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Short communication

Effect of the entomopathogenic nematode-bacterial symbiont complex on *Meloidogyne hapla* and *Nacobbus aberrans* in short-term greenhouse trials

Milena Caccia[a], Nicolás Marro[b], Juan Rondan Dueñas[c], Marcelo E. Doucet[a], Paola Lax[a,*]

[a] Instituto de Diversidad y Ecología Animal (CONICET-UNC) and Centro de Zoología Aplicada, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Rondeau 798, X5000AVP, Córdoba, Argentina

[b] Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, X5000AVP, Córdoba, Argentina

[c] Laboratorio de Biología Molecular, Pabellón CEPROCOR, Santa María de Punilla, X5164, Córdoba, Argentina

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ABSTRACT

Meloidogyne hapla and *Nacobbus aberrans* are plant-parasitic nematodes that form galls in the roots of infected plants and cause important economic losses. Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* infect and kill insects via toxins produced by their symbiotic bacteria. EPNs have shown to have an antagonistic effect on different plant-parasitic nematode species in field and greenhouse trials. The aim of the present work was to evaluate, in tomato plants in greenhouse, the effect of the application of three Argentine EPN isolates, their symbiotic bacteria and cell-free supernatants, on a population of *M. hapla* and two populations of *N. aberrans*. Sixty days after inoculation, the number of galls and egg masses, the nematode reproduction factor (RF) and plant biomass were calculated. With a few exceptions, biomass was not affected by the different treatments. None of the plant-parasitic nematode populations was reduced by infective juvenile inoculation of the different EPN isolates. Bacterial action differed among populations; *M. hapla* was the most susceptible one, with a significant reduction in the number of galls, egg masses and RF caused by the application of the three bacterial strains. The most significant effect was produced by the cell-free supernatants on nematode RF, with reductions of 62–90%, caused by bacterial metabolites. The different inoculation alternatives of the EPN-bacterial symbiont complex tested in the present work (infective juveniles, bacteria and cell-free supernatant) are compared for the first time for plant-parasitic nematode species.

1. Introduction

Plant-parasitic nematodes cause great damage to crops and, therefore, are a limiting factor in agriculture (Archana and Prasad, 2014), generating worldwide economic annual losses that have been estimated at $173 billion (Elling, 2013). In regions of tropical and sub-tropical climates, crop production losses caused by nematodes were estimated in 14.6% compared with 8.8% in developed countries (Nicol et al., 2011).

The root-knot nematode (*Meloidogyne* spp.) is one of the most damaging plant-parasitic nematodes in the world. This cosmopolitan genus comprises approximately 90 valid species (Jones et al., 2013); *M. arenaria*, *M. hapla*, *M. javanica* and *M. incognita* are polyphagous species that have the most severe effects on crops (Bent et al., 2008). On the other hand, the false root-knot nematode *Nacobbus aberrans* is native to the American continent and, to date, has been found in Argentina, Bolivia, Chile, Ecuador, USA, Mexico and Peru; it has quarantine importance and is characterized by a wide host range (EPPO, 2009).

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* infect and kill insects with the aid of toxins produced by their symbiotic bacteria. In *Steinernema* spp., bacteria belong to the genus *Xenorhabdus* whereas in *Heterorhabditis*, bacteria are of the genus *Photorhabdus*. The infective juvenile (IJ) enters the host and releases the bacteria in the hemocoel, where bacteria reproduce and kill the insect, generally within 48 h (Dillman et al., 2012). Bacteria produce cytolysin, hemolysin and toxins, some of which induce apoptosis or necrosis in the host cells (Nielsen Le-Roux et al., 2012).

Biological control for plant-parasitic nematodes management using antagonist microorganisms is an alternative to the application of chemical pesticides (Vagelas and Gowen, 2012). More than 30 years ago, an antagonism between plant-parasitic nematodes and EPNs was observed (Bird and Bird, 1986; Ishibashi and Kondo, 1986). EPNs have shown that effect in field and greenhouse trials on different species, such as *Criconemoides* spp., *Belonolaimus longicaudatus* (Grewal et al.,

* Corresponding author.
E-mail address: laxpaola@conicet.gov.ar (P. Lax).

