



RESEARCH ARTICLE

Candidate rhizobacteria as plant growth-promoters and root-knot nematode controllers in tomato plants

Karen Tatiana Chávez-Arteaga¹ ; Ángel V. Cedeño-Moreira² ;
Hayron Fabricio Canchignia-Martínez² ; Felipe Rafael Garcés-Fiallos^{3,*} 

¹ Departamento Bionintanga, NINTANGA S.A. Panamericana Norte km 10, Latacunga, Ecuador.

² Laboratorio de Microbiología del Departamento de Biotecnología, Ciencias Agrarias, Universidad Técnica Estatal de Quevedo. Campus Ing. Manuel Haz Álvarez, km 1.5 vía a Santo Domingo de los Tsáchilas, EC.120501. Quevedo, Ecuador.

³ Laboratory of Phytopathology, Experimental Campus La Teodomira, Faculty of Agronomic Engineering, Technical University of Manabí, Santa Ana EC130105, Ecuador.

* Corresponding author: felipe.garces@utm.edu.ec (F. R. Garcés-Fiallos).

Received: 7 July 2022. Accepted: 16 December 2022. Published: 31 December 2022.

Abstract

Meloidogyne incognita root-knot nematode is one of the main causes of tomato root damage and consequently crop production losses. Thus, in *in vitro* conditions, the number of nematodes hatched eggs (%) at 4 and 6 days and nematode mortality (J2 stage) at 8, 18, and 24 h, were evaluated in Petri dishes containing the candidate rhizobacteria *Enterobacter asburiae* (BA4-19 and PM3-14), *Acinetobacter calcoaceticus* (BM2-12), *Klebsiella variicola* (BO3-4) and *Serratia marcescens* (PM3-8). The well-known *Pseudomonas protegens* (CHA0) and *P. veronii* (R4) were used as controls. In greenhouse conditions, plant height, root weight, and symptoms, as well as gall and nematode numbers, were determined in tomato plants infected by *M. incognita* and treated with the seven rhizobacteria. In addition, all variables were correlated using Pearson's analysis. In general, a significant correlation was observed among the variables of both experiments, showing the antagonistic capacity of the strains against nematode. It seems, that PM3-8 and PM3-14 strains reduce hatching, and cause mortality of nematodes J2 if compared with CHA0 and R4 strains. Likewise, tomato treated with BM2-12 strain shows a higher height and root weight, as well as a smaller number of galls and nematodes in their roots. This study provides evidence that PM3-8 and PM3-14 strains reduce the *M. incognita* egg hatching, and that the BM2-12 strain can be a plant growth-promoter potential of tomato plants.

Keywords: *Lycopersicon esculentum* L.; *Meloidogyne incognita*; Ecuadorian rhizobacteria; antagonistic activity; biological control.

DOI: <https://dx.doi.org/10.17268/sci.agropecu.2022.038>

Cite this article:

Chávez-Arteaga, K. T., Cedeño-Moreira, A. V., Canchignia-Martínez, H. F., & Garcés-Fiallos, F. R. (2022). Candidate rhizobacteria as plant growth-promoters and root-knot nematode controllers in tomato plants. *Scientia Agropecuaria*, 13(4), 423-432.

1. Introduction

Tomato (*Solanum esculentum* L.) is one of the most important plant species of agricultural importance worldwide. Biotic factors such as diseases, especially root-knot nematodes (*Meloidogyne* spp.) cause severe damage and losses in different crops, mainly due to wide geographical distribution and their potential to infect a wide variety of plant species (Calderón et al., 2022). In Ecuador, the genus *Meloidogyne* is an important pathogen that can affect Solanaceae (*S. lycopersicum*, *S. betaceum*, *S. quitoense* and *Capsicum annuum* L.), as well as Musaceae (*Musa* sp.), rice (*Oryza sativa* L.), common bean (*Phaseolus vulgaris* L.), and lettuce

(*Lactuca sativa* L.) (Bridge, 1976; Erazo-Sandoval et al., 2020; Calderón et al., 2022).

Among the most important and most studied *Meloidogyne* species are *M. arenaria*, *M. javanica*, and *M. incognita* (dos Santos et al., 2012; Calderón et al., 2022; Wen et al., 2022). This latter species causes mainly root damage (galls), and other aerial parts symptoms *i.e.*, stunting, wilting, or chlorosis (yellowing) (Sikora et al., 2008; Calderón et al., 2022). As usual, infected plants root system is stunted leading to poorly growing crops (Muimba-Kankolongo, 2018). Symptoms are particularly stronger and severe when plants are infected soon after planting, being able to reduce crop productivity and cause between 68% and 100% fruit

production damage due to product's low quality (Hussey & Barker, 1973). *M. incognita* is a sedentary endoparasitic nematode that occurs in more than 2000 plant species (Jung and Wyss, 1999), which makes it difficult to control. A total of five *Meloidogyne* species have been identified in Ecuador, highlighting *M. incognita* as the most frequent, followed by *M. arenaria*, *M. graminicola*, *M. hapla* and, *M. javanica* (Calderón et al., 2022).

Root-knot nematode management is generally done by using nematicides. However, the prohibited use of methyl bromide, 1,3-dichloropropene and cadusafos, the main used pesticides, let the currently management of this pathogen with ineffective options (Sikora et al., 2008). Thus, the use of rhizobacteria is shown as an efficient strategy for phytopathogenic nematodes management (Hallmann et al., 1998; 1999; Sikora et al., 2008; Chávez-Arteaga et al., 2018). Agricultural soils have a high microbial diversity associated with different plant species root systems, highlighting the presence of Plant Growth Promoting Rhizobacteria (PGPR) (Cedeño-Moreira et al., 2020). These microorganisms can help in nitrogen fixation, increase the availability of nutrients in the rhizosphere, production of antibiotics, phytohormones, and siderophores (Siddiqui, 2005), as well as production of biofilms in different plant species (Haque et al., 2020). Nevertheless, secondary metabolites produced by PGPR in the plant rhizosphere also may cause direct negative effects on nematodes, for example, nematode eggs membrane rupture, and drastically reducing pathogen populations in underground tissue (El-Sherif et al., 1999; Siddiqui et al., 2005; Montes et al., 2016).

The diversity and number of existing PGPR are varied, as well as their use in different pathosystems. In recent years, rhizobacteria like *Pseudomonas protegens* (CHA0 strain) and *Pseudomonas veronii* (R4 strain) have shown a negative effect against nematodes (Canchignia-Martínez et al., 2016;

Montes et al., 2016). Both microorganisms produce the auxin indole-3-acetic acid (IAA), which can help develop the grape and soybean root system (Peñafiel-Jaramillo et al., 2016; Cedeño-Saavedra et al., 2017). Recently, seven rhizobacteria obtained from banana landraces rhizosphere in Los Ríos, Cotopaxi, and Bolívar provinces, Ecuador, and used in the present work, could be considered PGRP-like candidates due to their antagonistic activity (Chávez-Arteaga et al., 2018). Two of these strains, *Acinetobacter calcoaceticus* (BM2-12 strain) and *Serratia marcescens* (PM3-8 strain), showed negative effect *in vitro* and *in vivo* against *Phytophthora palmivora*, the causal agent of the black pod, as well as capacity plant growth-promoter on cocoa seedlings (Cedeño-Moreira et al., 2020).

