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Biofertilizers reduce *fusarium* wilt severity and improve early growth of 'maçã' banana seedlings under greenhouse conditions

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ABSTRACT

Banana cultivation is severely affected by *Fusarium* wilt, which compromises plant vigor and productivity. Biofertilizers have emerged as promising alternatives in integrated disease management by stimulating plant growth and activating defense mechanisms. This study evaluated the effects of different biofertilizers on vegetative development and disease severity in 'Maçã' banana seedlings inoculated with isolates of *Fusarium oxysporum* f. sp. *cubense* (Foc) belonging to Subtropical Race 4 (SR4). Four Foc SR4 isolates (CML3487, CML3488, CML3489, and CML3490) were evaluated in combination with the biofertilizers Agro-Mos®, Coppercrop®, Compost-Aid®, Nem-Out®, and a mixture of Compost-Aid® + Nem-Out®, under greenhouse conditions. Chlorophyll indices, plant height, pseudostem diameter, total leaf area, fresh and dry mass of shoot and root, as well as disease severity in aerial tissues, rhizome, vascular system, and roots were assessed, along with the area under the disease progress curve (AUDPC). Compost-Aid® promoted the highest chlorophyll indices and improved seedling growth, particularly in plants inoculated with isolates CML3488 and CML3490. For CML3488, shoot fresh mass increased from 42.54 g in untreated plants to 152.09 g with Compost-Aid®, while shoot dry mass increased from 4.68 g to 15.22 g. Disease severity in aerial tissues was substantially reduced by Compost-Aid®, Nem-Out®, and their combination, with reductions exceeding 60% in some isolate–treatment combinations compared with the corresponding inoculated controls without biofertilizer application. These biofertilizers were also associated with lower AUDPC values, indicating slower disease progression throughout the evaluation period. In contrast, Coppercrop® showed inconsistent performance and, in some cases, severity levels remained similar to or higher than those observed in untreated plants. These findings demonstrate that selected biofertilizers can reduce *Fusarium* wilt severity and improve the physiological performance and early growth of 'Maçã' banana seedlings under greenhouse conditions, supporting their use as complementary components of integrated *Fusarium* wilt management strategies.

Keywords: *Musa acuminata*; *Fusarium oxysporum* f. sp. *cubense*; induced resistance.

Biofertilizantes reduzem a severidade da murcha de *fusarium* e melhoram o crescimento inicial de mudas de banana 'maçã' em condições de estufa

RESUMO

O cultivo de banana é severamente afetado pela murcha de *Fusarium*, que compromete o vigor das plantas e a produtividade. Biofertilizantes têm emergido como alternativas promissoras no manejo integrado de doenças, por estimularem o crescimento vegetal e ativarem mecanismos de defesa. Este estudo avaliou os efeitos de diferentes biofertilizantes sobre o desenvolvimento vegetativo e na severidade da doença em mudas de bananeira 'Maçã' inoculadas com isolados de *Fusarium oxysporum* f. sp. *cubense* (Foc) pertencentes à raça subtropical 4 (R4ST). Quatro isolados de Foc R4ST (CML3487, CML3488, CML3489 e CML3490) foram avaliados em combinação com os biofertilizantes Agro-Mos®, Coppercrop®, Compost-Aid®, Nem-Out® e a mistura Compost-Aid® + Nem-Out®, em condições de casa de vegetação. Foram avaliados índices de clorofila, altura da planta, diâmetro do pseudocaule, área foliar total, massa fresca e seca da parte aérea e das raízes, além da severidade da doença em tecidos aéreos, rizoma, sistema vascular e raízes, bem como a área abaixo da curva de progresso da doença (AACPD). O biofertilizante Compost-Aid® promoveu os maiores teores de clorofila e favoreceu o crescimento das mudas, especialmente nas plantas inoculadas com os isolados CML3488 e CML3490. Para o isolado CML3488, a massa fresca da parte aérea aumentou de 42,54 g nas plantas sem biofertilizante para 152,09 g com a aplicação de Compost-Aid®, enquanto a massa seca da parte aérea aumentou de 4,68 g para 15,22 g. A severidade da doença nos tecidos aéreos foi substancialmente reduzida por Compost-Aid®, Nem-Out® e pela combinação desses produtos, com reduções superiores a 60% em algumas combinações entre isolados e tratamentos quando comparadas aos tratamentos de maior severidade. Esses biofertilizantes também proporcionaram menores valores de AACPD, indicando uma progressão mais lenta da doença ao longo do período de avaliação. Em contraste, Coppercrop® apresentou desempenho inconsistente e, em alguns casos, níveis de severidade semelhantes ou superiores aos observados nas plantas não tratadas. Esses resultados demonstram que biofertilizantes selecionados podem reduzir a severidade da murcha de *Fusarium* e melhorar o desempenho fisiológico e o crescimento inicial de mudas de bananeira ‘Maçã’ em condições de casa de vegetação, reforçando seu potencial como componentes complementares em estratégias integradas de manejo da doença do Panamá.

Palavras-chave: *Musa acuminata*; *Fusarium oxysporum* f. sp. *cubense*; resistência induzida.

