International Journal of Environment, Agriculture and Biotechnology
Vol-10, Issue-5; Sep-Oct, 2025
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Biological control of Plant-parasitic nematodes in Bananas using *Trichoderma atroviride* and cocoa-based organic amendments

Joaquim Paulo de Andrade Bernardo Cuvaca[1,2], Alex Mukiibi[3], Cleucilene Moura dos Reis[4], Alejandra Arroyo[5], Luis Ernesto Pocasangre Enamorado[6]

[1]Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal
[2]Chemical Process Engineering and Forest Products Research Centre, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Pólo II-Pinhal de Marrocos, 3030-790 Coimbra, Portugal
[3]University of Pretoria, Department of Plant and Soil Sciences, South Africa
[4]Universidad EARTH, San José 4442-1000, Costa Rica
[5]Escuela de Producción, Tecnología y Medio Ambiente, Universidad Nacional de Río Negro, Argentina
[6]Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica.
Corresponding author: jp.cuvaca@gmail.com / uc2022203881@student.uc.pt

Received: 01 Sep 2025; Received in revised form: 02 Oct 2025; Accepted: 07 Oct 2025; Available online: 16 Oct 2025

***Abstract*— *This study evaluates the combined use of the endophytic fungus Trichoderma atroviride (strains Endo 1 and Endo 2) and cocoa (Theobroma cacao) organic amendments for managing plant-parasitic nematodes in three banana cultivars: Boniface, Grand Nain, and Williams. Field experiments were conducted on a commercial banana farm in Costa Rica using a randomized block design. Treatments involved applying fungal spores and cocoa amendments bi-monthly over a six-week interval. Results revealed no statistically significant differences in total nematode populations among treatments; however, the combined application of T. atroviride and organic amendments reduced nematode populations more effectively than control plots. Improvements were also observed in root health and plant growth parameters over time. This suggests a potential, though not conclusive, synergistic effect of these biocontrol agents. Further long-term studies are recommended to better understand their independent and combined effects on nematode management in bananas.*

***Keywords*— endophytic fungi, organic amendments, plant-parasitic nematodes, root health, banana cultivation**

I. INTRODUCTION

Bananas (*Musa* spp.) play a vital role in global food security and serve as a staple food for over 400 million people. Bananas rank among the most significant global food crops, both in production and trade, with annual consumption exceeding 116.2 million tons. Latin America and the Caribbean account for approximately 80% of worldwide exports and nearly 30% of global production, which significantly influences their social development (UN, 2019; FAO, 2016; Dita *et al*., 2011).

Banana-producing regions face significant threats from climate change as well as outbreaks of pests and diseases (van Asten *et al*., 2011). Among the most critical diseases affecting bananas are the black leaf streak disease caused by the ascomycete fungus *Mycosphaerella fijiensis* (Morelet), the Panama disease as a result of *Fusarium oxysporum* Schelect f. sp. cubense infestation, the banana bunchy top virus (BBTV) transmitted by the aphid *Pentalonia nigronervosa* (Coquerel), the moko disease caused by the bacteria *Ralstonia solanacearum* (Smith), and several others such as reduced yield, toppling, reduced number, size, and yellowing of the leaves, etc., caused by plant-parasitic nematodes (PPN). The most common PPN are *H. multicinctus* (Cobb), *M. incognita* (Kofoid and White), *M. javanica* (Treub), *Pratylenchus goodeyi* (Sher and Allen), and *R. similis* (Cobb and Thorne) (Jones, 2007; Soto, 2008; Ploetz *et al*., 2003). Plant-parasitic nematodes inflict substantial economic losses on commercial banana plantations (Pocasangre *et al*., 2015; Soto, 2008; Ploetz *et al*., 2003). On average, worldwide losses due to PPN in bananas are estimated to be around 19.7%, with potential losses rising to 80% based on environmental conditions and agricultural practices used in the plantations (Araya, 2003; Niere *et al*., 1999). In Costa Rica, the primary drivers of a yield reduction of 30% to 50% in banana crops are *R. similis* and *H. multicinctus* (Castro *et al*., 2005).

Currently, conventional control methods for PPN involve two to three applications of nematicides annually. Traditional control methods include effective cultural practices such as soil management, optimal drainage, the use of cover crops, and the application of organic matter (Pocasangre, 2013). Research on the use of biological control agents such as bacteria, endophytic fungi, and mycorrhiza is currently underway. These biological agents form microbial communities in the rhizosphere with the capacity to improve root health and overall plant development through the suppression of banana PPN (Sikora *et al*., 2008; Sikora and Pocasangre, 2006; Zum Felde *et al*., 2005). Endophytic fungi are organisms with the ability to colonize internal plant tissue in a commensalism, mutualistic, or pathogenic relationship (Riveros, 2010; Sikora and Schuster, 1998). Several genera of fungi with antagonistic properties toward plant pathogens have been identified. Among those are *Trichoderma* and *Fusarium,* which are abundant in plant tissues and present high competition against PPN in bananas and plantains (Chaves, 2007; Zum Felde *et al*., 2006; Pocasangre *et al*., 2000).

To date, there is no evidence that specific nematode control can be achieved solely through the use of organic amendments (Timper, 2014). To anticipate and prevent yield losses in bananas due to PPN, more studies concerning the effects of biocontrol agents and cultural practices should be conducted. Therefore, this study aims to evaluate the combined effects of *Trichoderma atroviride* strains and cocoa-based organic amendments on the biocontrol of plant-parasitic nematodes in three Cavendish banana cultivars.

II. MATERIALS AND METHODS

Field site

To investigate these objectives, field trials were conducted from February to November 2015 (10 months) at the EARTH University commercial banana farm (Las Mercedes of Guácimo, Limón province), in the humid tropical region of Costa Rica (GPS coordinates 10° 12' 45" N 83° 35' 39" W), at 39 m above sea level. Meteorological stations at the farm recorded an average annual temperature of 25 °C, a relative humidity of 90%, and an average precipitation of 4315 mm (EARTH University, 2014).

Experimental design

The experimental area of approximately one hectare was divided into three equal blocks arranged in a continuous layout. Each block was further subdivided into three sub-blocks, each measuring 13.2 m by 45 m, separated by tertiary drains, resulting in a total of 9 sub-blocks. These sub-blocks were then divided into three small plots of the same size, leading to a total of 27 small plots. Each small plot was divided into three sections, which created nine sections per sub-block, corresponding to nine treatments, each treatment repeated three times (Table 1). Each sub-block contained six rows with 20 plants each, totaling approximately 120 plants. The plants were spaced 2.4 m apart between rows and 2.2 m apart within rows (Ochoa and Spiegeler, 2014).

Treatments

The experiment consisted of evaluating the combined application of *T, atroviride* strains Endo 1 (E1) and Endo 2 (E2) with organic amendments (OA). Fungi and OA were applied in front of the daughter plants from three cultivars of Cavendish bananas: Boniface (B), Grand Nain (GN), and Williams (W). Control plots were established for each cultivar without fungi or organic amendments. Treatments are shown in Table 1.

Table 1. The effects of the combined application of T. atroviride strains Endo 1 and Endo 2 with organic amendments on three cultivars of Cavendish bananas (Boniface, Grand Nain and Williams).

