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# Biological control of *Ralstonia solanacearum* and its effect on the vegetative growth of organic banana

Control biológico de *Ralstonia solanacearum* y su efecto en el crecimiento vegetativo de banano orgánico

Controle biológico de *Ralstonia solanacearum* e seu efeito no crescimento vegetativo da bananeira orgânica

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## Abstract

The banana (*Musa* AAA) is one of the main economically important crops worldwide. Currently, it faces a serious problem of plant death caused by the bacterium *Ralstonia solanacearum* (Smith) variety 2. The objective of this research was to evaluate the effect of biological control of Moko disease and its relationship with the vegetative growth of banana seedlings under field conditions. Four treatments were employed: absolute control (T0), *Trichoderma* spp. (T1), *Bacillus* spp. (Bio-remedy) (T2), and ADMF® (T3) with three replications in a completely randomized block design. The incubation period, the incidence percentage, and the area under the disease progress curve (AUDPC) were evaluated, along with plant survival and height, pseudostem diameter, and leaf emission rate. At 113 days after transplanting (dat), treatments T1, T2, and T3 showed higher seedling survival. Plant height (111-145 cm), pseudostem diameter (7.43-11.28 cm), and leaf emission rate (11.74-13.15 leaves) exhibited significant differences among treatments. Treated plants showed the lowest AUDPC (between 576.1 and 1435.4 units) compared to untreated plants (3156.55 units). *Trichoderma*, *Bacillus*, and ADMF®

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were demonstrated to reduce disease incidence and promote banana vegetative growth, proving to be viable sustainable alternatives for this crop.

## Resumen

El banano (*Musa* AAA) es uno de los principales cultivos de importancia económica a nivel mundial. Actualmente, enfrenta un grave problema de muerte de plantas causada por la bacteria *Ralstonia solanacearum* (Smith) raza 2. El objetivo de la presente investigación fue evaluar el efecto del control biológico de Moko y su efecto relación en el crecimiento vegetativo de plántulas de banano en condiciones de campo. Se emplearon cuatro tratamientos: ttestigo absoluto (T0), *Trichoderma* spp. (T1), *Bacillus* spp. (Bio-remedy) (T2) y ADMF® (T3) con tres repeticiones, en un diseño de bloques completamente al azar. Se evalúo el periodo de incubación, el porcentaje de incidencia y el área bajo la curva del progreso de la enfermedad (ABCPE), la sobrevivencia y altura de plantas, el diámetro del pseudotallo y la tasa de emisión foliar. A los 113 días después del trasplante (dtt), los tratamientos T1, T2 y T3 mostraron mayor sobrevivencia de plántulas. La altura de plantas (111-145 cm), el diámetro del pseudotallo (7,43-11,28 cm) y la tasa de emisión foliar (11,74-13,15 hojas) presentaron diferencias significativas entre tratamientos. Las plantas tratadas presentaron la menor ABCPE (entre 576,1 y 1.435,4 unidades) en comparación con las no tratadas (3.156,55 unidades).*Trichoderma*, *Bacillus* y ADMF® demostraron reducir la incidencia de la enfermedad y promover el crecimiento vegetativo de banano, constituyéndose en alternativas sostenibles viables para este cultivo.

**Palabras clave:** *Musa* AAA, *Trichoderma*, *Bacillus*, ADMF, *Ralstonia solanacearum*, ABCPE.

## Resumo

A banana (*Musa* AAA) é uma das principais culturas de importância econômica em todo o mundo. Atualmente, enfrenta um sério problema de mortalidade de plantas causado pela bactéria *Ralstonia solanacearum* (Smith) raça 2. O objetivo da presente pesquisa foi avaliar o efeito do controle biológico da doença Moko e sua relação com o crescimento vegetativo de mudas de banana em condições de campo. Foram utilizados quatro tratamentos: controle absoluto (T0), *Trichoderma* spp. (T1), *Bacillus* spp. (Bio-remedy) (T2) e ADMF® (T3), com três repetições, em um delineamento de blocos completamente ao acaso. Foram avaliados o período de incubação, a porcentagem de incidência e a área abaixo da curva de progresso da doença (ABCPD), além da sobrevivência e altura das plantas, o diâmetro do pseudocaule e a taxa de emissão foliar. Aos 113 dias após o transplante (dat), os tratamentos T1, T2 e T3 mostraram maior sobrevivência das mudas. A altura das plantas (111-145 cm), o diâmetro do pseudocaule (7,43-11,28 cm) e a taxa de emissão foliar (11,74-13,15 folhas) apresentaram diferenças significativas entre os tratamentos. As plantas tratadas apresentaram a menor ABCPD (entre 576,1 e 1.435,4 unidades) em comparação com as não tratadas (3.156,55 unidades). *Trichoderma*, *Bacillus* e ADMF® demonstraram reduzir a incidência da doença e promover o crescimento vegetativo do bananeiro, sendo alternativas sustentáveis viáveis para esta cultura.

