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ORIGINAL PAPER

Screening and identification of horticultural soil fungi for their evaluation against the plant parasitic nematode *Nacobbus aberrans*

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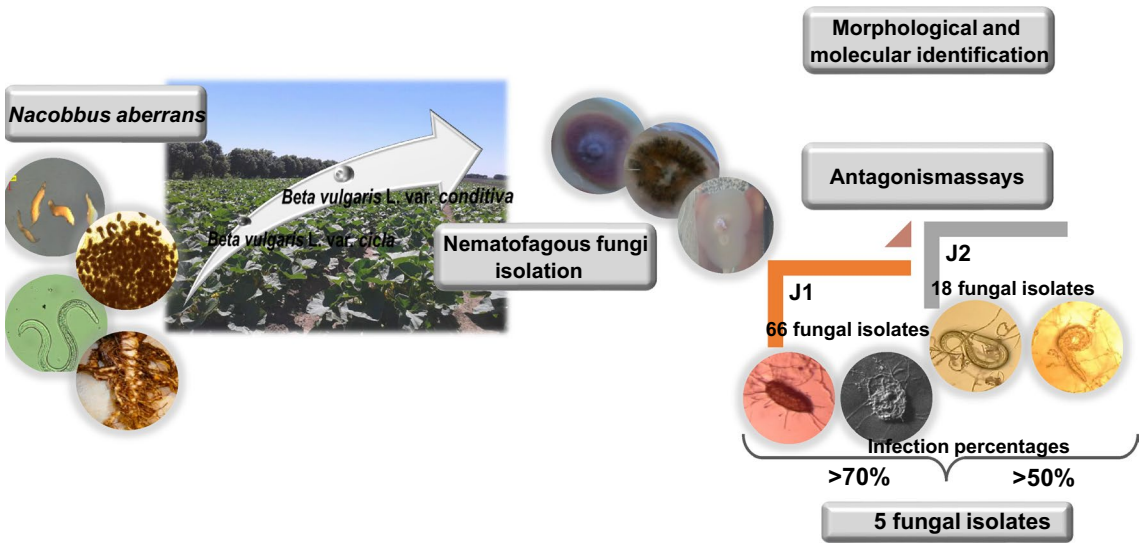
Abstract

The plant-parasitic nematode *Nacobbus aberrans* is an endoparasite causing severe losses to a wide range of crops from North to South America. The use of native antagonistic fungi may be considered as a possible biological control alternative to reduce the damages caused by this species. Antagonistic effects of 66 potential nematophagous fungi against eggs (J1) and second-stage juveniles (J2) of *N. aberrans*, were evaluated in vitro on water agar. DGC test showed significant differences ($p < 0.0001$) in the efficacy of some fungal isolates tested, with parasitism levels for J1 and J2 of 0–95 and 1–78%, respectively. Five isolates of *Purpureocillium lilacinum, Metarhizium robertsii* and *Plectosphaerella plurivora* appeared as the most effective antagonists of *N. aberrans*, relying on hyphae and adhesive conidia in host infection processes.

Graphical Abstract



Keywords False root-knot nematode · Horticultural agro-ecosystems · Nematophagous fungi · In vitro tests

Introduction

Córdoba is the second producer of vegetables in Argentina contributing with 16% of total production. In the region of Río Cuarto, chard (*Beta vulgaris* L. var *cicla*) and beet (*Beta vulgaris* L. var *conditiva*) represent, with 11 and 9% of all produced vegetables, the main horticultural crops

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(Fernández Lozano 2012). Some plant-parasitic nematodes, such as *Nacobbus aberrans*, cause severe damage to numerous crops during the growth season. This clade of nematodes has a wide host range comprising 18 plant families with approximately 84 species, including crops and weeds (Manzanilla-López et al. 2002). *N. aberrans*, also known as the false root-knot nematode, affects mainly potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), sugar beet (*B. vulgaris* L.), pepper (*Capsicum annuum* L.), and bean (*Phaseolus vulgaris* L.). Doucet (1989) initially reported a population of this parasite on *Chenopodium album* L. in the horticultural zone of Río Cuarto. However, in spite of the agricultural practices applied to reduce its density, the presence of *N. aberrans* on chard and beet continued to be reporting (Sosa et al. 2015). *N. aberrans* forms galls similar to those caused by species of the genus *Meloidogyne*. As a consequence, parasitized plant lose their capacity to take up water and nutrients, with reductions in growth and yield levels (Talavera et al. 2001). This nematode is also listed as an A1 quarantine pest, according to EPPO (2011).

Although some alternatives exist for nematode management based mainly on host plant resistance (Verdejo-Lucas et al. 2013), their integration with practices such as crop rotation, fallow, and soil sterilization are insufficient. Furthermore, the sustainability of these production systems is being seriously compromised by the effects of pesticides on biodiversity, or environmental and human health concerns (Nakajima and Ortega 2015).

Attention is increasingly turning towards an integrated management approach: incorporating the use of biocontrol agents with appropriate cultural practices, nematicidal or antagonistic plant extracts, and, where necessary, rational use of nematicides, to reduce nematode population densities below their economic thresholds (Desaeger and Rao 2000).

The biological components of the soil ecosystem are particularly important in limiting or regulating nematode populations. Mechanisms of action include competition, parasitism, predation or production of toxic metabolites. The presence of a great diversity of fungi with potential biocontrol activity has been reported in several soil environments, including non-cultured soils (Jaffee 1992; Lopez-Llorca and Jansson 2007).

Numerous studies focused on the effect of fungi that parasite J2, female or eggs of root-knot nematodes. Species belong to many genera, including *Fusarium, Drechslerella, Paecilomyces, Pochonia* and *Trichoderma*. Some species can also survive in absence of their hosts or proliferate as saprotrophs in soil (Peraza Padilla et al. 2014). Some fungi may also induce indirect benefits. Marro et al. (2014) reported that the application of *Glomus intraradices* at transplantation stage promoted the increase of aerial and root biomass of tomato, even in presence of *N. aberrans*, increasing productivity by enhancing resource uptakes when roots compete with soil pathogens. In addition, plants inoculated with *G. intraradices* at transplant showed a 56% reduction in the number of galls induced by *N. aberrans*.

Therefore, nematophagous fungi (NF) are an important group of soil microorganisms that can suppress populations of nematodes, including plant and animal parasitic species (Swe et al. 2011). The term "nematophagous fungi" (NF) is herein used to describe species with the ability to infect and parasitize nematodes to obtain nutrients. There are four groups of NF categorized according to their mode of action. These include: (i) nematode-trapping fungi using adhesive or mechanical hyphal structures, (ii) endoparasitic species using infective spores, (iii) egg-parasitic fungi invading eggs with their hyphal tips and (iv) toxin-producing species immobilizing nematodes before invasion (Swe et al. 2011). Based on the above considerations, the objectives of this study were to isolate and identify native NF from rhizosphere of horticultural crops and to evaluate their antagonistic activity against eggs and J2 of *N. aberrans*.