Treatment	Description
BE1OA	Boniface + Endo 1 + Organic Amendments
BE2OA	Boniface + Endo 2 + Organic Amendments
BC	Boniface Control
GNE1OA	Grand Nain + Endo 1 + Organic Amendments
GNE2OA	Grand Nain + Endo 2 + Organic Amendments
GNC	Grand Nain Control
WE1OA	Williams + Endo 1 + Organic Amendments
WE2OA	Williams + Endo 2 + Organic Amendments
WC	Williams Control

Fungal inoculum

The *T. atroviride* strains E1 and E2 were prepared from inocula preserved in the Cryo Bank of the Natural Sciences laboratory at EARTH University. Propagules from each fungal strain were cultured on 9-cm-diameter Petri dishes containing potato dextrose agar (PDA). Two weeks after incubation at 24 °C, mycelia were scraped from PDA dishes, washed with sterile distilled water, filtered with a piece of cloth to obtain a microspore solution, and transferred into 5 L jugs. Subsequently, the concentration of microspores in the final solution was determined using a hemocytometer under a light microscope.

Organic amendment

Cocoa fruit husks, as a remnant of the harvest (under organic plantations), were chopped into smaller pieces before being composted. The pH and temperature of the compost were monitored at two-week intervals until it was ready for use. The mixture was stirred every third night and watered as needed. The organic material was allowed to sit for three months and cured for an additional two weeks before being utilized.

Field application of *T. atroviride* strains and organic amendment

Five hundred milliliters of 4.0 x 10^4 cfu/mL *T. atroviride* E1 and E2 microspores and 1.5 kg of organic amendments were applied twice on 17/03/2015 and 30/04/2025 at the stem base of each selected plant.

Plant growth measurements

Plant measurements were carried out three times at a three-month interval (on 15/02/2015, 26/05/2015, and 25/08/2015). The first measurement was carried out one month before *T. atroviride* and organic amendment applications. Five of the 12 previously identified plants were randomly selected from each plot to measure plant height in meters and pseudostem circumference in centimeters. Height was taken from the plant base to the start point of the leaf flag, and pseudostem circumference was measured one meter from the plant base (Rosales *et al.*, 2008).

Root sampling and root health analyses

Twelve plants were selected from each plot, marked with identification tags, and grouped into four sets of three plants for root sampling. A small hole measuring 13 cm long, 13 cm wide, and 30 cm deep was excavated 10 cm from the succession sucker using a shovel, and roots were collected in plastic bags. Root samples were taken within 59 days interval between the first (29/04/2015) and second sampling (27/06/2015) and 54 days between the second and third samplings (10/09/2015). Roots were washed with tap water to remove soil and debris, weighted, and cut to separate functional roots from non-functional roots, and the root health index (RHI), root diameter, and necrosis index (NI) were assessed (Rosales *et al.*, 2008). The RHI is a parameter that measures the non-functional root percentage of a sample. A scale of 1 to 9 was used to evaluate RHI, where 1 represents a lower percentage of non-functional roots and 9 represents the highest percentage of non-functional roots, as shown in Table 2. For measuring root diameter and NI, five roots from each sample were randomly selected. The root diameter was measured using a graduated Vernier caliper, and the average diameter for the five roots was calculated. To assess root NI, the roots were cut into 5-cm pieces and dissected longitudinally with a scalpel. The root NI was estimated by measuring the percentage of damage in five root segments. Roots with no necrotic tissue received a score of 0%, roots with a quarter of the tissue affected by necrosis received a score of 5%, those with half of the tissue necrotic received a score of 10%, and a 20% score was attributed to fully necrotic root segments.

Table 2. Scale parameters used to measure root health index (Rosales et al., 2008).

Root health index (RHI)	Non-functional root percentage ranges (%)
1	0 - 10
2	11 - 20
3	21 - 30
4	31 - 40
5	41 - 50
6	51 - 60
7	61 - 70
8	71 - 80
9	> 80

Nematode extraction, identification and quantification

Ten grams of roots were weighed and blended at low speed for 10 seconds and then at high speed for five seconds with a Vitamix E310 Explorian (120V) blender. The blend was sieved through 60 µm, 140 µm, and 500 µm mesh sieves, and the resulting suspension was collected into a beaker to obtain a 100 mL root suspension in water. Two milliliters of aliquots were taken from the 100 mL and transferred into a counting plate. The number of *H. multicinctus, M. incognita,* and *R. similis* were counted under a light microscope (Rosales *et al.*, 2008).

Statistical analysis

Results were analyzed using InfoStat statistical software (Di Rienzo *et al.*, 2020) through analysis of variance (ANOVA) for each evaluated variable. An LSD Fisher comparison test at a 5% significance level was performed on variables that showed significant differences between treatments.

III. RESULTS

Effects on plant growth

Plant height and plant pseudostem girth increased over time, though treatments and controls did not differ significantly ($p > 0.05$) (Figure 1). Banana Cultivars showed a similar growth tendency over time. Plant height and pseudostem girth of Boniface, Grand Nain, and Williams were statistically similar over time ($p > 0.05$) (Figure 2). Associations of E1+OA and E2+OA with the cultivars showed no significant differences for both plant height and pseudostem girth variables ($p > 0.05$). Treatment BE1OA had greater growth in height, while WE1OA had lesser growth throughout time. On the other hand, treatments BE1OA and GNE1OA showed higher total averages of plant pseudostem girth superior to 47 cm (Table 3). Treatments with greater average height showed high pseudostem girth, which showed a positive and direct relationship between both growth parameters.

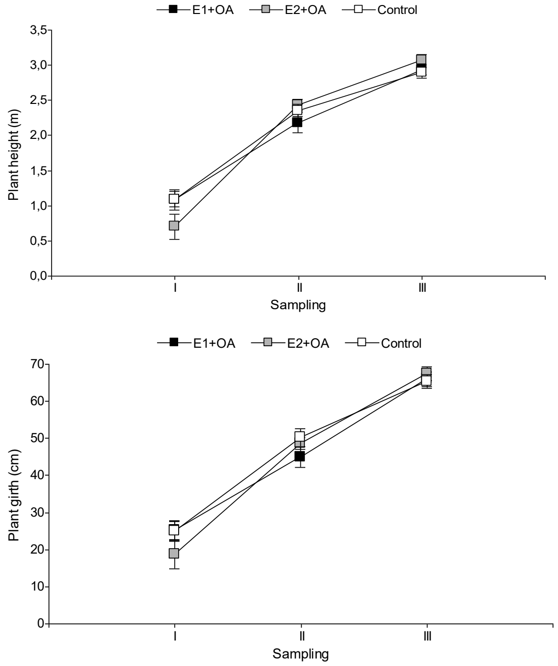

Fig.1. Effect of combined endophitic fungi and organic amendments (E1+OA and E2+OA) on plant height and pseudostem girth during 113 days.

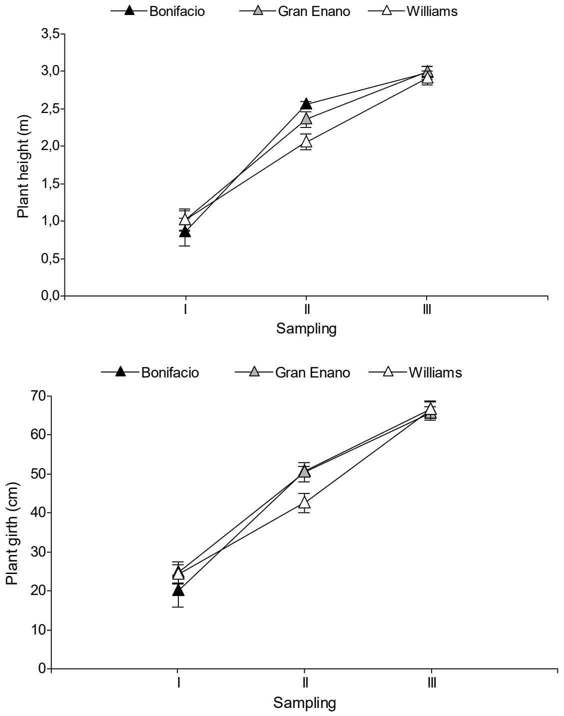

Fig.2. Banana height and pseudostem girth behavior over time.