**Palavras-chave:** *Musa* AAA, *Trichoderma*, *Bacillus*, ADMF, *Ralstonia solanacearum*, ABCPE.

## Introduction

Banana cultivation (*Musa* AAA) originated in the Asian continent and has now spread to many tropical regions of the planet (Brunda *et al*., 2023); It is a globally important cash crop, supporting the livelihoods and food security of millions of people (Debnath *et al*., 2019).

Banana cultivation is susceptible to diseases such as Moko or bacterial vascular wilt, caused by *Ralstonia solanacearum* (Smith) variety 2, which is characterized by affecting all the organs of the plant at any stage of development (Milk *et al*., 2022). The symptoms occur in one of the three youngest leaves, progressively advancing to the more senescent leaves, where the vascular tissues subsequently present vascular discoloration (Valencia *et al*., 2014). Also with the progression of the disease, the leaves turn yellowish to brown and pendulous, a stage in which the plant finally dies due to colonization and obstruction of the root system of the plants by *R. solanacearum* (Castillo, 2023; Ramirez *et al*., 2020).

Among the biological controls used worldwide against plant pathogens are some bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*, *Streptomyces* spp., among others), plant growth-promoting bacteria (*Azotobacter* spp., and *Azospirillum* spp.) and fungi (*Trichoderma* spp.) (Avozani *et al*., 2022; Crespo-Clas *et al*., 2023; Fernandes *et al*., 2020; Sabando-Ávila *et al*., 2017).

Some *Trichoderma* isolates can be considered plant growth-promoting rhizobacteria (PGPR) and inducers of plant defenses against plant pathogens, and they even can attack, parasitize, or simply occupy the ecological niche of parasites such as *R. solanacearum* (Avozani *et al.,* 2022; Quispe-Quispe *et al*., 2022). In general, biological control with *Bacillus* spp. and *Trichoderma* spp. has advantages in terms of farmers' economics and at the environmental level (Sabando-Ávila *et al*., 2017; Villegas-Escobar *et al*., 2018).

The use of these microorganisms and other measures more compatible with the environment are alternatives to reduce the populations of *R. solanacearum* in Musaceae. Therefore, this study aimed to evaluate the use of biological controls on Moko and their effect on banana vegetative growth.

## Materials and methods

### Description of the experimental area

This research was carried out in 2022, in the province of Esmeraldas (0°05´1.34´´ S and -79°26´25.0´´ W), at an altitude of 186 m a.s.l. The trial was set up in an organic banana plantation certified for this purpose 7 years ago by Quality Certification Services (QCS). Considering that the soils have been cultivated with cocoa and coffee, before carrying out the research, a physical-chemical analysis of the soil was carried out, whose results are presented in table 1.

The soil profile of the first site was deep, well-drained, with groundwater below 15 m, silty loam textured, classified as Eutrudox; while the second sandy clay site was classified as Oxisol, according to the USDA Soil Taxonomy (Santos *et al*., 2013; Soil Survey Staff, 2014).

In terms of climatic characteristics, the minimum rainfall was 7.2 mm in December and the maximum was 153.7 mm in June, with an average of 80.45 mm; the minimum temperature was 18 °C, while the maximum temperature was between 27.9 and 32.1 °C, in August and October, respectively.

**Table 1. Soil analysis of the organic banana trial, La Unión, Esmeraldas, Ecuador.**

| Parameters | Units | Result | Interpretation | Method of analysis |
|---|---|---|---|---|
| pH | | 6.2 | Slightly acidic | Soil: water ratio (1:2.5) |
| $NH_4$ | ppm | 24 | Average | Colorimetry technique |
| P | ppm | 65 | High | Colorimetry technique |
| K | Meq.100 $g^{-1}$ | 0.36 | Average | Atomic Absorption |
| Ca | Meq.100 $g^{-1}$ | 11 | High | Atomic Absorption |
| Mg | Meq.100 $g^{-1}$ | 39 | High | Atomic Absorption |
| S | ppm | 15 | Average | ICP method |
| Zn | ppm | 11.9 | High | Atomic Absorption |
| Cu | ppm | 8.2 | High | Atomic Absorption |
| Fe | ppm | 353 | High | Atomic Absorption |
| Mn | ppm | 6.1 | Average | Atomic Absorption |
| B | ppm | 0.48 | Low | ICP method |
| MO | % | 5.3 | High | Walkley-Black method |
| Ca/Mg | meq.100 $g^{-1}$ | 2.8 | | Atomic Absorption |
| Mg/K | meq.100 $g^{-1}$ | 11.14 | | Atomic Absorption |
| Ca+Mg/K | meq.100 $g^{-1}$ | 42.57 | | Atomic Absorption |
| Sand | % | 40 | | Bouyoucos method |
| Silt | % | 54 | | Bouyoucos method |
| Clay | % | 6 | | Bouyoucos method |
| Textural class | Silt loam | | | |

