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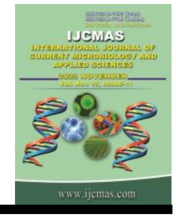

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# Banana (*Musa* AAA cv Williams) Response to Biological and Chemical Nematicides and their Rotation

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## A B S T R A C T

The effect of different biological and chemical nematicide cycles per year or their alternation was compared on banana (*Musa* AAA cv. Williams) root weight, root nematode control and crop yield in a commercial banana plantation in Ecuador testing 11 treatments in a randomized complete block design with five replicates. Treatments consisted of the rotation of two or three chemicals or even the rotation of four biological nematicide cycles by year or the rotation of chemical and biological nematicides in the year plus the untreated control. Averaging the 24 root nematode samplings, the rotation of two and three chemical nematicide cycles by year reduced *R. similis* (P= 0.0347) by 25 and 23%, *Helicotylenchus* spp. (P= 0.0009) by 31 and 18% and total nematodes (P= 0.0074) by 28 and 21%, respectively. The rotation of four biological nematicide cycles by year drops *R. similis* between 10 and 28%, *Helicotylenchus* spp. between 5 and 21% and total nematode between 5 and 23%. Although there is substantial, positive literature on nematode control with biological products, for us is the first time that some reduction was observed. Given the COVID-19 pandemic, it was not possible to do successive harvests, but at the final harvest, from 23 to 25 months after first treatment application, a difference in yield (P< 0.0001) was observed. The highest number of boxes per hectare per year was found in the treatment with 4 cycles by year of SeaMaxx® + Cronox® with 4149 followed closely by two chemical nematicides cycles by year with 4122 boxes of 18.14 kg. The additional net income in the treatments that increased yield, deducted the cost of packing for each additional box and the treatment cost was between US $327 and $3420 ha$^{-1}$ year$^{-1}$ 24 months after the first treatment was applied. However, the net profit for every dollar invested in nematode control was higher with two chemical nematicides with $20.2 compared to $6.9 returned for the four cycles of SeaMaxx® + Cronox® due to frequent of application and higher treatment cost.

**Keywords**

*Helicotylenchus* spp., *Radopholus similis*, root weight, yield

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## Introduction

Abiotic (soil texture, wind, radiation, temperature, rain) and biotic (black Sigatoka, nematodes, mealybugs, scales, black weevil) factors affect banana (*Musa* AAA) yield. Within the biotic factors, the nematodes are the main root pests of the crop. According to different authors (Chávez and Araya, 2001; 2010, Aguirre *et al.,* 2016a; 2016b) nematodes are common in the five provinces (Cañar, El Oro, Guayas, Los Ríos and Santo Domingo) where banana is cultivated in the country. Usually only polyspecific communities occur, consisting of a mixture mainly of *Helicotylenchus* spp. and *Radopholus similis*, with low frequency and numbers of *Pratylenchus* spp. and *Meloidogyne* spp. Nematodes delay foliar emission, lengthen the crop's vegetative cycle, reduce bunch weight and yield (Quénéhervé *et al.,*1991a, 1991b; Jaramillo *et al.,* 2019; Chávez *et al.,* 2020; Medina *et al.,* 2022).

Chemical nematicides are still feasible and economic option for banana root nematode control. Their applications are carried out when nematode analyzes indicate populations above the established economic threshold. The molecules approved for use in bananas are alternated according to their physico-chemical characteristics, considering the climatic condition to prevent their biodegradation. However, certifications, supermarkets and end users are looking for a final consumer fruit obtained with a low use of agrochemicals, especially of the toxicological profile IA and IB. The nematicides available to control nematodes in bananas belong mostly to these toxicological bands, which limits and restricts their application.

On the market there are fungal and bacterial nematicides (Abb-Elgawad and Hassan, 2018; Ruiu, 2018). Within these, *Trichoderma* species (Cumagun and Moosavi, 2015; Hernández *et al.,* 2016; Poveda *et al.,* 2020; D'Errico *et al.,* 2020) and different species of *Bacillus* (De Araujo and Pletto 2009, Cumagun and Moosavi 2015, Gao *et al.,* 2016, Villarreal-Delgado *et al.,* 2017; Radhakrishnan *et al.,* 2017) are applied to various crops to control nematodes. In the case of fungi, it is known that they reduce nematode populations infecting eggs and females of sedentary endoparasitic nematodes such as *Meloidogyne*, *Heterodera*, *Globodera* (Manzanilla *et al.,* 2013).

However, they have been also evaluated in migratory endoparasites such as *R. similis* (Vergara *et al.,* 2012) and their application had promoted plant growth (Hernández *et al.,* 2016). In the case of *Bacillus* spp., it is reported that secrete several metabolites that trigger plant growth and prevent pathogen infection, that induced physiological changes in plants as an adaptation to abiotic and biotic stresses, and degrading substances from *Bacillus* spp. damage pathogenic bacteria, fungi, nematodes, viruses, and pests (Radhakrishnan *et al.,* 2017; Li *et al.,* 2018; Borriss, 2020; Anckaert *et al.,* 2021). Bacteria and their metabolites affect both plant and microbial community (Burkett *et al.,* 2008; Berg *et al.,* 2017). Direct antagonistic effect can be achieved by parasitism, antibiosis, or competition for nutrients or infection sites. Indirectly, bacteria can enhance host defense mechanisms inducing systemic resistance (Raymaekers *et al.,* 2020; Borriss, 2020; Migunova and Sasanelli, 2021).

In addition, there are on the market, seaweed (*Ascophyllum nodosum*) extracts (Veronico and Melillo, 2021; Williams *et al.,* 2021; Wu *et al.,* 1998; Whapham *et al.,* 1994; Tarjan, 1977) that are applied to various crops to control sedentary nematodes (Manzanilla *et al.,* 2013) such as *Meloidogyne*, *Heterodera* and *Globodera* and in some cases for migratory endoparasites such as *Pratylenchus* (Tarjan, 1977). Wu *et al.,* (1997), Veronico and Melillo (2021) and Williams *et al.,* (2021) attribute the nematode control to the betaine content. Also, there are extracts of *Azadirachta indica* (Musabyimana and Saxena, 1999; Bartholomew *et al.,* 2014; Khan *et al.,* 2017) that are applied for root nematode control. The azadirachtin primarily interferes with the transformation of ecdysone to 20-hydroxy ecdysone disrupting chitin synthesis and other cascade events necessarily for the nematode to molt.

Medina *et al.*, (2022) testing on bananas products that contain some of the mentioned microorganisms, found a non-significant reduction of 6.9% of *R. similis*, 9% of *Helicotylenchus* spp. and 7.3% on the total nematode phytoparasitic population. Following the trend of consumers and the market, it is necessary to continue looking for available biological nematicides that be effective for banana nematode control. Therefore, the objective of the present experiment was to compare the effect of the rotation of chemical or biological nematicides or the rotation of chemical with biological nematicides or consecutive application of biological nematicides on nematode control, root system recovery and banana (*Musa* AAA) yield.

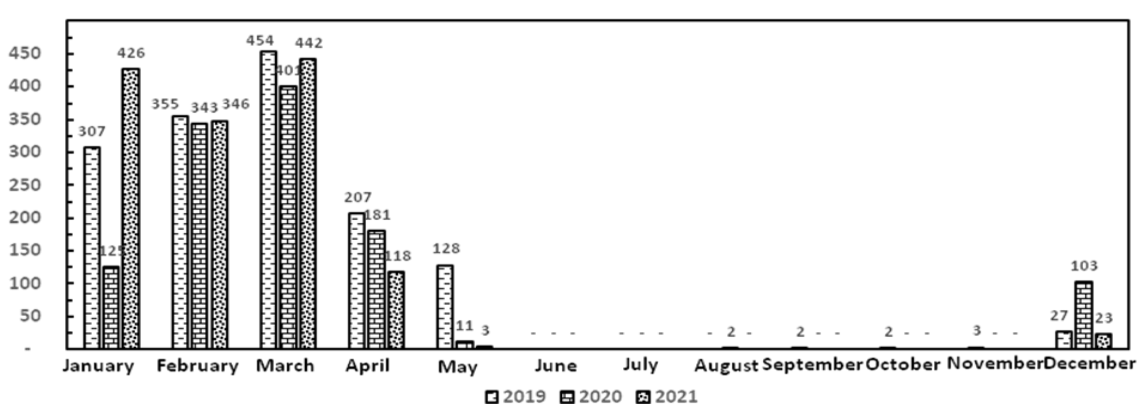
## Materials and Methods

The field experiment was carried out for 24 months within a nematode infected long-term ratoon commercial banana (*Musa* AAA cv. Williams) plantation, located in Milagro County, province of Guayas, Ecuador. The soil was taxonomically classified as an Inceptisol, and it had a silty clay loam texture (29% sand, 41% silt and 30% clay) with a pH of 6.5 and 1.6% organic matter.

The following concentrations of extractable cations were found, using Mehlich 3 (Mehlich, 1984) as the extractant: Ca 11.3, Mg 1.63, and K 0.67 cmol $L^{-1}$, and P 21.0, Zn 1.6, B 0.90, Cu 4.2, Fe 93.6 and Mn 29.0 µg $ml^{-1}$. The block or cable where the experiment was established had an annual yield of 3357 boxes of 18.14 kg per hectare for 2019, with a plant density of 1450 plants by hectare. The evaluation period was performed from September 2019 to October 2021 in which the density was reduced to 1267 plants by hectare.

De-suckering was carried out every eight weeks, leaving each production unit with a bearing mother plant, a large daughter sucker (follower) and a small grand-daughter (peeper) when possible. Shooting plants were propped with double polypropylene twine to the bottom of two well-developed adjacent plants. The follower sucker of each production unit was fertilized every 15 days with a mixture of nutrients at 100 kg $ha^{-1}$ adapted to the soil and crop requirements, consisting of 15-4-36 (N-$P_2O_5$-$K_2O$) fertilizers completing 385 kg N, 92 kg $P_2O_5$, 675 kg $K_2O$ in the year. Generally, during the rainy season, from January to May each year, water requirements was supplied by rainfall, where the annual precipitation was of 1482, 1164, and 1358 mm per year, for 2019, 2020 and 2021, respectively (Figure 1). A complex system of primary, secondary, and tertiary drains was provided to disperse excess rainfall and prevent water logging during heavy rains. From June to December each year, water was supplied mainly by sprinkling irrigation. Mean daily minimum/maximum temperatures for 2020 and 2021 were 19.1-19.4/35.1-33.7°C, respectively.