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1997), *Rotylenchulus reniformis* (Lone et al., 2014), *Globodera rostochiensis* (Perry et al., 1998) and *Meloidogyne* spp. (Khan et al., 2010, 2016; Raza et al., 2015; Kepenekci et al., 2016); the best results have been obtained with species of the latter genus (Lewis and Grewal, 2005). The application of IJs of different isolates has had a suppressive effect on *Meloidogyne* spp., both in the number of eggs (Pérez and Lewis, 2004) and egg masses (Kepenekci et al., 2016), and in the infection of second-stage juveniles (J2) in roots (Molina et al., 2007). Regarding *N. aberrans*, the only work conducted to date showed a reduction in nematode reproduction on tomato (*Solanum lycopersicum*) plants inoculated with IJs of *H. bacteriophora* and *S. rarum* (Caccia et al., 2013). Furthermore, the use of symbiotic bacteria and/or their metabolites has shown to have a nematicidal action against J2 of *Meloidogyne* spp. *in vitro* (Grewal et al., 1999; Hu et al., 1999; Aatif et al., 2012) as well as a reduction in host infection in greenhouse trials (Grewal et al., 1999; Sasnarukkit et al., 2002; Vyas et al., 2008; Kepenekci et al., 2016). Even in some treatments, the level of control was comparable to the chemical treatments (Vyas et al., 2008).

Some EPN isolates may be more effective against certain plant-parasitic nematode species, and some plant species may not be as attuned to the benefits of specific EPNs (Kenney and Eleftherianos, 2016). For this reason, it is important to consider new EPN isolates and compare the effect of different inoculation options of the nematode-bacterial symbiont complex. The aim of this work was to evaluate, under controlled conditions, the effect of the application of IJs of Argentine EPN isolates, as well as their symbiotic bacteria and metabolites, on *M. hapla* and *N. aberrans* populations and plant growth in tomato plants.

2. Materials and methods

2.1. Nematodes and bacterial cultures

The origin of the nematode species and symbiotic bacteria used are indicated in Table 1. Populations of *N. aberrans* and *M. hapla* were maintained on tomato plants cultivar Platense in a greenhouse. Egg masses were extracted from infected roots and placed in Petri dishes containing distilled water; they were kept at room temperature (20 ± 2 °C) until eggs hatched, and J2 were recovered with a pipette under stereoscopic microscope for inoculation. EPN isolates were multiplied on larvae of *Galleria mellonella* (Lepidoptera: Pyralidae), following the procedure described by Koppenhöfer (2007). IJs were collected using White traps (White, 1927) and maintained in water at 25 ± 1 °C until use, for no longer than 21 days (Pérez and Lewis, 2004).

To obtain symbiotic bacteria, 100 IJs were surface sterilized in 5% NaClO for 3 min and washed with sterile water. Externally sterilized nematodes were homogenized with a stick to release the symbiotic bacteria. A drop of the homogenate was streaked on to plates with brain-heart infusion agar as growth medium. Colonies were isolated after 48 h of incubation at 28 °C. Those colonies exhibiting uniform morphology and color were subcultured by incubating them at 25 °C for 24 h. Colonies were isolated and cultured in 50 ml of brain-heart liquid medium, which was incubated for 48 h at 30 °C, with agitation at 150 rpm. Cultures were centrifuged at 20000 g for 20 min at 4 °C and the supernatant was separated. The pellet was suspended in 50 ml of sterile physiological solution (bacterial suspension). Optical density of bacterial suspension was measured, and based on calibration curves, it was diluted in physiological solution to obtain a concentration of 10^6 CFU/ml; this dose has been used in similar experiments (Samaliev et al., 2000; Vagelas et al., 2007). Each supernatant containing metabolites was diluted in an equal proportion to that of its corresponding bacterial suspension and then passed through a 0.2-µm mesh filter (Millipore) (cell-free supernatant).