Materials and methods

Sampling site

In order to isolate the nematode and potential NF, soil and root samples were collected in *Nacobbus*-infested areas from an horticultural region located in Río Cuarto, Córdoba, Argentina (33°06′21″South, 64°15′41″ West, 418 m.s.n.m.) during the growing season in October 2015. In each site, soil samples (500 g each) were randomly collected from rhizosphere and non-rhizosphere soils of beet (*B. vulgaris* L. var. *conditiva*) and chard (*B. vulgaris* L. var. *cicla*) of *Nacobbus*-infested plants. A total of 20 soil samples were dried at 50 °C during 24 h. Root samples were conserved at 4 °C.

Nematode isolation, culture and identification

A single chard plant showing aboveground symptoms of parasitism (incipient wilting and stunting) was selected to get a nematode lineage for further assays. A single egg mass was removed from a single root gall containing only a female, to establish and multiplicate the inoculum of *N. aberrans* for the following experiments. The surface of the egg masses was disinfected with 1% NaOCl for 4 min, rinsed with sterilized water and placed onto roots of a susceptible tomato (*Solanum lycopersicum* L. var. *valouro*). Tomato plants were then inoculated and grown in plots with sterile soil and sand (1:1) in a glasshouse at 25 ± 5 °C, under strict quarantine conditions (Marro et al. 2014). After 55 days post inoculation, plants were renewed (three times for a total of 6 months) to increase the density of the nematode

population. Thereafter, the *N. aberrans* population was extracted for morphological (Manzanilla-López et al. 2002) and molecular (Rosso 2009) identification studies, and further antagonism assays.

Light microscopy observations

Females obtained from infected roots were handpicked and analyzed taking into account the detailed features proposed by Manzanilla-López et al. (2002). J2 and single egg masses were fixed in a hot solution of 4% formalin and transferred in pure anhydrous glycerol (Ryss 2002). Specimens were measured using light microscopy and specie designation was made considering the previous and posterior body portions, and bulb (Doucet and Di Rienzo 1991).

Molecular analysis

DNA was extracted from individual specimens by milling the nematodes with glass beads (400 µm diam., Sigma-Aldrich, USA) in buffer Tris–HCl (pH 8–10 mM) for 5 min (Atibalentja et al. 2004). PCR amplification of 18S rRNA was performed with specific primers NEMF1 (5′CGCAAA TTACCCACTCTC3′) and REV1 (5′AGTCAAATTAAG CCGCAG3′).

Polymerase chain reactions (PCR) were carried out in 25 µl volumes. PCR mix was added to each tube: 2.5 µl 10 × PCR buffer (Promega), 1.5 µl $MgCl_2$, 1 µl dNTP mixture (10 mM each) 1 µl of 10 pM forward primer, 1 µl of 10 pM reverse primer, 0.25 µl of Taq polymerase (Promega), 19.55 µl of distilled water and 5 µl of DNA. All PCR reactions were run in a C1000 Touch Thermal Cycler (BioRad) with 1 cycle of 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s. The last step was 72 °C for 7 min.

Amplified products were examined on 1.5% agarose gels. The fragments were purified using a commercial kit (Wizard® SV Gel and PCR Clean-Up System A9280a, Promega, USA) following the manufacturer instructions. Bidirectional sequencing was performed for each isolate on the purified PCR products by a commercial provider (Macrogen, NL Company, South Korea). Consensus sequences were produced through alignment by exploiting the overlap region between sensus and antisensus sequences, using BioEdit ver. 7.0.9.0. (Thompson et al. 1994). The sequences obtained were deposited in GenBank with accession numbers of MH000315, MH000316 and MH000317.

The nematode identity was confirmed through the construction of a phylogenetic tree. The dendogram included closest sequences (88% or higher identity and a 68% or higher query coverage) obtained by alignment through BLAST 2.2.32 (Zhang et al. 2000). The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.1/3.0 aLRT) (Guindon et al. 2010).

Fungal isolation and identification

The soil planting method was carried out according to the methodology proposed by Barra et al. (2013). Enumeration of fungal propagules was carried out on solid medium, by the surface spread method, by blending 10 g of soil from each of the 20 samples (10 from rhizosphere and 10 from non-rhizosphere soil) with 90 ml 0.1% peptone water solution. An aliquot (0.1 ml) of each sample dilution until 1×10^{-6} was inoculated on a semi-selective isolation medium (SM) (maize meal 17 g, NaCl 17.5 g, rose bengal 75 mg, triton X-100 0.3 ml, chloramphenicol 50 mg, streptomycin 0.1 ml, cycloheximide 2.5 ml, agar–agar 15 g, distilled water 1000 ml) (Barra et al. 2013) and incubated at 25 °C for 13 days. Fungal counts were expressed as $\log_{10}$ per g of soil. The fungi were sub-cultured on potato dextrose agar (PDA) to obtain pure cultures.

Light microscopy observations

All pure fungal cultures were identified at genus level by macroscopic and microscopic characteristics according to taxonomic keys of Humber (1998) and Samson et al. (1988). Fungal isolates belonging to genera *Metarhizium, Purpureocillium, Paecilomyces, Phialophora, Beauveria, Haplosporangium, Bionectria, Plectosphaerella* and *Verticiullium* were selected for biocontrol assays.

A total of 15 isolated fungi, which showed infection levels on J1 stages of *N. aberrans* higher than 70% (see below), were again analyzed by molecular parameters.

Molecular analysis

The 15 isolates that showed the highest nematocidal activity on J1 were maintained in sterile water. The fungal cultures were grown on PDA for 7 days at 25 °C. Mycelial biomass was collected for DNA analyses as described by Passone et al. (2010) with some modifications. A mycelium aliquot (50 mg) of each fungal isolate was transferred into eppendorf tubes and vortexed for 5 min in 1 ml of extraction buffer with glass beads (456 µm diameter, Sigma-Aldrich) to favor the disruption of fungal material. After incubation at 65 °C for 60 min, 1 vol of chloroform was added to the sample that was homogenized and centrifuged for 10 min at 13,000 rpm. The aqueous phase was then recovered and 1 vol of chloroform was added. The sample was homogenized and centrifuged again for 10 min at 13,000 rpm. The aqueous phase was precipitated with 1:10 volume of Na acetate (3M) and

500 µl of isopropanol. After incubation at room temperature for 20 min, it was centrifuged for 10 min at 13,000 rpm and the aqueous phase was discarded. Finally, the DNA pellet was washed with 70% ethanol and suspended in 25 µl of nuclease-free H_2O. The PCR (BioRad, Hercules, USA) was conducted in 50 µl reactions with the following concentrations: 5 µl de PCR buffer (10 × with 1. 5 mM Mg^{2+}), 2 U of *Taq* DNA polymerase, 300 µM each dNTPs and 200 nM of each primer. The primers used were the fungus-specific forward primer ITS1 (5′-TCCGTAGGTGAACCTGCG-3′) and the reverse primer ITS4 (5′-TCCTCCGCTTATTGA TATGC-3′) (White et al. 1998). The PCR was carried out with: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The reaction product was stored at 4 °C. The presence of PCR products was confirmed by gel electrophoresis on agarose gel, from which they were purified and sequenced, as previously described.