**Fig.1** Rainfall (mm) by month of the year during the time the experiment lasted from a weather station located within the farm where the experiment was set up.



Leaf fungi, especially black Sigatoka (*Pseudocercospora fijiensis*), was managed by deleafing weekly to reduce the pressure of black Sigatoka inoculum and by aerial spraying of alternate fungicides which resulted in 28 sprayings each year at 8 to 14 days intervals. The fungicides applied were: azoxystrobin, difenoconazole, epoxiconazole, tebuconazole, pyrimethanil, mancozeb, in emulsion with miscible oil (Spraytex) and water or in a water solution of 19 L $ha^{-1}$. Weeds were controlled spraying every 12 weeks a glyphosate solution of 1.5 L in 200 L of water with a manual knapsack sprayer. Before setting the experiment, nematodes were controlled every year by the rotation of one or two nematicide applications (Verango® 50SC-fluopyram-Bayer, Vydate® 24SL oxamyl-DuPont, Counter® 15GR-terbufos-AMVAC, Mocap® 15GR-ethoprophos-AMVAC) per year, based on the nematode population.

Eleven treatments were evaluated; 1: untreated control, 2. rotation of three chemical nematicide cycles by year, every four months (Verango®, Rugby®, Counter®, Vydate®, Mocap®, Counter®, Verango®), 3. rotation of two chemical nematicide cycles by year, every 6 months (Rugby®, Vydate®, Counter®, Mocap®, Rugby®), 4: rotation of chemical and biological nematicides, 4 cycles by year, every three months (Counter®, NemaRoot® 2 kg $ha^{-1}$, Mocap®, Cronox® 3 kg $ha^{-1}$ + Rhizomagic® 3 L $ha^{-1}$, Rugby®, Biosiembra products (Sirius® 1.25 L $ha^{-1}$ + Nutriaccion® 2.5 L $ha^{-1}$ + Mayestik® 1.25 L $ha^{-1}$), Vydate®, Cronox® 3 kg $ha^{-1}$+ Rhizomagic®3 L $ha^{-1}$, Mocap®), 5. Verango® one cycle by year plus the rotation of two biological nematicides by year, every four months (Verango®, Biosiembra products, Cronox® 3 kg $ha^{-1}$ + Rhizomagic® 3 L $ha^{-1}$, Verango®, NemaRoot® 2 kg $ha^{-1}$, Biosiembra products, Verango®), 6. Geosorganic (Biotrich® 1 L $ha^{-1}$+ Nutribacter® 1 L $ha^{-1}$+ Pochonia Root® 2 L $ha^{-1}$+ Nematex® 2 L $ha^{-1}$) four cycles by year every three months, 7. SeaMaxx® 3 L $ha^{-1}$ + Cronox® 3 kg $ha^{-1}$, four cycles by year every three months, 8. Ecozin Plus® 4.5 L $ha^{-1}$ four cycles by year every three months, 9. Cronox® 3 kg $ha^{-1}$ + Rhizomagic® 3 L $ha^{-1}$ four cycles by year every three months, 10. SeaMaxx® 10 L $ha^{-1}$ four cycles by year every three months, and 11. Biosiembra products four cycles by year every three months (Table 1).

The applied chemical nematicides were those available including Verango® fluopyram-Bayer, Counter® 15GR biodac-terbufos-AMVAC, Mocap® biodac-ethoprophos-AMVAC, Vydate® oxamyl-DuPont, and Rugby®-cadusafos-FMC (Table 1). The rates used per follower sucker were the recommended by the manufacturer in the product label of 0.3 g a.i. for Verango®, 3 g a.i. for Counter® and Mocap®, 2.4 g a.i. for Vydate® and 2 g a.i. for Rugby®. Verango® was applied in a water solution spreading 100 ml onto the soil surface with the manual knapsack hand sprayer (Protecno).

The biological nematicides applied were: NemaRoot® (*Paecilomyces lilacinus* 2 x $10^9$ ufc $g^{-1}$ – Innovak Global), SeaMaxx® (*Ascophyllum nodosum* extract 20%, N 3.8%, $P_2O_5$ 1.8%, $K_2O$ 3%, B 0.0136%, Cu 0.0036%, Fe 0.01%, Mn 0.024%, Mo 0.00087%, Zn 0.011% – FMC), Cronox® (*Trichoderma asperellun* 1 x $10^9$ ufc $g^{-1}$ -Biotor Labs), Sirius® (*Paecilomyces lilacinus* 1 x $10^9$ ufc $ml^{-1}$ - Biosiembra nature´s lab), Nutriaccion® (*Bacillus subtilis* 1.0 x $10^{13}$ ufc $ml^{-1}$ - Biosiembra nature´s lab), Mayestik® (*Paecilomyces fumosorosea* 1 x $10^9$ ufc $ml^{-1}$ - Biosiembra nature´s lab) 1.25 L $ha^{-1}$, Rhizomagic® (*Ascophylum nodosum* extract 46%, N 4.3%, $P_2O_5$ 3.8%, $K_2O$ 2.6%, B 800 ppm, Cu 100 ppm, Fe 300 ppm, Mn 850 ppm, Mo 50 ppm, Zn 800 ppm – FMC), Biotrich® (*Trichoderma harzianun* 1.0 x $10^9$ ufc $ml^{-1}$ - Geos Organic), Nutribacter® (*Bacillus subtilis* 1.0 x $10^{11}$ ufc $L^{-1}$ Geos Organic), Pochonia Root® (*Pochonia chlamydospora* 1.0 x $10^{10}$ ufc $L^{-1}$ - Geos Organic), Nematex® (*Paecilomyces lilacinus* 1 x $10^9$ ufc $ml^{-1}$ – Geos Organic).

The rate per hectare of each biological nematicide was divided by the plant density by hectare. Since the biological nematicides were applied with a manual knapsack hand sprayer (Protecno 20 L) calibrated to discharge 150 ml of solution in two pumpings, it was filled with 10 L of water, the amount of product corresponding to 120 plants was added and after shaken it was gauged to 18 L, then re-shaked again and thereafter 150 ml of the solution was applied in front of each follower sucker.

The rectangular plots for each treatment consisted of 150-175 production units. Plots were arranged in a randomized complete block design with five replicates. The application of the chemical or biological nematicides was made by spreading the product in a banded arc with a radius of approximately 0.40 meter around each follower sucker pseudostem, sprouting from the base of the sucker, using the Swissmex backpack equipment specific for Counter®, Mocap®, and Rugby®, the spotgun for Vydate® and the Protecno-20 L manual knapsack hand sprayer for Verango®, and the biological nematicides. Plant debris was removed from the soil surface prior to distributing the chemical and biological nematicides onto moist soil as directed by the product label. During the development of the experiment, no rooting or organic matter was applied in the experimental area.

One day before the nematicide application, and then every 30 days up to the 24 months that the experiment lasted, root samples were collected in each repetition. Each sample consisted of the roots of three follower suckers between 1.5-2.5 m height from recently flowered plants or prompt to bearing. In front of each follower sucker, a hole of 30 cm length, 15 cm wide and 30 cm depth (soil volume of 13.5 L) was dug at the plant base using a shovel. All the roots found were collected and placed in labelled (treatment and repetition) plastic bags and delivered to ANEMAGRO nematology laboratory in coolers.

In the laboratory, the root samples were registered and processed as soon as possible, and when it was necessary, stored in a refrigerator Indurama serie RS-10989-593 adjusted to 6-8 $^{o}$C until being processed. The roots were rinsed free of soil, separated in living roots (white or cream-colored roots), dead roots by nematodes (with symptoms of nematode damage, with light necrosis, but without root decay) and dead roots by other causes (rotten roots by excess water, snapping), left to dry off the surface moisture and weighed (Cas computing scale precision 5 kg ± 1 g). During the root separation process, in some roots, it was necessary to cut some damaged parts, which were classified accordingly. The total root weight corresponds to the sum of living roots, dead roots by nematodes and roots dead by other causes.

The three types of roots were cut into 1-2 cm long pieces separately and after homogenization, 25 g were randomly selected following the found proportion of each type of root. For example, in a sample of 52 g of total roots, with 44.5 g of living roots, 3 g of dead roots by nematodes, and 4.5 g of dead roots by other causes, there would be 85.5% of living roots, 5.7% of dead roots by nematodes, and 8.6 % of dead roots by other causes that multiplied by the used sample size of 25 g, would have 21.4 g of living roots, 1.4 g of dead roots by nematodes and 2.2 g of dead roots by others causes in the 25 g sample. These roots were macerated (Araya, 2002) in a kitchen blender (Osterizer; Sunbeam-Oster) for two periods of 10 seconds, at low and then at high speed, and nematode recovered in 0.025 mm (No 500) sieve. The nematodes were identified at the genus and species level, when possible, based on the morphological characteristics under a light microscope, following the key of Siddiqi (2000). The population densities of all plant-parasitic root nematodes present were recorded, and the values were converted to numbers per 100 g of roots. Total nematodes correspond to the sum of the phytoparasitic nematode species detected.

When starting the experiment, in each repetition, 20 production units selected randomly, excluding those from plot edges, edge drains, cable edges, dumpings, replanting plants or with double ratoon sucker, were progressively harvested from September 19, 2019, to November 21, 2019, which corresponded to the parent plant. The stem of each parent plant harvested was labelled with a code number (treatment, repetition, plant number 1 to 20; Ej: T-1, R-1, P-1), and date of harvest, bunch weight, number of hands by bunch were recorded separately for each production unit. Then the code number and date of harvest of each parent plant was passed to its follower sucker and at its harvest (first ratoon crop), date of harvest, bunch weight, number of hands by bunch were recorded separately for each plant but given the COVID-19 pandemic was impossible to do the harvest on all the ratoon suckers, reason why that sequence was lost. Then a final harvest was done at the end of the experiment, from August 22, 2021, to November 30, 2021, on another 20 production units, again selected randomly, excluding those from plot edges, edge drains, cable edges, dumpings, replanting plants or with double ratoon sucker, were also progressively harvested, starting 23 months after the first treatment application, and finishing 25 months from the first treatment application.