Studies on the use of PGPR in different crops of agricultural importance are very scarce. Thus, the present work aimed to evaluate the *in vitro* and *in vivo* antagonist activity of rhizobacteria against *M. incognita* and quantify the plant-growth promotion capacity in tomato plants infected by the nematode.

2. Materials and methods

Biological material

Tomato plants of cv. Floradade were used in all experiments. *Meloidogyne incognita* root-knot nematode (local strain Uteq-Mi1) used here, was identified using morphological (Taylor & Sasser, 1983) and molecular techniques (polymerase chain reaction – PCR according to Chávez-Arteaga et al., 2018). For selective amplification of target nucleotide sequences were chosen two primer pairs: Finc (5'CTCTGCCCAATGAGCTGTCC3') and Rinc (5'CTCTGCCCTCACATTAAG3') (Hesar et al., 2022), and Inc-K14F (5'CCCCTACACCCTCAACTTC3') and Inc-K14R (5'GGGATGTGTAATGCTCCTG3') (Wen et al., 2022). See Figure A1 (Appendix) for more details. The plant growth-promoting rhizobacteria (PGPR) shown in Table 1 were employed.

Table 1

Plant growth-promoting rhizobacteria used in the present research, and that produce (+) or not the secondary metabolites Protease (PR), hydrogen cyanide (HCN), pyrrolnitrine (Prn), diacetylfloroglucinol (2,4-DAPG), siderophores (Sd) and indole-3-acetic acid (IAA)

Strain identification	Rhizobacteria genera*	Antagonistic metabolites				Sd	IAA
		PR	HCN	Prn	2,4-DAPG		
Candidates**							
BA4-19	<i>Enterobacter asburiae</i>	+				+	
BM2-12	<i>Acinetobacter calcoaceticus</i>			+			+
BO3-4	<i>Klebsiella variicola</i>	+	+	+			+
PM3-8	<i>Serratia marcescens</i>	+	+				+
PM3-14	<i>E. asburiae</i>	+	+	+			+
Controls***							
CHA0	<i>Pseudomonas protegens</i>	+	+	+	+	+	+
R4	<i>P. veronii</i>	+		+		+	+

* Identity of 16S rRNA gene to information from GenBank NCBI.

** Chávez-Arteaga et al. (2018).

*** Cancchignia-Martínez et al. (2016); Montes et al. (2016); Peñafiel-Jaramillo et al. (2016); Cedeño-Saavedra et al. (2017).

***In vitro* experiments**

Assessment of nematode egg hatching

Egg masses of *M. incognita* were obtained of tomato roots with root-knot galls symptoms. These tissues were washed with sterile distilled water. Egg mass was collected with a sterilized tooth stick (65x2 mm) and placed on a sterile glass slide containing sterile distilled water and 50 µL of chloramphenicol. PGPR (100 µL at a concentration 1×10^8 cfu mL⁻¹) was placed on Petri dishes containing King B liquid medium. Subsequently, egg mass (± 100 eggs) was placed on these plates. The microorganisms were incubated at 27 ± 2 °C and 12-hour photoperiod for 6 days. On this day, the number of empty eggs in an area of 0.5 cm², an average of 50 eggs was evaluated using an optical stereo microscope at 5x magnification (model BX40, Olympus, Japan) coupled to a photographic camera with ISCapture Professional Imaging software.

Determination of nematodes mortality (J2 stage)

Like the methodology used in the previous variable, *M. incognita* egg masses were obtained from tomato roots with root-knot galls symptoms. These masses were placed into PCR Eppendorf tubes containing 50 µL of sterile water. Four days later, 5 µL of this suspension containing an average of 20 juvenile nematodes in J2 stage, were placed on Petri dishes containing King's B agar media and PGPR. At 8, 16 and 24 h after, nematodes were recovered and re-suspended in sterile water to verify their mobility or death.

Experiments in greenhouse conditions

Plant growth conditions

Seeds were sown in 12-cell expanded polystyrene (EPS) foam (Styrofoam) trays containing 46 cm³ of a sterilized mixture (121 °C for 20 min) of peat and clay loam soil (1:1, Novarbo seedling, black C1B). Cells were irrigated according to the crop needs. Seedlings were kept under greenhouse conditions (25 ± 4 °C and 12 h of light).

Inoculation of plant growth-promoting rhizobacteria

PGPR were grown on King's B agar media in dishes and maintained at 25 °C (± 1 °C) for 48 h. The bacterial culture (100 mL of a concentration 1×10^8 cfu mL⁻¹) was centrifuged (3000 g x 15 min), washed twice with sterile phosphate-buffered saline (pH 6.5), and finally re-suspended in the same solution for its inoculation.

Seedlings (19 days after sowing) were carefully taken out of the substrate, their roots thoroughly washed with tap water, surface disinfected in 0.1%

(v/v) NaOCl solution for 1 min and rinsed twice with sterile deionized water. Then, plants were inoculated by rhizobacteria suspension root dipping for 10 min. Plants whose roots were dipped in distilled water served as control. After inoculation, seedlings were immediately planted in 180 cm³ pots containing an infested mixture nematode of organic compost and vermiculite (1:1) and kept under the same conditions described before. The substrate was kept moist and irrigated twice every week with 1.7 mL nutrient solution per g of substrate. Subsequently, two applications were made at 4-day intervals with each of the strains (10 mL of a concentration 1×10^8 cfu mL⁻¹), placing the suspension at 5 cm of crown-root.

Nematode inoculum

M. incognita eggs and juveniles were obtained from tomato roots of cv. Floradade. These structures were extracted with NaOCl through the centrifugal flotation method (Hussey & Barker, 1973), using a shaking incubator (Incu-Shaker™ Shaking Incubators, Benchmark Scientific, U.S.A). Plants of this same cultivar were inoculated with 4000 nematodes (eggs and juveniles), grown for two months on the same sterilized substrate, and kept under the same conditions described before.

Seedlings at the first true leaf stage (15 days after inoculation) were planted in 180 cm³ pots containing sterilized substrate. One week after transplantation, plants were each inoculated with a suspension calibrated for 1000 eggs and J2 per mL of *M. incognita*, in holes in the soil around the stem of each plant. The plants were kept in a greenhouse at 25 ± 4 °C, 12 h of light and watered as needed.