1. Introduction

The cultivation of *Musa* spp. plays a fundamental socio-economic role in tropical and subtropical regions due to the high nutritional and commercial value of bananas (Kritsi et al., 2023). In Brazil, cultivars such as ‘Maçã’ are highly susceptible to *Fusarium oxysporum* f. sp. *cubense* (Foc), the causal agent of *Fusarium* wilt, also known as Panama disease. This disease compromises seedling quality and plant establishment, representing a serious threat to banana production systems worldwide (Tamang et al., 2024).

Fusarium wilt is considered one of the most challenging diseases in banana cultivation, as no fully effective control method is available under field conditions. Current management strategies rely mainly on the use of resistant cultivars, cultural practices, and chemical control (Ismaila et al., 2023). However, the high genetic variability of Foc, together with the emergence of new pathogenic strains, limits the long-term effectiveness of these approaches (Tamang et al., 2024). In this context, the use of healthy, vigorous seedlings is essential, as infected planting material is the primary pathway for disease dissemination and early establishment in production areas.

As a sustainable alternative, resistance induction involves activating plant defense mechanisms in response to biotic and abiotic stimuli (Kalaivani et al., 2021; Sharma et al., 2022). In this regard, biofertilizers have gained attention for their potential to stimulate plant growth, activate defense responses, and improve plant performance under biotic stress (Sallam et al., 2024). In addition, these inputs can enhance nutrient availability and soil biological activity, thereby reducing dependence on synthetic agrochemicals (Elnahal et al., 2022; Kumar et al., 2022). Such benefits are particularly relevant during the nursery phase, when adequate nutrition and physiological balance are crucial for ensuring initial crop vigor and tolerance to pathogens.

Commercial biofertilizers such as Agro-Mos®, Coppercrop®, Compost-Aid®, and Nem-Out® have been reported to promote growth and reduce disease severity in fruit crops (Carmo et al., 2023). The effectiveness of these products can be assessed using physiological and morphological indicators, including chlorophyll indices, plant height, pseudostem diameter, and biomass accumulation, which reflect the functional status of the photosynthetic apparatus and overall seedling vigor. The evaluation of these parameters allows a more integrated understanding of how biofertilizers influence early plant development under pathogen pressure.

Although several studies have demonstrated the potential of biofertilizers to promote plant growth and suppress soilborne diseases, information regarding their effectiveness against different *Fusarium oxysporum* f. sp. *cubense* SR4 isolates during the banana seedling stage remains scarce. In particular, little is known about how commercial biofertilizers influence physiological performance, vegetative development, and disease progression in ‘Maçã’ banana plants exposed to distinct pathogen isolates under nursery conditions. Therefore, this study aimed to evaluate the effects of different biofertilizers on chlorophyll indices, initial vegetative growth, seedling vigor, and *Fusarium* wilt severity in ‘Maçã’ banana seedlings inoculated with distinct Foc SR4 isolates under greenhouse conditions.

It was hypothesized that the evaluated biofertilizers would reduce *Fusarium* wilt severity and improve the vegetative development of 'Maçã' banana seedlings through growth promotion, enhanced nutrient availability, and activation of plant defense mechanisms against *Fusarium oxysporum* f. sp. *cubense*.

2. Material and Methods

The experiment was conducted in a greenhouse at the Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil, under natural photoperiod conditions. The average temperature during the experimental period ranged from 27 to 32 °C, with relative humidity ranging from 75 to 80%.

The biofertilizer treatments consisted of Agro-Mos® (3% phosphorus (P), 2.28% sulfur (S), 2% zinc (Zn), and 7.52% organic carbon), Coppercrop® (4.09% nitrogen (N), 3.41% organic carbon, and copper (Cu)), Compost-Aid® (containing *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*), *Bacillus subtilis*, and *Enterococcus faecium*; 1.25 × 108 CFU g^{-1}), Nem-Out® (containing *Bacillus licheniformis*, *Bacillus subtilis*, and *Trichoderma longibrachiatum*; 1.25 × 108 CFU g^{-1}), and a mixture of Compost-Aid® + Nem-Out®. A treatment without biofertilizer application was included as a control.

Application rates followed technical recommendations: Agro-Mos® (1 L ha^{-1}), Coppercrop® (0.5 L ha^{-1}), Compost-Aid® (10 kg ha^{-1}), and Nem-Out® (10 kg ha^{-1}). In the mixture, 50% of the recommended dose of each product was applied. Applications were made to the substrate at 0, 28, and 56 days after transplanting (DAT).

Four *Fusarium oxysporum* f. sp. *cubense* (Foc) isolates (CML3487, CML3488, CML3489, and CML3490), previously classified as Subtropical Race 4 (SR4), were obtained from the Mycological Collection of Lavras (CML), Department of Plant Pathology, Federal University of Lavras (UFLA), Brazil. The isolates were previously identified and characterized by Araújo et al. (2017) using morphological traits, phylogenetic analyses based on the TEF-1α gene, and pathogenicity tests. According to these authors, CML3488 and CML3489 belong to the same lineage, whereas CML3487 and CML3490 are grouped into a distinct, previously unreported lineage, indicating genetic variability among the isolates used in this study.