Harvesting of the parent plants, when the experiment was set up, and at the final harvest, was done by calibration, starting when bunches reached 10 weeks of age. When in the second hand, the central fruit of the outer whorl had a diameter of at least 35,7 mm-diameter the bunch was harvested. Bunch weight (Tru-Test electronic scale XR3000 kg ± 1g), and number of hands by bunch were recorder. The ratio, which is the number of boxes of 18.14 kg given by each bunch, was calculated considering a reduction of 23%, because was the average of the farm during the experimental time, which includes 12% of bunch stalk and 11% of non-marketable fruit. With the data of the number of bunches harvested in 2019 in the cable where the experiment was located, and the number of plants in that area, the initial ratooning was calculated in 1.52.

Since experimental plots were between 150 and 175 production units each, and the date of bunch harvest was registered for each production unit in the final harvest, which started in all plots and treatments in 08-22-2021 and finished for T-1 and T-7: 11-01-2021, T-2:11-15-2021, T-3: 11-21-2021, T-4 and T-9: 11-8-2021, T-5, T-6, T-8, T-10 and T-11: 11-30-2021, the ratooning in each repetition and treatment was calculated considering the difference in days to harvest compared to the untreated control. Root and nematode data were averaged by experimental plot across the 23 months excluding the first evaluation pre-treatment application. The composition of the nematode population was determined before treatment application and then for the average of the 23 root samplings. Data of root weights before treatment application, and thereafter for the average of the 23 root samplings, were subjected to ANOVA by Proc GLM of SAS and mean separation by LSD-test.

The number of nematodes was analyzed with generalized linear models, using the log transformation as link function and negative binomial distribution of the errors for the first nematode sampling alone, and thereafter for the average of the 23 nematode samplings together after the application.

Bunch weight, ratio, ratooning, and number of boxes of 18.14 kg per hectare per year (97% bunch recovery; 1406 initial 2019, 1229 bunches final 2021 * ratio * ratooning) were averaged for each repetition and harvest and subjected to ANOVA in PC-SAS® version 9.4.

## Results and Discussion

In the root sampling carried out pre-treatment application, no differences were found in the content of living roots (P= 0.1081), dead roots by nematodes (P= 0.5502), dead roots by other causes (P= 0.7628), total roots (P= 0.2669), nor in the percentage of living roots (P= 0.2961). The contents varied between 22.2 and 44.9 g for living roots, the dead roots by nematodes ranged between 8.6 and 16.8 g, the dead roots by other causes oscillated between 1.0 and 2.8 g, and the total roots between 32.1 and 59.7 g per follower sucker (Figure 2A-D). The percentages of living roots by follower sucker ranged between 60.2 and 76.4% (Figure 2E). Similarly, in this sampling, no difference was detected in the population per 100 g of roots per follower sucker for *R. similis* (P= 0.2730), *Helicotylenchus* spp. (P= 0.1821) and total nematodes (P= 0.2673) among the treatments (Figure 3A-F). Nematode populations among treatments fluctuated for *R. similis* between 5240 and 9240, for *Helicotylenchus* spp. between 7400 and 13160 and for total nematodes between 16040 and 27520 individuals per 100 g of roots by follower sucker. The composition of the phytoparasitic nematode population before treatments application was: 32.4% of *R. similis*, 45.3% of *Helicotylenchus* spp. 13.9% of *Pratylenchus* spp. and 8.4% of *Meloidogyne* spp. (Figure 4A).

Root content and nematode populations through the 24 samplings are presented in Figure 2A-E and Figure 3A-C. Across the different samplings, with few exceptions, the root content and nematode populations followed a similar pattern in all treatments. After treatments application, when comparing the average of the 24 samplings among treatments, no differences were found in living roots (P= 0.2399), dead roots by nematodes (P= 0.1065), dead roots by other causes (P= 0.2108) and total root weight (P= 0.3221), which ranged between 20.3 and 23.9 g, between 5.7 and 7.4 g, between 0.7 and 1.2 g and between 27.6 and 31.6 g per follower sucker, respectively (Figure 5A-D). The percentage of living roots was lower (P=0.0183) in the untreated follower suckers with 70.4% vs 76.9% in those treated with two chemical nematicide cycles by year (Figure 5E).

The highest nematode population of *R. similis* (P= 0.0347), *Helicotylenchus* spp. (P= 0.0009) and total nematodes (P= 0.0074) was found in the untreated suckers with 6234; 7869 and 17147 individuals by 100 g of roots by follower sucker, respectively (Figure 6A-C). Compared to the untreated suckers, the treatment with the rotation of two and three chemical nematicides reduced *R. similis* by 25 and 23%, *Helicotylenchus* spp. by 31 and 18%, and the total nematode population by 28 and 21%, respectively. In the suckers treated with the rotation of four biological nematicide cycles by year the reduction was for *R. similis* between 10 and 28%, *Helicotylenchus* spp. between 5 and 21% and the total nematode population between 5 and 23% (Figure 6A-C). The rotation with chemical and biological nematicide treatments drop *R. similis* by 12 and 15%, *Helicotylenchus* spp. by 13 and 22% and total nematode population by 12 and 19%.

Averaging the 23 samplings taken after treatments application, the nematode population composition maintains the same pattern, but changing the proportions, where *R. similis* increased to 36.4%, *Helicotylenchus* spp. to 46.3% and *Pratylenchus* spp. and *Meloidogyne* spp. was reduced to 11.4%, and *Meloidogyne* spp. to 5.9% (Figure 4B).

The initial ratooning in the experimental area was calculated with the total number of bunches harvested in the cable during 2019 with was divided by the number of plants (1450) by hectare in that cable, resulting in 1.52 bunches harvested in each banana stool per year, which was equivalent to an interval between harvests of 240 days. In the harvested done when the experiment was set up, bunch weight (P= 0.8391) was similar among treatments varying between 36.9 and 40.0 kg per bunch. In parallel, the number of hands (P= 0.8868) that varied between 9.1 and 9.6 per bunch, and the ratio (P= 0.8412), that fluctuated between 1.57 and 1.70 boxes (18.14 kg) per bunch, were also similar among treatments (Table 2). So, in congruence, the yield, which ranged between 3358 and 3638 boxes of 18.14 kg per hectare per year was similar (P= 0.8401) among treatments.

In the harvest carried out at the end of the experiment, which began 23 months after the first application of the treatments and ended 25 months after that first application, a difference (P< 0.0001) in ratooning, which varied between 1.44 and 1.95 bunches by production unit by year, and yield (P< 0.0001), with oscillated between 3026 and 4149 boxes (18.14 kg) per hectare per year, was found (Table 2). Bunch weight (P= 0.2272) which varied between 39.5 and 42.5 kg, the ratio (P= 0.2303) that fluctuated between 1.68 and 1.80 boxes per bunch, and the number of hands per bunch (P= 0.1320) that varied between 8.7 and 9.2 per bunch, were similar among treatments (Table 2).

In the sampling done before product application, no differences among treatments were found in root contents and nematode populations. In the production variables evaluated at the time of establishing this experiment, also no differences were found. This means that any difference that was found after applying the treatments, should be attributed to its effect. The four nematode genera detected are well known pathogens in banana roots (Gowen *et al.,* 2005; Quénéhervé, 2008; Dubois and Coyne, 2011; Volcy, 2011; Guzmán-Piedrahita, 2011a, 2011b; Sikora *et al.,* 2018), and agreed with those reported in Ecuador (Chávez and Araya, 2010; Aguirre *et al.,* 2016a, 2016b; Jaramillo *et al.,* 2019; Chávez *et al.,* 2020). Also, are in parallel with those found in the main banana producing exporting countries like Colombia (Castillo *et al.,* 2010), Philippines (Arceo, 2007), and Costa Rica (Vargas *et al.,* 2006; Vargas *et al.,* 2015; Araya and Vargas, 2018).

When the experiment began, the nematode population consisted mainly of *Helicotylenchus* spp. (45.3%) and *R. similis* (32.4%), maintaining such proportion until the end of the experiment with 46.3% of *Helicotylenchus* spp. and 36.4% of *R. similis* from the plant parasitic nematode community, as well *Meloidogyne* spp. and *Pratylenchus* spp. remained like the initial proportion with 5.9 and 11.4%, respectively. This agrees with that observed in Cavendish banana plantations from Ecuador, where greater proportion of *Helicotylenchus* has been found followed by *R. similis* (Chávez and Araya, 2010; Aguirre *et al.,* 2016a, 2016b; Jaramillo *et al.,* 2019; Chávez *et al.,* 2020).

*Helicotylenchus* spp. is an ecto-endoparasite (Blake, 1966; Orion and Bar-Eyal, 1995; Guzmán-Piedrahita, 2011b; Sikora *et al.,* 2018) that induces necrotic lesions on the surface of the roots. In contrast, *R. similis* is a migratory endoparasite that causes necrotic lesions along the entire root; in the epidermis, cortical parenchyma, and vascular cylinder (Blake, 1966; Orton and Siddiqi, 1973; Jackson *et al.,* 2003; Volcy, 2011; Guzmán-Piedrahita 2011a; Sikora *et al.,* 2018). The high population of *Helicotylenchus* spp. and *R. similis* was favored by the banana production system, that even though banana is an annual crop, its production is in perennial monoculture.

The reduction found in nematode population with the rotation of two and three chemical nematicide cycles by year was of 25 and 23% for *R. similis,* of 31 and 18% for *Helicotylenchus* spp. and of 28 and 21% for total nematodes, respectively. This means that 2 and 3 chemical nematicide cycles per year were very similar in nematode control which it is not reasonable. That more likely happened because in the treatment with 3 cycles, one nematicide application was done in January each year, with an excess of soil humidity, because it is within the rainy season, and it is known that nematicides works at soil field capacity (Bunt, 1987; Araya, 2004). Rainfall in January, February and March 2020 was of 125, 343 and 401 mm and for 2021, in those months was of 426, 346 and 442 mm, respectively.