Quantification of plant height and root weight

On the 30th day after transplanting, plant height was measured (distance between the ground surfaces to the point of growth of each plant) using a graduated ruler. After this, the tomato root system was carefully removed and washed with tap water, dried on a paper towel, and weighed (g) on a precision balance (Kern 440, China).

Assessment of root-knot symptoms in aerial and underground tissues

At 30 days, the effect of rhizobacteria in infected tomato plants with root-knot nematode *M. incognita* was assessed. Symptoms *i.e.*, root galls, dwarfism and leaf chlorosis were assessed qualitatively. Then, plants were collected, and their roots carefully removed. In these tissues, the gall numbers induced by nematodes were quantified. Immediately, root galls were liquefied and passed through sieve with a mesh size of 500, 90, and 25 µm. Eggs

and J2 nematodes recovered in the 25 µm sieves were observed, counted, and expressed per fresh weight (g) of tissues, using an optical stereo microscope at 10x magnification (Olympus, Japan).

Experimental design and statistical analysis

The experiments were carried out in a completely randomized design with eight treatments and three replications (each one containing two plants). After verification of homogeneity of the variances (Bartlett’s test) of the data sets, these were subjected to analysis of variance. Student’s t-test ($p \leq 0.05$) was used to compare treatments among the times evaluated of the variable Plant height, while Scott Knott’s test ($p \leq 0.05$) was used for the grouping of means of each variable. Also, Pearson’s analysis was performed to investigate the correlation among all variables evaluated. Statistical analyzes were performed using the software Prism Windows 5.04.

3. Results and discussion

Although root-knot nematode (*M. incognita*) is a major disease of tomato crops in Ecuador, little attention has been given to its identification, and even worse to its management using environmentally friendly technology. In our study, we compared the response of tomato plants

infected by *M. incognita* strain Uteq-Mi1, to the application or not with strains of seven rhizobacteria, some of these strains being Ecuadorian, evaluated both in *in vitro* and greenhouse conditions.

In vitro experiments

Nematode egg hatching

A higher number of nematode hatched eggs ($p < 0.01$) was observed in control dishes, at 4 (91 eggs) and 6 (98 eggs) days when compared to those treated with PGPR (4 days: 10 eggs; 6 days: 12 eggs, in average). Application of CHA0 (0.7 eggs in average), PM3-8 (2.8 eggs in average), R4 (3.0 eggs in average), and PM3-14 (6.8 eggs in average) strains significantly reduced the nematode egg hatching, in both times analyzed (Figure 1).

Nematode mortality

The mortality of J2 nematodes was significantly increased when applied all rhizobacteria at three times analyzed. Nevertheless, the dishes containing CHA0 strain showed a nematode mortality of 74% in average, when compared to their control (6% in average) and the other treatments (38% in average), analyzed over time (Figure 2). Additionally, regardless of the application or not of each strain, the nematode mortality increased over time.

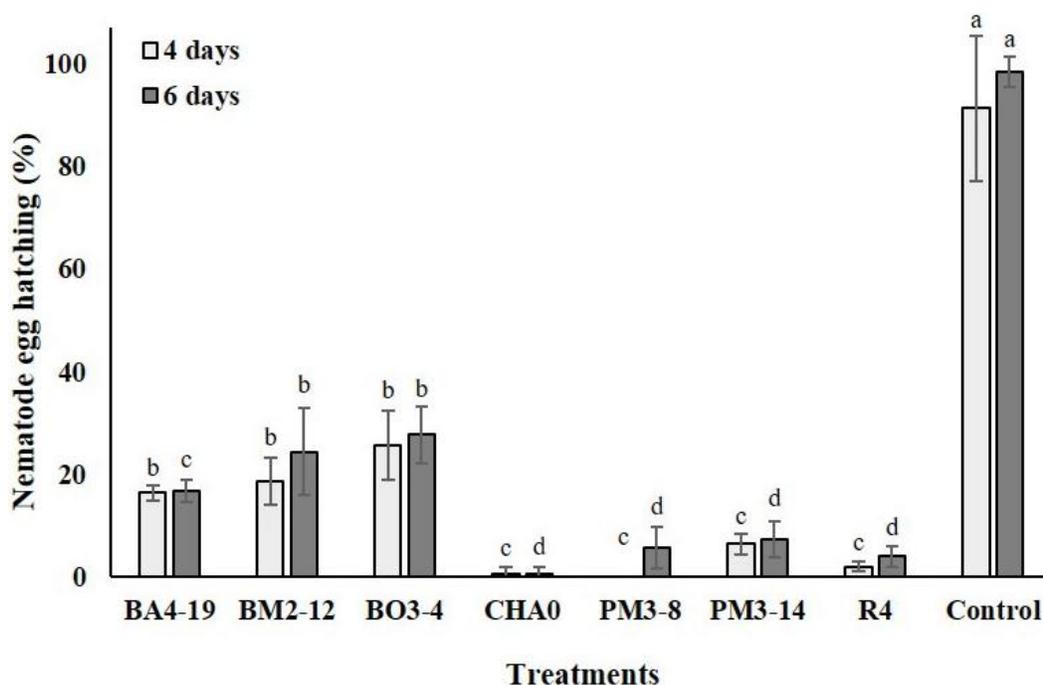


Figure 1. Number of nematode hatched egg (%) *Meloidogyne incognita* in Petri dishes containing *Enterobacter asburiae* (BA4-19), *Acinetobacter calcoaceticus* (BM2-12), *Klebsiella variicola* (BO3-4), *Pseudomonas protegens* (CHA0), *Serratia marcescens* (PM3-8), *E. asburiae* (PM3-14) and *P. veronii* (R4), or without rhizobacteria (only sterile distilled water), at 4 and 6 days.