Table 3. Effect of treatments on plant height and plant pseudostem girth.

Treatment	Height (m)	Pseudostem girth (cm)
BE1OA	2.27 a	48.38 a
BE2OA	1.93 a	41.98 a
BC	2.13 a	46.60 a
GNE1OA	2.07 a	47.22 a
GNE2OA	2.18 a	46.80 a
GNC	2.12 a	46.71 a
WE1OA	1.86 a	40.70 a
WE2OA	2.03 a	46.16 a
WC	2.09 a	46.78 a

† Averages with similar lowercase letters are not significantly different (p>0,05).

Effects on root health: root health index and necrotic index

The associations (E1+OA and E2+OA) showed no significant differences ($p > 0.05$) regarding RHI and NI when compared to control. Both associations reduced RHI and NI, being more efficient for the E2+OA (Figure 3).

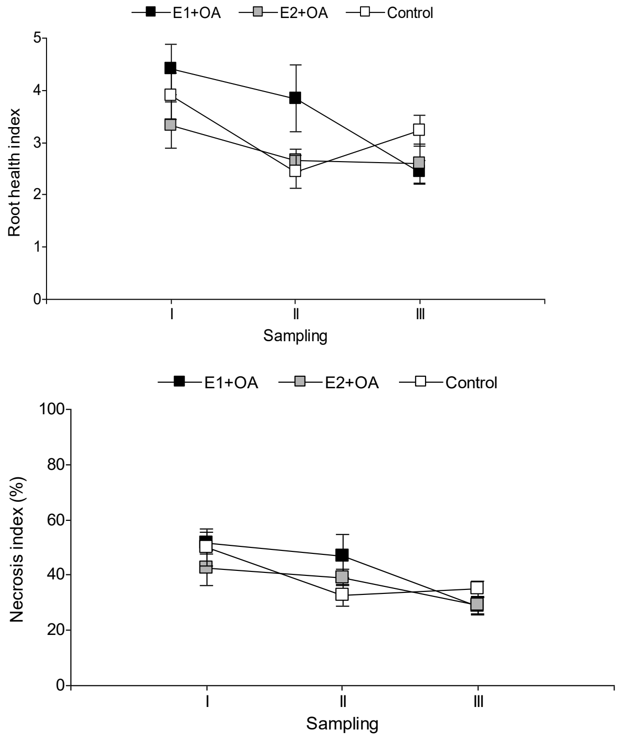

Fig.3. Effect of combined endophitic fungi and organic amendments (E1+OA and E2+OA) on banana root health and necrosis indices during three root sampling periods (p > 0.05).

The RHI and NI did not differ significantly among cultivars. The Boniface cultivar had a higher percentage of RHI than Williams and Grand Nain (Figure 4). The RHI showed a declining tendency over time. Treatments such as BE2OA, BC, and WE2OA indicated a lower total average of 2.7, 2.7, and 2.8 for RHI, respectively. Treatments GNE2OA and WE1OA showed a significant improvement in root health, lowering its RHI from 4.44 in the first sampling to 2.22 in the third sampling, achieving a 50.0% reduction (Table 4).

Table 4. Effect of treatments on the root health index.

Treatment	Days after application		
	0	59	113
BE1OA	4.78 a	3.56 a	2.78 ab
BE2OA	3.00 a	2.44 a	2.67 ab
BC	2.78 a	2.78 a	2.67 ab
GNE1OA	3.89 a	4.67 a	2.33 a
GNE2OA	4.44 a	2.56 a	2.22 a
GNC	4.56 a	2.22 a	3.00 ab
WE1OA	4.44 a	2.56 a	2.22 a
WE2OA	2.56 a	3.00 a	2.89 ab
WC	4.33 a	2.33 a	4.00 b

† Averages with similar lowercase letters are not significantly different (p>0,05).

Overall, there was a reduction in NI as a result of each treatment throughout time. In the treatments BC, BE2OA, and WE2OA, the average root area with necrotic damage decreased by 33.46%, 33.98%, and 36.94%, respectively. Treatment WE1OA showed the greatest reduction in necrotic damage, with its NI decreasing from 59.89% during the first sampling period to 25.67% in the third sampling, resulting in a 57.14% reduction. For the Boniface cultivar, the E2+OA treatment yielded the best result.

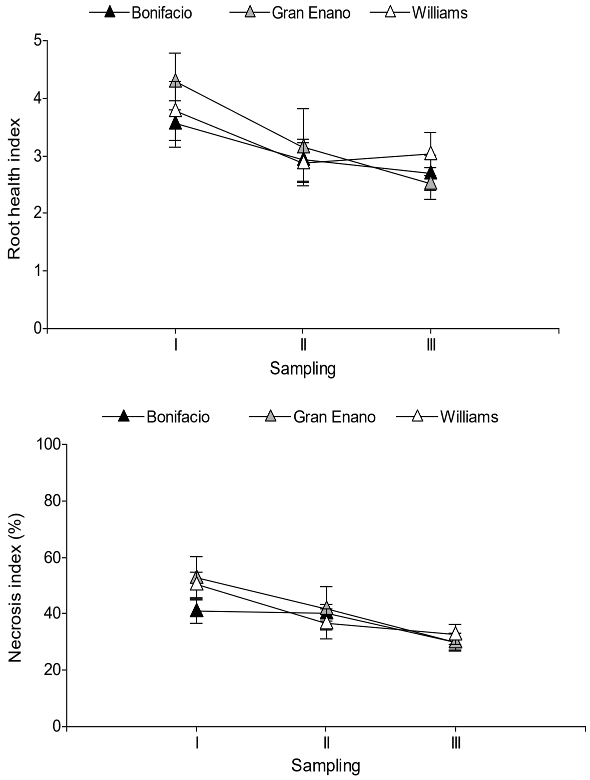

Fig.4. Root health and necrosis indices of banana cultivars over time.

Effects on root health: functional root weight and dead root weight

There was an increase in FRW over time for all treatments. Treatments and controls showed no significant difference throughout time. However, the plots treated with E2+OA and control had the highest functional roots compared to those treated with E1+OA (Figure 5). The FRW of banana cultivars increased over sampling time. Although there were no significant differences among cultivars, Boniface and Williams had the highest FRW, followed by the Grand Nain (Figure 6). While there were no significant differences in FRW among the banana cultivars and their interactions with *T. atroviride* during the first and third sampling periods, a significant difference was noted during the second sampling period. Treatments BE2OA and WC had the highest FRW over time (Table 7). Regarding dead root weight, treatments were significantly different during the first and third samplings and remained similar in the second sampling. Treatment GNE2OA had the highest and most significant reduction of the total percentage of dead roots of 74% (Table 8). Though FRW increased, there were no significant differences over time.