Those nematode population reductions agreed with results of Chávez *et al.,* (2020), also in Ecuador, who found drops between 22 and 49% for *R. similis*, between 23 and 40% for *Helicotylenchus* spp. and between 25 and 45% for total nematodes. Also are in parallel with Jaramillo *et al.,* (2019) as well in Ecuador, who reported reductions between 20 and 49% for *R. similis*, between 31 and 50% for *Helicotylenchus* spp. and between 29 and 49% for the total phytoparasitic nematode population.

In Costa Rica, Araya and Cheves (1997a, 1997b) found reductions between 22 and 63% for *R. similis* and between 25 and 89% for *Helicotylenchus* spp., and Moens *et al.,* (2004), also in Costa Rica, recorded drops between 18 and 59% for the total phytoparasitic nematode population. In Ivory Coast, Quénéhervé *et al.,* (1991a, 1991b, 1991c) indicated reductions of *R. similis* between 22.7 and 90.7% and between 32.5 and 100% for *Helicotylenchus* spp., and Castillo *et al.,* (2010) in Colombia found drops between 24 and 37% for *R. similis,* between 38 and 60% for *Helicotylenchus* spp., and between 25 and

33% for total nematodes. In Belize, Salguero *et al.*, (2016), found decreases between 33 and 47% for *R. similis*, between 36 and 65% for *Helicotylenchus* spp. and between 35 and 59% for total nematodes.

The reduction observed with the application of four biological nematicide cycles by year fluctuated between 10 and 28% for *R. similis*, between 5 and 21% for *Helicotylenchus* spp. and between 5 and 23% for the total phytoparasitic nematode population, which partially agreed with the non-significant drops reported by Medina *et al.*, (2022) of 6.9% of *R. similis*, 9% for *Helicotylenchus* spp. and 7.3% for the total phytoparasitic nematode population, testing biological products. The highest reduction with biological nematicides was obtained with four consecutive cycles of the mixture of Biosiembra nature´s lab products which included Sirius® (*Paecilomyces lilacinus*), Nutriaccion (*Bacillus subtilis*), and Mayestik (*Paecilomyces fumosorosea*) followed closely by the four consecutive cycles of SeaMaxx® (*Ascophyllum nodosum* extract 20%, N 3.8%, $P_2O_5$ 1.8%, $K_2O$ 3%, B 0.0136%, Cu 0.0036%, Fe 0.01%, Mn 0.024%, Mo 0.00087%, Zn 0.011% – FMC) with 28 and 25% for *R. similis*, 21 and 21% for *Helicotylenchus* spp. and 23 and 24% for the total phytoparasitic nematode population, respectively.

In the reduction observed with the mix of *Paecilomyces lilacinus*, *P. fumosorosea* and *Bacillus subtilis* it is impossible to know if all three equally decreased the nematodes or which microorganism was responsible for it. The reduction was lower than that reported by Araújo *et al.*, (2018), who with the immersion of banana plantlets into a *Bacillus subtilis* solution prevented up to 99% the infection by a mixed population composed of *R. similis*, *Meloidogyne* spp., *Pratylenchus* spp., and *Helicotylenchus* spp. in a pot experiment. On other crops, De Araujo and Pletto (2009) found that the application of the isolate PRBS-1 of *Bacillus subtilis* reduced the egg mases of *Meloidogyne* in tomato roots. For the application of *Paecilomyces lilacinus* there are many publications with positive results on sedentary endoparasite nematodes (Kiriga *et al.*, 2018; Grace *et al.*, 2019), however, Vargas *et al.*, (2015) did not found control against the migratory endoparasite *R. similis* with *P. lilacinus* applied on banana field conditions. In greenhouse conditions, Kepenekci *et al.*, (2017) reported reduction of the migratory endoparasite *Pratylenchus thornei*, with the application of *Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus*).

The control observed with the application of *Ascophyllum nodosum* was lower than that observed by Chacón and Cerda (2016) of 56% on the total phytoparasitic nematode population also on bananas in Ecuador and agreed with Tarjan (1977), who found reduction of the migratory endoparasite *Pratylenchus coffeae* in citrus nursery plants treated with *A. nodosum*. On sedentary endoparasites like *Meloidogyne*, Radwan *et al.*, (2012), reported 86.9% reduction in the number of root galling induced by *M. incognita* in tomato, Whapham *et al.*, (1994) indicated reductions in the fecundity of *M. javanica* in tomato, and Wu *et al.*, (1998) found that decreased the fertility of *M. javanica* in *Arabidopsis thaliana*, in all cases when were treated with extracts of the algae. Wu *et al.*, (1997) also reported reduction of *M. javanica* and *M. incognita* populations in tomato when treated with extracts of the algae.

The lack of banana nematode control with the application of *Pochonia chlamydospora* or *Trichoderma asperellun* disagreed with that reported by Castellanos (2016), who found significant reductions of *R. similis*, *Meloidogyne incognita* and *Helicotylenchus multicinctus* in three commercial banana (*Musa* AAA) plantations of Grande Naine, Bonifacio, and Williams of the Cavendish subgroup treated bimonthly with 2.5 g per plant of *P. chlamydosporia* var Catenulata or *T. asperellun*.

In the case of *Trichoderma* also dissented with Bruce da Silva *et al.*, (2022) who mentioned control of *R. similis* with *Trichoderma*. Likewise, differed with results in other crops, where Hernández (2014) and Hernández *et al.*, (2015) found that *T. asperellum* induced 90% of *M. incognita* mortality at 24 h *in vitro* conditions and in semi-controlled conditions it reduced the number of eggs by female in tomato plants and with Castro (2018), who did no found *Meloidogyne* spp. on soil cultivated with *Cucumis melo* when three applications of the mixture of *T. asperellum* and *Pochonia chlamydosporia* were done at planting and 30 and 50 days after sowing.

The no differences found in root weights more likely was due to the plant sampled and conditions of the experimental area. Root sampling was done on follower suckers between 1.5 and 2.5 m height which means that they were in active growth with young roots that if were infected with nematodes, the time maybe was not enough to develop symptoms. Additionally, the block or cable where the experiment was carried out have drainage channels to lower the water table level and prevent water logging which promotes root health.

So, snapping roots either by excess soil humidity or by the presence of number of pathogens (fungi-bacteria) in the nematode-induced lesions which hastens the destructions of roots, was prevented. Furthermore, the classification of functional roots is subjective (visual) and depends on root symptoms.

Roots infected by *R. similis* show reddish-brown lesions on the outer part of the roots penetrating throughout the cortex and then turns necrotic and *Helicotylenchus* spp. feeds on the outer cells of the root cortex, it produces a small-dashes reddish-brown to necrotic lesions. However, if the banana roots are still white and cream, it does not mean that they are free of nematodes. As indicated by Ayoub (1980); Mai (1985); McKenry and Roberts (1985) extensive loss of yield can occur when one or more nematode species may be feeding on a given plant, without showing obvious or specific plant symptoms. Here, maybe the nematode population, lower of 5000 per 100 g of roots, in some samplings, was not enough to develop root symptoms but it reduced ratooning and yield. This partially confirms the economic threshold suggested by INIAP (2018) of 2500 nematodes by 100 g of roots.

It is known that in white-cream roots infected with nematodes histological and physiological cell alterations occurs (Blake, 1966; Wyss, 2002; Grunewald *et al.,* 2009; Haegeman *et al.*, 2010; Jones *et al.*, 2016) which restrict water and nutrients uptake (Agrios, 2005; Haegeman *et al.*, 2010; Sikora *et al.*, 2018).

As expected in the production variables evaluated when the experiment was set up, no differences were found, which it is reasonable since bunches harvested came from stems with the commercial nematode control done before the treatment application, because nematicides are always applied in from of the follower suckers.

In the final harvest, a higher number of boxes per hectare per year was found in the treatments with 4 cycles by year of SeaMaxx® + Cronox® with 4149 followed by two chemical nematicides cycles by year with 4122 boxes. Other treatments with more than 4000 boxes were four cycles by year of Ecozin Plus® (4047), four cycles by year of Cronox® + Rhizomagic® (4040) and the alternation of two chemical nematicides with the alternation of two biological nematicides with 4035 boxes of 18.14 kg by hectare by year.

The significant difference in the number of boxes came from a non-significant increase in bunch weight and ratio, in all treatments, compared to the first harvest, and mainly from the significant increase in ratooning.

The highest improvement in ratooning was found with two chemical nematicide cycles by year with 0.36 units, followed by the four cycles by year of SeaMaxx® + Cronox® with 0.35 units, which means that the interval between harvest was reduced in 42 and 41 days, two year after the first nematicide application, in agreement with Quénéhervé *et al.,* (1991b), who found a cumulative reduction in time to harvest according to the cycle of 28 days in the first, 57 days in the second and 128 days in the third harvested cycle in plants treated with chemical nematicides.