The isolates were cultured on potato dextrose agar (PDA) for seven days. Subsequently, three mycelial discs (6 mm in diameter) from each isolate were transferred to liquid PDA and incubated under constant agitation (180 rpm) at 25 ± 2 °C for five days. The resulting suspension was filtered, and the conidial concentration was adjusted to 1 × 106 conidia mL^{-1} using a Neubauer hemocytometer.

Micropropagated 'Maçã' banana seedlings, approximately 30 cm in height, were transplanted into 3 L polyethylene bags filled with sterilized commercial substrate. At transplanting, 50 mL of the respective biofertilizer solution was applied to the substrate. The same volume (50 mL $plant^{-1}$) was reapplied at 28 and 56 days after transplanting (DAT). Seedlings were irrigated daily using a 10 L watering can and maintained near field capacity throughout the experimental period.

The experiment was conducted using a completely randomized design in a 6 × 5 factorial arrangement, consisting of six biofertilizer treatments (control without biofertilizer application, Agro-Mos®, Coppercrop®, Compost-Aid®, Nem-Out®, and Compost-Aid® + Nem-Out®) and five inoculation conditions (non-inoculated control, CML3487, CML3488, CML3489, and CML3490). Thus, the experiment included an absolute control without pathogen inoculation or biofertilizer application. Each treatment combination consisted of three replicates with two plants per experimental unit, resulting in six plants per treatment combination and a total of 180 plants evaluated in the experiment.

The following parameters were evaluated:

Chlorophyll indices: Chlorophyll indices were measured weekly in the central region of fully expanded leaves using a ClorofiLOG CFL 1030 chlorophyll meter. At the final evaluation, chlorophyll a, chlorophyll b, and total chlorophyll indices were recorded.

Vegetative growth: Plant height and pseudostem diameter were measured using a ruler and a digital caliper, respectively, whereas total leaf area (TLA) was estimated following Kumar et al. (2002). Fresh mass of shoot and root was recorded immediately after harvest, and dry mass was determined after drying in a forced-air oven at 65 °C until a constant weight was reached.

Disease severity: External symptoms were evaluated every two days throughout the experimental period. At the final evaluation (70 DAT), plants were removed from the substrate and longitudinally sectioned to assess internal symptoms in the rhizome, vascular tissue, and root system. Disease severity was determined using descriptive scales adapted from Mohamed et al. (1999). For rhizome discoloration, scores ranged from 1 (absence of discoloration) to 7 (dead plant). For vascular tissue discoloration, scores ranged from 1 (no discoloration) to 6 (complete vascular discoloration). For root symptoms, scores ranged from 1 (asymptomatic roots) to 6 (dead roots).

The area under the disease progress curve (AUDPC) was calculated according to the trapezoidal integration method described by Campbell and Madden (1990):

$$AUDPC= \sum[(Yi+Yi+1)x\ (ti+1-ti)]$$

where Yi and Yi+1 represent consecutive disease severity assessments, ti and ti+1 represent the corresponding evaluation times, and n is the total number of assessments.

Data were tested for normality (Shapiro–Wilk), homogeneity of variance (Bartlett), and independence (Durbin–Watson) at a 1% significance level. Analysis of variance (ANOVA) was performed at a 5% probability level, and means were compared using the Scott–Knott test ($p \leq 0.05$). ANOVA summaries, including degrees of freedom, F-values, and p-values, are provided in Supplementary Table S1. Statistical analyses were performed using R software (R Core Team, 2020). Although chlorophyll content and disease severity were evaluated over multiple time points, a conventional ANOVA approach was applied based on the final assessment of treatment effects. This decision was made to ensure consistency with the analysis applied to the remaining variables. However, it is acknowledged that repeated-measures ANOVA or mixed-effects models could provide a more detailed characterization of temporal dynamics, representing a methodological limitation of the study.

3. Results and Discussion

The biofertilizer factor significantly affected the chlorophyll a, chlorophyll b, and total chlorophyll indices in ‘Maçã’ banana seedlings (Figure 1). Among the evaluated products, Compost-Aid® resulted in the highest chlorophyll a value (Figure 1A), differing significantly from the control and Coppercrop® treatments. A similar pattern was observed for chlorophyll b (Figure 1B) and total chlorophyll content (Figure 1C), in which Compost-Aid® consistently promoted higher chlorophyll levels than the control, Coppercrop®, and Nem-Out® treatments.

Regardless of the treatment, total chlorophyll content increased progressively throughout the evaluation period (Figure 1D), reflecting seedling development under greenhouse conditions. However, seedlings treated with Compost-Aid® and the Compost-Aid® + Nem-Out® combination exhibited a more pronounced increase in chlorophyll accumulation over time, suggesting a potential benefit of these products in maintaining the photosynthetic pigment content of banana seedlings.