In vitro antagonism assays

J1 infection

Egg masses were removed by laceration of galls from parasitized tomato roots under a stereomicroscope. They were then disinfected (Tyler 1938) and placed in conical tubes with 10 ml of water and 105 µl of NaOCl, stirring vigorously for 2 min and then steeping for 5 min. The solution was removed and the eggs were rinsed with sterile distilled water with vigorous stirring (three times), and then transferred to streptomycin 0.1%, chloramphenicol 0.1% and cycloheximide 0.1% solutions (10 min for each one), stirring in each step. Finally, the eggs were diluted in 10 ml of sterile distilled water and counted in a Mc Master camera.

Pure cultures of 66 fungal isolates maintained on PDA were used for this study. Agar disks (2.5 mm in diameter) were cut from 1 week old cultures, placing each at the center of water agar (WA) (2% w/v) plates and incubated at 25 °C for 1 week when until the colony diameter reached approximately 2 cm. Twenty J1 of *N. aberrans*, previously surface disinfected, were then placed at the edge of the colonies. Two replicates for each fungus were used, with J1 in WA without fungi used as negative controls. Periodic observations were carried out under a microscope and the percentage of parasitized eggs was estimated according to Regaieg et al. (2011).

J2 infection

An aliquot of 100 µl of 1×10^6 spore suspensions of each isolate was placed at the center of WA (2% w/v) plates and incubated at 25 °C. J2s were obtained from egg masses of *N. aberrans* during 2 days incubation in water at room temperature. Disinfection was performed by suspending the J2s in a conical tube with 10 ml of water: NaClO (0.5% v/v) solution. Fifty J2s from freshly hatched eggs were inoculated after 1 week. Fifteen fungal isolated, from the 66 used in the egg infection assay, were selected according to the effect on J1. J2s on WA without fungi were used as negative control. The plates were maintained at 25 °C for 5 days. The antagonistic effect on J2 was analyzed again at two different times with five fungal isolates selected according to the effect on J2 observed in the first assay. The percent of parasitized J2 were evaluated under a stereoscope according to Peraza Padilla et al. (2014).

Statistical analysis

Data obtained were subjected to ANOVA test using InfoStat for Windows ver 2012 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina) (Di Rienzo et al. 2012). To determine the significance between the treatments a Formation of Excluding Groups (DGC) test; a cluster-based method for the identification of groups of non-homogeneous means (Di Rienzo et al. 2002); was also conducted (Time/Fungus).

Results

Nematode isolation and identification

Morphology of the observed specimens is according to the features previously described for *N. aberrans*. Figure 1a shows a sedentary mature female, endoparasitic in chard roots. Eggs deposition in the gelatin matrix was observed, with a variable number of eggs per sac (estimated between 37 and 833), with many eggs still retained inside the galls (Fig. 1b). The needle-shaped stylet morphology with small basal knobs was observed, ending in specialized musculature and two small nodules (Fig. 1c). Finally, the vulva was transversely located on the posterior portion of the body wall, surrounded by two slightly elevated cuticular lips (Fig. 1d).

The results obtained from molecular analysis of the partial sequences of the 18S rRNA gene confirmed the morphological identification. The phylogeny analysis showed that *N. aberrans* population associated with chard belonging to *N. aberrans* group, consisting of populations from Perú (AY820779, AY820780), Argentina (AY820777) and Bolivia (AY820778) (Fig. 2).

Fungal isolation and identification

The Log_{10} of colony forming units per gram of soil (Log_{10} cfu g^{-1}) varied widely in soils proceeding from areas with beet and chard cultivation (Fig. 3). The fungal count was significantly higher in CRS than other areas ($p < 0.05$).

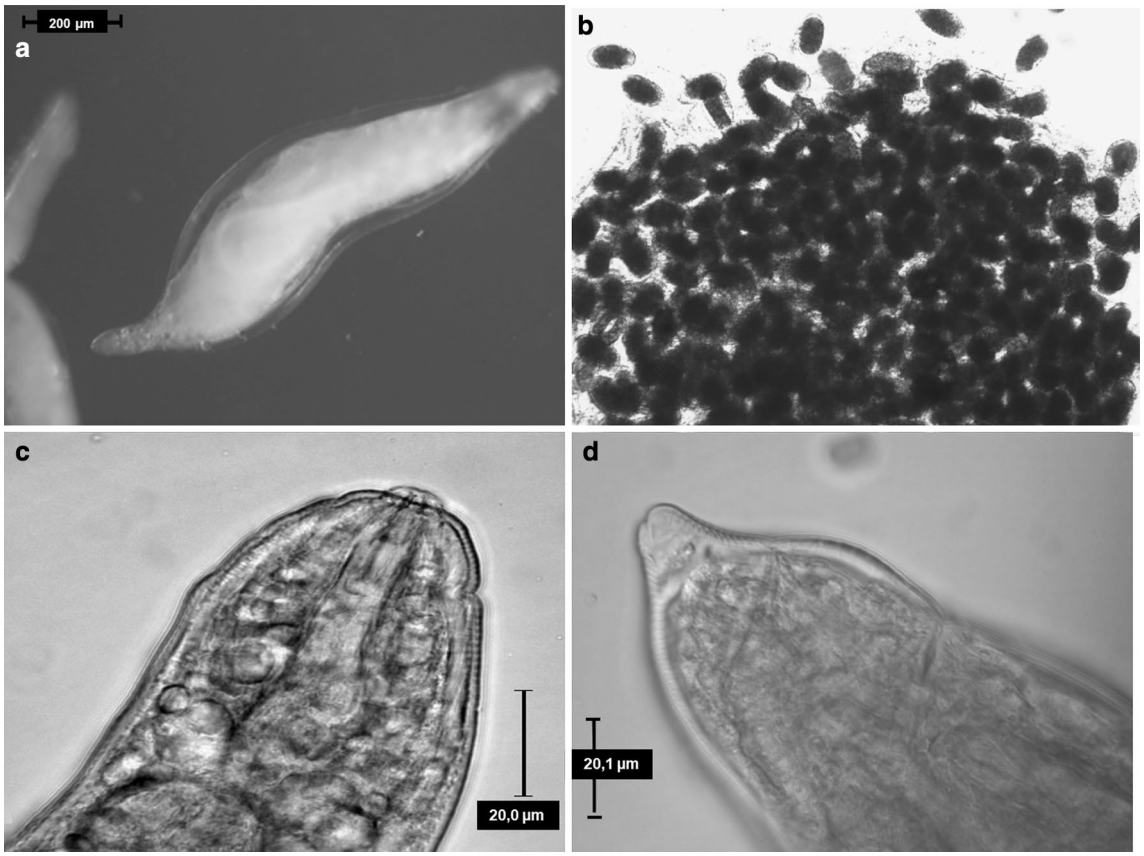

Fig. 1 **a** Mature females, **b** eggs masses, **c** anteriorly portion of the mature female, **d** posteriorly portion of the mature female