**Table.1** Description of the treatments evaluated with the sequence of the nematicides and month of application.

| Treatment | Nematicide and month of application | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| | 2019 | | | | 2020 | | | | | | | | | | | | 2021 | | | | | | | | |
| | S | O | N | D | J | F | M | A | My | J | Jl | A | S | O | N | D | J | F | M | A | My | J | Jl | A | S |
| **1. Untreated** | | | | | | | | | | | | | | | | | | | | | | | | | |
| **2. 3cc / year** | Ve | | | | Ru | | | | Co | | | | Vy | | | | Mo | | | | Co | | | | Ve |
| **3. 2cc / year** | Ru | | | | | | Vy | | | | | | Co | | | | | | Mo | | | | | | Ru |
| **4. R 2cc 2cb year** | Co | | | Nr | | | Mo | | | CR | | | Ru | | | Bi | | | Vy | | | CR | | | Mo |
| **5. Ve + 2bc / year** | Ve | | | | Bi | | | | CR | | | | Ve | | | | Nr | | | | Bi | | | | Ve |
| **6. Georg 4c year** | Go | | | Go | | | Go | | | Go | | | Go | | | Go | | | Go | | | Go | | | Go |
| **7. Sea + C 4c / year** | SC | | | SC | | | SC | | | SC | | | SC | | | SC | | | SC | | | SC | | | SC |
| **8. Eco-p 4c year** | EP | | | EP | | | EP | | | EP | | | EP | | | EP | | | EP | | | EP | | | EP |
| **9. C + R 4c year** | CR | | | CR | | | CR | | | CR | | | CR | | | CR | | | CR | | | CR | | | CR |
| **10. Sea 4c year** | Se | | | Se | | | Se | | | Se | | | Se | | | Se | | | Se | | | Se | | | Se |
| **11. Bi 4c year** | Bi | | | Bi | | | Bi | | | Bi | | | Bi | | | Bi | | | Bi | | | Bi | | | Bi |

Note: 0= September 2019 when the experiment was established and 24= October 2021 when the last application was done. T-2: 3 cc / year = rotation of 3 cycles of chemical nematicides by year, every four months, T-3: 2 cc / year = rotation of 2 cycles of chemical nematicides by year, every six months, T-4: R 2cc 2cb year= Rotation of 2 cycles of chemical nematicides alternated with the rotation of 2 cycles of biological nematicides by year, every three months, T-5: Ve + 2bc / year= Verango + rotation of two biological nematicides by year, every four months, T-6: Georg 4c year= Go= Geos organic products (Go= Biotrich® 1 L $ha^{-1}$ + Nutribacter® 1 L $ha^{-1}$ + Pochonia Root® 2 L $ha^{-1}$ + Nematex® 2 L $ha^{-1}$) four cycles by year, T-7: Sea + C 4c year = SC (SeaMaxx® 3 L $ha^{-1}$ - FMC + Cronox® 3 kg $ha^{-1}$- Biotor Labs) four cycles by year, T-8: Eco-p 4c year = EP= Ecozin Plus 4.5 L $ha^{-1}$- AMVAC 4 cycles by year, T-9: C + R 4c year= CR= Cronox® 3 kg $ha^{-1}$+ Rhizomagic® 3 L $ha^{-1}$ - FMC, four cycles by year, every three months, T-10: Sea 4c year= Se = SeaMaxx® 10 L $ha^{-1}$, four cycles by year every three months, and T-11: Bi 4c year= Bi=Biosiembra products (Sirius® 1.25 L $ha^{-1}$ + Nutriaccion 2.5 L $ha^{-1}$ + Mayestik 1.25 L $ha^{-1}$) four cycles by year every three months. Chemical nematicides; Ve= Verango® fluopyram 0.3 g a.i. -Bayer, Ru= Rugby® cadusafos 2 g a.i.– FMC, Co= Counter® 15GR terbufos 3 g a.i.-AMVAC, Vy= Vydate® 24SL oxamyl 2.4 g a.i. - DuPont, Mo= Mocap® 15GR ethoprofos 3 g a.i. AMVAC, all rates of chemical nematicides by follower sucker; biological nematicides: Nr= NemaRoot® 2 kg $ha^{-1}$ Innovak Global, CR= Cronox® 3 kg $ha^{-1}$+ Rhizomagic® 3 L $ha^{-1}$, Bi= Biosiembra products (Sirius® 1.25 L $ha^{-1}$ + Nutriaccion® 2.5 L $ha^{-1}$ + Mayestik® 1.25 L $ha^{-1}$), Geos organic products (Biotrich® 1 L $ha^{-1}$ + Nematex® 2 L $ha^{-1}$ + Nutribacter® 1 L $ha^{-1}$ + Pochonia Root® 2 L $ha^{-1}$). The rate of Verango was applied in a water solution of 100 ml and all biological nematicides in 150 ml by follower sucker.

**Table.2** Banana (*Musa* AAA cv. Williams) yield parameters according to the nematode management per year and cost benefit relationship at the final harvest, from 23 to 25 months after the first treatment application. Sell price of each box of 18.14 kg was US $6.50.

| Treatment | Bunch weight Kg | Number of hands / bunch | Ratio | Ratooning | Boxes $ha^{-1}$ $year^{-1}$ | Difference in boxes with untreated | Additional income US $ | Treatment cost US $ | Additional packing cost US $0.75 | Net income US $ | Net profit by dollar |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | First harvest at experiment establishment parent plant | | | | | | | | |
| **1. Untreated** | 37.1 | 9.5 | 1.58 | 1.52 | 3377 | | | | | | |
| **2. 3 Ch c/y** | 40.0 | 9.4 | 1.70 | 1.52 | 3638 | | | | | | |
| **3. 2 Chc/y** | 38.0 | 9.4 | 1.61 | 1.52 | 3457 | | | | | | |
| **4. 4 c/y2Ch-2B** | 36.9 | 9.3 | 1.57 | 1.52 | 3358 | | | | | | |
| **5. 3 c/y1Ch-2B** | 38.2 | 9.4 | 1.62 | 1.52 | 3471 | | | | | | |
| **6. 4c/y Geos organic** | 38.0 | 9.5 | 1.61 | 1.52 | 3458 | | | | | | |
| **7. 4 c / y Sea + C** | 39.1 | 9.5 | 1.66 | 1.52 | 3557 | | | | | | |
| **8. 4 c / y Ecozin** | 38.9 | 9.6 | 1.65 | 1.52 | 3534 | | | | | | |
| **9. 4 c / y C + R** | 37.9 | 9.3 | 1.61 | 1.52 | 3441 | | | | | | |
| **10. 4 c / y Sea** | 37.2 | 9.4 | 1.58 | 1.52 | 3378 | | | | | | |
| **11. 4 c / y Bi** | 37.7 | 9.1 | 1.60 | 1.52 | 3424 | | | | | | |
| **Probability** | P= 0.8391 | P= 0.8868 | P= 0.8412 | | P= 0.8401 | | | | | | |
| | | | Final harvest from 23 to 25 months after first treatment application | | | | | | | | |
| **1. Untreated** | 42.3 | 9.2 | 1.79 | 1.59 | 3498 | 0 | | | | | |
| **2. 3 Ch c / y** | 42.5 | 9.0 | 1.80 | 1.63 | 3606 | 108 | 702 | 294 | 81 | 327 | 1.1 |
| **3. 2 Ch c / y** | 40.6 | 9.0 | 1.72 | 1.95 | 4122 | 624 | 4057 | 169 | 468 | 3420 | 20.2 |
| **4. 4 c / y 2Ch-2B** | 40.2 | 8.7 | 1.71 | 1.92 | 4035 | 537 | 3492 | 352 | 403 | 2737 | 7.8 |
| **5. 3 c / y 1Ch-2B** | 41.0 | 9.1 | 1.74 | 1.76 | 3764 | 266 | 1728 | 265 | 199 | 1263 | 4.8 |
| **6. 4 c / y Geos organic** | 41.0 | 9.1 | 1.74 | 1.61 | 3443 | -55 | | 196 | | | |
| **7. 4 c / y Sea + C** | 41.1 | 9.0 | 1.74 | 1.94 | 4149 | 651 | 4230 | 476 | 488 | 3266 | 6.9 |
| **8. 4 c / y Ecozin** | 42.0 | 9.1 | 1.78 | 1.85 | 4047 | 549 | 3570 | 628 | 412 | 2531 | 4.0 |
| **9. 4 c / y C + R** | 40.5 | 8.8 | 1.73 | 1.90 | 4040 | 542 | 3522 | 486 | 406 | 2629 | 5.4 |
| **10. 4 c / y Sea** | 39.5 | 8.9 | 1.68 | 1.59 | 3283 | -215 | | 302 | | | |
| **11. 4 c / y Bi** | 40.2 | 8.8 | 1.71 | 1.44 | 3026 | -472 | | 194 | | | |
| **Probability** | P= 0.2272 | P=0.1320 | P= 0.2303 | P<0.0001 | P< 0.0001 | | | | | | |

Ratio= number of boxes of 18.14 kg per bunch [77% of the bunch weight was packet (23% rejection that includes 12% bunch stalk and 11% rejected bananas) / 18.14 kg by box]. 2019: 1450 plants per hectare from which 97% of the bunches were processed (1406 bunches), 2021:1267 plants per hectare from which 97% of the bunches were processed (1229 bunches), ratooning= number of bunches harvested by each banana stool by year, boxes per hectare per year= (1406 or 1229 bunches * ratio * ratooning). Each value is the mean of five replicates and in each replicate 20 bunches were harvested. Ch: chemical nematicide, B: biological nematicide. Net profit= (additional income – treatment control cost – banana box packing cost of US $0.75 each). Product cost by hectare: Counter® 15GR $150 $ha^{-1}$, Verango® 50SC $180 $ha^{-1}$, Rugby® 10GR $150 $ha^{-1}$, Vydate® 24SL $150 $ha^{-1}$, Mocap® 15GR $170 $ha^{-1}$, SeaMaxx® $12 $L^{-1}$; T-7 $36 $ha^{-1}$ and T-10 $120 $ha^{-1}$; Cronox® $195 $ha^{-1}$, NemaRoot® $200 $ha^{-1}$, Sirius® $17.5 $ha^{-1}$, Nutriaccion® $35 $ha^{-1}$, Rhizomagic $36 $ha^{-1}$, Mayestik® $17.5 $ha^{-1}$, Biotrich® $15 $ha^{-1}$, Nutribacter® $15 $ha^{-1}$, Pochonia Root® $30 $ha^{-1}$, Nematex® $30 $ha^{-1}$, application cost by cycle $20. The treatment cost was obtained dividing the product cost of each treatment by year by the ratooning of each treatment.

**Fig.2A-C** Root content (g) by follower sucker in banana plants (*Musa* AAA cv Williams) treated with different number of chemical and biological nematicides cycles per year or their rotation. Each point is the average of five repetitions. In each repetition, three follower suckers from 1.5-2.5 m height were excavated at its base and in front of it, making a hole of 30 cm length, 15 cm wide and 30 cm depth from where all the roots were collected.