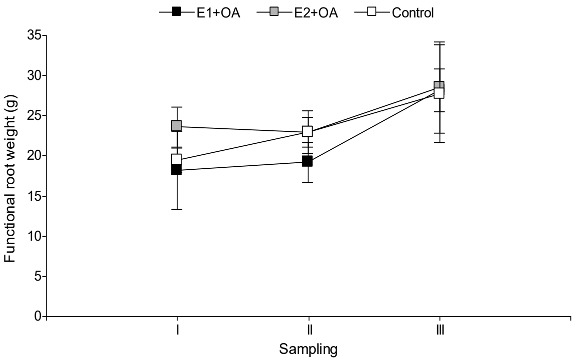

Fig.5. Effect of combined endophitic fungi and organic amendments (E1+OA and E2+OA) on functional root weight over three sampling periods.

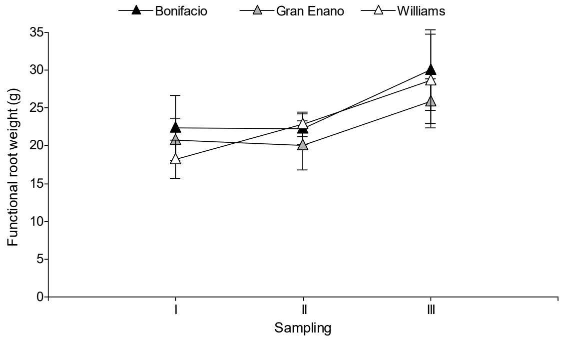

Fig.6. Functional root weight behavior for Boniface, Grand Nain, and Williams over time.

Table 7. Functional root weight of each treatment throughout time.

Treatment	Sample (g)		
	I	II	III
BE1OA	24.56 a	19.36 ab	27.09 a
BE2OA	22.08 a	28.90 b	37.79 a
BC	20.50 a	18.41 ab	25.14 a
GNE1OA	19.28 a	15.49 a	31.35 a
GNE2OA	25.55 b	18.79 ab	26.17 a
GNC	17.32 a	25.76 ab	20.06 a
WE1OA	10.73 a	22.75 ab	26.05 a
WE2OA	23.13 a	21.13 ab	21.63 a
WC	20.71 a	24.61 ab	38.07 a

† Averages with similar lowercase letters are not significantly different (p>0,05).

Table 8. Effect of treatments on dead root weight throughout time.

Treatment	Days after application		
	0	42	84
BE1OA	17.58 abc	17.32 a	10.68 ab
BE2OA	12.22 ab	17.23 a	9.86 ab
BC	11.70 ab	14.38 a	10.71 ab
GNE1OA	8.31 a	13.63 a	8.04 ab
GNE2OA	23.40 c	14.93 a	6.00 a
GNC	14.46 abc	8.41 a	10.84 ab
WE1OA	8.50 a	16.91 a	7.15 a
WE2OA	9.18 a	11.70 a	11.29 ab
WC	19.69 bc	8.70 a	14.31 b

† Averages with similar lowercase letters are not significantly different ($p > 0.05$).

Effects on plant-parasitic nematodes

The root population densities of *R. similis*, *M. incognita,* and *H. multicinctus* varied depending on the treatments applied. Although there were no significant overall differences in *R. similis* population densities, the combined application of E1+OA and E2+OA significantly reduced the *R. similis* populations at 84 and 42 days after application compared to the control (Figure 7). In the case of *M. incognita,* there were differences among treatments during the three sampling periods. The E1+OA and E2+OA treatments resulted in slight reductions in the population, with decreases of 33% and 36%, respectively, compared to the control. The population densities of *M. incognita* remained constant in the control treatment throughout all three sampling periods (Figure 7). For *H. multicinctus*, no significant differences between treatments were observed. However, the application of E1+OA consistently suppressed the population. Both E2+OA and control treatments showed a reduction in *H. multicinctus* densities from the first to the second sampling period; however, the population density increased again from the second to the third sampling (Figure 7).

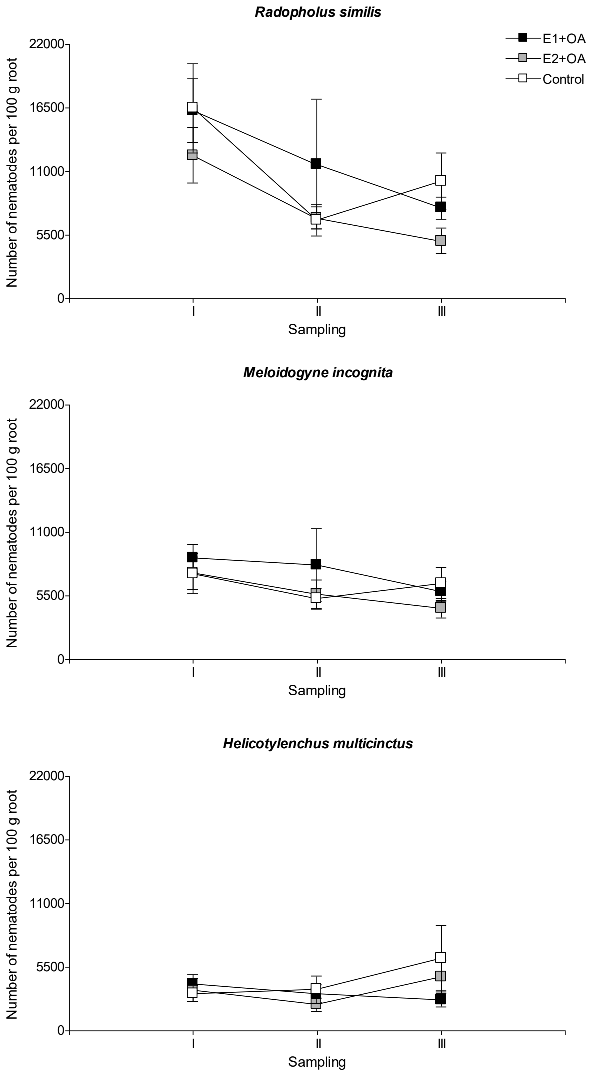

Fig.7. Effect of combined endophitic fungi and organic amendments (E1+OA and E2+OA) on plant-parasitic nematode populations during three sampling periods (p > 0.05)

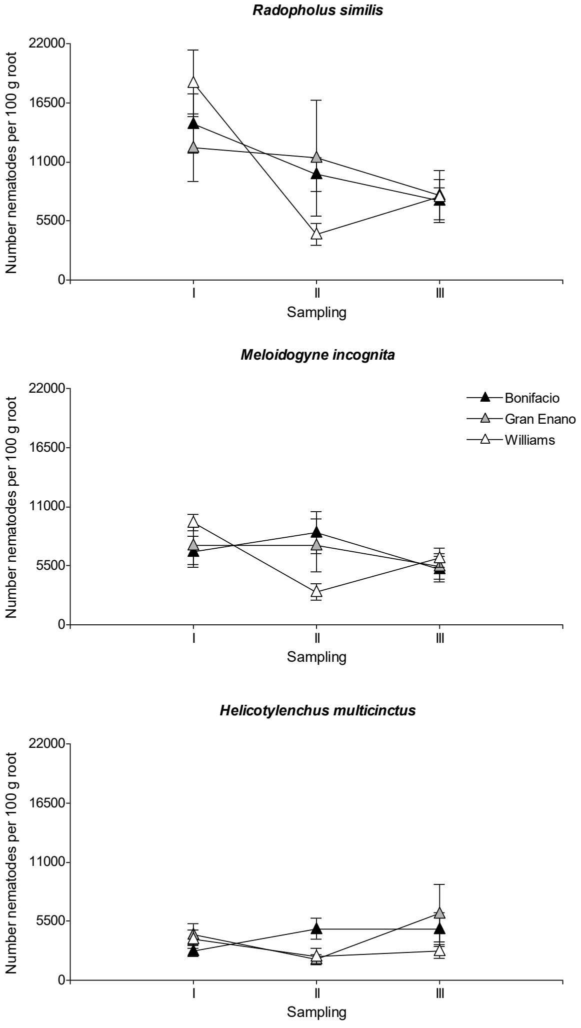

Fig.8. Plant-parasitic nematode populations behaviour on B, GN, and W cultivars throughout time (averages with similar lowercase letter are not significantly different; p > 0.05).