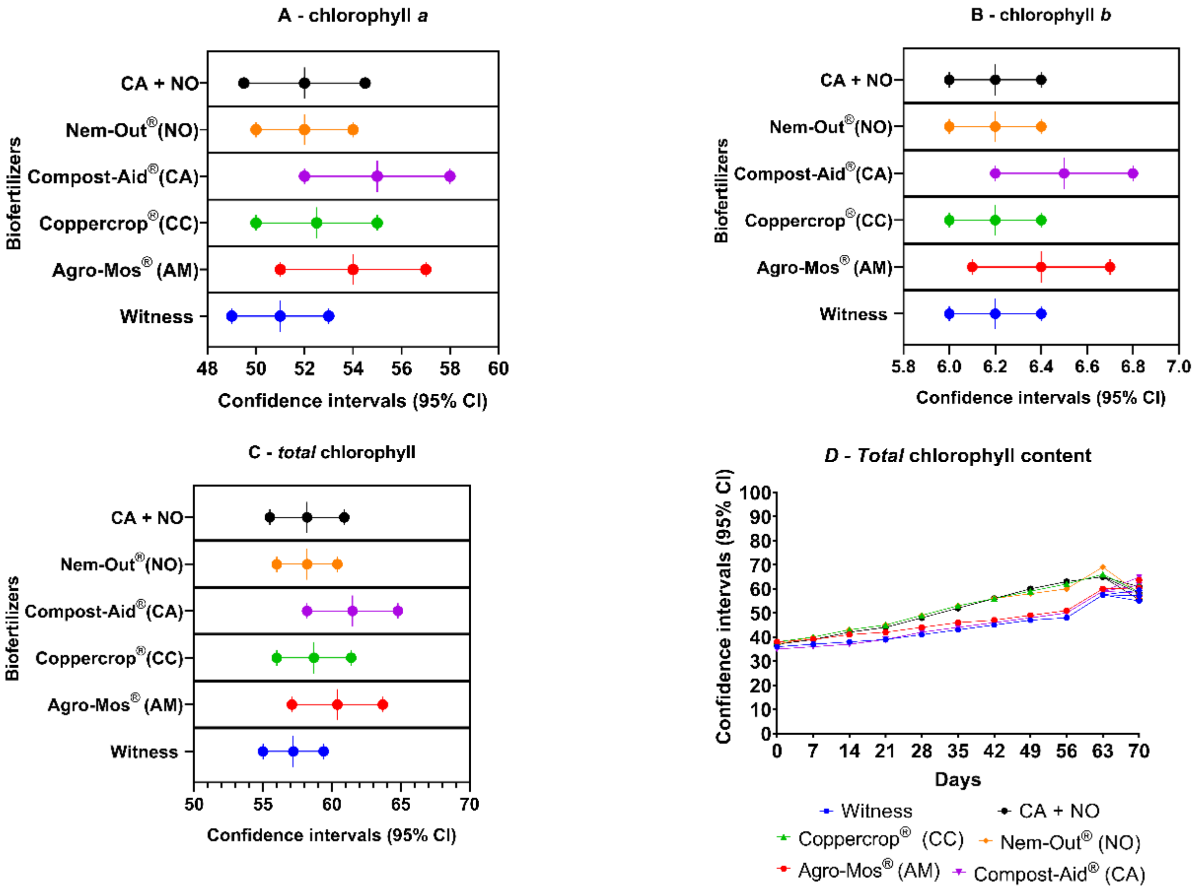

Figure 1. Confidence intervals (95% CI) of chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and temporal dynamics of total chlorophyll (D) in ‘Maçã’ banana seedlings. Means followed by the same letter do not differ according to the Scott–Knott test (*p* ≤ 0.05).

Chlorophyll plays a central role in plant growth and defense, contributing not only to photosynthetic activity but also to the biosynthesis of defense-related compounds such as phenolics, flavonoids, and alkaloids (Manghwar et al., 2021). It is also associated with maintaining cellular integrity and reinforcing structural barriers against pathogen entry (Khan et al., 2024).

In the present study, Compost-Aid® consistently increased chlorophyll levels, which may be associated with the presence of *Lactobacillus plantarum*, a bacterium known to produce siderophores and indolic compounds that can enhance nutrient availability, particularly iron, an essential element for chlorophyll biosynthesis (Shivay and Mandi, 2020). Similar positive effects of siderophore-producing microorganisms on chlorophyll accumulation have been reported in common bean (Sharma et al., 2003; Sun et al., 2022) and in tomato plants treated with *L. plantarum* (Szilagyi-Zecchin et al., 2015).

Significant biofertilizer × Foc isolate interactions were detected for pseudostem diameter, shoot fresh mass, and shoot dry mass ($p \leq 0.05$) (Table 1).

Seedlings inoculated with isolates CML3488 and CML3490 exhibited greater pseudostem diameter when treated with Agro-Mos®, Compost-Aid®, Nem-Out®, or the Compost-Aid® + Nem-Out® mixture, differing significantly from the untreated control and Coppercrop®. For isolate CML3489, increases in pseudostem diameter were mainly observed with Agro-Mos® and Compost-Aid®. In contrast, seedlings inoculated with CML3487 maintained a high pseudostem diameter regardless of biofertilizer application, with values similar to those of the non-inoculated control.