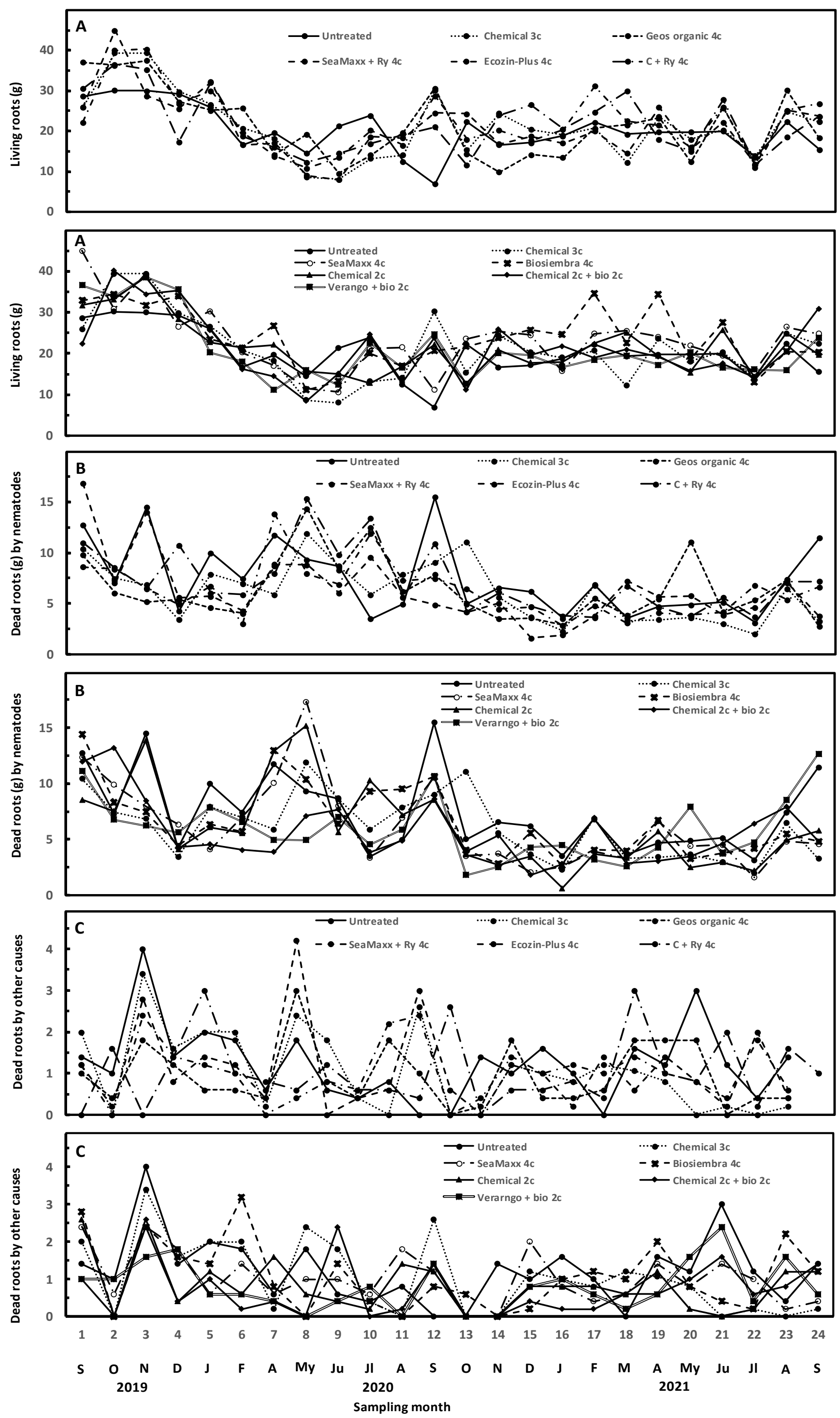


**Fig.2D-E** Total root weight (g) and percentage of living roots by follower sucker in banana plants (*Musa* AAA cv Williams) treated with different number of chemical and biological nematicides cycles per year or their rotation. Each point is the average of five repetitions. In each repetition, three follower suckers from 1.5-2.5 m height were excavated at its base and in front of it, making a hole of 30 cm length, 15 cm wide and 30 cm depth from where all the roots were collected.

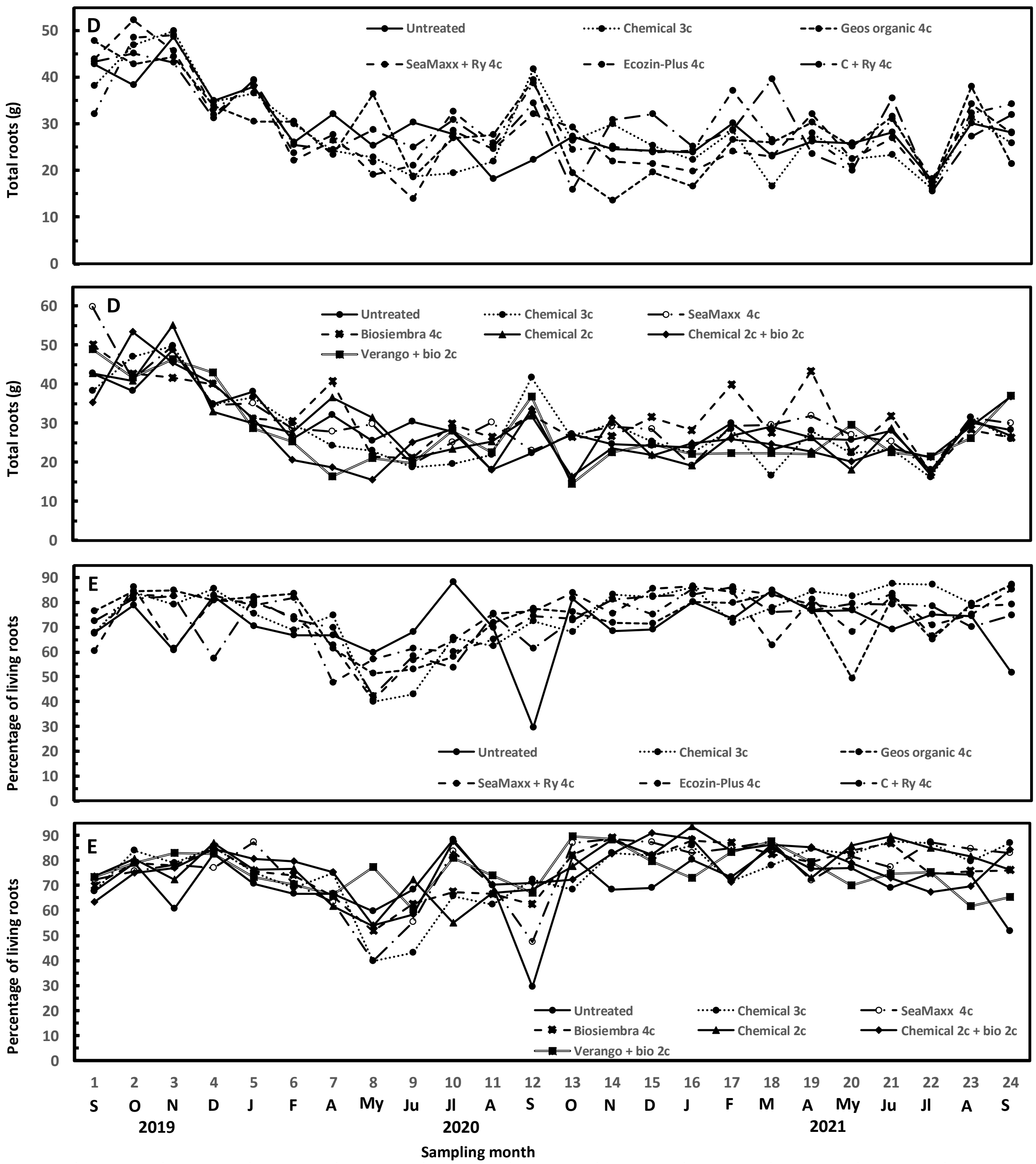


**Fig.3A-F** Number of nematodes per 100 g of banana (*Musa* AAA cv. Williams) roots per follower sucker treated with different number of chemical and biological nematicides cycles per year or their rotation. Each point is the average of five repetitions. In each repetition, three follower suckers of 1.5-2.5 m height were dug in their base and in front, making a hole of 30 cm length, 15 cm wide and 30 cm depth from where all roots were collected.

