SILVEIRA, F. A.; GOMES, L. B.; PIO, L. A. S.; RIBEIRO, C. H. M.; GUIMARÃES, P. H. S.; RODRIGUES, M. A.; ARAÚJO, N. A. F. Biofertilizers inhibit the mycelial growth of *fusarium oxysporum* f. sp *cubense*. **Revista de Agricultura Neotropical**, Cassilândia-MS, v. 12, n. 2, e9189, Apr./June, 2025. ISSN 2358-6303. DOI: https://doi.org/10.32404/rean.v12i2.9189.

Biofertilizers inhibit the mycelial growth of *fusarium oxysporum* f. sp *cubense*

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Received: 11/11/2024; Accepted: 04/04/2025.

ABSTRACT

The objective of this study was to evaluate the effect of different concentrations of the biofertilizers Agro-Mos® (0.0, 1.0, 2.0, 4.0, and 8.0 mL L^{-1}), Compost-Aid® (0, 0.5, 1.0, 2.0, and 4.0 mg L^{-1}), and Nem-Out® (0, 2.5, 5.0, 10.0, and 20.0 mg L^{-1}) on the mycelial growth and inhibition rate of various isolates (CML3387, CML3488, CML3489, and CML3490) of *Fusarium oxysporum* f. sp. *Cubense* (*Foc*) under in vitro conditions. The isolates CML3487 and CML3490 had a higher inhibition rate than the isolates CML3488 and CML3489. It was concluded that the biofertilizer concentrations tested under in vitro conditions inhibited the mycelial growth of *Foc*, except for the 1.0 mL L^{-1} concentration of Agro-Mos®.

Keywords: Biological control, *Fusarium* wilt, *In vitro* assay, Spore germination.

Os biofertilizantes inibem o crescimento mycelial de *fusarium oxysporum* f. sp *cubense*

RESUMO

Objetivou-se avaliar o efeito de doses dos biofertilizantes Agro-Mos® (0,0; 1,0; 2,0; 4,0; 8,0 ml L^{-1}); Compost-Aid® (0; 0,5; 1,0; 2,0; 4,0 mg L^{-1}) e Nem-Out® (0; 2,5; 5,0; 10,0; 20,0 mg L^{-1}) no crescimento micelial e taxa de inibição de diferentes isolados (CML3387, CML3488, CML3489 e CML3490) de *Fusarium oxysporum* f. sp. *Cubense* in vitro. Os isolados CML3487 e CML3490 apresentaram maior taxa de inibição que os isolados CML3488 e CML3489. Conclui-se que as doses dos biofertilizantes estudados in vitro inibiram o crescimento micelial de *Foc*, exceto a dose 1,0 mL^{-1} de Agro-Mos®.

Palavras-chave: Controle biológico, Murcha de *fusarium*, Ensaio *in vitro*, Germinação de esporos.

1. Introduction

Bananas (*Musa* spp), originating from Southeast Asia and the Western Pacific, are typically cultivated in tropical and subtropical regions. In poorer countries, where a large part of the population is malnourished, bananas become a basic source of nutrients due to their rich composition of minerals and vitamins, as well as providing readily available energy (Scott, 2021).

However, banana production faces several obstacles, one of the main ones being Panama disease, caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Panama disease is among the most destructive threats to banana cultivation, significantly impacting crop productivity worldwide (Tamang et al., 2024).

Several studies have sought strategies to control Panama disease, caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*). However, no effective control method is currently available. Conventionally, the most commonly adopted approaches include chemical, cultural, and genetic control measures (Cannon et al., 2022; Ismaila et al., 2023). However, conventional strategies are often ineffective for pathogens like *Foc*, which have a high evolutionary capacity and genetic variability. Therefore, it is important to conduct research aimed at alternative disease control measures to minimize the production losses caused by the pathogen (Sasseron et al., 2020).

Among the alternatives, the induction of plant resistance, which involves activating inactive and latent mechanisms, has been widely studied, and the results are promising. The activation of these mechanisms occurs through biotic or abiotic agents (Fontana et al., 2021).

The use of biofertilizers for inducing resistance in plants offers advantages, such as assisting in plant growth, participating in soil nutrient recycling, and reducing the use of agrochemicals, thus contributing to environmental decontamination (Kumar et al., 2020; Wang; Bi, 2021). Various products are being tested for their inducing action, among which Agro-Mos®, Compost-Aid®, and Nem-Out® stand out.

In this context, the present study aimed to evaluate the effect of different concentrations of biofertilizers on the mycelial growth of four *Foc* isolates under in vitro conditions.

2. Material and Methods

Four *Foc* isolates corresponding to Race 1 were used: CML3487, CML3488, CML3489, and CML3490. The characterization and identification of the isolates were carried out by Araújo et al. (2017). Isolates CML3488 and CML3489 were classified within the same lineage, while isolates CML3487 and CML3490 were assigned to a newly identified lineage not previously described. The isolates were cultured on potato dextrose agar (PDA) medium (Acumedia, Michigan, USA).