Table 1
Origin of plant-parasitic nematodes, entomopathogenic nematodes and bacterial cultures involved in the present study.

Nematodes/Bacteria	Code	Locality (Department, Province)
Plant-parasitic nematode		
Meloidogyne hapla	LT	Las Tapias (San Javier, Córdoba)
Nacobbus aberrans	LUL	Lules (Lules, Tucumán)
	RC	Río Cuarto (Río Cuarto, Córdoba)
Entomopathogenic nematode/bacterial symbiont		
Heterorhabditis bacteriophora/ Photorhabdus luminescens	CBA	Córdoba (Córdoba, Córdoba)
Steinernema sp./*Xenorhabdus* sp.	LB	Villa La Bolsa (Santa María, Córdoba)
S. rarum/X. szentirmaii	RACA	Rama Caída (San Rafael, Mendoza)

2.2. Experimental design

Two experiments were conducted under controlled conditions in a greenhouse. Seeds of tomato cv Platense were germinated in plastic trays containing a mixture (3:1) of sterile soil and vermiculite (autoclaved at 1.5 atm for 30 min). Soil physicochemical properties were as follows: organic matter = 4.06%; organic carbon = 2.36%; N = 0.22%; P = 116.7 ppm; pH = 6.6. After five weeks, seedlings with four true leaves were extracted and placed in plastic pots (3.8 cm in diameter x 15 cm in height) containing 190 g sterilized soil and sand (3:1). Immediately after transplanting, for both experiments, 1.5 ml of water containing 100 J2 were inoculated on roots (Initial population = Pi); then, they were covered with the same substrate. Immediately after inoculation, depending on the experiment, IJs, bacterial suspensions, or cell-free supernatants were applied on the surface soil in each pot, as follows. In the first experiment, the effects of IJs of each EPN isolate on the different plant-parasitic nematode populations were analyzed. In each treatment (n = 7), 25 IJ/cm^2 contained in a final volume of 4 ml of water were inoculated with a pipette (Molina et al., 2007); this is the dose usually used for insect control in the field (Georgis and Hague, 1991). In the second experiment (n = 6), 4 ml of the bacterial suspension (10^6 CFU/ml) or of the cell-free supernatant, depending on the treatment, was applied. In both experiments, controls (plants inoculated only with the phytoparasitic nematode) were treated with the same amount of water or sterile culture medium (in the second experiment). The plants were grown at 25 ± 1 °C, with a 12-h photoperiod; automatic irrigation was applied daily. After 60 days, the plants were uprooted and the roots were carefully washed to remove adhered soil particles. The number of galls and egg masses was counted under stereoscopic microscope. Egg masses were removed and immersed in a 1% NaClO solution during 4 min to dissolve the gelatinous matrix (Hussey and Barker, 1973); the number of eggs was counted under light microscope. The soil of each pot was processed using the centrifugal-flotation technique (Jenkins, 1964) to obtain filiform individuals. For each replicate, the final population (Pf) of *N. aberrans* and *M. hapla* was calculated by summing the total number of eggs and the nematodes extracted from the soil; with these values, the reproduction factor was calculated (RF = Pf/Pi). After making all the observations, the roots and the aerial part of each plant were dried in a heater to estimate biomass. Both experiments had a completely randomized design and were repeated twice.

2.3. Data analysis

The variables RF and biomass of tomato plants were analyzed using Linear Mixed Models. The best model fitting heterogeneous variances was selected using the Akaike and Bayesian criteria (Zar, 1999). The number of galls and egg masses was analyzed using Generalized Linear Mixed Models, considering a Poisson distribution. In all cases, treatments and replications were defined as fixed and random effects,

Table 2
Tomato plant dry weight (g) 60 days after inoculation with *Meloidogyne hapla, Nacobbus aberrans* and infective juveniles of different entomopathogenic nematode isolates.