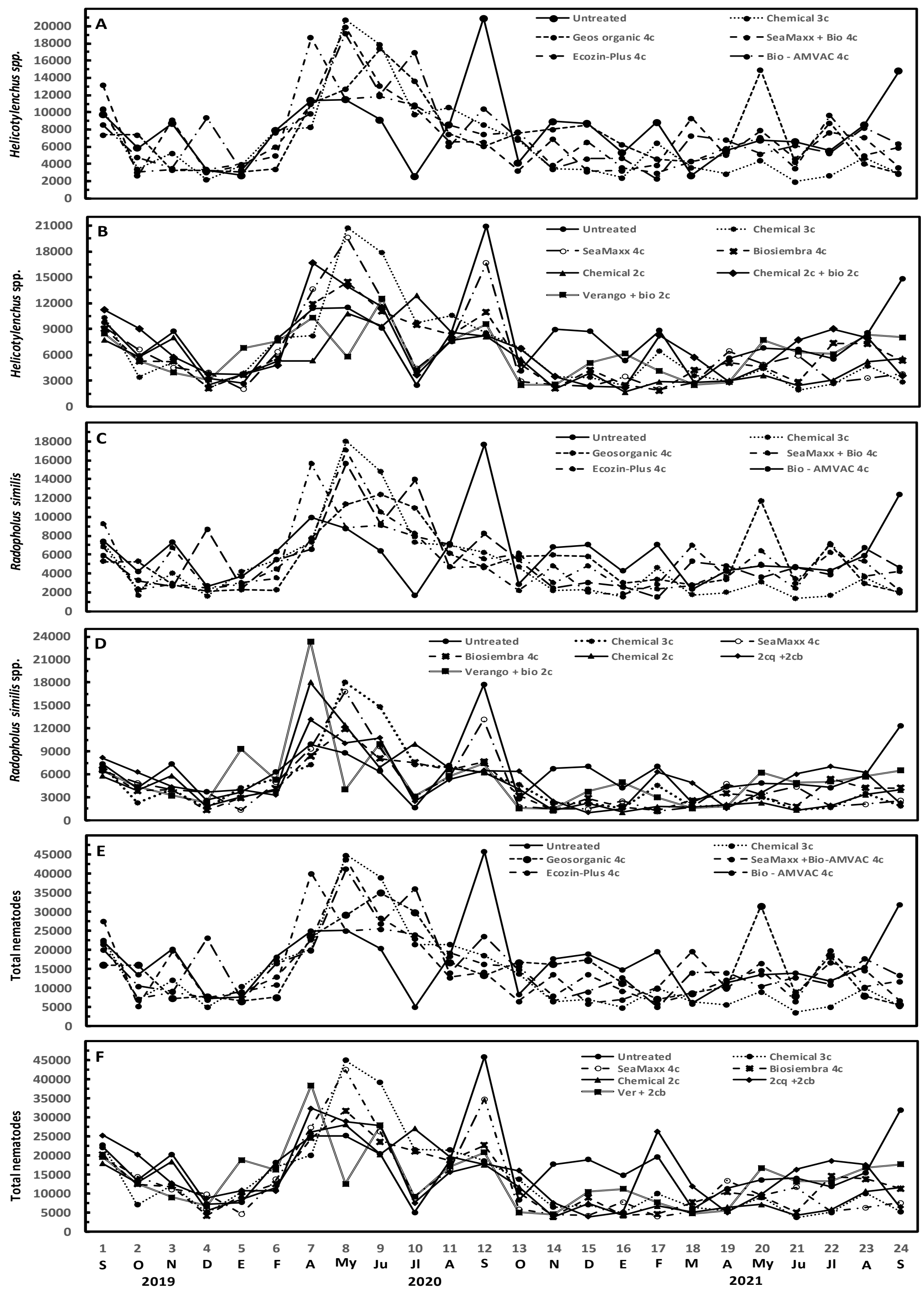


**Fig.4A-B** Composition of the plant-parasitic nematode population by follower sucker in banana plants (*Musa* AAA cv Williams) treated with different number of chemical and biological nematicides cycles per year or their rotation. **A)** pre-treatment and **B)** post treatment application. Pre-treatment application each slice is the average of 55 repetitions (11 treatments by 5 repetitions) and post-treatment application each slice is the average of 1265 observations (23 sampling * 11 treatments * 5 repetitions). In each repetition the value comes from a composed root sample of three follower suckers.

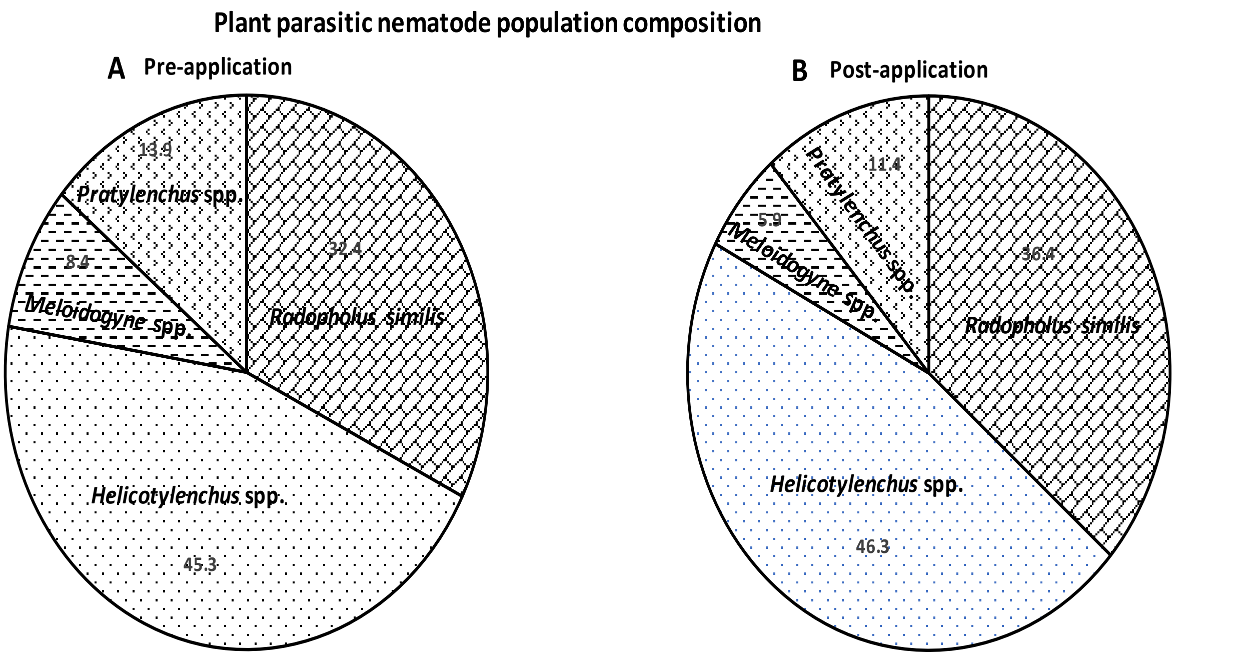


**Fig.5A-E** Living roots (g), dead roots (g) by nematodes, dead roots (g) by other causes, total roots (g) and percentage of living roots by follower sucker in banana plants (*Musa* AAA cv Williams) treated with different number of chemical and biological nematicides cycles per year or their rotation. Each bar is the average of 120 observations (24 samplings * five repetitions) and in each repetition the value is the average of three follower suckers. In each follower sucker, a hole of 30 cm length, 15 cm wide and 30 cm depth was excavated at the base, and all roots were collected.

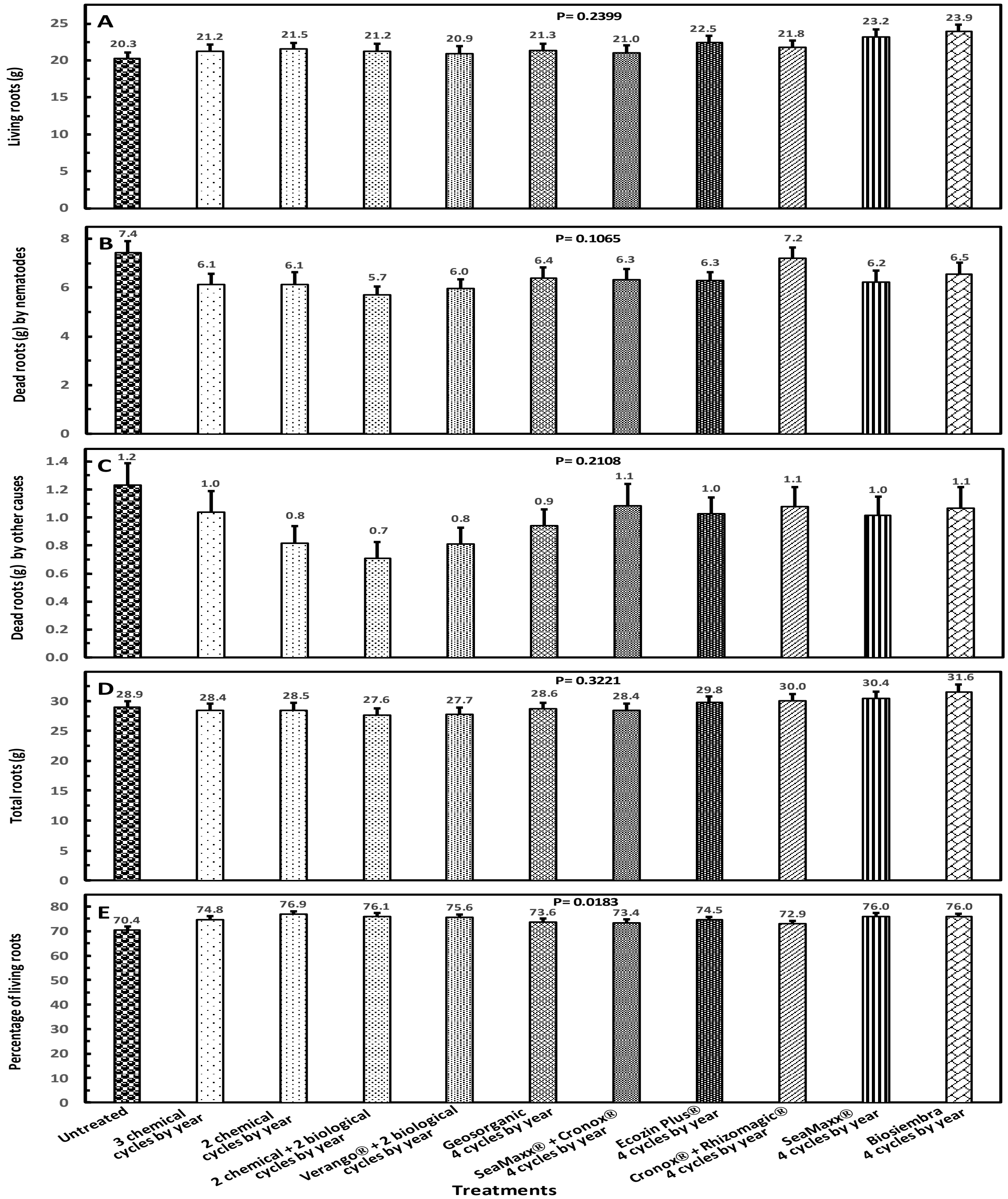
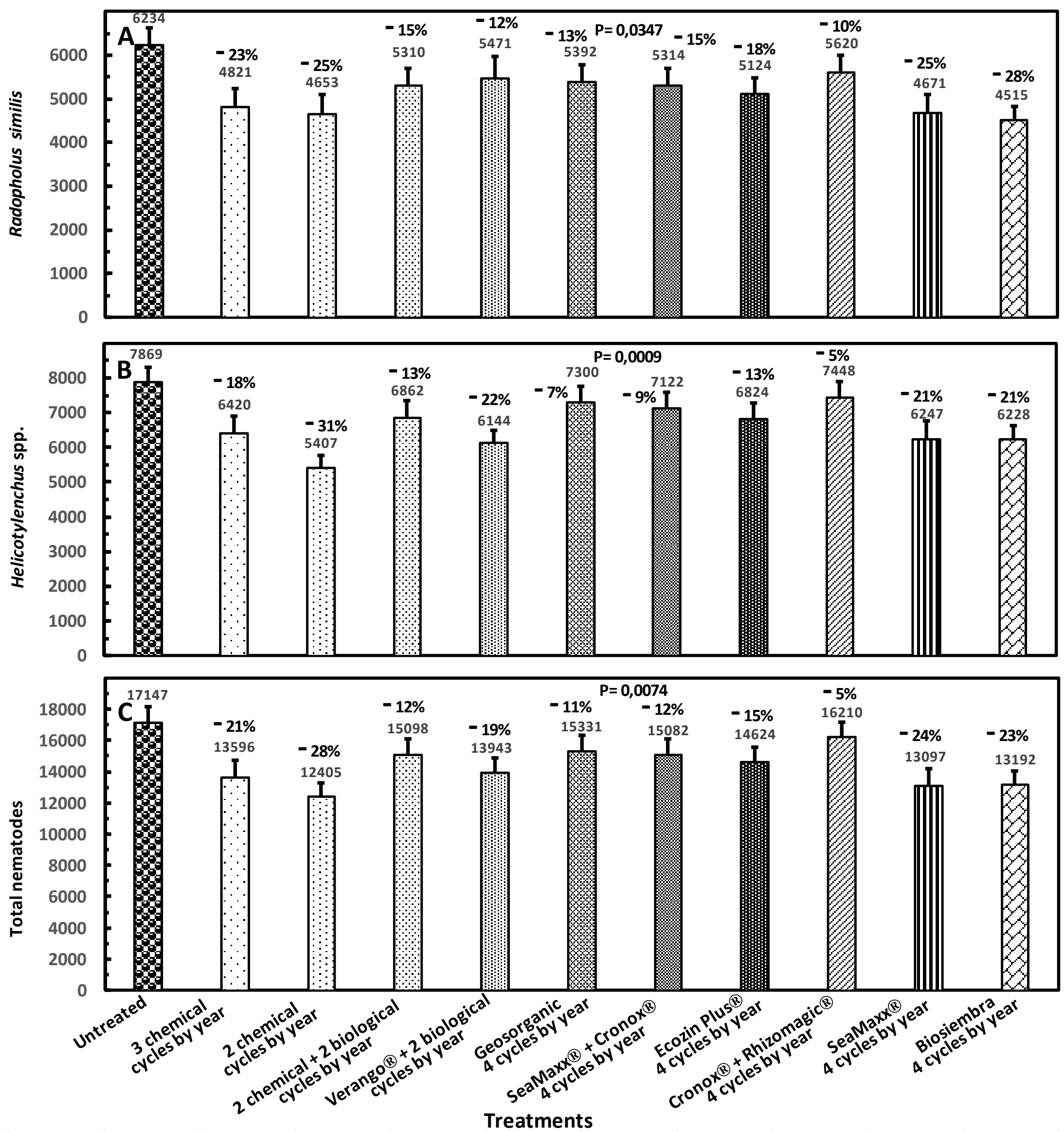