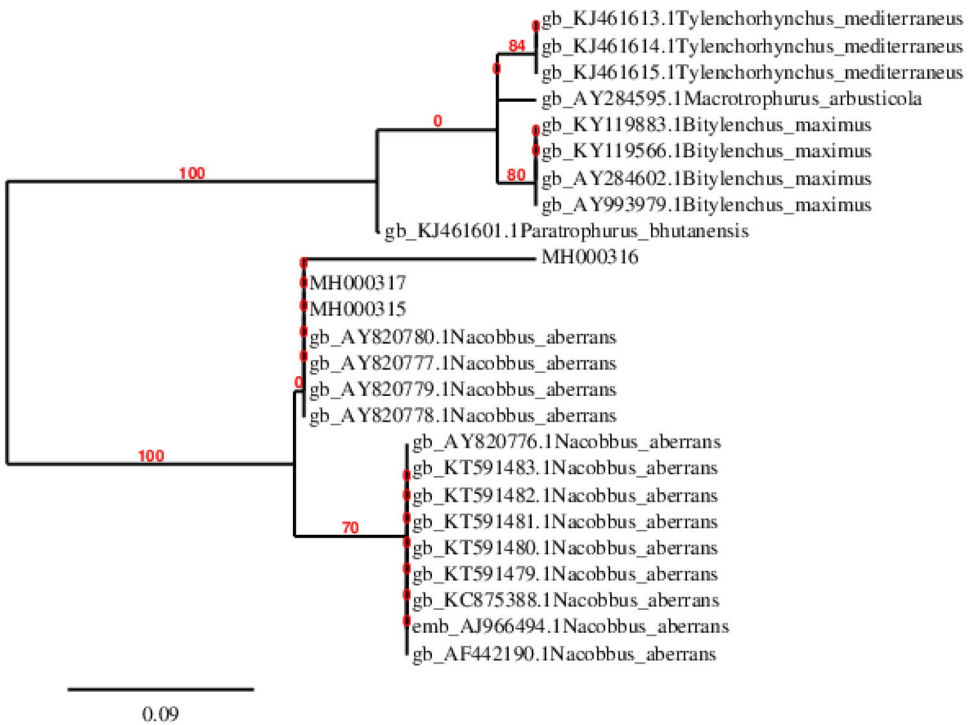

Fig. 2 Phylogenetic tree produced with the sequence obtained from single specimens of *N. aberrans* associated with the chard plant, using the alignment of a 304 nt fragment of the 18S rRNA region related to NCBI entries. The scale bar represents the nucleotide differences of the sequence

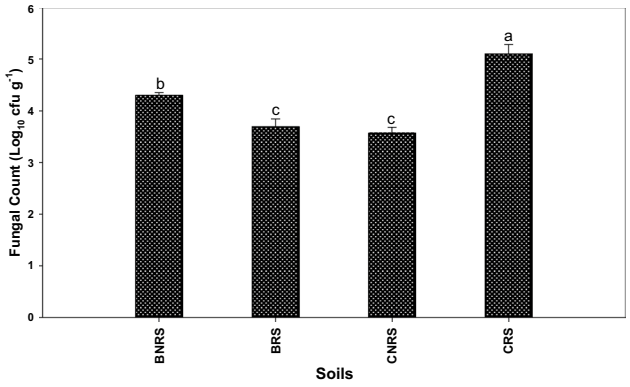

Fig. 3 Fungal count (Log_{10}) from different substrates of the horticultural agroecosystem. Data with the different letter are significantly different ($p < 0.05$) according to DGC test. Mean values based on five data ± standard error (SE)

Morphological identification revealed that the greatest fungal diversity occurred in soils proceeding from areas with beet cultivation, with presence of 16 genera. Meanwhile, eight fungal genera were found in soils of chard. Mycological analyses showed that 52% of the total fungi isolated belonged to genera with potential nematophagous activity.

Through molecular analysis, the fungi isolated were identified as *Purpureocillium lilacinum* (isolates SR, SR6, SR7, SR10, SR13, SR14, SR15, SR38, SR59, SR64); *Bionectria* sp. (SRR35); *Metarhizium robertsii* (SR51, SRR51); *Plectosphaerella plurivora* (SRA14) and *Chrysosporium lobatum* (SRR54) (Table 1).

In vitro J1 parasitism studies

In the selection test, the effect of 66 fungal isolates on J1 was evaluated in vitro, determining the percent of parasitized eggs and the adherence mechanisms. The ANOVA test showed statistically significant differences (df = 65, $F = 237.25$, $p < 0.0001$) in the ability to parasitize the eggs, with 60% of the fungi tested that showed levels of egg infection greater than 50% (Fig. 4). No parasitized eggs were observed in the control plates. Isolates from chard non-rhizosphere soil (CNRS) and chard rhizosphere soil (CRS) were able to parasite eggs of *N. aberrans* in the order of 68 and 58%, respectively. In beet non-rhizosphere (BNRS) and beet rhizosphere soil (BRS) isolates evaluations, the parasitized eggs ranged from 0 to 95 and 0 to 84%, respectively.

The mechanism of adherence used by the NF to parasitize the *N. aberrans* J1 varied among the isolates studied. In general, the mechanism most frequently and effectively used was that of adhesive hyphae, followed by the formation of hyphae network and adhesive conidia. The latter, in some interactions, were combined with adhesive hyphae, showing a greater efficiency to parasitize J1 (Fig. 5) as for isolates of *P. lilacinum* SR6, SR59 and *Metarhizium* sp. SR19, SR20, SR25, SR32.

Based on the infection obtained in egg parasitism assay (Fig. 4), 15 fungal isolates were selected to evaluate the effect on J2. They were *P. lilacinum* SR (infection percentage: 86%), SR6 (78%), SR7 (84%), SR10 (69%), SR13 (73%), SR14 (73%), SR15 (86%), SR38 (86%), SR59 (74%), SR64 (95%). Other species included *Bionectria* sp. SRR35 (75%); *M. robertsii* SR51 (83%), SRR51 (88%); *P. plurivora* SRA14 (77%) and *C. lobatum* SRR54 (85%).

In vitro J2 parasitism

A first preliminary analysis was carried out with the 15 selected fungi in order to establish their infection capacity on J2. Non-parasitized motile and immotile J2 were observed on the control plates, indicating that the disinfection treatment used was adequate. Infection capacity on J2 was variable, ranging from 1 to 78% (Fig. 6). Five isolates showed

Table 1 Molecular identification of the 15 hyphomycete fungal isolates

Isolate	Fungi	GenBank accesion no.	Identity (%)	Coverage (%)
SR	*Purpureocillium lilacinum*	MF996809	100	100
SR6		MF996810		
SR7		MF996811		
SR10		MF996817		
SR13		MF996812		
SR14		MF996818		
SR15		MF996815		
SR38		MF996813		
SR59		MF996816		
SR64		MF996814		
SR51	*Metarhizium robertsii*	MF996819	100	99
SRR51		MF996820		
SRR35	*Bionectria* sp.	MF996821	99	99
SRR54	*Chrysosporium lobatum*	MF996822	98	99
SRA14	*Plectosphaerella* sp.	MF996823	99	99