Treatments	*M. hapla* LT	*N. aberrans* LUL	*N. aberrans* RC
Control	1.5 ± 0.9a	2.4 ± 1.3a	1.4 ± 0.4b
Steinernema sp. LB	1.6 ± 0.7a	2.3 ± 0.9a	1.7 ± 0.7b
S. rarum RACA	1.2 ± 0.6a	2.4 ± 1.2a	2.1 ± 0.8b
Heterorhabditis bacteriophora CBA	1.4 ± 0.7a	1.8 ± 0.6a	2.7 ± 1.4a

Data are a mean of 14 replicates. Columns with different letters are significantly different according to DGC test ($p \leq 0.05$).
Abbreviations. LT: Las Tapias, LUL: Lules, RC: Río Cuarto, LB: La Bolsa, RACA: Rama Caída, CBA: Córdoba.

respectively. Means were compared using an *a posteriori* DGC test ($p \leq 0.05$). The analyses were performed using the software Infostat and its interface with the software R (Di Rienzo et al., 2013).

3. Results

The application of IJs on tomato biomass had different effects according to the plant-parasitic nematode population (Table 2). There were no differences in biomass between treatments and control for plants inoculated with *M. hapla* LT ($p = 0.34$) or with *N. aberrans* LUL ($p = 0.33$). In *N. aberrans* RC, the highest biomass was recorded in the treatment with *H. bacteriophora* CBA ($p = 0.0037$). Furthermore, IJ inoculation did not have significant effects on any of the nematode populations in number of galls (*M. hapla* LT: $p = 0.06$; *N. aberrans* LUL: $p = 0.30$; *N. aberrans* RC: $p = 0.08$), egg masses (*M. hapla* LT: $p = 0.06$; *N. aberrans* LUL: $p = 0.58$; *N. aberrans* RC: $p = 0.06$), or RF (*M. hapla* LT: $p = 0.49$; *N. aberrans* LUL: $p = 0.30$; *N. aberrans* RC: $p = 0.75$) (data not shown).

The application of bacterial suspensions and cell-free supernatants did not affect the biomass (Table 3) of plants parasitized by *M. hapla* LT ($p = 0.80$) or *N. aberrans* RC ($p = 0.40$), whereas the biomass of tomato infected with *N. aberrans* LUL was increased by the bacterial suspension of *Xenorhabdus* sp. LB ($p = 0.0008$) and reduced by the supernatant of *P. luminescens* CBA ($p = 0.0008$). Regarding their effects on phytoparasitic nematodes (Table 4), the number of galls ($p < 0.0001$) and egg masses ($p < 0.0001$) was significantly reduced in all *M. hapla* LT treatments as well as the RF ($p = 0.005$), whereas cell-free supernatants were the most efficient, especially that of *Xenorhabdus* sp. LB. In the *N. aberrans* LUL population, only bacterial suspension of *X. szentirmaii* RACA reduced the number of galls significantly ($p < 0.0001$). The application of the three supernatants reduced the number of galls and egg masses, with *P. luminescens* CBA being the most efficient ($p < 0.0001$); RF was affected only by the latter supernatant and that of *Xenorhabdus* sp. LB ($p = 0.0016$). For *N. aberrans* RC, bacterial suspension of *Xenorhabdus* sp. LB did not affect any variable with respect to control, whereas the remaining treatments with bacterial suspensions or supernatants reduced the number of galls ($p < 0.0001$) and egg masses ($p < 0.0001$), with the supernatant of *Xenorhabdus* sp. LB being the most efficient. The three supernatants and the bacterial suspension of *P. luminescens* CBA reduced RF of *N. aberrans* RC population significantly ($p < 0.0001$).

Table 3
Tomato plant dry weight (g) 60 days after inoculation with *Meloidogyne hapla, Nacobbus aberrans*, symbiotic bacterial suspensions and their cell-free supernatants.

