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Nematodes and root system are affected by rhizobacterial consortium in the third generation of commercial banana plants

Ángel Mauricio Crespo-Clas[1], Hayron Fabricio Canchignia-Martínez[2], Felipe Rafael Garcés-Fiallos[3]

[1] Technical University of Manabí, Postgraduate Institute, Portoviejo, Manabí, Ecuador. E-mail: acrespo2397@utm.edu.ec

[2] State Technical University of Quevedo, Facultad de Ciencias Agrarias, Santo Domingo de los Tsachilas, Quevedo, Los Ríos Ecuador. E-mail: hcanchignia@uteq.edu.ec

[3] Technical University of Manabí, Faculty of Agronomic Engineering, Laboratory of Phytopathology, Experimental Campus La Teodomira, Santa Ana, Manabí, Ecuador. E-mail: felipe.garces@utm.edu.ec

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ABSTRACT

Rhizobacteria has shown promising results in managing nematodes and improving the root system of banana plants. However, their effects on commercial banana plants in different generations remain unclear. In this study performed under field experiment, we evaluated the effect of three types (injection, edaphic and foliar) of a local rhizobacterial consortium application on the system root, nematode populations, and their damage in the third-generation plants. Only mother and daughter plants were treated twice with the rhizobacteria. Plants not treated with rhizobacteria constituted the control treatment. Our results show that rhizobacteria affect different underground tissues and nematodes in third-generation banana plants. *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus similis*, and *Rhabditis* spp. were the nematodes found in banana roots. *R. similis* population and the damage in the root system of banana plants were reduced significantly by the rhizobacteria edaphic application. The application of rhizobacteria could be a sustainable management strategy over time to improve the root system and suppress nematodes, and their damage in commercial banana plants.

Keywords: *Musa acuminata* AAA, *Radopholus similis,* Root system damage, Nematode biological control, Rhizobacteria.

Nematoides e sistema radicular são afetados pelo consórcio de rizobactérias na terceira geração de bananeiras comerciais

RESUMO

As rizobactérias têm apresentado resultados promissores no controle de nematoides e na melhoria do sistema radicular de plantas de banana. Entretanto, seus efeitos sobre as bananeiras comerciais em diferentes gerações ainda não estão claros. Neste estudo realizado em condições de campo, avaliamos o efeito de três tipos (injeção, edáfico e foliar) de aplicação de um consórcio local de rizobactérias no sistema radicular, populações de nematoides e seus danos nas plantas da terceira geração. Somente as plantas mãe e filha foram tratadas duas vezes com as rizobactérias. As plantas não tratadas com rizobactérias constituíram o tratamento controle. Nossos resultados mostram que as rizobactérias afetam diferentemente os tecidos subterrâneos e nematoides em bananeiras da terceira geração. *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus similis* e *Rhabditis* spp. foram os nematoides encontrados nas raízes das plantas de banana. A população de *R. similis* e os danos no sistema radicular das bananeiras foram reduzidos significativamente pela aplicação edáfica de rizobactérias. A aplicação de rizobactérias pode ser uma estratégia de manejo sustentável ao longo do tempo para melhorar o sistema radicular e suprimir os nematoides e seus danos em plantas comerciais de banana.

Palavras-chave: *Musa acuminata* AAA, *Radopholus similis*, Danos no sistema radicular, Controle biológico de nematoides, Rizobactérias.

1. Introduction

Banana (*Musa acuminate* AAA) belongs to the Monocotyledonous class, Zingiberales order, and the Musaceae family, composed of the *Musa* and *Ensete* genera (Simpson et al., 2019). This plant species is a crucial staple food produced in over 130 countries situated in diverse tropical and subtropical regions worldwide (Churchill, 2011; Drenth and Kema, 2021). Countless biotic problems affect banana crops worldwide, including diseases such as Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*), Moko (*Ralstonia solanacearum*), Yellow sigatoka (*Pseudocercospora musae*), Black leaf streak (BLS) (*Pseudocercospora fijiensis*), and those caused by nematodes (*Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., and *Radopholus similis*) (Chávez–Velazco et al., 2009; Churchill, 2011; Chávez-Arteaga et al., 2020; Drenth and Kema, 2021; Kisaakye et al., 2023).