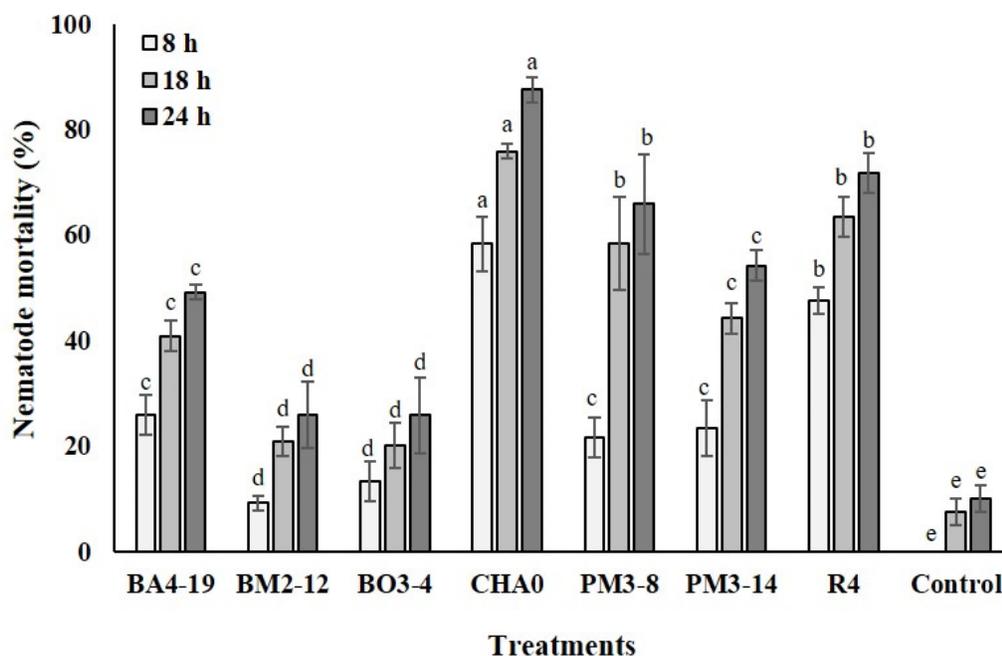


Figure 2. Mortality of *Meloidogyne incognita* nematodes J2 stage (%) in Petri dishes containing *Enterobacter asburiae* (BA4-19), *Acinetobacter calcoaceticus* (BM2-12), *Klebsiella variicola* (BO3-4), *Pseudomonas protegens* (CHA0), *Serratia marcescens* (PM3-8), *E. asburiae* (PM3-14) and *P. veronii* (R4), or without rhizobacteria (only sterile distilled water), at 8, 18 and 24 h.

All rhizobacteria significantly reduced and increased the nematode egg hatching and its mortality (J2 stage), respectively, showing even a relation between both variables in all analyzed times in *in vitro* conditions. Nevertheless, CHA0, R4, PM3-8, and PM3-14 strains were shown to be more effective in inhibiting the nematode egg hatching, while the first cited strain had the ability to kill a greater number of nematodes, in comparing to control and the other rhizobacteria, analyzed at over time. To our best knowledge, the effectiveness of CHA0 and R4 strains in killing nematodes has already been verified, but mostly in other crops. Indeed, CHA0 strain can inhibit egg hatching by 60%, as well as it can kill between 55 and 63% of juvenile J2 nematodes of *M. javanica* (Hamid et al., 2003; Siddiqui and Shaukat, 2003). On the other hand, R4 strain can affect the viability of *Xiphinema index* (Canchignia-Martínez et al., 2016; Montes et al., 2016). It is known that *M. incognita* is affected by strains of *Serratia marcescens* and *Pseudomonas fluorescens* in cotton (Hallmann et al., 1998; 1999). Therefore, this is the first report of CHA0, R4, PM3-8, and PM3-14 strains showing antagonistic activity against *M. incognita* isolated from tomato roots in *in vitro* conditions.

We attribute the antagonist effect against *M. incognita* shown by the strains, to the production of volatile compounds. It is known that rhizobacteria produce diacetylfloroglucinol (2,4-DAPG), hydro-

gen cyanide (HCN), pyrrolnitrine (PrnR), iron-chelating siderophores (ICS), antibiotics, protease (PR), and indole-3-acetic acid (IAA) (Hamid et al., 2003; Siddiqui & Shaukat 2003; Cancignia-Martínez et al., 2016; Montes et al., 2016). These substances have been implicated in the reduction of deleterious effects and pathogenic rhizosphere microorganisms, creating an environment more favorable for the plants root growth (Siddiqui, 2005). For example, HCN inhibits the cytochrome oxidase of nematode cells, inhibiting its mitochondrial respiration causing paralysis and rapid death (Cunningham et al., 1997). This substance and 2,4-DAPG and PR can even function as nematicides, by degrading the cell wall of both eggs and J2 stage nematodes, finally causing lysis (Siddiqui and Shaukat, 2003; Timper et al., 2009). Therefore, it is plausible to assume that volatile bioactive compounds produced by the rhizobacteria under study, can affect the nematode egg hatching and its mortality (J2 stage).

Our results showed that the nematode mortality increased over time, regardless of the application or not of each strain. If we observe the control treatment, we realize that the mortality also increased. In this way, we can infer that in addition to the negative effect induced by volatile bioactive compounds on the nematodes (Siddiqui & Shaukat 2003; Siddiqui, 2005), these tend to die over time.

Experiments in greenhouse conditions

Plant height and root weight

Both plant height (20 days: $p < 0.05$; 30 days: $p < 0.01$) and root weight ($p < 0.01$) varied among treatments (Figure 3). Tomato plants treated with BM2-12 and R4 strains obtained a higher height, at 20 (32 cm in average) and 30 (55 cm in average) days, in comparison to other treatments (Figure 3A). Additionally, an increase in height was observed in tomato plants' time course, but not in plants treated with the BA4-19 strains and the untreated ones. Interestingly, only R4 strain generated a negative effect against nematodes, while the BM2-12 strain was less effective in the *in vitro* experiments. It is known that some rhizobacteria isolates like *P. fluorescens* produce phytohormones, generating greater plant growth (Siddiqui & Shaukat, 2003; Hashem & Abo-Elyousr,

2011). Perhaps the rhizosphere of tomato plants is being stimulated by some volatile compound present in both bacteria, resulting in greater height. Tomato plants treated with CHA0, PM3-14 and R4 strains showed at lower root weight (values less than 3 g), compared to their control and the other treatments (4.2 g in average, Figure 3B). Although this result is contrary to expectations, Pearson's analysis shows a significant correlation among root weight with gall and nematode numbers (Table 2). Therefore, the number of galls and J2 stage nematodes influenced the weight of underground tissues. On the other hand, the null correlation between plant height and the other variables (Table 2) shows a differentiated response by tomato plants to each of the rhizobacteria, and different metabolic pathways can be activated. However, more studies are needed to understand these processes.