The GN cultivar exhibited the lowest incidence of *R. similis*, followed by the B and W cultivars (see Figure 8). However, these differences were not significantly different from the control group. While there were no significant variations in the *M. incognita* population among the banana cultivars, both the B and GN cultivars demonstrated a greater reduction in this phytonematode between the second and third sampling periods. The behaviors of *M. incognita* and *R. similis* were similar in the W cultivar. For the *H. multicinctus* nematode, its population increased over time in the B cultivar. In contrast, the GN cultivar showed a decrease in population between the first and second samplings, followed by an increase between the second and third samplings. The *H. multicinctus* population in the Williams cultivar remained stable throughout the study.

Regarding the effects of fungal strains and cocoa amendment application on the total PPN densities, there were no significant differences between treatments. However, the association between *T. atroviride* and cocoa organic amendment reduced the total population PPN. Treatment E1+OA had more impact in reducing PPN (41%), followed by treatment E2+OA and control with 34% and 23% reductions, respectively. There were no significant differences in total PPN between B, GN, and W cultivars. The total number of PPN was slightly reduced for both B and GN over time, while for the W cultivars, it decreased between the first and second samplings followed by an increase between the second and third samplings (Figure 9).

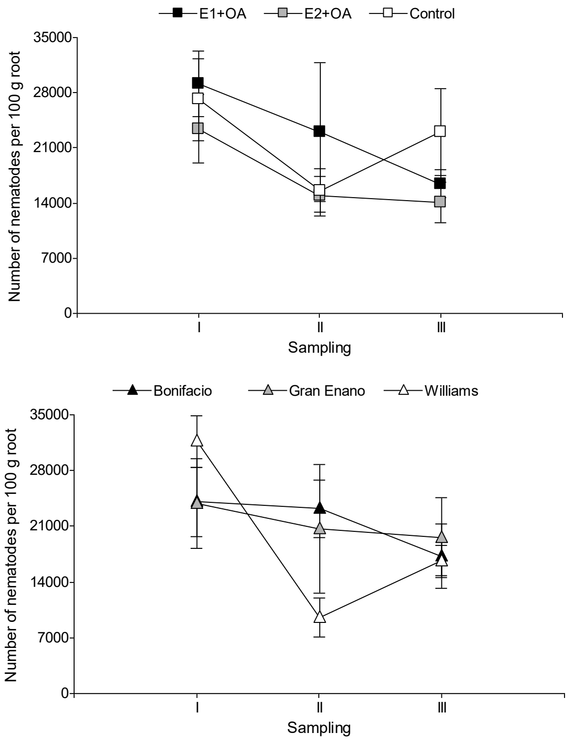

Fig.9. Effect of combined endophitic fungi and organic amendments (E1+OA and E2+OA) and banana cultivars on total plant-parasitic nematode populations during three sampling periods ($p > 0.05$)

Table 4. Population density of PPN per 100g of B, GN, and W cultivars under different treatments.

Treatment	PPN/100 g root			
	R. similis	*M. incognita*	*H. multicinctus*	Total
BE1OA	12611 a	8926 b	3889 c	25426 d
BE2OA	7370 a	5630 b	3981 c	16981 d
BC	11759 a	5981 b	4389 c	22130 d
GNE1OA	12000 a	7074 b	3130 c	22204 d
GNE2OA	8315 a	5500 b	3871 c	17686 d
GNC	11241 a	7630 b	5426 c	24296 d
WE1OA	11185 a	6815 b	2907 c	20907 d
WE2OA	8704 a	6407 b	2596 c	17707 d
WC	10426 a	5611 b	3241 c	19278 d

† Averages with similar lowercase letter are not significantly different ($p > 0.05$).

No significant differences in plant-parasitic nematode (PPN) populations over time ($p > 0.05$) were observed among the treatments. However, interesting interactions were noted between cultivars and control treatments. The treatments BE2OA, GNE2OA, and WE2OA exhibited lower populations of *R. similis*, averaging fewer than 9,000 nematodes per 100 grams of roots. For *M. incognita* the treatments GNE2OA, WC, and BE2OA recorded the lowest population densities, with an average below 6,000 nematodes per 100 grams of roots. In contrast, *H. multicinctus* had populations of fewer than 5,000 nematodes per 100 grams of roots across all treatments. Additionally, the treatments WE1 and WE2 demonstrated even lower PPN populations, averaging below 3,000 nematodes per 100 grams of roots over time (see Table 4).

IV. DISCUSSION

Growth Promotion

Height and pseudostem circumference increased during the experiment, which coincides with the normal plant growth dynamics promoted by division and cell expansion with time (Taiz and Zeiger, 2006). The growth rate of each banana cultivar is a function of its genetics and the influence of abiotic conditions such as nutrition, climate, and crop cultural management.

Treatments showed no statistical differences with respect to plant height and pseudostem circumference. These results are not in line with those who found that banana plants treated with *T. atroviride* presented greater plant height than the absolute control (Cassambai *et al.*, 2012; Chaves *et al.*, 2009; Menjivar *et al.*, 2006). In another study conducted by Pocasangre et al. (2006), a six-centimeters plant height increase was recorded in a banana plant nursery protected with *T. atroviride* and *Fusarium oxysporum.* Pocasangre *et al.* (2004) found that endophytic fungi application in banana plants promotes growth and leads to an increase in length and root weight, in addition to an increase in pseudostem diameter. Radical biomass production and phytonematode biocontrol provided by endophytic fungi increase the radical efficiency of nutrient exploration (Sikora and Pocasangre, 2006). This factor is largely due to the antagonistic characteristics of *T. harzianum* 1295-27, which promote phosphate solubilization and other micronutrient availability necessary for plant growth (Altomare *et al.*, 1999).

There is evidence that *T. atroviride* produces indole-3-acetic acid (IAA), which is used to promote plant growth (Gupta *et al.*, 2014). Association of organic amendments with endophytic fungi increases secretion and production of organic acids such as gluconic, citric, and fumaric acids and phenols, which solubilize and increase the availability of nutrients such as phosphorus, zinc, iron, and manganese, which are necessary for plant development (Yedidia *et al.*, 2011; Altomare *et al.*, 1999). Apart from nutrition improvement, organic amendments also provide a favorable environment for endophytic fungi colonization on plant tissue, thus ensuring maximum growth promotion (Stirling, 2014). The difference in the results of this experiment might be related to incompatibilities between fungi and organic amendments. Therefore, it is necessary to study the compatibility of *T. atroviride* and cacao fruit husk organic amendments so as to obtain expected results (Guetsky *et al*., 2001).

In this study, there was a noticeable reduction in PPN and root damage in the third sample compared to the earlier samplings, indicating that fungi and amendments need time to establish properly in the rhizosphere, and their effect is not immediate but more sustainable over time. Therefore, it is advisable to monitor similar experiments for an extended period to comprehensively determine the biocontrol effects of the fungi and organic amendments on phytonematodes over an extended period.