OM: organic matter

**Experimental conditions**

The sowing distance in the field was 1.5 x 1.5 m. Additionally, preventive disinfection measures were applied during and after the installation of the experiment (using quaternary ammonium for the disinfection of footwear and tools), because the soil had a history of the presence of *R. solanacearum*.

**Experimental design and statistical analysis**

A randomized complete block design (RCBD) was used, with four treatments, established in three blocks (table 2). The experimental units were plots of 10 m x 8.7 m, containing 18 banana plants, totaling 216. An analysis of variance (ANOVA) was applied and Duncan's test ($P\leq0.05$) was used for the comparison of means, using the INFOSTAT statistical program version 2020.

**Table 2. Treatments evaluated in biological control of *Ralstonia solanacearum* causing Moko in banana plants.**

| Symbol | Treatments | Description |
|---|---|---|
| T0 | Absolute control | No product application |
| T1 | *Trichoderma* spp. | *T. asperellum, T. harzianum* and *T. virens* |
| T2 | *Bacillus* spp. (Bio-remedy) | *Bacillus subtilis, B. megaterium* and *Paenibacillus polymyxa* |
| T3 | ADMF® | Product of the maceration of plant remains (de-stressing action of maximum performance) |

**Evaluated biological controls and their application in the field**

*Trichoderma* spp. The isolates of *Trichoderma* spp. (*T. harzianum*, *T. asperellum* and *T. virens*) came from the collection of the Phytopathology Laboratory of the Department of Plant Protection of the EETP of INIAP, Ecuador. The inoculum of the biological control was seeded in Petri dishes containing the potato dextrose agar (PDA) culture medium that was incubated for 7 days. Subsequently, 5 $mL.L^{-1}$ of sterile water was added to each Petri dish, and the conidia were detached from the mycelium with a spatula. The solution obtained from each isolate was filtered through sterile gauze and adjusted to a concentration of $1\times10^7$ $conidia.mL^{-1}$ in a Neubauer chamber (Agamez-Ramos *et al*., 2008).

For field application, a mixture was prepared using 16 mL of *Trichoderma* spp. suspension, with a concentration of $1\times10^7$ conidia.$mL^{-1}$, in one liter of water.

**The mixture was evenly distributed in the experimental unit, with a dose of 56 mL of suspension per plant.**

BIO-REMEDY® is a commercial product containing *B. subtilis, B. megaterium,* and *P. polymyxa*, at a concentration of $1x10^8$ $CFU.g^{-1}$. The application of the product in the field was carried out at a rate of 3 $kg.ha^{-1}$, uniformly in each experimental unit.

ADMF® is a commercial product classified as a biostimulant, obtained from the maceration of plant remains containing N (4%), P (2 %), K (11 %), Ca (1.5 %), Mg (1 %), Fe (0.4 %) and OM (56 %), which was applied uniformly in the experimental units in doses of 2 $L.ha^{-1}$.

**Variables evaluated**

Soil microbial load was assessed at the beginning and end of the experiment by collecting 12 soil samples, one for each treatment (4) and repetition (3). Each soil sample (1 g) was homogenized and subjected to serial dilution ($10^{-1}$ to $10^{-5}$) using a PDA culture medium (potato, dextrose, agar; Difco brand). Dilutions were seeded in Petri dishes and incubated at laboratory room temperature (24 ± 5 °C) for six days, to allow the growth of filamentous fungal colonies. Subsequently, the observation and counting of the developed colonies was carried out.

The incubation period of the disease was quantified daily in seedlings transplanted in the field (21 days of age), until the appearance of the first symptom.

The percentage of incidence of the disease was obtained weekly, using the formula suggested by Castaño-Zapata (1989):

$$\% \text{ Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants evaluated}} x\,100$$

With the incidence data obtained during the evaluation, the area under the disease progression curve (AUDPC) was calculated using the formula proposed by Shaner and Finney (1977):

$$\mathrm{ABCPE} = \sum_{i=1}^{n-1}\left[\frac{X_{i+1} + Xi}{2}\right](t_{i+1} - ti)$$

Where:

Xi: intensity of disease damage at the ith evaluation.

t*i*: time in days of the ith evaluation.

n: number of evaluations.

The percentage of plant survival was recorded weekly, quantifying the number of living plants in each plot, and then transforming the values obtained into a percentage.