Treatments	*M. hapla* LT	*N. aberrans* LUL	*N. aberrans* RC
Control			
Water	1.8 ± 0.7a	3.6 ± 1.0b	1.2 ± 0.3a
Culture medium	1.8 ± 0.7a	3.4 ± 0.8b	1.1 ± 0.3a
Bacterial suspension			
Xenorhabdus sp. LB	1.8 ± 0.7a	5.7 ± 1.7a	1.3 ± 0.5a
X. szentirmaii RACA	2.1 ± 0.8a	3.4 ± 1.0b	1.7 ± 0.7a
P. luminescens CBA	1.3 ± 0.3a	3.4 ± 1.0b	1.5 ± 0.7a
Cell-free supernatant			
Xenorhabdus sp. LB	1.9 ± 1.0a	3.5 ± 2.0b	1.2 ± 0.5a
X. szentirmaii RACA	1.6 ± 0.7a	3.5 ± 2.0b	1.4 ± 0.6a
P. luminescens CBA	1.7 ± 0.9a	1.4 ± 0.4c	1.7 ± 0.7a

Data are the mean of 14 replicates. Columns with different letters are significantly different according to DGC test ($p \leq 0.05$).
Abbreviations. LT: Las Tapias, LUL: Lules, RC: Río Cuarto, LB: La Bolsa, RACA: Rama Caída, CBA: Córdoba.

4. Discussion

The antagonism between plant-parasitic nematodes and EPNs is of particular interest for two reasons: first, for the search of new alternatives to supplant or complement the use of chemical products to control nematode pests; secondly, the fact that EPNs are already commercially available for the control of insect pests in various countries (Said et al., 2015). This work analyzed the effect of the application of IJs, symbiotic bacteria and cell-free supernatants of EPNs on Argentine populations of *N. aberrans* and *M. hapla,* as well as on the biomass of host plants. No previous studies have compared the different inoculation possibilities of the EPN-bacterial symbiont complex tested in the present study on plant-parasitic nematodes.

Most of the studies conducted to date have used the direct application of IJs in aqueous suspension and, in some cases, it had an antagonistic effect on different phytoparasitic nematode species (Pérez and Lewis, 2002; Molina et al., 2007; Maru et al., 2011, 2013; Aatif et al., 2012; Caccia et al., 2013). However, EPNs may not have the same effect on all nematodes (Lewis and Grewal, 2005) and, occasionally, this reduction has not proven to be effective (Fallon et al., 2002; LaMondia and Cowles, 2002; Nyczepir et al., 2004). This finding is in agreement with our present observations, since none of the *N. aberrans* or *M. hapla* populations was affected by IJ inoculation of the different EPN isolates tested. In a previous study in our laboratory, in tomato plants, we found that IJs of *H. bacteriophora* and *S. rarum* reduced the multiplication of a *N. aberrans* population by 53 and 57%, respectively; however, the number of galls and egg masses was not reduced (Caccia et al., 2013). This divergence in the results could be caused by differences between the EPN isolates tested, since they had a different origin than those used in the present work. Pérez and Lewis (2004) applied 25 JI/cm^2 of *S. feltiae* and *S. riobrave* and observed a reduction in penetration of J2 *M. incognita* and *M. hapla* in peanut (*Arachis hypogaea*) roots, while IJs of *H. bacteriophora* did not produce that effect. Those authors explained this since species of *Steinernema,* but not *H. bacteriophora*, could enter the roots releasing their bacteria. This difference was not observed in our study. Kepenekci et al. (2016) neither observed divergences between genera of EPNs, both reduced egg masses of *Meloidogyne* spp. in tomato using the same dose than in the present study. Furthermore, in the present work, EPNs did not affect tomato plant biomass, except for the interaction between *N. aberrans* RC and IJs of *H. bacteriophora* CBA, which produced a 92% increase in tomato dry weight. Previous studies involving IJs of different EPN species have shown inconsistent results in their effects on plant dry weight (Fallon et al., 2002; Shapiro-Ilan et al., 2006) as well as increases in biomass (Hussaini et al., 2009; Maru et al., 2011, 2013).