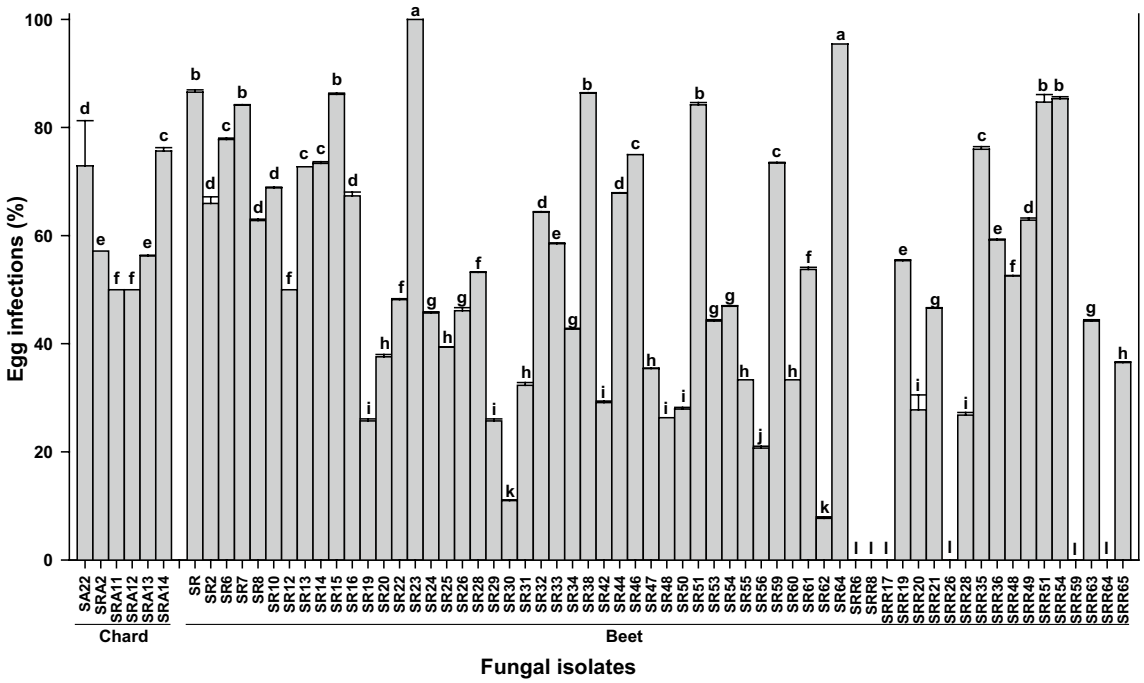

Fig. 4 *Nacobbus aberrans* egg infections (%) by 66 fungal isolates from chard and beet rhizospheric and non-rhizospheric soils. Data with the same letter are not significantly different ($p < 0.05$) according to DGC test. Mean values based on two data ± standard error (SE)

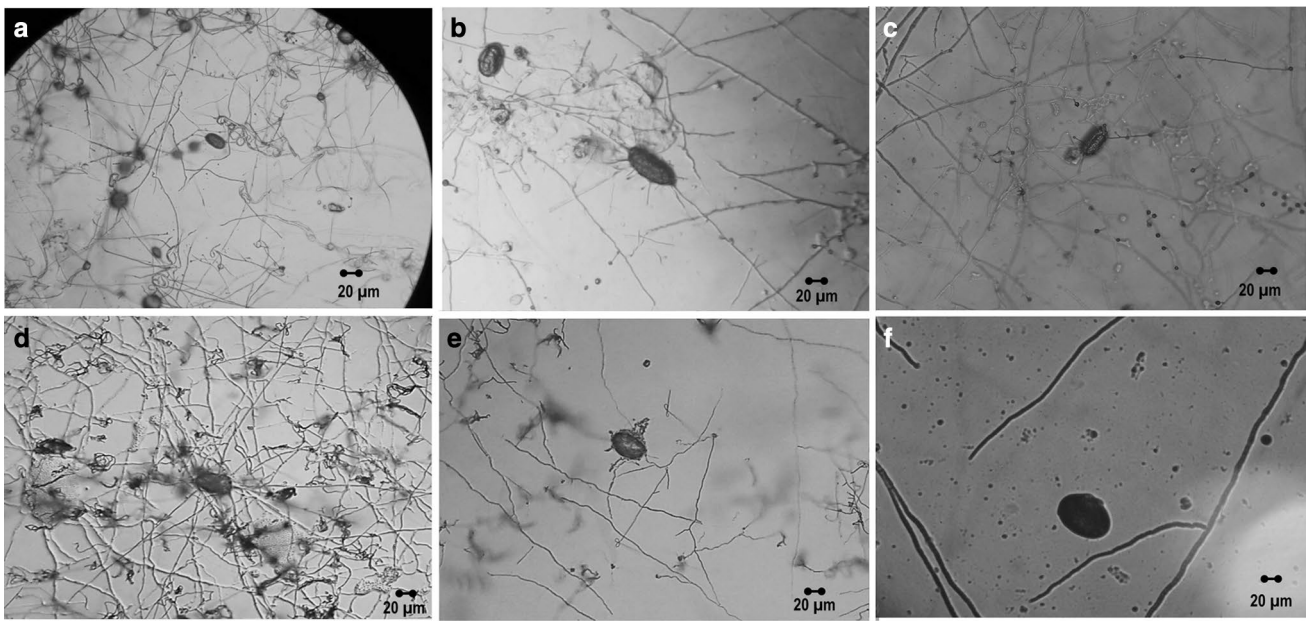

Fig. 5 Mechanism of adherence: **a, b** adhesive hyphae, **c** surrounding hyphae, **d** network of hyphae and fructification inside the egg, **e** adhesive conidia, **f** negative infection

a greater infection capacity (df = 14, $F = 21.64$, $p < 0.0001$): *P. plurivora* SRA14 (78%), *M. robertsii* SR51 (72%) and *P. lilacinium* isolates SR7, SR14, SR38 (60, 64 and 59%, respectively). These isolates also showed infection levels on the J1 higher than 70%. The other isolates tested showed low infection levels, between 2 and 41%, and were not considered in the J2 infection test repeated over time.

Results obtained from infection over time assay of the 5 fungal isolates showed that two independent variables, time (df = 2, $F = 33.69$, $p < 0.0001$) and fungus (df = 4, $F = 11.11$,

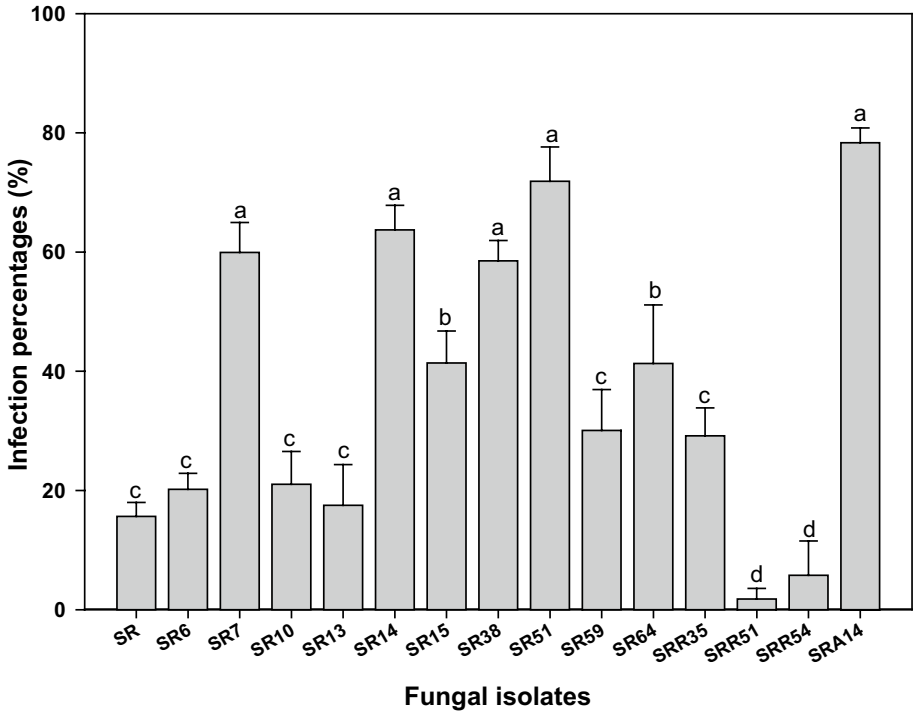

Fig. 6 *Nacobbus aberrans* larval infection (%) by the 15 selected fungi as potential biocontrol agents. Data with different letters are significantly different according to DGC test ($p < 0.05$). Mean values based on two data ± standard error (SE)