Root Health

Root health depends on many factors and is highly related to phytonematode population density. In this research, it was quite notorious that the necrosis index, as well as root health, improved substantially in the third sample in comparison with the first sample that reported the highest root damage percentages. Root health improvement and a decrease in root necrosis were observed over time. Pocasangre *et al*. (2004) stated that radical health improvement could be associated with biocontrol and root biomass production enhanced by endophytic fungi and organic amendments, which increase radical nutrient exploration efficiency. Root health improvement implies a greater ability of the plant to tolerate pathogens.

Although no statistically significant differences were obtained between treatments in terms of functional and dead root parameters, functional root weight increases and a decrease in dead root weight were registered over the three samples. This indicates that endophytic fungi and organic amendments have the possibility of improving radical health, regardless of the banana cultivar. Similar results were found by Ochoa and Spiegeler (2014), who obtained functional root weight increases in Boniface and Grand Nain cultivars with Endo 1 application. Similarly, Menjivar *et al*. (2006) found that banana plants inoculated with endophytic fungi on Bananita and Carmen farms in Costa Rica had a superior functional root weight than the absolute control, although there were no significant differences within treatments. In the same investigation, *Trichoderma* sp. and *Fusarium* sp. fungi had statistically superior functional root weight than the absolute control at the FORMOSA farm in Costa Rica. The functional root weight parameter is closely related to plant growth and shows water and nutrient absorption capacity as well as plant anchorage. Rosales (2008) established that there exists a direct relationship between functional root weights and banana productivity. The weight values of functional roots of BE2OA (37.8 g per plant) and GNE1OA (31.4 g per plant) are in the root weight ranges reported by Rosales (2008), who studied the relationship between functional roots and productivity in six banana-growing cantons in Costa Rica. In three cantons of higher banana productivity, functional root weight obtained in Siquirres was between 36 g and 135 g per plant; in Matina, it was between 35 g to 143 g per plant; and in Talamanca, it was between 31 g to 114 g per plant.

For the dead roots variable, treatments showed values that differed from the absolute control for the first and third samples. The third sample stands out a lot, having treatments with the lowest dead root weight values: GNE2OA with 6.00 g, WE1OA with 7.15 g, and GNE1OA with 8.04 g. Similar results were found by Menjivar *et al*. (2006), who obtained 7.1 g of dead roots in the Valery banana cultivar applied with *T. atroviride* on the FORMOSA farm in Costa Rica. This reduction in the number of dead roots coincides with the increase in functional root weight obtained in this research, which shows a positive effect of treatments on radical health. Dead root weight is a radical health indicator that reflects the damage caused by nematodes, rot caused by excess water in the soil, and physical damage to the roots. Per 10 g of dead roots, banana productivity is reduced by 88 boxes/ha on average (Rosales, 2008).

The association of *T. atroviride* with organic amendments did not affect root diameter and total root weight. These results differ from those obtained by Cassambai *et al*. (2012), Chaves *et al*. (2009), and Chaves (2007), who found a significant increase in the total root weight of bananas in greenhouses with *Trichoderma* sp. application. Several studies have shown that endophytic fungi promote growth in banana plants through increased root system development (Chaves *et al*., 2009; Chaves, 2007; Pocasangre *et al*., 2004; Meneses, 2003; Pocasangre, 2003, 2002). Endophytic fungi inoculations increased the total weight of Grand Nain roots by 39 % (Meneses, 2003). The differences in the obtained results of this study may be caused by failure or miniature fungi colonization of root tissue, modified ecology with the use of cocoa-based organic amendments, and nitrogen fertilizer and herbicide application in the experimental field (Guetsky *et al*., 2001). According to Luc *et al*. (2005), antagonistic agents' potential can be increased by the use of organic amendments and green manure in various ways. Amendments play a central role in soil fertility improvement in physical, chemical, and biological aspects. This provides an ideal environment in the rhizosphere that stimulates the colonization of plant tissue by endophytic fungi (Stirling, 2014). Good colonization of *Trichoderma* sp. allows this fungus to cause substantial changes in plants' metabolism, radical health promotion, and nutrient availability, hence increasing crop growth. Khan *et al*. (2012) also reported an increase in root biomass compared to the absolute control treatment. In the same experiment, Khan *et al*. (2012) postulate that organic amendments improve organic matter composition in the soil, which promotes radical health improvement.

Effects on plant-parasitic nematodes

Although there were no statistically significant differences between the treatments, notable variations in the *R. similis* population were observed among the samples. Specifically, the *R. similis* population in the third sample was lower than in the first, indicating a 49% reduction between these two samples. This suggests that endophytic fungi and the applied organic amendments require time to colonize and exert their biocontrol effects. According to Pocasangre (2000), endophytic fungi exhibit antagonistic effects on *R. similis* in the roots of several banana genetic groups, including Grand Nain, Williams, Gros Michel, FHIA 01, and FHIA 23, leading to a population reduction of 79% to 90%. In a further study, Pocasangre (2002) reported a decrease in the *R. similis* population of between 21% and 83% with the application of *Trichoderma* sp. and Fusarium spp. on in vitro plants of the Grand Nain cultivar. Additionally, a field study conducted by Pocasangre *et al*. (2006) demonstrated that inoculation with *T. atroviride* on banana plants reduced the *R. similis* population. This reduction is associated with a significant decrease in the number of females in the root systems of the affected plants (Sikora, 1992). Studies on endophytic bacteria and mycorrhiza have shown that these bacteria enhance plant-mycorrhiza interactions, which in turn stimulate vegetative growth and activate plant defense mechanisms against attacks by phytonematodes (Niere *et al*., 1999; Alamri et al., 2022). The low effectiveness of endophytic fungi observed in this study may be attributed to rhizosphere soil contamination caused by management practices, such as the application of nematicides and fertilizers. These practices are known to affect biodiversity and disrupt interactions within the soil (Altieri, 1992; Tiwari, 2024), which can significantly reduce the antagonistic potential of the microflora in the rhizosphere (Carroll, 1990).

While there were no statistically significant differences between treatments concerning the reduction of *M. incognita* populations, the combination of endophytic fungi with organic amendments reduced the population of this phytonematode by 33% to 36%. Similarly, there was a trend towards reduced *H. multicinctus* populations with the E1+OA application. Boniface and Grand Nain cultivars also exhibited trends of phytonematode population reduction, despite no statistically significant differences between them. These findings are consistent with those reported by Ochoa and Spiegeler (2014), who studied phytonematode biocontrol using *T. atroviride* in Boniface, Grand Nain, and Williams cultivars. Other research has shown that applying endophytic fungi can achieve phytonematode population reductions ranging from 15% to 90% in bananas (Cassambai *et al*., 2012; Chaves *et al*., 2009; Chaves, 2007; Pocasangre *et al*., 2004). To optimize the control of plant-parasitic nematodes, it is essential to identify synergistic effects between biocontrol agents and different banana cultivars (Hallmann and Sikora, 2011; Sikora *et al*., 2010). In this study, no statistically significant differences were observed in the overall total population of plant-parasitic nematodes (PPN) when using the endophytic fungus *T. atroviride* and organic amendments. However, it has been documented that applying organic amendments increases the presence of nematode antagonists in the soil (Stirling, 2011; Luc *et al*., 2005). Decomposing organic matter produces secondary metabolites such as phenols, nitric acid, and ammonia gas, which can interfere with nematode activity (Hallmann and Sikora, 2011). Khan *et al*. (2012) discovered that combining the *T. harzianum* fungus with organic amendments from neem (*Azadirachta indica*) leaves reduces the population of *M. incognita* in eggplants. Similar findings were reported by Stirling *et al*. (2005), who combined *T. harzianum* with sawdust amendments, successfully decreasing the *M. javanica* population in tomatoes over a two-year experiment. Additionally, incorporating sugar cane residue amendments into the field 23 weeks before planting resulted in an 85% reduction in the *Pratylenchus zeae* nematode population in sugar cane roots 24 weeks after planting (Stirling *et al*., 2005). These studies indicate that high carbon-to-nitrogen (C/N) ratio amendments act slowly in suppressing nematode populations. Consequently, the organic amendments used in this study did not achieve the expected nematode control, likely due to insufficient time for decomposition and the influence of other agricultural practices, such as chemical fertilizers and insecticides.