Table 1. Pseudostem diameter, shoot fresh mass, and shoot dry mass in 'Maçã' banana seedlings as affected by Foc isolates and biofertilizers. Means followed by the same lowercase letter within a column and the same uppercase letter within a row do not differ according to the Scott–Knott test ($p \leq 0.05$)

Biofertilizers	Pseudostem diameter (mm)				
	Isolate				
	Inoculated control without biofertilizer	CML3487	CML3488	CML3489	CML3490
Inoculated control without biofertilizer	23.80 aA	25.90 aA	18.86 bB	23.78 bA	17.28 bB
Agro-Mos®	30.05 aA	27.51 aA	25.98 aA	27.73 aA	27.52 aA
Coppercrop®	28.25 aA	25.79 aA	18.68 bB	24.14 bA	18.97 bB
Compost-Aid®	24.44 aA	25.70 aA	27.96 aA	30.58 aA	28.35 aA
Nem-Out®	25.36 aA	26.02 aA	26.23 aA	19.93 bA	25.76 aA
Compost-Aid®+Nem-Out®	27.71 aA	24.62 aA	26.64 aA	25.39 bA	25.22 aA
CV (%)	13.71				
Biofertilizers	Shoot fresh mass (g)				
	Isolate				
	Inoculated control without biofertilizer	CML3487	CML3488	CML3489	CML3490
Inoculated control without biofertilizer	65.07 bA	91.14 aA	42.54 cA	73.92 aA	41.88 bA
Agro-Mos®	168.77 aA	109.33 aB	95.79 bB	102.28 aB	99.76 aB
Coppercrop®	125.73 aA	96.28 aA	41.25 cB	76.35 aB	49.21bB
Compost-Aid®	113.03 aA	114.46 aA	152.09 aA	132.03 aA	149.24 aA
Nem-Out®	136.62 aA	122.49 aA	93.90 bB	64.03 aB	117.11 aA
Compost-Aid®+Nem-Out®	125.95 aA	104.70 aA	133.66 aA	98.78 aA	105.12 aA
CV (%)	30.19				
Biofertilizers	Shoot dry mass (g)				
	Isolate				
	Inoculated control without biofertilizer	CML3487	CML3488	CML3489	CML3490
Inoculated control without biofertilizer	5.75 bA	9.55 aA	4.68 cA	8.89 aA	4.37 bA
Agro-Mos®	17.16 aA	12.23 aA	10.11 bA	11.38 aA	12.14 aA
Coppercrop®	11.56 aA	10.43 aA	4.60 cA	8.66 aA	5.53 bA
Compost-Aid®	10.28 aB	7.21 aB	15.22 aA	12.23 aA	14.76 aA
Nem-Out®	12.54 aA	11.72 aA	9.66 bA	6.19 aA	13.32 aA
Compost-Aid®+Nem-Out®	11.70 aA	10.72 aA	15.36 aA	9.70 aA	9.85 aA
CV (%)	34.46				

Shoot biomass accumulation followed a pattern consistent with pseudostem development. In inoculated plants that received no biofertilizer, shoot fresh and dry masses were generally reduced, highlighting the negative effect of pathogen infection on seedling growth. Under inoculation with CML3488, the highest shoot fresh and dry mass values were observed in treatments with Compost-Aid® and the Compost-Aid® + Nem-Out® mixture, followed by Agro-Mos® and Nem-Out®. In contrast, Coppercrop® resulted in lower biomass accumulation across most isolates. The greater biomass production observed in seedlings treated with Compost-Aid® and the Compost-Aid® + Nem-Out® mixture may be related to the combined effects of growth-promoting microorganisms on nutrient acquisition, plant metabolism, and suppression of *Fusarium* wilt development.

Biofertilizers significantly affected plant height, total leaf area, and root biomass (Figure 2). Among the evaluated products, Compost-Aid® and the Compost-Aid® + Nem-Out® combination generally promoted greater vegetative growth and root development across the evaluated isolates. Agro-Mos® also improved some growth variables compared with the untreated control, although its effects were less consistent among isolates. At the same time, Coppercrop® and Nem-Out® did not differ statistically from untreated plants in several of the evaluated variables.

Non-inoculated seedlings showed the highest growth values, confirming the negative effect of *Fusarium* wilt on plant development. Differences among isolates were observed, although biofertilizer application partially mitigated the suppressive effects of infection, suggesting that these products may help maintain seedling growth under pathogen pressure and could serve as complementary tools in integrated disease management programs.

Biofertilizers are widely recognized for enhancing vegetative growth in fruit crops (Kumar et al., 2022). Similar responses have been reported in passion fruit (Diniz et al., 2022) and in banana studies showing improved soil microbial balance and suppression of Foc (Wang et al., 2022). The presence of growth-promoting microorganisms enhances nutrient recycling and availability (Chakraborty and Akhtar, 2021) while promoting plant development through phytohormone production and microbiome structuring (Singh et al., 2021).

Fusarium wilt severity in aerial tissues varied according to the biofertilizer × isolate interaction (Figure 3A). Compost-Aid® resulted in the lowest severity levels in plants inoculated with CML3488. Nem-Out® and Compost-Aid® + Nem-Out® were effective against CML3487 and CML3490. The highest severities were observed in the negative control (CML3487 and CML3490) and in plants treated with Coppercrop® (CML3488 and CML3489).

Agro-Mos® reduced severity for CML3487 and CML3488, but was associated with higher severity values for CML3489 (Figure 3B). Coppercrop® reduced severity only for CML3487 and CML3490, while higher severity levels were observed for the other isolates.