Table 2 ANOVA test

Fact	Df	SRA14		SR38		SR51		SR14		SR7	
		CM	*F*	CM	*F*	CM	*F*	CM	*F*	CM	*F*
T	2	1341.4	24.8*	847.5	11.4*	1143.7	15.9*	6.1	0.03	915.0	9.4*
Error	9	54.1		74.2		72.0		179.5		97.5	
Total	11										

Effect of time—T on the infective capacity of larvae J2 of *N. aberrans* of each isolate fungi evaluate

Df degrees of freedom, *CM* mean square

*Significant at $p < 0.05$

$p < 0.0001$), significantly affected the dependent variable under study. Moreover, the time/fungus interaction was statistically significant (df = 8, $F = 2.72$, $p < 0.0156$). An individual analysis was hence performed, to elucidate the time effect on the infective capacity of each NF (Table 2). The analyses showed that *P. lilacinum* SR14 was the only fungal isolated that maintained its infective capacity on J2 without statistically significant modifications in the time.

Figure 7 shows that all fungal isolates, with the exception of SR14, significantly reduced ($p < 0.05$) their infective capacity in the order of 31% at T2. However, the percentage of infected larvae by SRA14, SR38, SR51 and SR7 in the last evaluated period (T3) increased at similar levels to T1, which varied between 49 and 78%. *P. lilacinum* SR14 maintained its infective capacity around of 63% at the three evaluation times.

In the same trial, we attempted to elucidate the mechanisms of adherence used by isolates to infect *N. aberrans* J2. When the interactions between isolates of *P. lilacinum* SR38,

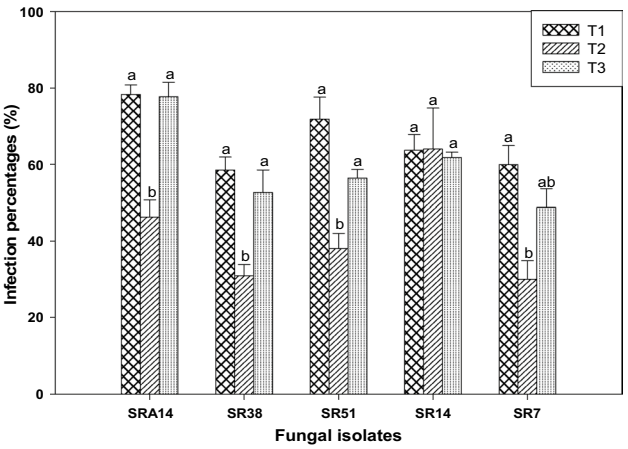

Fig. 7 *Nacobbus aberrans* larval infection (%) by the 5 selected fungi as potential biocontrol agents. Data with different letter for each fungal isolated at each time evaluated (T1/T2/T3) are significantly different according to DGC test ($p < 0.05$). Mean values based on four data ± standard error (SE)

SR7 and SR14 and *M. robertsii* SR51 with *N. aberrans* J2 were evaluated, the mechanism observed was that of enveloping hyphae (Fig. 8 a, b, c and d). Adhesive conidia was the mechanism of adherence used by *P. plurivora* SRA14 (Fig. 8e).

Discussion

Based on molecular and morphological studies, the false root-knot nematode population isolated from Río Cuarto corresponds to *N. aberrans* as described by Manzanilla-López et al. (2002). The identification was confirmed by the 18S rRNA partial sequences that revealed the highest similarity with *N. aberrans* populations of Perú, Argentina and Bolivia (Anthoine and Mugniéry 2005). This study