Of all the diseases that affect bananas, those caused by nematodes are among the most important. These animals can affect the banana root system, causing a 100% reduction in fruit yield (Chávez-Velazco et al., 2009). The damage to the root system impairs the absorption of water and nutrients, ultimately hindering the plant's growth (Lara-Posadas et al., 2016). A group of plant parasitic nematodes affects the development of plants, considerably reducing the yield of the banana crops. The four main genera of nematodes that damage banana roots and reduce their yield are *Radopholus similis*, *Helicotylenchus* spp., *Pratylenchus* spp., and *Meloidogyne* spp., the former burrowing nematode being the most abundant (Chávez and Araya, 2010). Though no less important, *Meloidogyne* spp. are animals particularly noteworthy due to their broad geographic range and ability to infect a wide range of crops, resulting in severe economic losses (Calderón et al., 2022).

While synthetic nematicides are currently the most effective approach to controlling plant parasitic nematodes, their usage is constrained by environmental and legislative factors, so the biological control agents should be a strategy that is both environmentally sustainable and economically feasible (Pires et al., 2022). It must be considered that many banana producers have changed the conventional production system to an organic one, which produces higher economic benefits for them due to its lower production cost and higher fruit sale price (Jimenez et al., 2007).

In fact, in a recent study by Fotso et al. (2022), they verify that biological methods use is more effective than chemical ones when handling nematodes such as *R. similis*. Thus, the use of plant growth-promoting rhizobacteria (PGPRs) could be an alternative for disease management in Musaceae (Yang et al., 2021). PGPRs promote unfavorable conditions for the reproduction of the burrowing nematode and more favorable conditions for the development and production of the banana crop, which could contribute to more sustainable production (Torres-Asuaje et al., 2023). Recently, our research group isolated and identified some rhizobacteria in the rhizosphere of local Ecuadorian banana varieties, and due to their antagonistic activity, they could be considered a PGPR (Chávez–Arteaga et al., 2018; 2020).

Some of these rhizobacteria, such as *Pseudomonas protegens* (strain CHA0), *Serratia marcescens* (strain PM3-8), *Enterobacter asburiae* (strain BA4-19), and *P. veroni* (strain R4) reduce the number of eggs and J2 individuals of *M. incognita*, and *Acinetobacter calcoaceticus* (strain BM2-12), CHA0, and R4 reduced the number of galls and nematodes in tomato roots (Chávez–Arteaga et al., 2022). The current understanding is limited on the rhizobacteria used in banana crops in Ecuador and whether its effect on nematodes is long-lasting over time. Thus, our work aimed to evaluate the effect of the application of a consortium of potential PGPRs on nematodes infecting roots of the third generation of commercial banana plants cv. Williams.

2. Material and Methods

The research was carried out between 2020 and 2022, on a cacao fallow, in a farm at La Maná City, Cotopaxi province, Ecuador, at an altitude of 125 meters above sea level, and characterized as a humid forest. Cavendish banana plants (*Musa acuminata* AAA group) cv. Williams belonging to the first generation were used in this work. The bacterial consortium used in the research was composed of the rhizobacteria *Acinetobacter calcoaceticus* (strain BM2-12), *Enterobacter absuriae* (strain BA4-19), *Pseudomonas protegens* (strain CHA0), *P. veroni* (strain R4), and *Serratia marcescens* (strain PM3-8) (Table 1). These microorganisms were isolated from the rhizosphere of Ecuadorian banana and plantain landraces (farms at Los Ríos, Cotopaxi, and Bolívar provinces) and morphologically and biochemically identified by Chávez–Arteaga et al. (2018).

Cavendish banana plants were transplanted in August 2020, at a spacing of 3 x 2.5 meters, resulting in a population density of 1333 plants. Within the farm, all banana plants were culturally managed (removal of surplus and unwanted suckers, deleafing, and propping with bamboo poles, among other field activities). A single phytosanitary deleafing was carried out to eliminate the leaf area affected by BLS in each generation of plants. Narrow and broad-leaf weeds in the crops was manually controlled using a motor scythe (KM 280 R Multi-Tool Powerhead, Stihl, EUA).