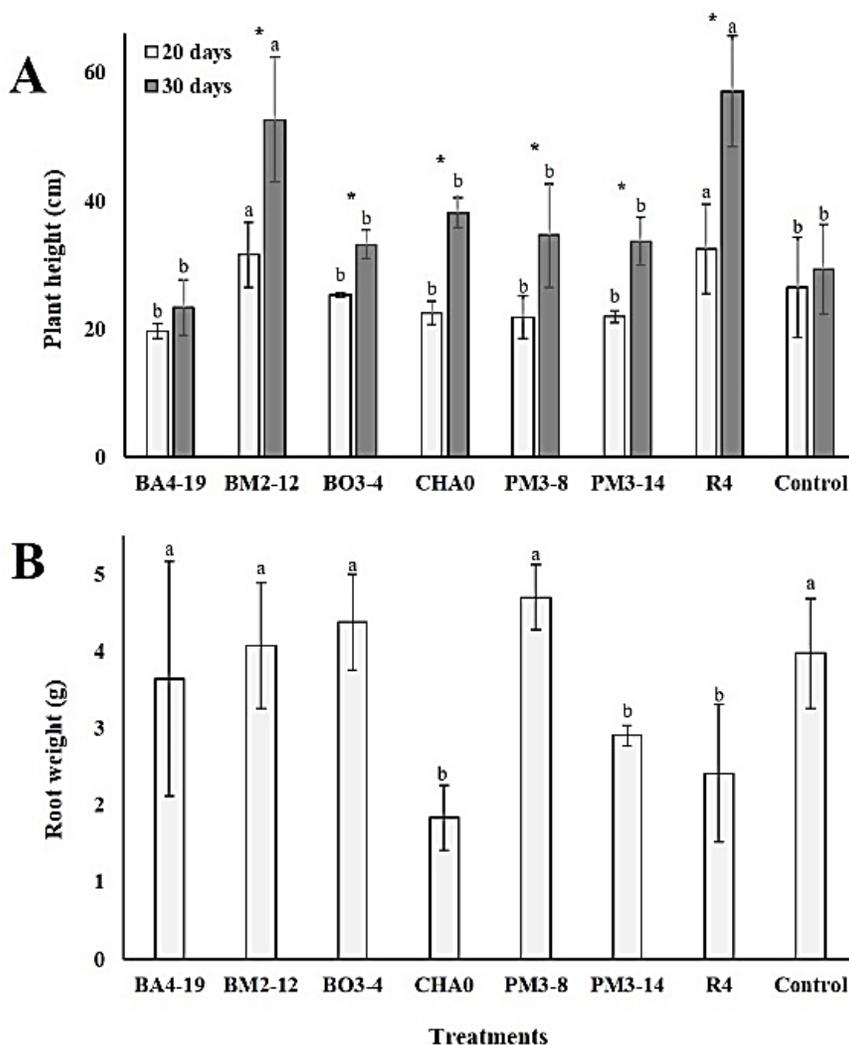


Figure 3. Plant height (cm, A) and root weight (g, B) in tomato plants infected by *Meloidogyne incognita* and treated or not with *Enterobacter asburiae* (BA4-19), *Acinetobacter calcoaceticus* (BM2-12), *Klebsiella variicola* (BO3-4), *Pseudomonas protegens* (CHA0), *Serratia marcescens* (PM3-8), *E. asburiae* (PM3-14) and *P. veronii* (R4). Plants whose roots were dipped in distilled water served as control.

Assessment of root-knot symptoms in aerial and underground tissues

Independent of the application or not of rhizobacteria, all tomato plants showed symptoms caused by root-knot nematode *M. incognita*, at 30 days after inoculation (Figure 4). However, these symptoms (root galls, dwarfism, and leaf chlorosis) were stronger and intense in infected plants without rhizobacteria (Figure 4 C and F), in comparison with their respective plants treated with Bm2-12 (Figure 4 A and D), CHA0 (Figure 4 B and E) and the rest of strains. All symptoms have been observed in tomato plants and other plant species in Ecuador (Bridge 1976; Erazo-Sandoval et al., 2020; Calderón et al., 2022), leading to poorly growing and low fruit production in crops (Muimba-Kankolongo, 2018).

Roots of untreated plants with any of the strains showed a higher number of galls (Figure 5A) and nematodes (J2 stage) (Figure 5B), when compared to those treated ($p < 0.01$). Practically, the behavior was similar in both variables. A lower number of galls (Figure 5A) were observed in plants treated with BM2-12 (318 galls), CHA0 (59 galls) and R4 (300 galls) strains, compared to those control (3099 galls) or the rest of the treatments (1341 galls on average). On the other hand, the application of BM2-

12 (233 nematodes g^{-1} fresh weight) and CHA0 (173 nematodes g^{-1} fresh weight) strains (Figure 5B), significantly reduced the number of nematodes in the roots of tomato plants, compared to those control (1467 nematodes) or with the rest of treatments (771 nematodes g^{-1} fresh weight on average).

Lower galls and nematode numbers were observed in roots of tomato plants treated with BM2-12 and CHA0 strains. However, the R4 strain also induced a reduction of gall numbers in underground tissues, compared to those in control or with the rest of the strains. BM2-12, CHA0, and R4 strains produce the enzymes protease and catalase, as well as HCN and PLT (Siddiqui & Shaukat 2003; Canchignia-Martínez et al., 2016; Montes et al., 2016). It is also known that some strains of *P. fluorescens* can reduce galling by *M. incognita* and their population in roots (Siddiqui et al., 2005; Hashem & Abo-Elyousr, 2011). Although many strains used in the present experiments showed a negative effect against the nematode *M. incognita*, the metabolite compounds role in tomato plants remains poorly understood (El-Sherif et al., 1999). Hence, elucidating the effect of these substances on the pathogen, as well as in tomato plants will be an exciting challenge for future research.