Significant interactions were observed for disease severity in rhizome and vascular tissues (Table 2). Nem-Out®, Compost-Aid®, and their combination consistently reduced severity across most isolates, whereas Agro-Mos® and Coppercrop® showed isolate-dependent and, in some cases, less favorable responses, particularly for CML3489, which exhibited the highest severity.

In vascular tissues, Compost-Aid®, Nem-Out®, and their mixture generally resulted in lower severity values across the evaluated isolates, whereas CML3488 exhibited the highest severity levels, particularly under Coppercrop® treatment.

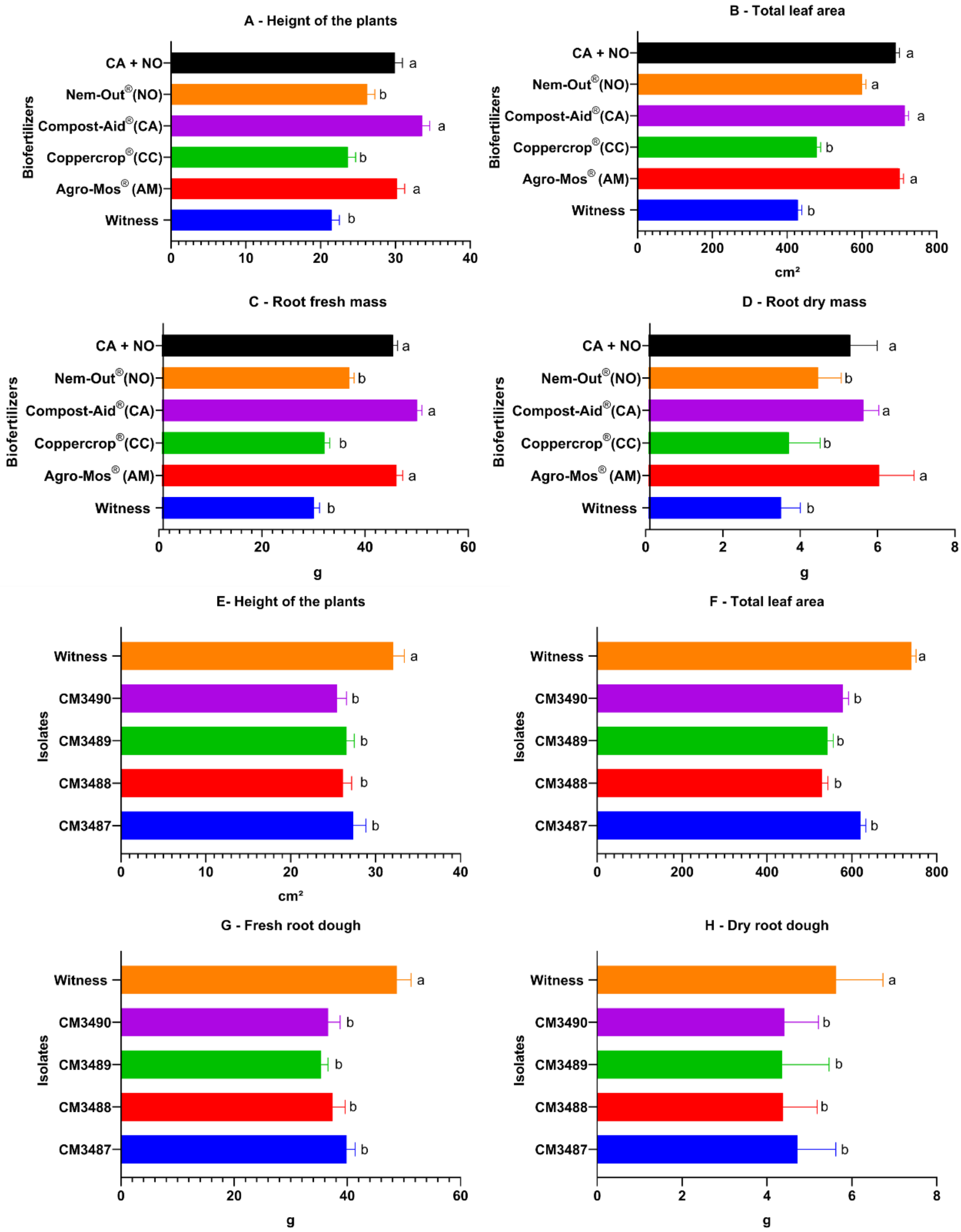

Figure 2. Plant height (A, E), total leaf area (B, F), root fresh mass (C, G), and root dry mass (D, H) of ‘Maçã’ banana seedlings inoculated with different *Fusarium oxysporum* f. sp. *cubense* (Foc) isolates and treated with biofertilizers. Panels A–D show the main effects of biofertilizer treatments, whereas panels E–H show the main effects of Foc isolates. Means followed by the same letter do not differ according to the Scott–Knott test ($p \leq 0.05$).