**Fig.6A-C** Number of nematodes per 100 g of banana roots (*Musa* AAA cv Williams) by follower sucker treated with different number of biological and chemical nematicide cycles per year or their alternation. Each bar is the mean ± standard error of 120 observations (24 samplings * five repetitions) and in each repetition the value is the average of three follower suckers of 1.5-2.5 m high. A hole of 30 cm length, 15 cm wide and 30 cm depth was dug in front of each follower sucker and all roots were collected. Percentage changed with respect to the untreated control.




Similarly, Quénéhervé *et al.,* (1991a) and Gowen (1995) reported an increase in the harvest period from 13 to 32 and from 22 to 40 days, respectively, in plants infected with nematodes that were not treated compared with those applied with chemical nematicide. In congruence with this extension in the period to harvest, Roderick *et al.,* (2012) reported an increase of 13.6 more days to harvest in Mbwazirume banana plants to which they added nematodes compared to plants without the addition of nematodes.These results confirmed that banana nematodes are serious threat to banana production in

Ecuador as was found by Jaramillo *et al.,* (2019) and Chávez *et al.,* (2020) and agreed with Dita *et al.,* (2013) thoughts, that nematodes continue to be a serious threat to banana production in Latin America and the Caribbean. The percentages of yield increase varied between 15.3 and 18.6% at 24 months after the first treatment application, which agreed with Jaramillo *et al.,* (2019), who found that nematode control with chemical nematicides increased yield between 16 and 31% and is in line with Chávez *et al.,* (2020), who reported an increase between 5 and 17%, both in Ecuadorian conditions. Likewise, the percentage increase in yield is in parallel with that compiled by Gowen and Quénéhervé (1990), who mentioned increases between 14 and 263% and Gowen (1995), who cited increases between 5 and 275% and were lower than that reported by Stanton and Pattisson (2000) of 46%.

Yield was increased between 108 and 651 boxes of 18.14 kg (2 – 11.8 mt) by hectare by year which is in line with that reported earlier in Ecuador by Jaramillo *et al.,* (2019) who found an increase between 538 and 1045 boxes (9.7 – 18.9 mt) by hectare by year and with Chávez *et al.,* (2020) who cited increases between 226 and 730 boxes (4 – 13.2 mt) by hectare by year. The yield increased also agreed with Quénéhervé *et al.,* (1991b), who indicated increases in production between 523 and 1157 boxes (9.5-21 tm), with Pattison *et al.,* (1999) who reported increases between 655 and 953 boxes of 13 kg (8.5-12.3 tm), with Salguero *et al.,* (2016), who found increases between 545 and 832 boxes of 18.14 kg (9.9-15.1 tm), and was lower than that reported by Araya and Lakhi (2004), who cited increases of 1245 boxes of 18.14 kg (22,6 tm) per hectare per year, controlling nematodes through the application of chemical nematicides.

The highest yield (number of boxes per hectare per year) was observed in plants treated with four cycles per year of SeaMaxx® + Cronox® followed closely by the treatment of the rotation of two chemical nematicide cycles by year. In the case of the mix of SeaMaxx® + Cronox® the improvement in yield did not match with the nematode control observed of 15% for *R. similis*, 9% for *Helicotylenchus* spp. and 12% for total nematodes, since in other biological treatments a higher nematode control was obtained, but with lower yield. The yield increased with the two chemical treatments agreed with the nematode control achieved of 25% for *R. similis*, 31% for *Helicotylenchus* spp. and 28% for the total phytoparasitic nematode population. Then, more likely a biostimulant effect could occurred with the application of the mix of SeaMaxx® + Cronox®.

The high population of *Helicotylenchus* spp. and the increased achieved in production with the application of nematicide indicated that their parasitism reduces growth, development and production in accordance with observations by McSorley and Parrado (1986); Gowen and Quénéhervé (1990); Chau *et al.,* (1997); Barekye *et al.,* (1998, 2000); Gowen (2000); Ssango *et al.,* (2004); Guzmán-Piedrahita (2011b); Coyne *et al.,* (2013) and Salguero *et al.,* (2016), who reported that *H. multicinctus* and *H. dihystera* damaged the banana root system and reduced yield between 19% (Speijer and Fogain, 1999) and 34% (Reddy, 1994). Additionally, Sijmons *et al.,* (1994) indicated that the induction and maintenance of feeding sites of *Helicotylenchus* spp. causes physiological changes in the structure of cells. In the case of *R. similis* it is well supported that it reduced the yield in banana (Gowen and Quénéhervé, 1990; Gowen, 1993, 1995; Araya, 2004; Roderick *et al.,* 2012; Coyne *et al.,* 2013). The presence of nematodes with different parasitic habits; *R. similis* migratory endoparasite and *Helicotylenchus* spp. an ecto-endoparasite most likely exacerbates root damage since lesions can develop at feeding sites and through root tissue. In addition, plants often activate post-infection resistance mechanisms, even in cases where the population of nematodes increases over time, and the nematode-plant interaction is compatible. Therefore, together these processes can represent a high energy expenditure for plants that can interfere with the filling and development of the bunch. Given that both nematode genera cause damage to the crop, for the implementation of options for their management, the population of all the phytoparasitic nematodes present should be considered, as has been suggested by Araya (2004); Ramclam and Araya (2006); Salguero *et al.,* (2016) and Aguirre *et al.*, (2016a, 2016b).

During the development of the experiment, the market price of a box of 18.14 kg of bananas was US $6.30 and of a nematicide application cycle was for Counter® 15FC $150, Verango® $180, Vydate® 24SL $150, Rugby® 10GR $150, Mocap® 15GR $170, SeaMaxx® T-3 $36, T-10 $120, Cronox® $195, NemaRoot® $200, Sirius® $17.5, Nutriaccion® $35, Rhizomagic® $36, Mayestik® $17.5, Biotrich® $15, Nutribacter® $15, Pochonia root® $30, and Nematex® $30 per hectare. The cost of the fertilizer, control of black Sigatoka and weeds, and other tasks was the same for the control plots and those treated with nematicide, since the increase recorded was mainly for ratooning. The additional net income from the increase in yield, deducted the cost of labor of $0.75 of packing for each additional box and the treatment cost was between US $327 and $3420 per hectare per year 24 months after the first treatment was applied (Table 2).

This net gain agrees with that indicated in Ecuador by Jaramillo *et al.,* (2019) who found amounts between $2550 and $5759 and with that reported by Chávez *et al.,* (2020), who mentioned values between $1050 and $3432 by hectare by year and it is in parallel with data from Australia, where Pattison *et al.,* (1999) reported amounts between $2494 and $5910 per hectare per year. Although, a higher additional income was obtained with the four cycles of SeaMaxx® + Cronox® by year, the net profit for every dollar invested in nematode control was higher with two chemical nematicides with $20.2 compared to $6.9 return for the treatment with four cycles of SeaMaxx® + Cronox®. For us it is the first time that some effect of biological nematicides it is observed, despite a substantial, positive literature on this topic. The evaluation of these products must continue to determine consistency in their performance under different ecological conditions. If the effectiveness of these products is proven, their demand can be stimulated that allows them to be purchased at more competitive prices that lead to increasing their economic return when they are applied.

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