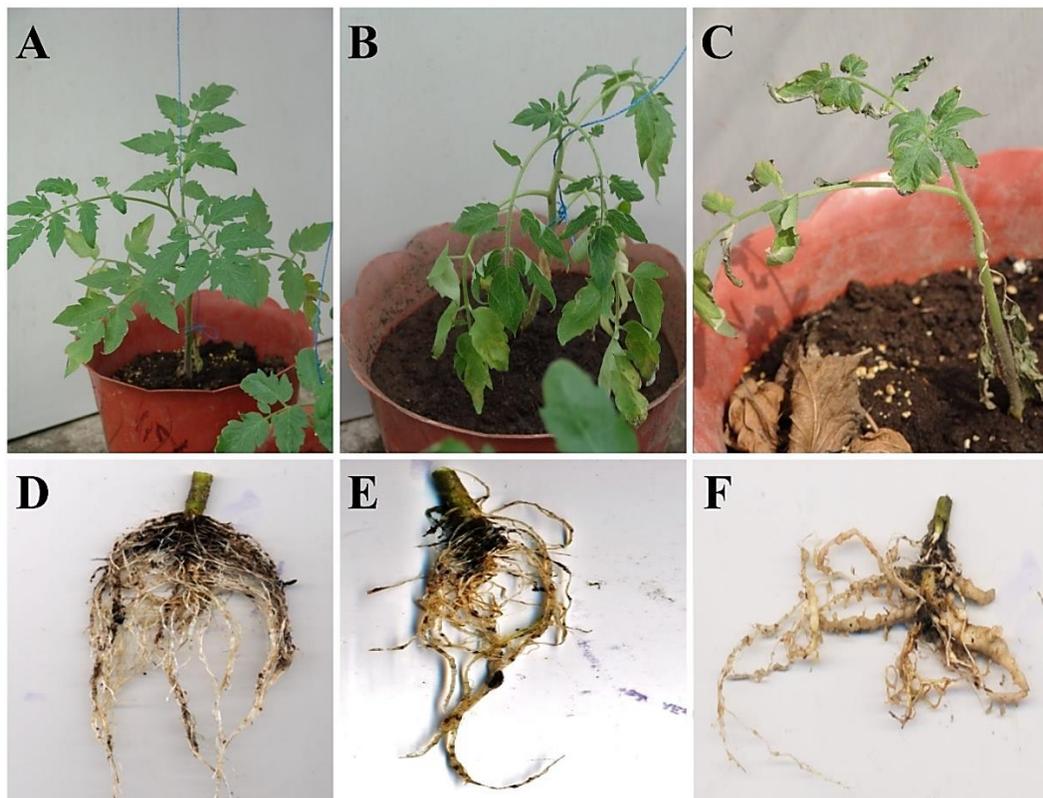


Figure 4. Symptoms in tomato plants infected by root-knot nematode *Meloidogyne incognita* and treated or not with rhizobacteria strains, at 30 days. A and D, slight leaf wilting and low growth of galls in root of plants infected and treated with *Acinetobacter calcoaceticus* strain Bm2-12. B and E, slight leaf wilting, epinasty, chlorosis and root galls in plants infected and treated with *Pseudomonas protegens* strain CHA0. C and F, dwarfism, chlorosis and root galls stronger and intense in plants only infected with nematodes.

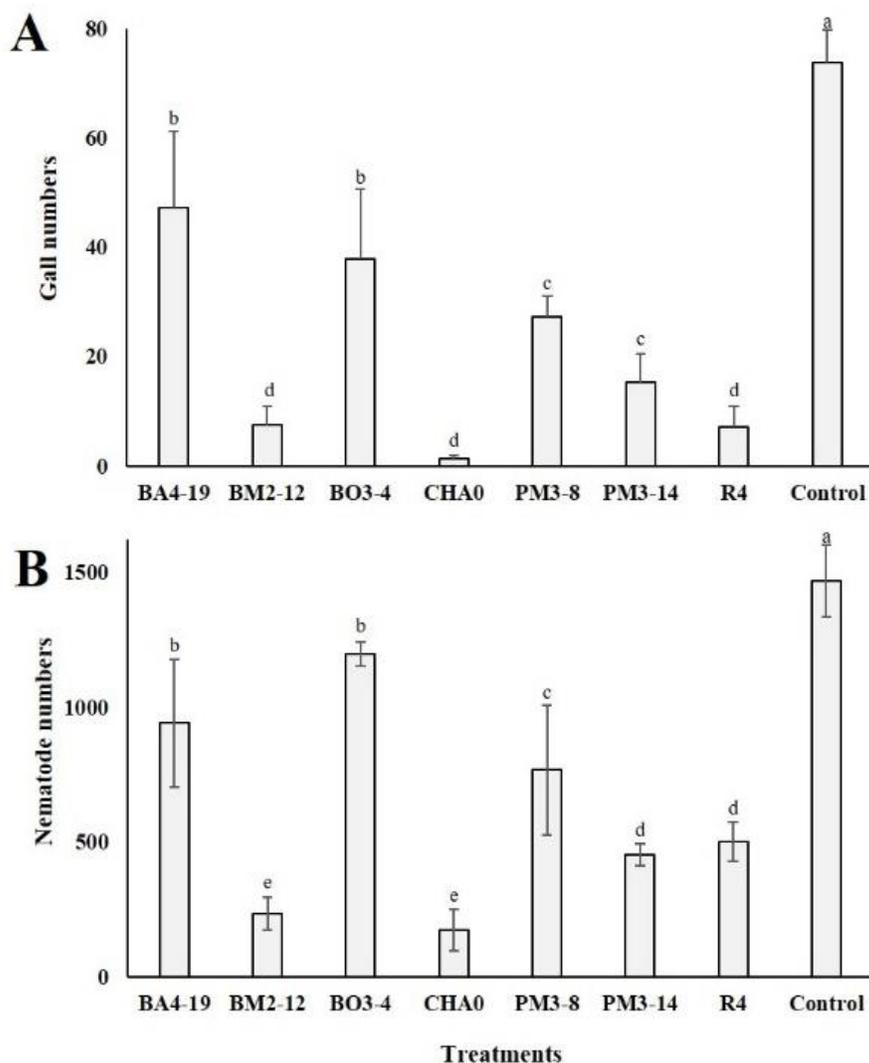


Figure 5. Gall and nematode numbers in roots of tomato plants infected by *Meloidogyne incognita* tomato plants treated or not with *Enterobacter asburiae* (BA4-19), *Acinetobacter calcoaceticus* (BM2-12), *Klebsiella variicola* (BO3-4), *Pseudomonas protegens* (CHA0), *Serratia marcescens* (PM3-8), *E. asburiae* (PM3-14) and *P. veronii* (R4). Plants whose roots were dipped in distilled water served as control.

Pearson's correlations among variables

Results of Pearson's correlation analysis among all variables can be observed in **Table 2**. Nematode egg hatching at 4 and 6 days was correlated with nematode mortality at 8, 18 and 24 days (*p*-values among 0.0005 and < 0.0001). The variables plant height at 20 and 30 days, only correlated with each other (< 0.0001). The nematode numbers at 8, 18 and 24 h were correlated with each other (< 0.0001), but also with root weight (*p*-values among 0.0003 and 0.0077), as well as with gall (*p*-values among 0.0006 and 0.0007) and nematode (*p*-values among 0.0008 and 0.0380) numbers. The gall numbers in root tissues were correlated with nematode egg hatching at 4 and 6 days (< 0.0001), as well as with plant height at 30 days (0.0030) and nematode J2 numbers (0.0399). Finally, root weight was correlated with gall (0.0081) and nematode

stage J2 numbers (< 0.0001). These responses observed in *M. incognita* caused by rhizobacteria in both conditions show the importance of Ecuadorian strains with antagonistic capacity against this little-studied pathogen. However, more studies are needed to understand the pathogen-rhizobacteria-plant interaction and the effect of strains on tomato fruit production.

In this work, we report for the first time the ability of *S. marcescens* (PM3-8 strain) and *E. asburiae* (PM3-14 strain) to inhibit egg hatching, and to kill a greater number of nematodes (J2 stage), comparing herself with the strains CHA0 and P4. Also, we verified that the roots of tomato plants treated with *A. calcoaceticus* (BM2-12 strain) showed a higher height, as well as a fewer number of galls and nematodes in underground tissues.