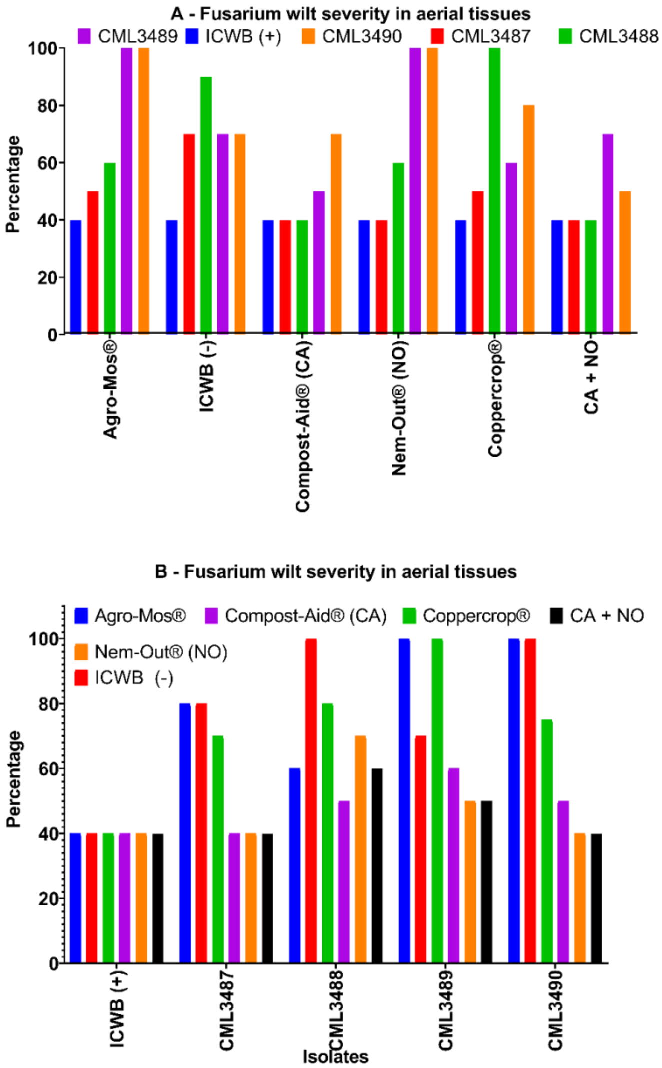

Figure 3. Severity of *Fusarium wilt* in the aerial tissues of 'Maçã' banana seedlings inoculated with different *Fusarium oxysporum* f. sp. *cubense* isolates and treated with biofertilizers. Legend: ICWB: Inoculated control without biofertilizer.

Table 2. *Fusarium* wilt severity in the rhizome and vascular tissues of 'Maçã' banana seedlings as affected by Foc isolates and biofertilizer treatments. Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ according to the Scott–Knott test ($p \leq 0.05$)

Biofertilizers	*Fusarium* wilt severity in the rhizome				
	Isolate				
	Inoculated control without biofertilizer	CML3487	CML3488	CML3489	CML3490
Inoculated control without biofertilizer	28.57 aB	85.72 aA	95.24 bA	71.43 aA	119.05 aA
Agro-Mos® (AM)	28.57 aB	47.62 aB	61.9 bB	90.47 aA	80.95 bA
Coppercrop®(CC)	28.57 aB	66.67 aA	38.09 bB	95.24 aA	66.66 bA
Compost-Aid® (CA)	28.57 aC	47.62 aC	147.62 aA	90.47 aB	57.14 bC
Nem-Out® (NO)	28.57 aA	47.62 aA	85.71 bA	61.9 aA	61.9 bA
CA + NO	28.57 aA	33.33 aA	57.14 bA	52.38 aA	47.6 bA
CV (%)	41.46				
Biofertilizers	*Fusarium* wilt severity in the vascular system				
	Isolate				
	Inoculated control without biofertilizer	CML3487	CML3488	CML3489	CML3490
Inoculated control without biofertilizer	33.33 aB	50.00 aB	83.33 bA	66.67 bB	105.56 aA
Agro-Mos® (AM)	33.33 aB	50.00 aB	55.56 bB	111.11 aA	61.11 bB
Coppercrop® (CC)	33.33 aA	50.00 aA	33.33 bA	77.78 bA	55.56 bA

Compost-Aid® (CA)	33.33 aA	33.33 aA	55.56 bA	38.89 bA	38.89 bA
Nem-Out® (NO)	33.33 aC	50.00 aC	138.89 aA	100.00 aB	61.11 bC
CA + NO	33.33 aA	38.89 aA	66.67 bA	55.55 bA	38.89 bA
CV (%)	38.23				

Root severity and AUDPC were significantly affected by biofertilizers and isolates, but no interaction was detected (Figures 4A-D). Disease severity in roots was higher in CML3488 and was reduced by all biofertilizers except Coppercrop®. The lowest AUDPC values were obtained with Compost-Aid®, Nem-Out®, and their combination, indicating slower disease progression throughout the evaluation period, whereas Agro-Mos® and Coppercrop® displayed higher values.