The height of plants (cm) was evaluated monthly with the help of a ruler graduated in m, measuring the height from the base of the soil to the point of insertion of the true leaf. The diameter of the pseudostem was also assessed monthly, using a digital caliper (Tacklife DC02, Singapore). Initially, the diameter was measured at the base of the plants, but when the plants grew, it was measured at 1 m from the soil. Finally, for the leaf emission rate, at 30 days after transplanting (dat), the flag leaf was marked on each plant, and at the end of the study, the number of leaves emitted by each plant was quantified.

## Results and discussion

**Microbial load**

The presence of *Penicillium* sp., *Cladosporium* sp., *Colletotrichum* sp., *Fusarium* sp., *Rhizopus* sp., and *Colletotrichum* sp. was observed at the beginning of all treatments (table 3), while four months after the first evaluation, the microorganisms *Penicillium* sp., *Colletotrichum* sp., and *Fusarium* sp. were found in the control. *Trichoderma* spp. and *Penicillium* sp. were found in treatment with *Trichoderma* spp.; *Penicillium* sp. and *Bacillus* sp. were found in treatment with Bio-remedy, and *Cladosporium* sp., *Colletotrichum* sp., and *Rhizopus sp.* were found in treatment with ADMF®.

**Table 3. Determination of microbial load of soil organisms, analyzed at the beginning and the end of the research trial.**

| Treatments<br>Start of trial | | Presence of microorganisms<br>End of trial | |
|---|---|---|---|
| T0 | Absolute control | *Penicillium* sp.<br>*Cladosporium* sp.<br>*Colletotrichum* sp.<br>*Fusarium* sp. | *Penicillium* sp.<br>*Colletotrichum* sp.<br>*Fusarium* sp. |
| T1 | *Trichoderma* spp. | *Penicillium* sp.<br>*Cladosporium* sp.<br>*Colletotrichum* sp.<br>*Fusarium* sp.<br>*Rhizopus* sp. | *Trichoderma* sp.<br>*Penicillium* sp. |
| T2 | Bacillus spp.<br>(Bio-remedy) | *Penicillium* sp.<br>*Colletotrichum* sp.<br>*Fusarium* sp.<br>*Rhizopus* sp. | *Penicillium* sp.<br>*Bacillus* sp. |
| T3 | ADMF® | *Cladosporium* sp.<br>*Colletotrichum* sp.<br>*Fusarium* sp.<br>*Rhizopus* sp. | *Cladosporium* sp.<br>*Colletotrichum* sp.<br>*Rhizopus* sp. |

ADMF®: Product of the maceration of plant remains (de-stressing action of maximum functioning).

The presence of *Trichoderma* in the soil at the end of the experiment could be due to the application of one of the products based on this biological control, inferring that the fungus practically occupied the ecological niche, displacing the rest (Quispe-Quispe *et al*., 2022; Sabando-Ávila *et al*., 2017).

**Incubation period**

The first symptom of Moko, recognized as the yellowing of newly emerged leaves, was determined at 48 dat in the control treatment (T0). Conversely, in plants treated with *Bacillus* spp. (T2) and ADMF® (T3), symptoms of the disease were observed at 60 dat. It manifested later (69 dat) in plants treated with *Trichoderma* (Figure 1). Although its cycle lasts about 21 days until the plant dies (He *et al*., 1983), the appearance of Moko symptoms, in Williams banana cultivar (*M. acuminata*) seedlings of the Cavendish subgroup, can be observed between 6 and 30 days after inoculation with *R. solanacearum* (Creencia *et al*., 2022; Ramírez *et al*., 2020).

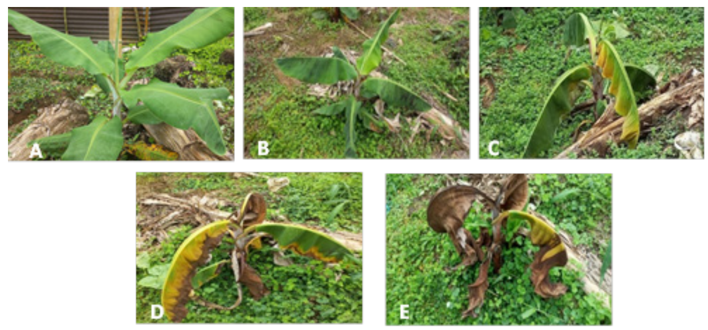


**Figure 1. Symptoms caused by Ralstonia solanacearum.** A = no symptoms, B = one wilted leaf, C = two to three wilted leaves, D = four or more wilted leaves, E = completely dead plant.