Table 2

Pearson's correlation among nematode egg hatching at 4 (NEH4) and 6 (NEH6) days, nematode mortality at 8 (NM8), 18 (NM18) and 24 (NM24) hours, plant height at 20 (PH20) and 30 (PH30) days, root weight (RW), and gall (GN) and nematode numbers (NM), variables evaluated in laboratory or greenhouse conditions. The values shown for each Pearson's correlation are the coefficient (r) and the value resulting from regression analysis (p -values between 0.0001 and 0.05). Cells with significant correlations were highlighted with gray-colored

	NEH4	NEH6	NM8	NM18	NM24	PH20	PH30	RW	GN	NM
NEH4	-	0,9771	0,4284	0,5696	0,5850	0,0040	0,1034	0,0631	0,6424	0,0273
	-	<0,0001	0,0005	<0,0001	<0,0001	0,7698	0,1254	0,2365	<0,0001	0,4398
NEH6		-	0,4640	0,5851	0,6088	0,0162	0,0802	0,0940	0,6256	0,0509
		-	0,0002	<0,0001	<0,0001	0,5529	0,1801	0,1452	<0,0001	0,2893
NM8			-	0,8084	0,8056	0,0058	0,0649	0,4630	0,4042	0,4061
			-	<0,0001	<0,0001	0,7241	0,2298	0,0003	0,0008	0,0008
NM18				-	0,9859	0,0175	0,0556	0,2891	0,4188	0,1813
				-	<0,0001	0,5380	0,2675	0,0067	0,0006	0,0380
NM24					-	0,0185	0,0583	0,2814	0,4110	0,1843
					-	0,5264	0,2555	0,0077	0,0007	0,0363
PH20						-	0,6794	0,0201	0,0300	0,0003
						-	<0,0001	0,5092	0,4185	0,9310
PH30							-	0,0101	0,3362	0,0264
							-	0,6410	0,0030	0,4478
RW								-	0,2782	0,8666
								-	0,0081	<0,0001
GN									-	0,1781
									-	0,0399
NM										-
										-

4. Conclusions

In laboratory conditions, all rhizobacteria negatively affected the nematodes, standing out *P. protegens* (CHA0), *S. marcescens* (PM3-8), *E. asburiae* (PM3-14), and *P. veronii* (R4) by reducing the nematode egg hatching, as well as CHA0 strain for being the most effective in killing nematodes of *M. incognita*. In greenhouse conditions, tomato plants treated with *A. calcoaceticus* (BM2-12) and R4 strains showed a higher height, and the plants treated with CHA0, PM3-14 and R4 strains showed a lower root weight. Independent of the application or not of rhizobacteria, all tomato plants showed symptoms caused by root-knot nematode, but these were stronger and more intense in infected plants without rhizobacteria. A lower number of galls was observed in plants treated with BM2-12, CHA0 and R4 strains. Also, the number of nematodes in roots was reduced with the application of BM2-12 and CHA0 strains. To the best of our knowledge, this is the first report on the capacity of *S. marcescens* and *E. asburiae* to plant growth-promoter and root-knot nematode controller, respectively, in tomato plants. Although many variables, both laboratory and greenhouse conditions, were significantly correlated in this research, some exceptions were observed, for example, the null correlation between plant height and the other variables. Even though further studies are needed to understand the pathogen-rhizobacteria-plant interaction and the effect of strains on tomato fruit production, our study shows practical results about the biological control of *M. incognita* in tomato crops.

Conflict of interest

The authors express no conflict of interest associated with this work.

Acknowledgments

This work was supported by the Universidad Técnica Estatal de Quevedo (UTEQ-FOCICYT-2020-N7). The authors are grateful to Dr. Maddela Naga Raju for to revised the manuscript. We also thank anonymous reviewers and academic and section editor for improving the manuscript.

ORCID

K. T. Chávez-Arteaga  <https://orcid.org/0000-0002-7615-2145>
 Á. V. Cedeño-Moreira  <https://orcid.org/0000-0002-6564-5569>
 H. F. Canchignia-Martínez  <https://orcid.org/0000-0003-1195-5446>
 F. R. Garcés-Fiallos  <https://orcid.org/0000-0002-1795-4439>

References

- Bridge, J. (1976). Other contributions: plant parasitic nematodes from the lowlands and highlands of Ecuador. *Nematropica*, 6, 18-23.
- Calderón, J., Miño-Castro, G., Llumiquinga, P., Abbas, M., Gutiérrez-Gutiérrez, C., Segovia-Salcedo, M., & Proaño, K. (2022). Distribution of *Meloidogyne* spp. in agricultural crops of Ecuador: a literature review (1976–2021). *CABI Reviews*, 17, 028.
- Canchignia-Martínez, H. F., Altimira, F., Montes, C., Sánchez, E., Tapia, E., et al. (2017). Candidate nematocidal proteins in a new *Pseudomonas veronii* isolate identified by its antagonistic properties against *Xiphinema index*. *The Journal of General and Applied Microbiology*, 63, 11-21.
- Cedeño-Moreira, A. V., Romero-Meza, R. F., Auhing-Arcos, J. A., Mendoza-León, A. F., Pacheco, F. A., & Canchignia-Martínez, H. F. (2020). Caracterización de *Phytophthora* spp. y aplicación de rizobacterias con potencial en biocontrol de la enfermedad de la mazorca negra en *Theobroma cacao* variedad CCN-51. *Scientia Agropecuaria*, 11, 503-512.
- Cedeño-Saavedra, D. A., Canchignia-Martínez, H. F., Cruz-Rosero, N., Guerra-Cuenca, F. F., Gaibor-Fernández, R., & Cedeño-Moreira, A. V. (2017). Rizobacterias que promueven el

desarrollo e incremento en productividad de *Glycine max* L. *Ciencia y Tecnología*, 10, 7-15.

Chávez-Arteaga, K., Guato-Molina, J., Peñafiel-Jaramillo, M., Mestanza-Uquillas, C., & Canchignia-Martínez, H. F. (2018). Bacterias fluorescentes productoras de metabolitos antagónicos de cultivos nativos de *Musa* sp. y su diversidad filogenética al gen ARNr 16S. *Ciencia y Tecnología*, 11, 17-29.

Cunningham, L., Pitt, M., & Williams, H. D. (1997). The *cioAB* genes from *Pseudomonas aeruginosa* code for a novel cyanide-insensitive terminal oxidase related to the cytochrome *bd* quinol oxidases. *Molecular Microbiology* 24, 579-91.

dos Santos, M. F. A., Furlanetto, C., Almeida, M. R. A., Carneiro, M. D. G., Mota, F. C., et al. (2012). Biometrical, biological, biochemical and molecular characteristics of *Meloidogyne incognita* isolates and related species. *European Journal of Plant Pathology* 134, 671-684.

El-Sherif, M. A., Ali, A. H., & Barakat, M. I. (1999). Suppressive bacteria associated with plant parasitic nematodes in Egyptian agriculture. *Japanese Journal of Nematology*, 24, 55-59.