The application of bacteria and/or their supernatants have shown the highest antagonistic effect on plant-parasitic nematodes (Vyas et al., 2008; Aatif et al., 2012; Kepenekci et al., 2016). Here, the action of bacterial suspensions differed among populations; *M. hapla* LT was the most susceptible, showing a significant reduction in the number of galls (51–67%), egg masses (48–68%) and RF (55–62%) after the application of the bacterial strains. In *N. aberrans*, *X. szentirmaii* RACA reduced the number of galls by 21% (LUL) and 28% (RC), and of egg masses by 25%

Table 4
Effect of the application of symbiotic bacterial suspensions and their cell-free supernatants on populations of *Meloidogyne hapla* and *Nacobbus aberrans* in tomato plants.

Treatments	*M. hapla* LT			*N. aberrans* LUL			*N. aberrans* RC		
	Galls	Egg masses	RF	Galls	Egg masses	RF	Galls	Egg masses	RF
Control									
Water	39.8 ± 29.3a	25.7 ± 18.3a	56.7 ± 27.3a	40.1 ± 10.6a	10.2 ± 3.7a	28.4 ± 2.9a	47.5 ± 8.7a	17.1 ± 3.9a	41.7 ± 10.2 a
Culture medium	40.8 ± 28.1a	24.1 ± 15.3a	57.7 ± 21.8a	36.8 ± 12.2a	10.0 ± 3.0a	25.2 ± 5.8a	47.0 ± 10.3a	18.2 ± 3.8a	37.1 ± 16.4a
Bacterial suspension									
Xenorhabdus sp. LB	19.4 ± 10.2b	12.5 ± 6.2b	25.6 ± 14.8b	31.6 ± 7.5a	9.3 ± 5.4a	24.4 ± 13.9a	47.0 ± 10.3a	18.8 ± 7.0a	53.1 ± 21.1a
X. szentirmaii RACA	14.0 ± 7.5c	8.7 ± 1.7c	21.5 ± 7.5b	26.6 ± 13.6b	8.8 ± 6.1a	17.8 ± 14.2a	37.3 ± 10.3b	12.8 ± 4.1b	27.2 ± 16.7a
P. luminescens CBA	13.3 ± 8.0c	7.6 ± 5.0c	22.7 ± 10.2b	35.1 ± 11.4a	9.6 ± 4.5a	21.9 ± 8.9a	36.3 ± 14.5b	9.0 ± 4.0c	13.7 ± 8.8b
Cell-free supernatant									
Xenorhabdus sp. LB	2.9 ± 2.4e	2.3 ± 1.9e	5.7 ± 2.4c	26.1 ± 13.6b	5.2 ± 2.6b	5.9 ± 4.2b	12.3 ± 4.4d	2.5 ± 1.8e	9.5 ± 8.6b
X. szentirmaii RACA	8.4 ± 7.5d	5.4 ± 3.6d	10.9 ± 9.0c	26.6 ± 12.0b	4.8 ± 4.2 b	13.8 ± 10.3a	20.0 ± 8.7c	5.0 ± 3.1d	14.1 ± 9.7b
P. luminescens CBA	9.0 ± 7.5d	7.5 ± 6.6c	9.9 ± 7.1c	8.3 ± 6.5c	2.3 ± 1.8c	5.8 ± 4.1b	23.5 ± 16.4c	5.3 ± 3.1d	13.8 ± 7.9b

Data are the mean of 14 replicates (average per root). Columns with different letters are significantly different according to the DGC test ($p \leq 0.05$).
Abbreviations. LT: Las Tapias, LUL: Lules, RC: Río Cuarto, LB: La Bolsa, RACA: Rama Caída, CBA: Córdoba. RF: reproduction factor.

