357.50 ± 44.81 a	60.25 ± 12.50 a	1.62 ± 0.12 a	89.75 ± 30.78 a	30.00 ± 4.69 ab	2.00 ± 0.00 a	65.35 ± 47.50 a
NC	382.50 ± 57.32 a	63.00 ± 9.52 a	1.78 ± 0.19 a	89.75 ± 45.03 a	36.68 ± 6.47 a	0.25 ± 0.50 a	13.37 ± 26.74 a
T	336.00 ± 30.28 a	58.25 ± 4.57 a	1.63 ± 0.20 a	84.50 ± 37.93 a	36.63 ± 7.25 a	1.75 ± 2.87 a	29.04 ± 34.09 a
C2	198.50 ± 46.74 b	29.00 ± 9.83 b	1.53 ± 0.28 a	38.81 ± 11.20 b	40.48 ± 12.21 a	0.00 ± 0.00 a	0.00 ± 0.00 a
T2	197.25 ± 73.97 b	29.75 ± 12.09 b	1.70 ± 0.16 a	30.25 ± 6.55 b	21.25 ± 5.56 b	0.25 ± 0.50 a	4.83 ± 9.66 a

[a] Mean values based on four data. *SE* standard error. Values in the same column with different letters are significantly different according to LSD test ($p < 0.05$)

in the ecosystem. The ability to develop in different environmental conditions has allowed species of *Plectosphaerella* to be isolated from diverse hosts throughout the world. Its isolation has been reported mainly from the rhizosphere of plants, but also insects, crustaceans, or nematodes (Giraldo & Crous, 2019; Yu & Coosemans, 1998), and some members are known for their alkali-tolerant properties (Grum-Grzhimaylo et al., 2013, 2016; Okada et al., 1993).

On the other hand, considering that the production of extracellular enzymes may be included among the parasitic properties of NF (Gortari & Hours, 2008; Khan et al., 2003; Park et al., 2004), the ability of *P. plurivora* SRA14 to produce chitinase and protease enzymes was evaluated under inductive conditions. According to Bonants et al., (1995) and Tikhonov et al., (2002), these enzymes may be involved in the decomposition of nematode eggshells. These tests evidenced a low enzyme production capacity (chitinases and proteases) of the isolate of *P. plurivora* SRA14 whose nematophagous capacity on eggs (76.0%) and J2 (78.3%) of *N. aberrans s.l.* was evidenced in preliminary studies (Sosa et al., 2018).

The establishment of a fungus with biocontrol potential in the field depends largely on its ability to survive and proliferate in the soil (Magan, 2001). In the studies on target (tomato) and non-target (basil) plants for *N. aberrans s.l.*, it was possible to recover *P. plurivora* SRA14 from the rhizospheric soil samples after 30 days of inoculation (10^3 CFU g^{-1} of soil). In addition, a decrease in fungal density was observed, compared to the initial inoculum (10^6 conidia g^{-1} of soil), which would suggest a limited capacity of *P. plurivora* SRA14 to colonize the rhizosphere of the tomato crop in a saprophytic manner. However, significant fungal counts were recorded in the tomato endorhizosphere (3.5×10^4 CFU g^{-1} of roots), which indicates a good capacity for endophytic colonization, since no symptoms of wilting or browning were observed in plants. This result is relevant since most of the life cycle of *N. aberrans s.l.* occurs within the roots of plants, so the ability of the BCA to develop endophytically could indirectly favor the antagonism against the nematode. Similarly, some authors have reported that species of the *Plectosphaerella* genus presented endophytic lifestyles, colonizing plant tissue without causing visible symptoms (D'Amico et al., 2008; García et al., 2013; Götz et al., 2006; Junker et al., 2012; Thiergart et al., 2020) or as epiphytes showing antagonistic effects against bacterial pathogens (Zhou et al., 2017). At the same time, the effect of the inoculation of *P. plurivora* SRA14 was evaluated in basil plants, a non-target plant belonging to the horticultural agro-ecosystem on which pathogenicity of some *Plectosphaerella* spp. has been reported (Raimondo & Carlucci, 2018). Basil plants inoculated with *P. plurivora* SRA14 did not show symptoms of browning of the roots and neck, nor alterations in the growth parameters of the plants, although the fungus was able to colonize both the rhizospheric and endorizospheric zones of the plants. Therefore, these results differ from those reported in recent studies in which some species of the *Plectosphaerella* genus cause browning (Carlucci et al., 2012; Raimondo & Carlucci, 2018), wilting (Xu et al., 2014), and rotting of fruits, roots, and neck in different crops (Cannon et al., 2012; Carrieri et al., 2014; Durán et al., 2018; Giraldo et al., 2017; Hyde et al., 2014; Su et al., 2017;

