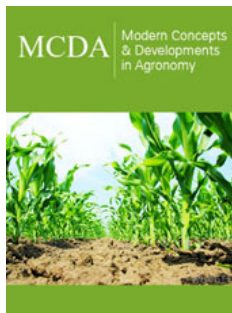


Effect of Rachis Leachate on populations of Root- Knot Nematodes of the Genus *Meloidogyne* Spp.

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Abstract

More than 40 genera of phytonematodes act as obligate parasites of higher plants. Considered hidden enemies, difficult to control and responsible for large losses in crops of economic importance. Currently, efforts are dedicated to its management through the search for biocontrollers and the use of organic fertilizers of plant or animal origin, whose action allows increasing antagonistic organisms and/or inducing unfavorable conditions for their development. In this context, the Research Institute of Tropical Roots and Tubers Crops (INIVIT) has validated, with the participation of producers, the benefits of banana rachis leachate obtained at the institution as a nutritional supplement in foods, vegetables and grains. In this sense, observations and results of its application during the years 2022 and 2023 lead to the present work with the objective of reporting the effect of rachis leachate on populations of Root-Knot Nematodes (RKN) of the genus *Meloidogyne* spp. The method used consisted of visual analysis of the root system and determination of the galling index in treated and untreated plants. The root system of untreated plants exhibited grade 4 (30 to 99 galls per plant). The first communication is made on the biocontrol potential of banana rachis leachate (10%) in reducing visible symptoms of infection by root-knot nematodes, showing a regulatory effect on population levels of phytonematodes in tomato and pepper plants. Studies aimed at understanding the mechanisms of action of the bioproduct as biological control of nematodes of the genus *Meloidogyne* spp. are recommended.

Keywords: Bionematicides; Management; Root-knot nematodes; Tomato; Bell pepper

Introduction

Cultivated plants are subjected to different factors exerted by the environment, whether abiotic or biotic, which contribute to reduce potential yields, decrease the quality of agricultural products, increase production costs and even cause losses due to severe pest infestations [1]. In this context, soil phytopathogens constitute an important group of biotic factors, including several species of fungi, nematodes and bacteria, whose effective management is considered of vital importance for the successful development of crops, due to the potential damage they can cause [2]. Among the different threats to a wide range of economically important crops worldwide, plant pathogenic nematodes are one of the most important. They are very small worms that feed on plant roots and are considered silent enemies, as they remain hidden inside the roots or in the soil, making it difficult to detect their presence early and, therefore, to take effective actions to contain them [3].

Meloidogyne species, known as Root-Knot Nematodes (RKN), are a group of plant parasitic nematodes with a worldwide distribution [4]. It is the most damaging of all phytonematode genera attacking tomatoes in tropical and subtropical regions, causing yield losses estimated at up to 30% [5].

Nematode management has often been based on the application of nematicidal molecules, despite their reasonable efficacy; the use of nematicidal chemical compounds is becoming a growing concern due to various environmental aspects, risks to human health and high cost to farmers [6]. In the last 20 years, studies exploring the use of antagonistic and competitive microorganisms against RKNs have gained great attention [7].

Chemical compounds can control this pest faster and more effectively, however, the damage caused to the ecosystem as well as the residual effects have led to increased interest in finding safer substitutes. In this sense, the application of novels and methods that are more selective to control *Meloidogyne* spp. is promoted. The search for natural microbial antagonists can offer new alternatives for the biological control of nematodes. In Cuba several microbial species, such as *Pochonia chlamydosporia* [8], which are used in different crops have shown great efficacy in controlling this type of phytopathogen under certain management conditions and soil types. Other isolates (*Stenotrophomona* ssp. CIGB G1 and *Sphingobacterium* sp. CIGBTb) have shown some activity against zoonematodes in *in vitro* assays and have shown potential as biocontrols [9].

The production of organic fertilizers is an economic alternative for the future for small producers, which guarantees its use to improve soil properties, nutrients for the crop and its health [10]. In this context, the Research Institute of Tropical Roots and Tuber Crops (INIVIT), has developed a bioproduct for agricultural use obtained from harvest residues from banana and plantain production [11].

The studies corresponding to the quality and identity of substances of the bioproduct developed by the institution in late 2019 and early 2020 were carried out by Cuban institutions with up-to-date analytical methodologies and techniques available, recognized for the commercial registration of biofertilizers and biopesticides in Cuba. The studies affirm that the bioproduct obtained does not generate unacceptable risks to human health and the environment [12,13]. The use of rachis leachate in vegetables, tropical roots and grains has been validated by INIVIT's research-development team with the participation of producers who have evidenced the benefits of the bioproduct as a nutritional supplement, by contributing to crop nutrition, provoking the growth of roots, stems and leaves and increasing plant vigor [14]. In this sense, observations and results of its application during the years 2022 and 2023 lead to the present work with the objective of reporting the effect of the bioproduct developed from rachis leachate on populations of gill-forming nematodes of the genus *Meloidogyne* spp.

Materials and Methods

The research is part of the validation process (years 2022-2023) of the use of the bioproduct developed at INIVIT from banana residues (rachis leachate), for which polygons were established to evaluate its effect on vegetable production among them: tomato (*Solanum lycopersicum* L. var. HA-3019) and pepper (*Capsicum annum* L. var. California Wonder st), in areas of INIVIT in the period November-May of the years 2022 and 2023 in a carbonated brown fluffy soil [15].