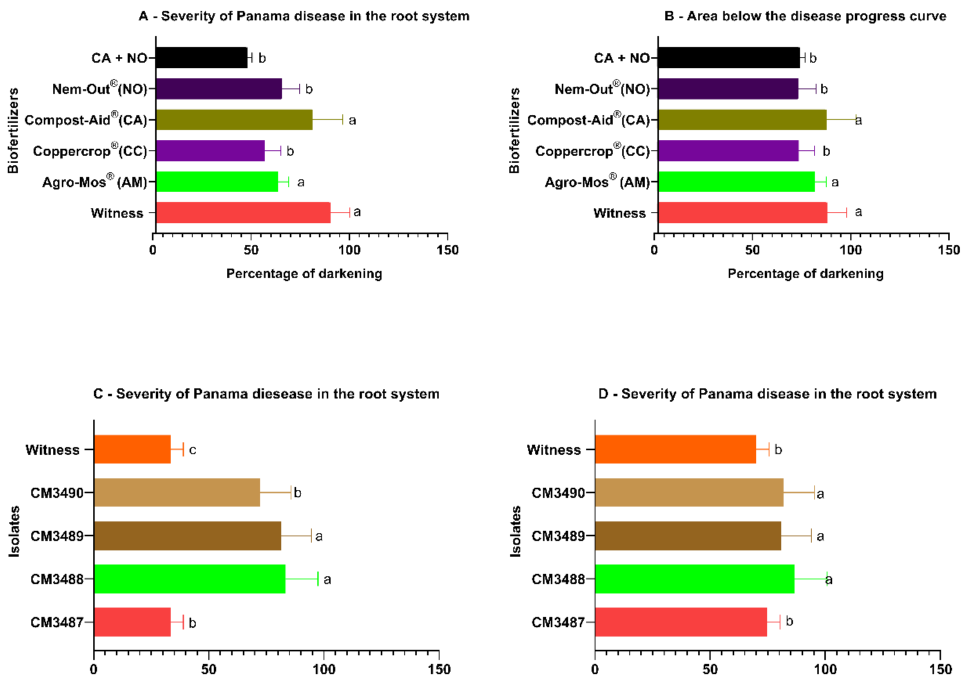

Figure 4. *Fusarium* wilt severity in the root system (A and C) and area under the disease progress curve (AUDPC; B and D) of 'Maçã' banana seedlings as affected by biofertilizer treatments and Foc isolates. Panels A and B show the main effects of biofertilizer treatments, whereas panels C and D show the main effects of Foc isolates. Means followed by the same letter do not differ according to the Scott–Knott test ($p \leq 0.05$).

Compost-Aid® and Nem-Out® were the most effective treatments for reducing disease severity. Their performance may be associated with the presence of *Bacillus subtilis*, which exhibits well-documented antifungal activity through chitinase production and secretion of metabolites that disrupt fungal cell integrity (Gacitúa et al., 2009; Zhu et al., 2020). In addition, this bacterium efficiently colonizes the rhizosphere, enhances nutrient availability, and activates systemic resistance in plants (Orberá et al., 2014; Sidorova et al., 2018; Blake et al., 2021).

Compost-Aid® also contains *Lactobacillus plantarum*, known for producing bacteriocins with antimicrobial activity (Roselló et al., 2013; López-Seijas et al., 2019), and *Enterococcus faecium*, which contributes to pathogen suppression through organic acid production (Fhoula et al., 2013). The observed increases in chlorophyll indices may further suggest elicitor activity, since induced resistance is commonly associated with changes in photosynthetic metabolism (Nowicka et al., 2018).

Although Agro-Mos® and Coppercrop® were less effective against *Fusarium* wilt, previous studies have reported their potential application in other plant pathogen systems (Kunwar et al., 2023). The inconsistent performance of Coppercrop® among Foc isolates suggests that its effectiveness may depend on specific host-pathogen interactions. In some situations, disease severity remained similar to or even higher than that observed in untreated plants, indicating that this product may not provide stable protection against *Fusarium* wilt under all infection scenarios. Such variability may be associated with differences in pathogen aggressiveness or in the responsiveness of the plant–microorganism interaction. Therefore, additional studies are needed to clarify the mechanisms involved and to determine the conditions under which Coppercrop® may contribute more effectively to disease suppression. Overall, the superior performance of Compost-Aid®, Nem-Out®, and their combination highlights the importance of formulations containing multiple biological agents, as synergistic microbial interactions may help suppress complex soilborne pathogens such as *F. oxysporum* f. sp. *cubense* (Nowicka et al., 2018).

4. Conclusions

The combined application of Compost-Aid® and Nem-Out® effectively reduced *Fusarium* wilt severity and enhanced vegetative growth of 'Maçã' banana seedlings, highlighting their potential as complementary components of integrated disease management strategies during the early developmental stage. Although field validation is still necessary, the greenhouse results demonstrate that biofertilizers represent a promising complementary strategy for the integrated management of *Fusarium* wilt disease in banana production systems.

Authors' Contribution

Silveira, F. A.: Conceptualization, investigation, methodology, data curation, formal analysis, writing – original draft, and writing – review and editing. Ribeiro, C. H. M.: Writing – original draft and writing – review and editing. Gomes, L. B.; Rodrigues, M. A.; Araújo, N. A. F.; Piñango, D. O. P.: Investigation, evaluations, data collection, writing – original draft, and writing – review and editing. Guimarães, P. H. S.: Statistical analysis and interpretation of results. Pio, L. A. S.: Conceptualization, funding acquisition, methodology, formal analysis, supervision, project administration, writing – review and editing, and final approval of the manuscript.

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