The non-inoculated plants remained green and asymptomatic during the development of this research; in addition, the incubation period obtained, contrasted with the results found by Creencia *et al*. (2022) and Ramírez *et al*. (2020), these differences would be given by the conditions in which each research was carried out. Regardless of this fact, all biological treatments applied, especially *Trichoderma*, seem to have a negative effect against Moko in banana plants.

**Incidence of the disease**

The incidence of Moko was different between treatments ($P \leq 0.014$) from 48 to 113 dat (Figure 2). Although microorganisms and ADMF® significantly reduced the disease, a superiority of *Trichoderma* and ADMF® was observed. For example, disease incidence at 113 dat (end of the experiment) was 86 % in untreated plants, while in those treated with *Bacillus* spp., ADMF®, and *Trichoderma* spp. it was 43 %, 27 %, and 17 %, respectively (Figure 2).

According to Bautista-Montealegre *et al*. (2016), the incidence of Moko in untreated plants fluctuated between 60 and 77 %, which was lower than what was found in the present research. In both bananas and tomatoes, it has been possible to verify the efficacy of other commercial products based on *Trichoderma* and *Bacillus*, such as Ecoterra® for the management of vascular wilt caused by *R. solanacearum* (Ceballos *et al*., 2014; Zhou *et al*., 2021). However, the low incidence of Moko found in the present study (17 %) was lower than that obtained by Zhou *et al*. (2021) in tomato plants treated with *B. velezensis* (36 %). This further denoted the importance of the results obtained in the present research.

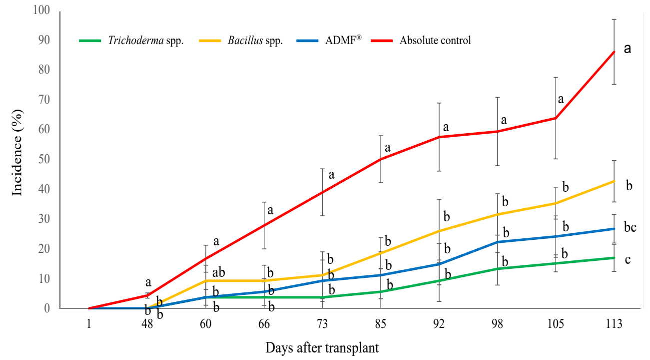


**Figure 2. Incidence of the disease caused by *Ralstonia solanacearum* in bananas and its response to biological and cultural control treatments.** Means followed by letters in common are not significantly different for Duncan's test (P<0.05). The vertical lines at each of the points in the figure refer to the standard deviation.

**Area under the disease progression curve**

The AUDPC was different among treatments (P≤0.05), with a higher value (3,197 units) found in the untreated plants (Figure 3). Plants treated with *Bacillus* spp., ADMF®, and *Trichoderma* spp. had an incidence of 1,435, 936, and 576 units, respectively. If it is considered that *R. solanacearum* is a fairly aggressive bacterium that is easily dispersed and difficult to control, the results presented here would be important in future decisions in the management of Moko in Musaceae. It should be considered that all products used in the research have been formulated from microorganisms, including ADMF® that has organic matter. Thus, biological controls may be inhibiting the growth of phytobacteria directly or through the action of bioactive compounds, or simply occupying the microcosm of the soil (Quispe-Quispe *et al*., 2022; Ramírez *et al*., 2020; Villegas-Escobar *et al*., 2018), thus considerably reducing Moko's AUDPC in banana plants.

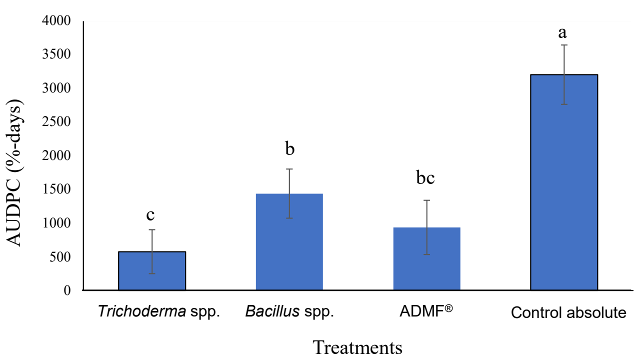


**Figure 3. Area under the disease progression curve (AUDPC) caused by *Ralstonia solanacearum* in banana and its response to biological and cultural control treatments.** Different letters indicate significant differences according to Duncan (P<0.05). The intervals on each bar represent the standard error.

**Plant survival**

Although plant survival varied significantly between treatments (*P≤0.005)* from 48 dat, this variable was always lower in untreated plants at 14 %. The most discrepant answers were found at 105 and 113 dat. At both times, plants treated with *Bacillus* spp., ADMF®, and *Trichoderma* spp. averaged 57 %, 73 %, and 83 %, respectively. Bautista-Montealegre *et al.* (2016) indicated that the survival of banana plants ranged between 13 and 40 %; the lowest value (13 %) was similar to that obtained in the present research. The positive effect on the survival of banana plants obtained with ADMF® and *Trichoderma* spp. was relevant, considering that it was obtained under field conditions.