Erazo-Sandoval, N. S., Echeverría-Guadalupe, M. M., Jave-Nakayo, J. L., León-Reyes, H. A., Lindao-Córdova, V. A., Manzano-Ocaña, J. C., & Inca-Chunata, N. M. (2020). Effect of *Pleurotus ostreatus* (Jacq.) and *Trichoderma harzianum* (Rifai) on *Meloidogyne incognita* (Kofoid & White) in tomato (*Solanum lycopersicum* Mill.). *Acta Scientiarum. Biological Sciences*, 42, e47522.

Haque, M. M., Mosharaf, M. K., Khatun, M., Haque, M. A., Biswas, M. S., et al. (2020). Biofilm producing rhizobacteria with multiple plant growth-promoting traits promote growth of tomato under water-deficit stress. *Frontiers in Microbiology*, 11, 542053.

Hallmann, J., Quadt-Hallmann, A., Rodríguez-Kábana, R., & Kloepper, J. W. (1998). Interactions between *Meloidogyne incognita* and endophytic bacteria in cotton and cucumber. *Soil Biology and Biochemistry*, 30, 925-937.

Hallmann, J., Rodríguez-Kabana, R., & Kloepper, J. W. (1999). Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. *Soil Biology and Biochemistry*, 31, 551-560.

Hamid, M., Siddiqui, I. A., & Shahid Shaukat, S. (2003). Improvement of *Pseudomonas fluorescens* CHA0 biocontrol activity against root-knot nematode by the addition of ammonium molybdate. *Letters in Applied Microbiology*, 36, 239-44.

Hashem, M., & Abo-Elyousr, K. A. (2011). Management of the root-knot nematode *Meloidogyne incognita* on tomato with combinations of different biocontrol organisms. *Crop Protection*, 30, 285-292.

Hesar, A. M., Rostami, M., Ghaderi, R., Danesh, Y. R., Jalal, A., da Silva, C. E., & Teixeira, M. C. M., (2022). Population genetic structure of *Meloidogyne javanica* recovered from different regions of Iran. *Agriculture*, 12, 1374.

Hussey, R. S., & Barker, K. R. (1973). Comparison of methods for collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*, 57, 1025-1028.

Jung, C., & Wyss, U. (1999). New approaches to control plant parasitic nematodes. *Applied Microbiology and Biotechnology*, 51, 439-446.

Montes, C., Altimira, F., Canchignia, H., Castro, Á., Sánchez, E., et al. (2016). A draft genome sequence of *Pseudomonas veronii* R4: a grapevine (*Vitis vinifera* L.) root-associated strain with high biocontrol potential. *Standards in Genomic Sciences*, 11, 76.

Muimba-Kankolongo, A. (2018). Vegetable Production. In: Food Crop Production by Smallholder Farmers in Southern Africa. Challenges and Opportunities for Improvement. Academic Press. pp. 205-274.

Peñafiel-Jaramillo, M., Barrera-Álvarez, A. E., Torres-Navarrete, E. D., Canchignia-Martínez, H. F., Prieto-Encalada, H., & Morante-Carriel, J. (2016). Producción de ácido indol-3-acético por *Pseudomonas veronii* R4 y formación de raíces en hojas de vid "Thompson seedless" *in vitro*. *Ciencia y Tecnología*, 9, 31-36.

Siddiqui, Z. A. (2005). PGPR: Prospective Biocontrol Agents of Plant Pathogens. In: Siddiqui Z. A. PGPR: Biocontrol and Biofertilization. Springer, Dordrecht. pp. 111-142.

Siddiqui, I. A., & Shaukat, S. S. (2003). Endophytic bacteria prospect and opportunities for the biology control of plant-parasitic nematodes. *Nematología Mediterránea*, 31, 111-120.

Siddiqui, I., Hass, D., & Heeb, S. (2005). Extracellular protease of *Pseudomonas fluorescens* CHA0, a biocontrol factor activity against the root-knot nematode *Meloidogyne incognita*. *Applied and Environmental Microbiology*, 71, 5646-5649.

Sikora, R., Niere, B., & Dababat, A. (2008). Mutualistic endophytic fungi and in-planta suppressiveness to plant parasitic nematodes. *Biological Control*, 46, 15-23.

Timper, P., Koné, D., Yin, J., Ji, P., & Gardener, B. B. M. (2009). Evaluation of an antibiotic-producing strain of *Pseudomonas fluorescens* for suppression of plant-parasitic nematodes. *Journal of Nematology*, 41, 234-40.

Taylor, A., & Sasser, J. (1983). Biología, identificación y control de los nematodos de nódulo de la raíz. En *Especies de Meloidogyne*. Carolina del Norte. p. 109.

Wen, Y., Chen, K., Cui, J. K., Wang, T., Zhang, H., et al. (2022). First report of the root-knot nematode *Meloidogyne incognita* on *Salvia miltiorrhiza* Bunge in Henan Province, China. *Plant Disease*, 2022.

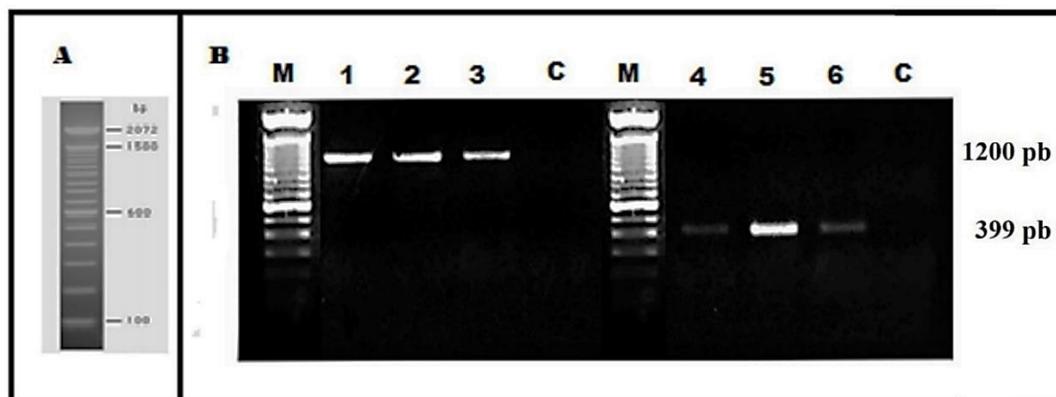


Figure A1. PCR amplification of *Meloidogyne incognita* using the primer pairs Finc / Rinc and Inc-K14F / Inc-K14R amplifies 1200 and 399 bp, respectively. A, 100 bp DNA Ladder. B, Lanes: DNA molecular weight marker (M); Amplification of *M. incognita* using the primer pairs Finc / Rinc (samples 1, 2 and 3) and Inc-K14F / Inc-K14R (samples 4, 5 and 6); Negative control *Radopholus similis* (C).