V. CONCLUSIONS

This study indicates that combining *Trichoderma atroviride* strains with cocoa-based organic amendments has potential for managing plant-parasitic nematodes in banana cultivation. While no statistically significant reductions were observed, trends toward improved root health and reduced nematode populations were notable, particularly over time. These findings suggest a delayed but possibly sustained biocontrol effect. Further research is needed to isolate the independent and combined impacts of these treatments and to assess their compatibility under varying agricultural conditions.

ACKNOWLEDGMENT

We gratefully acknowledge EARTH University and Staff for their unwavering support and provision of resources throughout the course of this research.

REFERENCES

[1] Alamri, S., Nafady, N. A., El-Sagheer, A. M., El-Aal, M. A., Mostafa, Y. S., Hashem, M., & Hassan, E. A. (2022). Current utility of arbuscular mycorrhizal fungi and hydroxyapatite nanoparticles in suppression of tomato root-knot nematode. *Agronomy*, *12*(3), 671.

[2] Altieri, M. A. (1992). Biodiversidad, agroecologia y manejo de plagas. Valparaiso (CL): Centro de Estudios en Tecnologías Apropiadas para América. 162 p.

[3] Altomare, C., Norvell W. A., Bjorkman, T. & Harman, G. E. (1999). Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. *Applied and Environmental Microbiology* 65(7):2926-2933.

[4] Araya, M. (2003). Situación actual del manejo de nematodos en banano (*Musa* AAA) y plátano (Musa AAB) en el trópico americano. In Rivas, G. y Rosales, F. (eds.). *Manejo convencional y alternativo de la sigatoka negra, nematodos y otras plagas asociadas al cultivo de Musáceas*: actas del taller realizado en Guayaquil. Ecuador, 11-13 de agosto de 2003. Guayaquil (EC): *INIBAP*, 79-102.

[5] Cassambai, E. L., Lekidayo, L. & Pocasangre, L. E. 2012. Uso de hongos endófitos como promotores de crecimiento en el cultivo de banano. Tierra Tropical [en línea]. Vol. 8, no. 19. [consultado 19agosto 2015], p. 9-18. Disponible en el World Wide Web: <http://webcache.googleusercontent.com/search?q=cache:vd8I7kG1qcgJ:tierratropical.org/wp-content/plugins/download-monitor/download.php%3Fid%3D163+&cd=1&hl=en&ct=clnk>.

[6] Castro, M., Duarte, R., Portillo, C. & Gonzales, J. (2005). Banana yield response to nematode control in Dole farms Costa Rica. In Turner, WD. y Rosales, FE. Banana root system towards a better understanding for its productive management. Proceedings of an international symposium held in San Jose, Costa Rica. San José (CR). p. 24-25.

[7] Chaves, N. (2007). Utilización de bacterias y hongos endofíticos para el control biológico del nematodo barrenador *Radopholus similis* (Cobb, Thorn) [Tesis Mag. Sc.]. Turrialba (CR): CATIE. 82 p.

[8] Chaves, N. P., Pocasangre, L. E., Fritz, E., Rosales, F. E. & Sikora, R. A. (2009). Combining endophytic fungi and bacteria for the biocontrol of *Radopholus similis* (Cobb) Thorne and for effects on plant growth. *Scientia Horticulture,* 122(3),472 -478.

[9] Di Rienzo J. A., Casanoves F., Balzarini M. G., Gonzalez L., Tablada M. & Robledo C. W. InfoStat versión (2020). Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar

[10] Dita, M. A., Garming, H., Van den Bergh, I., Staver, C., & Lescot, T. (2011, October). Banana in Latin America and the Caribbean: current state, challenges and perspectives. In *VII International Symposium on Banana: ISHS-ProMusa Symposium on Bananas and Plantains: Towards Sustainable Global Production 986* (pp. 365-380).

[11] FAO-Food and Agricultural Organization of the United Nations. 2016. Crops. FAOSTAT. http://www.fao.org/faostat/ en/#data/QC.

[12] Guetsky, R., Shtienberg, D., Elad, Y., & Dinoor, A. (2001). Combining biocontrol agents to reduce the variability of biological control. *Phytopathology*, *91*(7), 621-627.

[13] Gupta, V. K., Schmoll, M., Herrera, A. E., Upadhyay, R. S., Druzhinina, I. and Tuohy, M. G. (2014). Biotechnology and biology of *Trichoderma*. 1ª ed. Oxford (GB): Elsevier. 405 p.

[14] Hallmann, J., & Sikora, R. A. (2011). Endophytic fungi. In Davies, K. y Spiiegel, Y. (eds.). Biological control of plant – parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms. New York (US): Springer Science & Business media B.V. 305 p.

[15] Jones, D. R. (2007, September). Disease and pest constraints to banana production. In *III International Symposium on Banana: ISHS-ProMusa Symposium on Recent Advances in Banana Crop Protection for Sustainable 828* (pp. 21-36).

[16] Khan, M. R., Mohiddin, F. A., Ejaz, M. N., & Khan, M. (2012). Management of root-knot disease in eggplant through the application of biocontrol fungi and dry neem leaves. *Turkish Journal of Biology*, *36*(2), 161-169.

[17] Luc, M., Sikora, R. A. & Bridge, J. 2005. Plant parasitic nematodes in subtropical and tropical agriculture. Vol. 2. Londres (GB): CABI. 877 p. ISBN 085199 727 9.

[18] Meneses, A. 2003. Utilización de hongos endofíticos provenientes de banano orgánico para el control biológico del nematodo barrenador *Radopholus similis* Cobb, Thorne. [Tesis Mag. Sc.]. Turrialba (CR): CATIE. 67 p.

[19] Menjivar, R. D., Pocasangre, L. E., Zum Felde, A., Riveros, A. S., Rosales, F. E. & Sikora, RA. 2006. Hongos endofíticos como agentes biológicos de control de fitonematodos en banano. In 17 Reunión Internacional de Asociación para la cooperación de Pesquisas sobres Banana en el Caribe y América Tropical, 15 a 20 de octubre de 2006-Joineville-Santa Catarina-Brasil. Joinville (BR): ACORBAT. p 249-254.

[20] Niere, B. I., Speijer, P. R. & Sikora, R. A. (1999). A novel approach to the biological control of banana nematodes. In Deutscher Tropentag 1999 in Berlin. Session: Sustainable Technology Development in Crop Production. 6 p.