These biological controls increased the survival rate of the plants compared to the untreated plants (control), thus highlighting their potential for effective disease management in bananas. Future studies should optimize the large-scale application of these antagonistic microbial agents in commercial plantations, as current trials indicate that they can increase yields by reducing losses caused by bacterial wilt.

**Plant height and pseudostem diameter**

Both the height of the plants (Figure 4A) and the pseudostem diameter (Figure 4B) showed differences between treated and untreated plants, only at 90 and 113 dat (P≤0.05). In both times, the application of *Trichoderma* spp. and ADMF® generally increased plant height (greater than 140 cm) and pseudostem diameter (greater than 10 cm) by 14 and 20 %, respectively, compared to *Bacillus* spp. and the control.

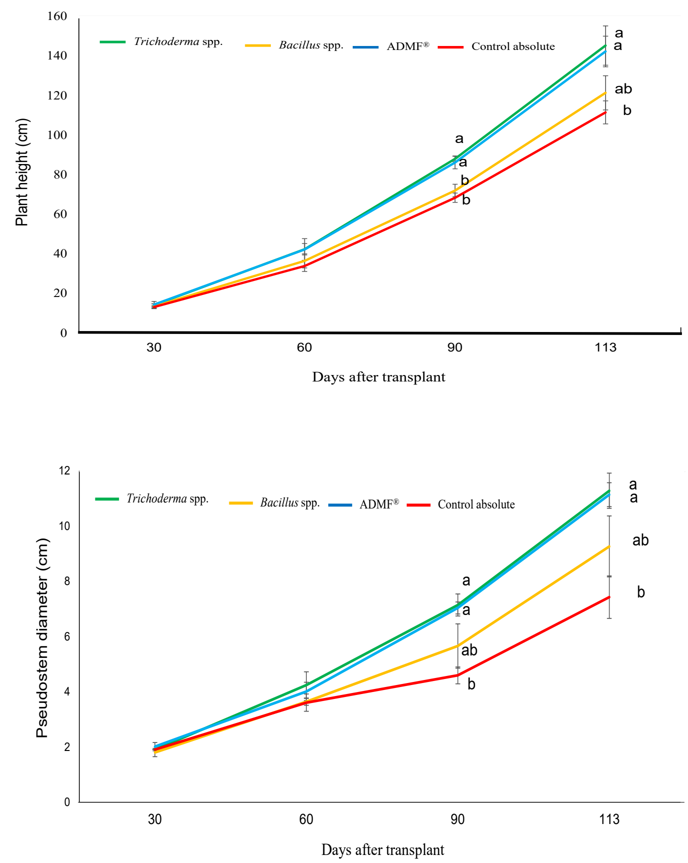


**Figure 4. Plant height (cm) (A) and pseudostem diameter (cm) (B) of banana and their response to biological and cultural control treatments for *Ralstonia solanacearum*.** Different letters indicate significant differences according to Duncan (P<0.05). The intervals on each bar represent the standard error.

The use of both bacterial and fungal biological controls can induce increased height in tomato plants infected with *R. solanacearum* and *Meloidogyne incognita*, respectively (Chávez-Arteaga *et al*., 2022; Zhou *et al*., 2021). Biological controls would not only positively affect plant height and pseudostem diameter, but also chlorophyll content, leaf area, leaf thickness, shoot biomass, and roots of banana seedlings infected with *F. oxysporum* f. sp. *cubense* variety 4 tropical (Li *et al*., 2021). Even though, the mechanism by which *Trichoderma* spp., ADMF®, or in a certain way *Bacillus* spp. induced increased plant height and pseudostem diameter in banana plants was not studied, it could be inferred that these inputs could be acting as biostimulants or plant growth promoters (Chavez-Arteaga *et al*., 2022; Quispe-Quispe *et al*., 2022).

**Leaf emission rate**

Although the leaf emission rate assessed up to 113 dat was similar in plants treated with *Trichoderma* spp., *Bacillus* spp., and ADMF® compared to those not treated, only the *Trichoderma* spp. treatment differed positively from the control (13.3 and 11.8 leaves on average, respectively). The increase generated by *Trichoderma* spp. in banana plants could subsequently also have an impact on fruit production. However, this and other aspects may be addressed in future research. In this context, Vargas-Calvo *et al*. (2015) noted that the number of total leaves emitted (filiform and true) in tall and short banana cultivars was 39 leaves on average throughout their life cycle.