Table 1. Plant growth-promoting rhizobacteria (PGPR) consortium used in this research, with their respective code, pH, and estimated bacterial concentration in colony-forming units (cfu mL^{-1})

Bacterial species	Code	pH	Concentration (cfu mL^{-1})
Pseudomonas protegens	CHA0	5.1	6.63E+09
Pseudomonas veroni	R4	5.1	7.03E+08
Enterobacter absuriae	BA4-19	5.4	5.90E+08
Acinetobacter calcoaceticus	BM2-12	5.2	6.68E+08
Serratia marcescens	PM3-8	5.0	9.70E+08

Plants were irrigated using a sprinkler irrigation system smooth drive (21 mm of river water every week). Plants were fertilized with the macro- and micro-elements Potassium (K), Nitrogen (N), Phosphorus (P), Magnesium (Mg), Calcio (Ca), Zinc (Zn) and Bore (B) in 459, 400, 90, 90, 90, 15, and 5 Kg concentrations per ha per year, respectively. BLS disease management within the banana crop consisted in average every ten days foliar sprays of Triazoles and morpholines in a mixture with Pyrimethanil and Mancozeb in Winter, and biweekly foliar sprays of Mancozeb in the summer, using in all cases a motor pump. The Plants were irrigated using a sprinkler irrigation system smooth drive (21 mm of river water every week). Plants bunches were harvested manually.

Bacterial inoculum was obtained through bioreactors consisting of plastic canisters (50 L) coupled to a motor (95 GPH 5 W, 1.8 x 1.8 x 1.4 ", 1.5 GPM Max Flow Rate, Pulaco, USA) and gasoline filter to maintain the aerobic capacity of the bacteria and avoid contamination by external agents, respectively. The pre-inoculum of each of the five PGPRs grown for 48 h in King B liquid medium (King et al. 1954), was placed in the canisters (1×10^8 bacterial cells in 500 mL of sterilized distilled water) containing molasses, flour corn (a rich carbon source) and mineral salts (sulphates and phosphates). The bacterial suspension, with a pH of 5.2, was kept at 25ºC ± 3 with constant shaking for 15 h per day for four days.

Banana mother and daughter (return) plants (n=10) were inoculated with the PGPR consortium at the bacterial concentrations indicated in Table 1. Inoculations were performed 90 days after transplanting and 60 days after emission of the suckers in mother and daughter plants, respectively. Plants were inoculated in three ways: a) injection in the zone where the leaf sheaths originate (an approximate distance of 40 cm counted from the base of the pseudostem) using an automatic loading syringe (10 mL); b) edaphic drench type using a manual pump (200 mL); and c) foliar using a motor pump covering the total foliar area of the plants. Plants not treated with the rhizobacterial consortium constituted the control treatment.

For the root system analysis, the number of principal and secondary roots, root mass weight, and functional roots (%) were determined only in underground tissues of four granddaughter banana plants (third generation) per treatment. In order, to quantify the nematodes and root system damage, in January 2022, root samples were collected from four granddaughter plants of four banana clones per treatment, identified, and taken to the laboratory, where the nematodes were extracted using the method of Sarah and Boisseau (2008).

Each sample was then morphologically identified and quantified using a binocular microscope (model BA310, Motic, Germany). In these four banana plants, also root system damage (%) in banana plants was determined by estimating the percentage of the root system affected by nematodes. The experiment was carried out in a randomized complete block design, with four repetitions. After verification of homogeneity of the variances of the data sets, data were subjected to analysis of variance. Scott Knott test ($P \leq 0.05$) was used to separate the means. Statistical analyzes were performed using the ASSISTAT software, version 7.7 (Silva and Azevedo, 2002).

3. Results and Discussion

Management strategies like the rhizobacteria use that are friendly to the environment and human health would be one of the most appropriate tools to manage biotic factors in banana crops. In this study, we assessed the nematode populations and their damage in the roots of third generation of commercial banana plants, whose predecessors (grandmother and mother) were treated with a rhizobacterial consortium.

In general, the root system of banana granddaughter plants was significantly affected by the application of the rhizobacterial consortium in mother and daughter plants (Table 2). The number of principal and secondary roots, root mass weight, and functional roots percentage were an average of 28, 60, 38, and 25% higher in granddaughter plants whose predecessors were treated with PGPR, respectively, compared to the untreated. System root tissues in third generation of banana plants were improved with the application of the rhizobacterial consortium, regardless of the type of application.