The bioproduct used was obtained at the INIVIT at from banana rachis leachate (Musa AAA, Cavendish subgroup, cultivar 'Gran enano') according to the methodology proposed by Morales & Maza [11]. The application of the bioproduct was carried out

weekly at a concentration of 10% and in all study cases; treatment without application (absolute control) was used. The foliar and soil applications were carried out in the morning hours (9:00am) with aspray backpack (Spain, Matabi) of 16L capacity, with a Lurmark AN 2.5 flood-jet nozzle with a pressure from 1.5 to 2.0 bar.

Taking into account the objectives of communication, only results obtained from root analysis (number of galls) using the visual method without resorting to laboratory analysis, using the scale proposed by Taylor & Sasser [16] (Table 1), will be referred to. Fifty percent of the treated and untreated plants were sampled.

Table 1: Aging index scale proposed by Taylor & Sasser [16].

Scale Degree	No. of Guts
0	0
1	De 1 a 2
2	De 3 a 10
3	De 11-30
4	de 30-99
5	>100

Results and Discussion

The effect of banana rachis leachate (10%) on the reduction of visible symptoms of infection by gill-forming nematodes was evident (Figures 1-3), showing a regulating effect on population levels of phytonematodes of the genus *Meloidogyne*. Visual observation and the use of a damage scale (0-5 degrees), according to the number of galls (derived from the functional alterations caused by the juveniles in the tissues during their growth), made it possible to determine the minimum reduction (degree 0) in the root system of treated plants and a degree 4 in the roots of untreated plants, corresponding to 30 to 99 galls. Bello et al. [17] reports that the visual analysis method allows assessing the effectiveness of the techniques used in the control of *Meloidogyne* without the need to resort to laboratory analysis.



Figure 1: Visible symptoms of infection by gill-forming nematodes on untreated plants.

Note: tomato roots with different nodulation rates. Photo taken by L. Morales, INIVIT, 2023.



Figure 2: Root system of tomato plants treated with plantain rachis leachate (10%) obtained at INIVIT.

Note: reduction to a minimum of nodules or absence of typical galls. Photo taken by L. Morales, INIVIT, 2023.



Figure 3: Root system of untreated bell pepper plant showing grade 4 (number of galls 30-99).

Note: Photo taken by V. Ventura, INIVIT, 2023.

In the untreated plants of the two crops, symptoms representative of the *Meloidogyne* genus were observed in the roots. The roots thickened in the invasion zone and the development of galls or nodules typical of this pathogen, which had diameters two to three times larger than healthy roots. Heavily infested roots were much shorter than healthy roots and exhibited fewer lateral roots and root hairs. Diseased plants with roots showing typical galls or nodules showed poor growth, small leaves, and pale green or yellowish color. Verdejo-Lucas [18] points out that *Meloidogyne* species are the nematodes that mainly affect tomato root functionality by interfering with water uptake and nutrient transport. RKNs establish an intimate relationship with their hosts, as they induce the formation of typical galls on roots, which are the first visible symptoms of this kind of infection Rehman et al. & Kyndt T et al. [19].

The regulatory effect on population levels of root-knot nematodes could be attributed to the benefits of rachis leachate as a nutritional supplement in crops that stimulates defense

mechanisms that favor resistance to pathogens, an action that is favored by the microbial load determined in the composition of the leachate developed at the INIVIT (4.4×10^6 CFU/mL) of bacteria of the genus *Bacillus* sp. [11].

Research on the potential for control of phytopathogenic fungi through leachate applications has suggested the viability of these products [20]. In Costa Rica, research has been conducted with leachates, mainly to control black sigatoka (*Mycosphaerella fijiensis* Morelet) [21,22].

Rachis leachates have been evaluated in tomato crops, finding greater microbial activity in the flowering and harvest stage [23,24]. They have also been effectively tested in the control of powdery mildew and in the production of roses [22,25]. Its use combined with *Tagetes patula* are alternatives that can be used to mitigate moko (*Ralstonia solanacearum* Smith) infestations in plantains and bananas [26].

The results obtained constitute findings of great importance and constitute the first communication on the biocontrol potential of plantain rachis leachate in the reduction of visible symptoms of infection by gill-forming nematodes in tomato and bell pepper crops and suggest the need to take full advantage of the benefits of leachate obtained from plantain rachis in the management of root-knot nematodes. In the current situation, in which numerous active substances of phytosanitary action are being withdrawn from the market because they are highly toxic and leave residues in the harvested products, the results obtained through the use of banana rachis leachate in the production of vegetables such as tomatoes and peppers could contribute to mitigate the incorporation of high concentrations of fertilizers and allow ecological recovery of nutrients to the soil and the communities of organisms that inhabit it, which is promising for the development of integrated strategies for the management of phytopathogenic nematodes and suggest studies aimed at understanding the mechanisms of action of its use as a biological control of nematodes of the genus *Meloidogyne* spp [27,28].

Conclusion

The use of banana rachis leachate suggests the possibility of its use and represents a novel alternative for the biocontrol of soil phytoparasite communities by significantly reducing the nodulation rate of gill-forming nematodes of the genus *Meloidogyne*. The results exhibited with the use of the bioproduct obtained at INIVIT corroborate its usefulness as a nutritional and sanitary supplement for agricultural use and arouse the interest of in-depth studies on the mechanisms of action involved in the search for biocontrollers of phytopathogenic nematodes.

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