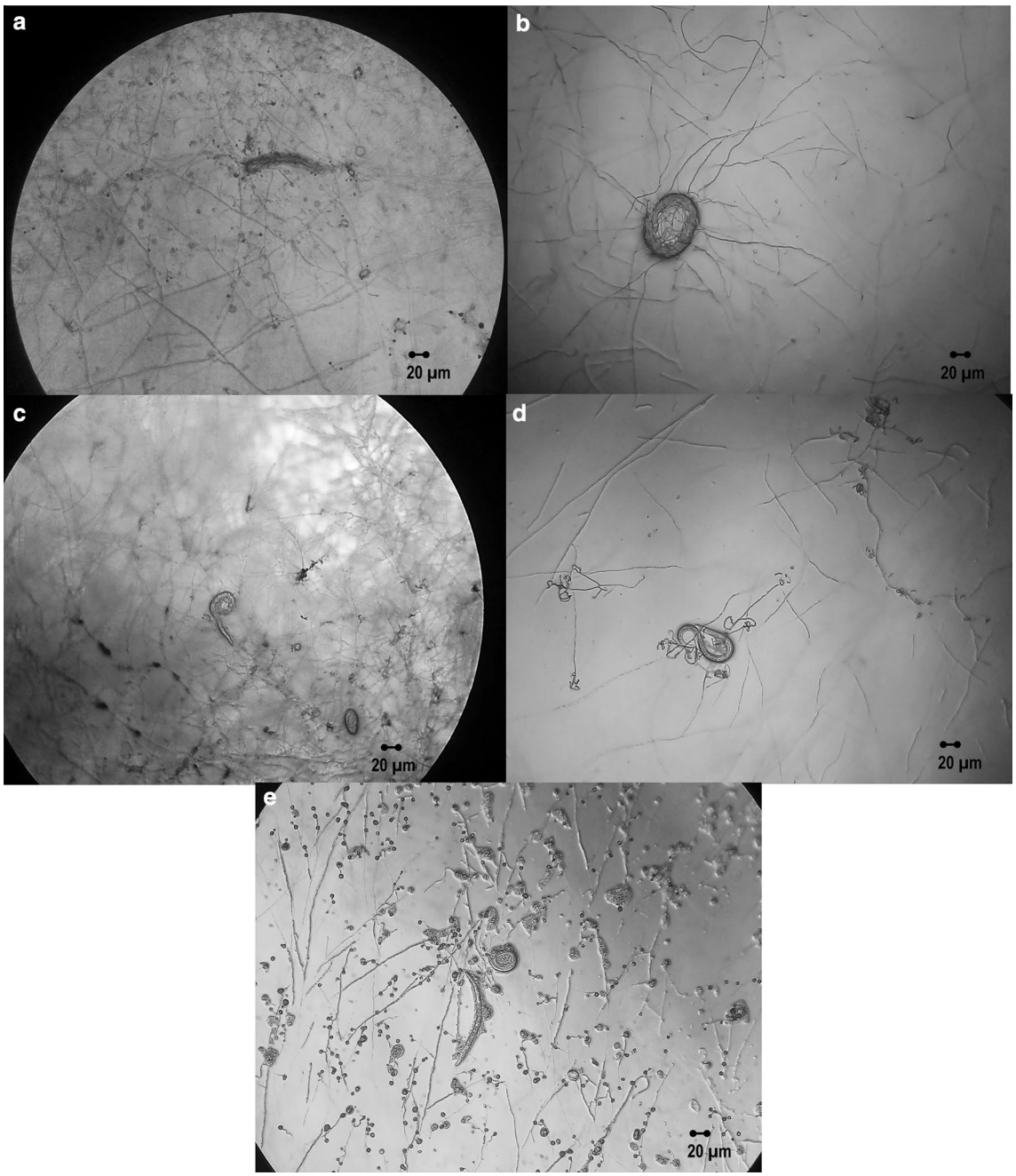

Fig. 8 Mechanism of adherence: enveloping hyphae **a** SR38, **b** SR7, **c** SR14 and **d** SR51, adhesive conidia, **e** SRA14

corroborates previous works carried out by Doucet and Lax (2005) who reported that *N. aberrans* is the main nematode pest that parasitizes the horticultural crops of our region.

Mycological analyses revealed that the greatest fungal diversity was present in soils from areas destined to beet cultivation, and that 52% of the total mycoflora belonged to genera with potential nematophagous activity, which were selected to evaluate the antagonist activity towards *N. aberrans*.

As almost all phytopathogenic nematodes attack roots, it is important to consider the biology of NF in the rhizosphere to implement effective biological control strategies.

This study showed that of the total fungi selected (66), 60% were able to infect eggs with percentages greater than or equal to 50%. Many NF are able to develop specific mycelial structures, commonly called traps that capture nematodes and extract nutrients from them (Yang et al. 2007). However, in this study, the mechanism of adherence on eggs varied among isolates, being adhesive hyphae, hyphal networks and adhesive conidia the most frequently used by most effective isolates selected. Peraza Padilla et al. (2014) evaluated the effect of 14 fungal isolates on *Meloidogyne javanica* eggs, showing that *Trichoderma* sp. Tri2 (96%) and Tri1 (95%) had greater antagonistic activity, while 99% of eggs without parasite were observed in presence of a *Monacrosporium* sp. (Mo1 and Mo2).

Our antagonistic assay on J2 showed that five fungal isolates (*P. lilacinum* SR38, SR7 and SR14; *M. robertsii* SR51 and *P. plurivora* SRA14) were able to infect larvae in percentages greater than or equal to 58%. A priori, when comparing the results obtained in T1 and T2, it could be inferred that strains SRA14, SR38, SR51 and SR7 reduced their infective capacity. However, when the experiment was again repeated (T3), it was observed that all fungal antagonists recovered their infective capacity on J2, reaching parasitism levels similar to those of the first trial. These results suggest that the fungal development at T2 could be influenced by some external factor (incubation temperature, substrate water availability, among others) that did not allow the fungi to achieve an optimal growth, consequently affecting their infective capacity. It is noteworthy the robustness of *P. lilacinum* SR14 that kept the ability to parasitize J2 in the three evaluated times, without significant variations. The results of this study demonstrated that *P. lilacinum* SR38, SR7 and SR14; *M. robertsii* SR51 and *P. plurivora* SRA14 strains have biocontrol potential towards *N. aberrans*. They showed in vitro effective biocontrol on J1 and J2. This result could be compared with those obtained by Dávila and Hío (2005), who observed that *Paecilomyces* sp. (1×10^6 conidia ml^{-1}) was able to parasitize > 50% of a *M. javanica* population, under controlled conditions, after 72 h. The variability in infection levels observed among the different NF studied could be directly related to their mechanisms of adherence to the host. In fact, it is important to emphasize that *P. plurivora*, which showed a high infective activity (around 80%), was the only fungus using adhesive conidia as a mechanism of infection, while the other isolates relied on enveloping hyphae.

The NF mechanisms of host attack are the result of long-term evolutive adaptations, and appear as a key factor in the evaluation of their biocontrol potential. In an in vitro study, Méndoza de Gives et al. (1994) evaluated the ability to generate hyphal traps of *Arthrobotrys conoides* for *N. aberrans* J2 predation, at 25 °C. In other NF such as *Hirsutella rhossiliensis*, the adhesive conidia germinated after passive adhesion to ectoparasitic nematodes moving in soil pores, then developing the mycelium inside the host (Jaffee 1992). A close fungus, *Hirsutella heteroderae*, was found also in association to sedentary cyst nematodes showing, however, low biocontrol efficacy (Sturhan and Schneider 1980). Jaffee (2003) was also able to produce a formulation based on the predatory fungus *Dactylellina ellipsospora*, characterized by forming hyphal networks with adhesive buttons that immobilize the nematodes. Other fungi used for formulations include *A. dactyloides* (Stirling 2014) and *Pochonia chlamydosporia*, a widespread nematode egg parasite also found in association to *N. aberrans* populations in Mexico (Manzanilla-López et al. 2013; Franco Navarro et al. 2008).

The results of the antagonism tests allow advancing in the knowledge about the potential biocontrol of the new isolations of *P. lilacinum, M. robertsii* and *P. plurivora*, because these fungal species were tested, for the first time, on both stages (J1 and J2) of *N. aberrans*. In a previous study, Gortari and Hours (2016) did not evidenced a conclusive activity of *P. lilacinum* on J1 of *N. aberrans*.

In conclusion, this study shows that *N. aberrans* was present on chard culture, and that rhizosphere and non-rhizosphere soils of chard and beet crops were important reservoirs of NF. Moreover, some selected fungi (SRA14, SR51, SR14, SR7 and SR38) showed high antagonist activity on *N. aberrans* J1 and J2 stages in vitro. Therefore, in future studies it is important to deepen our knowledge on the nematode–fungi-host interactions to elucidate their biological control and application potential.