(only in RC), whereas *P. luminescens* CBA induced a significant reduction in galls (23%), egg masses (47%) and RF (63%) of RC population. In a greenhouse experiment, the number of *Meloidogyne* spp. females in tomato roots treated with 10^3 and 10^6 cell/ml suspensions of *Pseudomonas oryzihabitans* (associated with *Steinernema abassi*), decreased by 22% and 82%, respectively (Vagelas et al., 2007). In addition, a reduction in egg masses caused by those treatments was reported. While *Xenorhabdus* sp. LB bacterial suspension did not have a significant effect on *N. aberrans*, the application of its supernatant proved to be efficient in reducing the nematodes variables. Here, the most significant effect of the three supernatants was observed on nematode multiplication, with reductions that ranged between 62% (*N. aberrans* RC) and 90% (*M. hapla* LT). No previous studies have evaluated the effect of the application of cell-free supernatants of symbiotic bacteria on the reproduction factor of plant-parasitic nematodes. However, in studies with supernatants of EPN bacterial symbionts, a decrease in host root penetration of *Meloidogyne* spp. (Grewal et al., 1999; Sasnarukkit et al., 2002) or a reduction in the gall index (Vyas et al., 2008) were observed. Furthermore, Kepenekci et al. (2016) tried different inoculum options (IJs of *H. bacteriophora* and *Steinernema* spp., infected cadavers of insect larvae and cell-free supernatants of their symbiotic bacteria) and found than the supernatant treatment was the most effective option. Nevertheless, these authors did not specify the concentrations of bacteria by which they obtained the supernatants; that makes it difficult to compare the effectiveness of those isolates with the ones we analyzed.

In the present work, the application of bacteria and their supernatants had no significant influence on plant biomass, except in tomato infected with *N. aberrans* LUL, in which biomass decreased with the supernatant of *P. luminescens* CBA and increased with the bacterial suspension of *Xenorhabdus* sp. LB. This last result agrees with previous findings showing increases of biomass with the application of cell-free supernatants of different isolates of EPN bacteria (Vyas et al., 2008; Kepenekci et al., 2016). The latter authors attributed this effect, at least partially, to a decrease in plant-nematode infection rate. However, care must be taken when comparing plant growth parameters since there may be a fertilizer effect of the bacterial culture medium treatments (Kepenekci et al., 2016). Also, Vyas et al. (2008) drenched the roots as an application method, which could have produced more effective results on the plants. Besides, the original concentration of the medium culture from which they obtained the supernatant was nor specified in that work.

While the three supernatants had a considerable activity on *N. aberrans* and *M. hapla*, those corresponding to *Xenorhabdus* sp. LB and *P. luminescens* CBA were the most efficient, possibly due to the metabolites produced by those bacteria. Strains of *Xenorhabdus* spp. and *Photorhabdus* spp. produce several metabolites, some of which have insecticidal (Bowen et al., 1998; Yang et al., 2006; Nielsen Le-Roux et al., 2012; Castagnola and Stock, 2014), antifungal (Eleftherianos et al., 2007; Houard et al., 2013), antibiotic (Li et al., 1995; Ji et al., 2004; Morales-Soto and Forst, 2011; Song et al., 2011) and antiparasitic activity (Nollmann et al., 2012). So far, however, the only metabolites identified as having nematicidal activity are indole derivatives and hydroxystilbene produced by *P. luminescens* (Hu et al., 1999) and ammonia by *Xenorhabdus* spp. (Grewal et al., 1999). Other studies evidenced *in vitro* a nematicidal (Grewal et al., 1999; Sasnarukkit et al., 2002; Maru et al., 2011) or nematostatic effect (Aatif et al., 2012) on J2 *Meloidogyne* spp.

The fact that most nematicidal activity was recorded in cell-free supernatants might be beneficial in economic terms, since *in vitro* bacterial production is more cost-efficient and easier than that of EPN or the application of infested cadavers; in turn, it would involve less transportation, durability and formulation problems (Kepenekci et al., 2016). Efforts to identify the bacterial metabolite/s that would have nematicidal effect should be encouraged. Furthermore, additional research is still needed to determine their efficacy, especially under field conditions.

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