Ton & Mauch-Mani, 2004; Usami & Katagiri, 2017). Species in this family are primarily soil saprobes or weak to virulent, facultative, or obligate plant pathogens (Giraldo & Crous, 2019). Recently, studies based on the characterization of the genomes and the transcriptome of *Plectosphaerella* spp. have revealed that the diverse lifestyles and pathogenicity of the strains are determined by the expression of specific sets of fungal genes in the plant (Muñoz-Barrios et al., 2020).

Finally, to corroborate the antagonism results obtained in the in vitro studies, the *in plant* assay was carried out under greenhouse conditions with sterile and naturally-infested soils. The presence of *Plectosphaerella* spp. was confirmed in both substrates with counts of 10^3 CFU g^{-1}, verifying the ability to grow and colonize the horticultural soil, thus exercising an "inoculative" type control strategy. These results differ from those obtained by Jacobs (2000) in which they determined that a strain of *P. cucumerina* with biocontrol capacity against PCN was a poor saprophytic competitor against other soil fungi. The results obtained in both substrates showed that biocontrol strategies (FC and T2) did not affect the development of tomato plants. First, it was determined that the values corresponding to the vigor parameters (length and weight of the root, large and weight of the aerial part) of the tomato plants were similar to the control, without presenting signs of phytotoxicity. Secondly, the *P. plurivora* SRA14 inoculation produced increases in the biomass of tomato fruits, estimated at 64.5% compared to that of the control cultures. Similarly, Girardi et al. (2022) reported that the inoculation of *Purpureocillum lilacinum* SR14 incremented plant growth (root and aerial part) with a 47% reduction of the *N. aberrans s.l.* egg masses. The effectiveness of *P. plurivora* SRA14 control on the *N. aberrans s.l.* population was evidenced in greenhouse pot experiments. The application of the NF reduced the "reproduction factor" of the phytonematode, both in sterile and naturally-infested soil by 65 and 42%, respectively. Atkins et al., (2003) have detected and isolated *P. cucumerina* from two UK sites where PCN (potato cyst nematodes) populations have been shown to be in decline. Although in these studies it could not be determined if this fungus was the cause of the decrease in PCN, this species has been shown in previous studies to have potential as a BCA, reducing PCN populations by up to 60% in field trials after from their incorporation into alginate granules (Jacobs, 2000). Furthermore, some species of the genus *Plectosphaerella* demonstrated fungicidal or pesticidal properties (Batista and Silva 1959; Domsch et al., 2007; Duc et al., 2009; Gräfenhan et al., 2011). The general effect of *P. plurivora* SRA14 seems to imply the interruption of embryonic development and the death of juveniles (Sosa et al., 2018), which results in the reduction of the nematode population, besides ensuring an initial fungal inoculum for the next crop.

Thus, this study evidenced the ability of *P. plurivora* SRA14 to grow under a range of environmental conditions, endophytically colonize the roots of the tomato plant – a crop of economic importance affected by *N. aberrans s.l.* – without affecting either the target or non-target (basil) cultures. *In plant* antagonism tests demonstrated that the application of the NF reduced the population of the PPN without affecting the host crop. Furthermore, this strategy could be applied as a preventive biological control practice since SRA14 could effectively colonize and establish at levels that ensure low loads of the *N. aberrans s.l.* in the soil horticultural ecosystem. Therefore, it is a plausible alternative to be adopted in the horticultural production area of Río Cuarto because it safeguards the health of crops with environmental care, and contributes to the growing demand for safe food. Its parasitic effects on the soil nematofauna and other PPN should be discussed in future.

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Author contributions All authors conceived and designed the research; NG, AS, JLG and MP conducted experiments, and analyzed the data; all authors discussed the results; AP, AS, and NG wrote the article; all authors read and approved the manuscript.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare they have no financial interests.

Human and animal rights No human and/or animal participants were involved in this research.

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