Table 2. Number of principal and secondary roots, root mass weight (g), and percentage of functional roots, in banana plants cv. Williams whose predecessors (mother and daughter plants) were treated with a plant growth-promoting rhizobacteria (PGPR) consortium applied either by pseudostem injection, soil drench (edaphic) or foliar spray.

Treatments	Root numbers		Root mass weight (g)	Functional roots (%)
	Principal	Secondary		
Injection	51.8±1.7 a*	67.5±2.6 a**	304.5±12.8 a	80.0±12.3 a
Edaphic	42.0±1.8 a	81.3±3.3 a	242.0±18.9 a	92.5±7.2 a
Foliar	50.0±1.4 a	78.0±5.4 a	273.3±17.4 a	93.3±8.3 a
Control	34.5±2.4 b	30.5±3.1 b	169.8±24.5 b	66.5±9.2 b
Mean	44.6	64.3	247.4	83.1
P-value	0.0105	0.0227	0.0162	0.0149

* Means with the same letters in the column belong to the same cluster (Scott Knott, $P \leq 0.05$). ** The value next to the mean represents the standard deviation.

Two points of discussion can be identified: a) the effect of rhizobacteria as promoters of banana plant growth, and b) the effect of rhizobacterial consortium as nematicide reaching the third generation of plants. Rhizobacteria could prevent infection by nematodes, increase the nutrient content and diversity of the bacterial community in the rhizosphere, and promoted the growth of banana plants (Yang et al., 2021).

Biostimulants based on microorganisms incorporated into the soil can promote the growth and suppress the burrowing nematode population in the roots of banana successional sucker in farm commercial conditions (Torres-Asuaje et al., 2023). However, these effects were observed only in plants (first generation) treated with rhizobacteria, contrary to our work that the system root of granddaughter plants improved their morphological characteristics over time.

The nematode population fluctuated between 0 and 9180±2475 individuals per 100 g of banana roots (Table 3). In these underground tissues were identified *Helicotylenchus* spp., *Meloidogyne* spp., *Pratylenchus* spp., *R. similis*, and *Rhabditis* spp. Of all these nematodes found and described in our research and in that of Chávez–Velazco et al. (2009) and Chávez and Araya (2010), the burrowing nematode *R. similis* is the major problematic and destructive nematode of banana plants in Ecuador. In fact, this nematode is the most abundant on banana roots. Generally, 95% of commercial banana roots can be affected by *R. similis*, compared to others such as *Helicotylenchus* spp., *Pratylenchus* spp., *Meloidogyne* spp., with 79, 31, and 7%, respectively (Chávez and Araya, 2010).

Only the edaphic application of the PGPRs reduced the population of *R. similis* in the roots on average three-fold compared to those of plants whose predecessors were treated with an injection and foliar application, or untreated (Table 3). It should be noted that the rhizobacteria used in our research were isolated from native Ecuadorian cultivars of *Musa* spp. (Chávez-Arteaga et al., 2018). These rhizobacteria have *in vitro* activity against *M. fijiensis* and *M. incognita* in banana and tomato, respectively (Chávez-Arteaga et al., 2020; 2022). As observed, the natural environment of our rhizobacteria is the banana rhizosphere. Moreover, when applied edaphically, the prokaryotes significantly reduced the *R. similis* population by acting directly on the pathogen, for example, by producing its antagonistic metabolites. The effect of these potential PGPRs against *R. similis* in banana roots under field conditions would be the first report in Ecuador.

The edaphic application with the rhizobacterial consortium on mother and daughter plants also reduced the damage produced by nematodes in the root system of banana granddaughter plants by an average of 60% compared to those other treatments (Figure 1). This result would show only the local non-systemic effect exerted by the PGPRs in the long term. It is known that our rhizobacteria produce bioactive metabolites *i.e.*, protease (PR), hydrogen cyanide (HCN), pyrrolnitrin (Prn), diacetylphloroglucinol (2,4-DAPG), siderophores (Sd) or indole-3-acetic acid (IAA) (Chávez-Arteaga et al., 2022), directly affecting individuals of *R. similis* and inducing a change and reorganization of microflora in the rhizosphere of banana daughter plants (Yang et al., 2021).