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Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

References

Anthoine G, Mugniéry D (2005) Variability of the ITS rDNA and identification of *Nacobbus aberrans* (Thorne, 1935) Thorne and Allen, 1944 (Nematoda: Pratylenchidae) by rDNA amplification. Nematol 7:503–516

Atibalentja N, Noel GR, Ciancio A (2004) A simple method for the extraction, PCR-amplification, cloning, and sequencing of *Pasteuria* 16S rDNA from small numbers of endospores. J Nematol 36:100–105

Barra P, Rosso LC, Nesci A, Etcheverry MG (2013) Isolation and identification of entomopathogenic fungi and their evaluation against *Tribolium confusum, Sitophilus zeamais*, and *Rhyzopertha dominica* in stored maize. J Pest Sci 86:217–226

Dávila L, Hío JC (2005) Evauación biocontroladora de *Arthrobotrys* sp. y *Paecilomyces* sp. sobre *Meloidogyne javanica* in vitro y bajo condiciones de invernadero en crisantemo (*Drendranthema grandiflora* Anderson). Agron Colomb 23:223–229

Desaeger J, Rao MR (2000) Infection and damage potential of *Meloidogyne javanica* on *Sesbania sesban* in different soil types. Nematology 2:169–178

Di Rienzo JA, Guzmán AE, Casanoves F (2002) A multiple-comparisons method based on the distribution of the root node distance of a binary tree. J Agric Biol Environ Stat 7:129–142

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW (2012) "Infostat". FCA, Universidad Nacional de Córdoba, Córdoba

Doucet ME (1989) The genus *Nacobbus* Thorne Allen, 1944 in Argentina. 1. Study of a population of *N. aberrans* (Thorne, 1935) Thorne & Allen, 1944 on *Chenopodium album* L. from Rio Cuarto, province of Cordoba. Rev Nématol 12:17–26

Doucet ME, Di Rienzo J (1991) El género *Nacobbus* Thorne & Allen, 1944 en Argentina. Caracterización morfológica y morfométrica de poblaciones de *N. Aberrans* (Thorne, 1935) Thorne & Allen, 1944. Nematropica 21:19–34

Doucet ME, Lax P (2005) El género *Nacobbus* Thorne & Allen, 1944 en la Argentina. 6. La especie *N. Aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Nematoda: Tylenchida) y su relación con la agricultura. Acad Nac AyV LIX:5–45

EPPO (2011) PQR-EPPO database on quarantine pests. http://www.eppo.int

Fernández Lozano J (2012) La producción de hortalizas en Argentina. (Caracterización del sector y zonas de producción) Corporación del mercado central de Buenos Aires, 29. Informe técnico. http://www.central-servicios.com.ar/cmcba/ziptecnicas/la_produccion_de_hortalizas_en_argentina.pdf

Franco Navarro F, Vilchis Martinez K, Miranda Damian J (2008) New records of *Pochonia chlamydosporia* from mexico: isolation, root colonization and parasitism of *Nacobbus aberrans* eggs. Nematropica 39:133–142

Gortari MA, Hours RA (2016) *Purpureocillium lilacinum* LPSC # 876: producción de conidias en cultivos sobre sustratos sólidos y evaluación de su actividad sobre *Nacobbus aberrans* en plantas de tomate. Revista Fac Agron 115:2

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321

Humber RA (1998) Entomopathogenic fungal identification. Center for Agriculture and Health, Ithaca

Jaffee BA (1992) Population biology and biological control of nematodes. Can J Microbiol 38:359–364

Jaffee BA (2003) Correlations between most probable number and activity of nematode-trapping fungi. Phytopathology 93:1599–1605

Lopez-Llorca LV, Jansson HB (2007) Fungal parasites of invertebrates: multimodal biocontrol agents? In: Robson GD, van West P, Gadd GM (eds) Exploitation of fungi. Cambridge University Press, Cambridge, pp 310–335

Manzanilla-López RH, Costilla MA, Doucet ME, Franco J et al (2002) The genus *Nacobbus* Thorne & Allen, 1944 (Nematoda: Pratylenchidae): systematics, distribution, biology and management. Nematropica 32:149–228

Manzanilla-López RH, Esteves I, Finetti-Sialer MM, Hirsch PR, Ward E et al (2013) *Pochonia chlamydosporia*: advances and challenges to improve its performance as a biological control agent of sedentary endo-parasitic nematodes. J Nematol 45:1–7

Marro N, Lax P, Cabello M, Doucet ME, Becerra AG (2014) Use of the arbuscular mycorrhizal fungus *Glomus intraradices* as biological control agent of the nematode *Nacobbus aberrans* parasitizing tomato. Braz Arch Biol Technol 57:668–674

Méndoza de Gives P, Zavaleta Mejía E, Herrera Rodriguez D, Quiroz Romero H (1994) In vitro trapping capability of *Arthrobotrys* spp. on infective larvae of *Haemonchus contortus* and *Nacobbus aberrans*. J Helminthol 68:223–229

Nakajima ES, Ortega E (2015) Exploring the sustainable horticulture productions systems using the emergy assessment to restore the regional sustainability. J Clean Prod 96:531–538

Passone MA, Rosso LC, Ciancio A, Etcheverry MG (2010) Detection and quantification of *Aspergillus* section *flavi* spp. in stored peanuts by real-time PCR of nor-1 gene, and effects of storage conditions on aflatoxin production. Int J Food Microbiol 138:276–281

Peraza Padilla W, Orozco Aceves M, Esquivel Hernández A (2014) Evaluación in vitro de hongos nematófagos en zonas arroceras de Costa Rica contra el nematodo agallador *Meloidogyne*. Agron Costarric 38:19–32

Regaieg H, Ciancio A, Raouani NH, Rosso L (2011) Detection and biocontrol potential of *Verticillium leptobactrum* parasitizing *Meloidogyne* spp. World J Microbiol Biotechnol 27:1615–1623

Rosso LC (2009) Cloning, sequence, and expression analysis of a new MnSOD-encoding gene from the root-knot nematode *Meloidogyne incognita*. J Nematol 41:52–59

Ryss AY (2002) Express technique to prepare permanent collection slides of nematodes. Zoosyst Rossica 11:257–260

Samson RA, Evans HC, Latgé JP (1988) Atlas of entomopathogenic fungi. Springer, Berlin

Sosa AL, Passone MA, Rosso LC, Salusso F, Etcheverry M (2015) Aislamiento de nematodos y hongos con potencial actividad nematófaga en cultivos hortícolas del cinturón verde de la ciudad de Río Cuarto, Córdoba, Argentina. III Congreso Argentino de Microbiología Agrícola y Ambiental (CAMAYA), Buenos Aires

Stirling GR (2014) Biological control of plant parasitic nematodes: soil ecosystem management in sustainable agriculture, 2nd edn. CABI, Wallingford

Sturhan D, Schneider R (1980) *Hirsutella heteroderae*, a new nematode-parasitic fungus. Phytopathol Zeitschrift 99:105–115

Swe A, Li J, Zhang KQ, Pointing SB, Jeewon R, Hyde KD (2011) Nematode-trapping fungi. Curr Res Environ Appl Mycol 1:1–26

Talavera M, Itou K, Mizukubo T (2001) Reduction of nematode damage by root colonization with arbuscular mycorrhiza (*Glomus* spp.) in tomato *Meloidogyne incognita* (Tylenchida: Meloidogynidae) and carrot *Pratylenchus penetrans* (Tylenchida: Pratylenchidae) pathosystems. Appl Entomol Zool 36:387–392

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific Gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tyler J (1938) Egg output of the root-knot nematode. Proc Helminthol Soc Wash 5:49–54

Verdejo-Lucas S, Blanco M, Cortada L, Sorribas FJ (2013) Resistance of tomato rootstocks to *Meloidogyne arenaria* and *Meloidogyne javanica* under intermittent elevated soil temperatures above 28 °C. Crop Prot 46:57–62

White JH, Wise A, Main MJ, Green A et al (1998) Heterodimerization is required for the formation of a functional GABAb receptor. Nature 396:679–682

Yang J, Baoyu Tian LL, Ke QZ (2007) Extracellular enzymes and the pathogenesis of nematophagous fungi. Appl Microbiol Biotechnol 75:21–31

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203–214

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