## Conclusions

*Trichoderma* spp. (T1), *Bacillus* spp. (T2) and ADMF® (T3) have a beneficial effect on the management of Moko caused by *R. solanacearum* in bananas. These inputs evidenced their biocontrol capacity, which allows to reduce the incidence and progress of the disease in banana plants under field conditions. Likewise, their application promotes a greater vegetative development of banana plants. Considering these results, *Trichoderma*, *Bacillus,* and the ADMF® product are promising sustainable alternatives to be implemented in the integrated management of Moko in organic banana crops. However, further studies are needed to confirm its effectiveness on a larger scale.

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## Literature cited

Agamez Ramos, E. Y., Zapata Navarro, R. I., Oviedo Zumaqué, L. E., & Barrera Violeth, J. L. (2008). Evaluation of substrates and fermentation solid process for spores production of *Trichoderma* sp. *Revista Colombiana de Biotecnología*, *10*(2), 23-34. https://www.redalyc.org/articulo.oa?id=77610204

Avozani, A., Tumelero, A. I., Baldiga Tonin , R., Denardin, N., Gomes Silva, A., & Garcés-Fiallos, F. R. (2022). Biological control of *Corynespora cassicola* and *Drechslera tritici-repentis*. *Revista de Agricultura Neotropical*, *9*(4), e7111. https://doi.org/10.32404/rean.v9i4.7111

Bautista-Montealegre, L. G., Bolaños-Benavides, M. M., Abaunza-González, C. A., Arguelles-Cárdenas, J. H., & Forero-Camacho, C. A. (2016). Moko de plátano y su relación con propiedades físicas y químicas en suelos del departamento de Quindío Colombia. *Revista Colombiana de Ciencias Hortícolas, 10*(2), 273-283. https://doi.org/10.17584/rcch.2016v10i2.5066

Brunda, N. B., Chirag, D., Jilen, M., & Menaka, M. (2023). Effect of blending proportion on sensory appeal of the blended squash using banana pseudostem sap with mango, papaya and *Aloe vera. IJCS, 11*(1), 06-11. https://www.chemijournal.com/archives/2023/vol11issue1/PartA/10-6-32-941.pdf

Castaño-Zapata, J. (1989). Estandarización de la estimación de daños causados por hongos, bacterias y nematodos en fríjol (*Phaseolus vulgaris* L.). *Fitopatología Colombiana*, *13*(1), 9-19.

Castillo, T. (2023). Effect of shade on the severity of Moko (*Ralstonia solanacearum*) *on Guineo* (*Musa balbisiana* ABB). *European Journal of Agriculture and Food Sciences, 5*(1), 1-5. https://doi.org/10.24018/ejfood.2023.5.1.611

Ceballos, G., Álvarez, E., & Bolaños, M. M. (2014). Reduction in populations of *Ralstonia solanacearum* race *2* in plantain (*Musa* AAB Simmonds) with extracts from *Trichoderma* spp. and antagonistic bacteria. *Acta Agronomica, 63*(1), 83-90. https://doi.org/10.15446/acag.v63n1.43121

Chávez-Arteaga, K.T., Cedeño-Moreira, Á.V., Canchignia-Martínez, H.F., & Garcés Fiallos, F.R. (2022). Candidate rhizobacteria as plant growth-promoters and root-knot nematode controllers in tomato plants. *Scientia Agropecuaria*, *13*(4), 423-432. https://doi.org/10.17268/sci.agropecu.2022.038

Creencia Armi, R., Alcantara, E. P., Opulencia, R. B., Diaz, M. G. Q., & Monsalud, R. G. (2022). A preliminary screening of philippine mangrove soil bacteria exhibit suppression of *Ralstonia solanacearum* (Smith) Yabuuchi et al. causing moko disease of banana (*Musa acuminata Cavendish* subgroup) under laboratory conditions*. Journal of the International Society for Southeast Asian Agricultural Sciences, 28*(1), 135-148. http://issaasphil.org/wp-content/uploads/2022/06/11.-Creencia-et-al-2022-Mangrove-soil-bacteria-FINAL.pdf

Crespo-Clas, Á.M., Canchignia-Martínez, H.F., & Garcés-Fiallos, F.R. (2023). Nematodes and root system are affected by rhizobacterial consortium in the third generation of commercial banana plants. *Revista de Agricultura Neotropical*, *10*(3), e7725. https://doi.org/10.32404/rean.v10i3.7725

Debnath, S., Khan, A. A., Das, A., Murmu, I., Khan, A., & Mandal, K. K. (2019). *Genetic Diversity in Banana.* 217-241. https://doi.org/10.1007/978-3-319-96454-6_8