[21] Ochoa, M. P. M. & Spiegeler, J. E. S. 2014. Evaluación de dos cepas de *Trichoderma atroviride* para el biocontrol de nematodos en tres líneas Cavendish. [Proyecto de Graduación Lic. Ing. Agr.]. Guácimo (CR): Universidad EARTH. 39 p.

[22] Ploetz, R. C., Thomas, J. E. & Slabaugh, W. R. 2003. Diseases of banana and plantain. In Ploetz, RC. (ed.). Diseases of tropical fruits crops. Wallingford (GB): CABI Publishing. 227 p.

[23] Pocasangre, L. E. (2000). Biological enhancement of banana tissue culture plantlets with endophytic fungi for the control of the burrowing nematode *Radopholus similis* and the Panama disease (Fusarium oxysporum f. sp. cubense) [Tesis Ph. D.] Bonn (DE): University of Bonn. 95 p.

[24] Pocasangre, L. E. (2002). Mejoramiento biológico de vitro plantas de banano mediante la utilización de hongos endofíticos para el control del nematodo barrenador *Radopholus similis*. In Riveros, AS.; Pocasangre, LE.; Rosales, FE. (eds.). In Taller internacional sobre Inducción

de Resistencia y Uso de Tecnologías Limpias para el Manejo de Plagas en Plantas, Turrialba, Costa Rica, 27-30 Agosto de 2002. Turrialba (CR). p. 33-39.

[25] Pocasangre, L. E. (2003). Nuevas estrategias para el manejo de nematodos en musáceas. In Rivas, G. y Rosales, F. (eds.). Manejo convencional y alternativo de la Sigatoka negra, nematodos y otras plagas asociadas al cultivo de Musáceas en los trópicos. In Actas del taller, Guayaquil, Ecuador, 11-13 de agosto de 2003. Guayaquil (EC): INIBAP. p.121.

[26] Pocasangre, L. E. (2013). Manejo sostenible de fitonematodos en plantaciones comerciales de banana. In Pocasangre, LE. (ed.). Producción sostenible de banano: Mejoramiento genético, seguridad alimentaria, manejo agronómico y carbono neutralidad, Tributo Dr. Phil Rowe. In Congreso Internacional de Banano. Realizado el 29-31 de Julio, 2013, Las Mercedes de Guácimo, Costa Rica. Limón (CR). 47 p.

[27] Pocasangre, L. E., Pérez-Vicente, L., & Ferris, H. (2015). CHAPTER 5.8: Organic Banana Disease Management. In *Plant Diseases and their Management in Organic Agriculture* (pp. 351-365). The American Phytopathological Society.

[28] Pocasangre, L., Sikora, R. A., Vilich, V., & Schuster, R. P. (1999, June). Survey of banana endophytic fungi from Central America and screening for biological control of Radopholus similis. In *II ISHS Conference on Fruit Production in the Tropics and Subtropics 531* (pp. 283-290).

[29] Pocasangre, L., Zum Felde, A., Meneses, A., Cañizares, C., Riveros, A., Rosales, F. & Sikora, R. 2004. Manejo alternativo de fitonematodos en banano y plátano. In Memorias: 16 Reunión Internacional de Asociación para la cooperación de Pesquisas sobres Banana en el Caribe y América Tropical, 2004 realizado en Oaxaca, México. Oaxaca (MX): ACORBAT. p. 106-112. Publicación especial.

[30] Riveros, A. S. A. 2010. Inducción de resistencia en plantas: interacción: planta-patógeno. San José (CR): IICA. 261 p. ISBN 978-92-9248-185-8.

[31] Rosales, F. E., Pocasangre, L. E., Trejos, J., Serrano, E., Peña, W. & Brown, D. (2008). Guía de diagnóstico de la calidad y salud de suelos bananeros. Bioversity International. 38 p.

[32] Sikora, R. A. (1992). Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. *Annual review of phytopathology*, *30*(1), 245-270.

[33] Sikora, R. A., & Pocasangre, L. (2006, October). The concept of a suppressive banana plant: root health management with a biological approach. In *Proceedings of the XVII ACROBAT International Congress* (Vol. 1, pp. 241-248).

[34] Sikora, R. A. & Schuster, R. P. (1998). A novel approach to nematode IPM. In Frison, EA, Gold, CS.; Karamura, EB. y Sikora, RA. (ed). In Mobilizing IPM for sustainable banana production in Africa. Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28 November 1998. Nelspruit (SA). p. 27-137.

[35] Sikora, R. A., Zum Felde, A., Mendoza, A., Menjivar, R., & Pocasangre, L. (2008, October). In planta suppressiveness to nematodes and long term root health stability through biological enhancement-Do we need a cocktail?. In *IV International Symposium on Banana: International Conference on Banana and Plantain in Africa: Harnessing International 879* (pp. 553-560).

[36] Soto, M. (2008). Bananos. Técnicas de producción, manejo postcosecha y comercialización. 2ª.ed. San José (CR). 1100 p.

[37] Stirling, G. R. (2011). Biological control of plant-parasitic nematodes: An ecological perspective, a review of progress and opportunities for further research. In Davies, K. y Spiiegel, Y. (eds). Biological control of plant – parasitic nematodes: Building coherence between microbial ecology and molecular mechanisms. New York (US): Springer Science & Business Media B. V. 305 p. ISBN 978-1-4020-9647-1.

[38] Stirling, G. R. (2014). Biological control of plant–parasitic nematodes: soil ecosystem management in sustainable agriculture. Vol. 2. Oxfordshire (GB). CABI. 430 p.

[39] Stirling, G. R., Wilson, E. J., Stirling, A. M., Pankhurs, C. E., Moody, P. W., Bell, M. J., & Halpin, N. (2005). Amendments of sugarcane trash induce suppressiveness to plant-parasitic nematodes in a sugarcane soil. *Australasian Plant Pathology*, *34*(2), 203-211.

[40] Taiz, L., & Zeiger, E. 2006. Fisiología vegetal. Vol. 2. Sunderland (US): Universitat Jaume. 1180 p. ISBN 978-84-8021-600-5.

[41] Timper, P. 2014. Conserving and enhancing biological control of nematodes. Journal of Nematology 46(2),75-89.

[42] Tiwari, S. (2024). Impact of nematicides on plant-parasitic nematodes: Challenges and environmental safety. *Tunisian Journal of Plant Protection*, *19*(2).

[43] UN-United Nations Department of Economic and Social Affairs (2019). UN Comtrade Database. https://comtrade.un.org/data/

[44] van Asten, P. J., Fermont, A. M., & Taulya, G. (2011). Drought is a major yield loss factor for rainfed East African highland banana. *Agricultural water management*, *98*(4), 541-552.

[45] Yedidia, I., Srivastva, A. K., Kapulnik, Y., & Chet, I. (2001). Effect of Trichoderma harzianum on microelement concentrations and increased growth of cucumber plants. *Plant and soil*, *235*(2), 235-242.

[46] Zum Felde, A., Pocasangre, L. E. & Sikora, R. A. (2005). The potential use of microbial communities inside suppressive banana plants for banana root protection. In Rosales, FE. y Turner, WD. (eds.). Banana root system: towards a better understanding for its productive management. Proceedings of an international symposium held in San José, Costa Rica, 3-5 November 2003. San José (CR). 169-177.

[47] Zum Felde, A., Pocasangre, L. E., Carñizares-Monteros, C. A., Sikora, R. A., Rosales, F. E., & Riveros, A. S. (2006). Efecto de inoculaciones combinadas de hongos endofíticos en el biocontrol de Radopholus similis. *Infomusa*, *15*(1-2), 12-18.