Indeed, PGPRs produce bioactive compounds such as enzymes and other low molecular weight compounds that play an important role in the ecological strategy in the competition for space and nutrients with other microorganisms (Gionco et al., 2017; Marcano et al., 2016; Gamez et al., 2019). Additionally, the suppression of *R. similis* in banana roots could also be associated with the defense mechanisms induction by the rhizobacteria. For instance, many biocontrollers can upregulate the defense-related gene expression (Kisaakye et al., 2023), and produce antibiotics and biofilms (Yuan et al., 2015; 2018). Thus, we could infer that the reduction of *R. similis* individuals would be associated with a suppressive effect and another inducing resistance by the bacterial consortium in banana plants.

Table 3. Nematode populations in roots (100g) of banana granddaughter plants cv. Williams, whose predecessors (mother and daughter plants) were treated with a plant growth-promoting rhizobacteria (PGPR) consortium applied either by pseudostem injection, soil drench (edaphic) or foliar spray.

Treatments	*Helicotylenchus* spp.	*Meloidogyne* spp.	*Pratylenchus* spp.	*Radopholus similis*	*Rhabditis* spp.
Injection	2135±1850*	2030±1055	0±0	7650±1650 a***	1035±1080
Edaphic	1350±1010**	0±0	0±0	2570±2155 b	0±0
Foliar	2050±1155	1020±1090	2030±2100	6030±1490 a	2130±1945
Control	4155±1985	1110±935	2350±2050	9180±2475 a	1570±1120
Mean	6573.8	1040.0	1095.0	3964.1	1183.8
P-value	0.2284	0.3584	0.4754	0.0395	0.3284

* The value next to the mean represents the standard deviation. ** In the nematode species when there are no letters, there was no significant difference. *** Means with the same letters in the column belong to the same cluster (Scott Knott, $P \leq 0.05$).

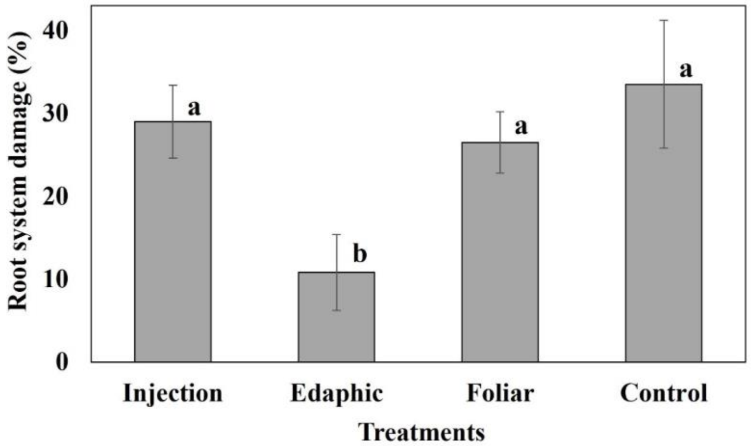

Figure 1. Root system damage (%) in granddaughter banana plants cv. Williams, whose predecessors (mother and daughter plants) were treated with a plant growth-promoting rhizobacteria (PGPR) consortium applied by pseudostem injection, soil drench (edaphic) or foliar spray. Plants not inoculated with the rhizobacteria constituted the control treatment. Bars with the same letters in the Figure belong to the same cluster (Scott Knott, $P \leq 0.01$). The error bars represent the standard deviation of the mean.

4. Conclusions

The rhizobacterial consortium applied by pseudostem injection, soil drench (edaphic) or foliar spray to mother and daughter banana plants, increases the number of principal and secondary roots, root mass weight (g) and percentage of functional roots in banana granddaughter plants cv. Williams (third generation). Additionally, the rhizobacterial consortium applied as a soil drench is effective in reducing both the population of *R. similis* in banana roots, and the damage to the root system of these banana plants. This knowledge is valuable in the development of integrated nematode management strategies for banana crops, which would have a positive impact on the sustainability of the crop worldwide, benefiting farmers and consumers.

Authors' Contribution

Hayron Fabricio Canchignia-Martínez and Felipe Rafael Garcés-Fiallos conceived and designed research. Ángel Maurício Crespo-Clas and Hayron Fabricio Canchignia-Martínez performed the experiments. Hayron Fabricio Canchignia-Martínez contributed new reagents or analytical tools. Ángel Mauricio Crespo-Clas analyzed data and wrote the manuscript. Felipe Rafael Garcés-Fiallos processed the experimental data, performed the analysis, and drafted the manuscript. All authors read and approved the manuscript.

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