Fernandes Domingues Duarte, C., Cecato, U., Trento Biserra, T., Mamédio, D., & Galbeiro, S. (2020). *Azospirillum* spp. en gramíneas y forrajeras. *Revisión. Rev. Mex. Cienc. Pecu*., *11*(1), 223-240. https://doi.org/10.22319/rmcp.v11i1.4951

He, L. Y., Sequeira, L., & Kelman, A. (1983). Characteristics of strains of *Pseudomonas solanacearum* from China. *Plant Disease*, *67*, 1357-1361. https://www.apsnet.org/publications/plantdisease/backissues/Documents/1983Articles/PlantDisease67n12_1357.PDF

Leite Pais, A. K., Silva dos Santos, L. V., Rodrigues Albuquerque, G. M., Gomes de Farias, A. R., Silva Junior, W. J., de Queiroz Balbino, V., Freire Silva, A. M., Siqueira da Gama, M. A., & Barbosa de Souza, E. (2022). Comparative genomics and phylogenomics of the *Ralstonia solanacearum* Moko ecotype and its symptomatological variants. *Genetics and Molecular Biology, 45,* 4, e20220038. https://doi.org/10.1590/1678-4685-GMB-2022-0038

Li, X., Li, K., Zhou, D., Zhang, M., Qi, D., Jing, T., Zang, X., Qi, C., Wang, W., Xie, J. (2021). Biological control of banana wilt disease caused by *Fusarium oxyspoum* f. sp. *cubense* using *Streptomyces* sp. H4. *Biological Control*, *155*, 104524. https://doi.org/10.1016/j.biocontrol.2020.104524

Quispe-Quispe, E., Moreira-Morrillo, A.A., & Garcés-Fiallos, F.R. (2022). Una revisión sobre biocontroladores de *Phytophthora capsici* y su impacto en plantas de *Capsicum*: Una perspectiva desde el exterior al interior de la planta. *Scientia Agropecuaria*, *13*(3), 275-289. https://doi.org/10.17268/sci.agropecu.2022.025

Ramírez, M., Neuman, B. W., & Ramírez, C. A. (2020). Bacteriophages as promising agents for the biological control of Moko disease (*Ralstonia solanacearum*) of banana. *Biological Control, 149*, 104238. https://doi.org/10.1016/j.biocontrol.2020.104238

Sabando-Ávila, F., Molina-Atiencia, L.M., & Garcés-Fiallos, F.R. (2017). Trichoderma harzianum en pre-trasplante aumenta el potencial agronómico del cultivo de piña. *Revista Brasileira De Ciências Agrárias*, *12*(4), 410-414. https://doi.org/10.5039/agraria.v12i4a5468

Santos, H. G., Jacomine, P. K. T., Anjos, L. H. C., Oliveira, V. A., Oliveira, J. B., Coelho, M. R., Lumbreras, J. F., & Cunha, T. J. F. (2013). *Sistema brasileiro de classificação de solos*. 3. ed. *Rev. Ampl*. Rio de Janeiro: Embrapa Solos.

Shaner, G., and Finney R. E. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology, 67*, 1051-1056. https://doi.org/10.1094/Phyto-67-1051

Soil SurveyStaff. (2014). Keys to soil taxonomy. *Washington: Natural Resources Conservation Service and Agriculture Department*.

Valencia, L., Álvarez, E., & Castaño, J. (2014). Resistencia de treinta y cuatro genotipos de platano (*Musa* AAB) y banano (*Musa* AAA) a cinco cepas de *Ralstonia solanacearum* Raza *2* (Smith). *Agronomía, 22*(2), 21-34. http://agronomia.ucaldas.edu.co/downloads/Agronomia 22(2)_3.pdf

Vargas-Calvo, A., Acuña-Chinchilla, P., & Valle-Ruiz, H. (2015). La emisión foliar en plátano y su relación con la diferenciación floral. *Agronomía Mesoamericana*, *26*(1), 120-128. https://doi.org/10.15517/am.v26i1.16935

Villegas-Escobar, V., González-Jaramillo, L.M., Ramírez, M., Moncada, R.N., Sierra-Zapata, L., Orduz, S., & Romero-Tabarez, M. (2018). Lipopeptides from *Bacillus* sp. EA-CB0959: Active metabolites responsible for *in vitro* and *in vivo* control of *Ralstonia solanacearum*. *Biological Control*, *125*, 20-28. https://doi.org/10.1016/j.biocontrol.2018.06.005

Zhou, Y., Yang, L., Wang, J., Guo, L., & Huang, J. (2021). Synergistic effect between *Trichoderma virens* and *Bacillus velezensis* on the control of tomato bacterial wilt disease. *Horticulturae*, *7*(11), 439. https://doi.org/10.3390/horticulturae7110439