The isolates were combined with different concentrations of the biofertilizers Agro-Mos® (composition: 3% Cu, 2.28% S, 2% Zn, and 7.52% organic C), Compost-Aid® (composition: *Lactobacillus plantarum*, *Bacillus subtilis*, and *Streptococcus faecium* at a concentration of 1.25 x 108 CFU g^{-1}), and Nem-Out® (composition: Bacillus licheniformis, *Bacillus subtilis*, and *Trichoderma longibrachiatum* at a concentration of 1.25 x 108 CFU g^{-1}), provided by the company Alltech. The biofertilizers were studied individually at their respective concentrations: Agro-Mos® 0.0, 1.0, 2.0, 4.0, and 8.0 ml L^{-1}, Compost-Aid® 0.0, 0.5, 1.0, 2.0, and 4.0 mg L^{-1}, and Nem-Out® 0.0, 2.5, 5.0, 10.0, and 20.0 mg L^{-1}.

The culture medium was PDA, adjusted to pH 6.0, then autoclaved for 20 minutes at 121 °C. While still warm, the biofertilizer concentrations were added to the medium in a flow chamber and homogenized with a rod. Petri dishes with a diameter of 9 cm were used, and 20 mL of the medium was poured inside each dish. After the medium solidified, a 6 mm diameter disc of mycelium from the corresponding isolate, grown on PDA for seven days at 25 °C ± 2 °C, was transferred to the center of the Petri dish.

The cultures of *Foc* isolates were maintained in a BOD incubator with controlled temperature and photoperiod, being 25 °C ± 2 °C and 12 hours, respectively, for eight days. The experiment was conducted using a completely randomized design (CRD), arranged in a 4 × 5 factorial scheme (isolates × concentrations), with six replications. Each experimental unit consisted of one Petri dish. The control treatment consisted of the absence of biofertilizers in the PDA medium (zero concentration) for each *Foc* isolate.

The diameter of the mycelial growth of *Foc* isolates was measured every two days for eight days using a millimeter ruler. The colony diameter was determined by averaging two measurements in diametrically opposite directions. At the end of the experiment, the total mycelial growth, daily mycelial growth, and the inhibition rate of mycelial growth of the isolates were obtained.

The data were subjected to analysis of variance at a 5% significance level. For those that showed significant differences, a Skott-Knott test was performed for qualitative factors at a 5% significance level, and regression analysis was conducted for quantitative factors. The analyses were performed using R software (The Development Core Team, 2019).

For the variable daily mycelial growth, analysis of variance (ANOVA) was performed using a completely

randomized design in a 4×5×4 factorial scheme (isolates × concentrations × days), with a split-plot arrangement over time. The subplots were observations made over days 2, 4, 6, and 8. The analysis was performed using a mixed-effects model (Cameron; Trivedi, 2013), with a Box-Cox transformation applied to the data due to the non-normality of the residuals. The study was conducted using the predicted model presented below:

$$y_s = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_{ijk} + \tau_l + (\alpha\tau)_{il} + (\beta\tau)_{jl} + (\alpha\beta\tau)_{ijl}$$

where s = 1, ..., n; (n observations); μ is the effect associated with the general mean (constant); αi is the effect of the i-th factor (isolate), i = 1, 2, 3, 4; βj is the effect associated with the j-th concentration (0, 1, 2, 4, 8), j = 1, 2, 3, 4, 5; γk is the effect associated with the replications, k = 1, 2, 3; δijk is the effect associated with the plot error; τl is the effect associated with the l-th time in days (2, 4, 6, 8), l = 1, 2, 3, 4, $(\alpha\beta)_{ij}$, $(\alpha\tau)_{il}$, $(\beta\tau)_{jl}$, $(\alpha\beta\tau)_{ijl}$, and the effects associated with the interactions.

3. Results and Discussion

The three biofertilizers showed a significant interaction effect for mycelial growth and inhibition rate variables. The interactions of concentrations at each isolate level were analyzed, and all effects were significant.

The study of the daily mycelial growth variable was carried out using graphs of the predicted model.

Regarding the inhibition rate (Figure 1A), a decrease in mycelial growth with increasing concentrations of Agro-Mos® was observed. The highest growth inhibition was for isolate CML3487, followed by isolate CML3490. The lowest growth inhibition was for isolate CML3488.

The isolates showed increased mycelial growth at 1.0 and 2.0 ml L^{-1} concentrations, declining until the highest concentration of 8.0 ml L^{-1}. All isolates showed the least mycelial growth at the concentration of 8.0 ml L^{-1}. The lowest growth was observed for isolates CML3487 and CML3490 (Figure 1B).

Figure 1C shows the temporal response of *Foc* isolates to Agro-Mos®. All isolates showed an increase in average growth over the days, with isolate CML3489 showing the highest growth, followed by isolates CML3488 and CML3487. The lowest average growth over the days was observed for isolate CML3490.

Over time, the average growth of the *Foc* isolates was lower at concentrations of 2.0 mL^{-1} and above compared to the control (Figure 1D). The 8.0 mL^{-1} concentration resulted in the lowest average mycelial growth across all time points. Interestingly, at the 1.0 mL^{-1} concentration, *Foc* growth exceeded that of the control, indicating that this lower concentration actually promoted the mycelial development of *Foc* over time.

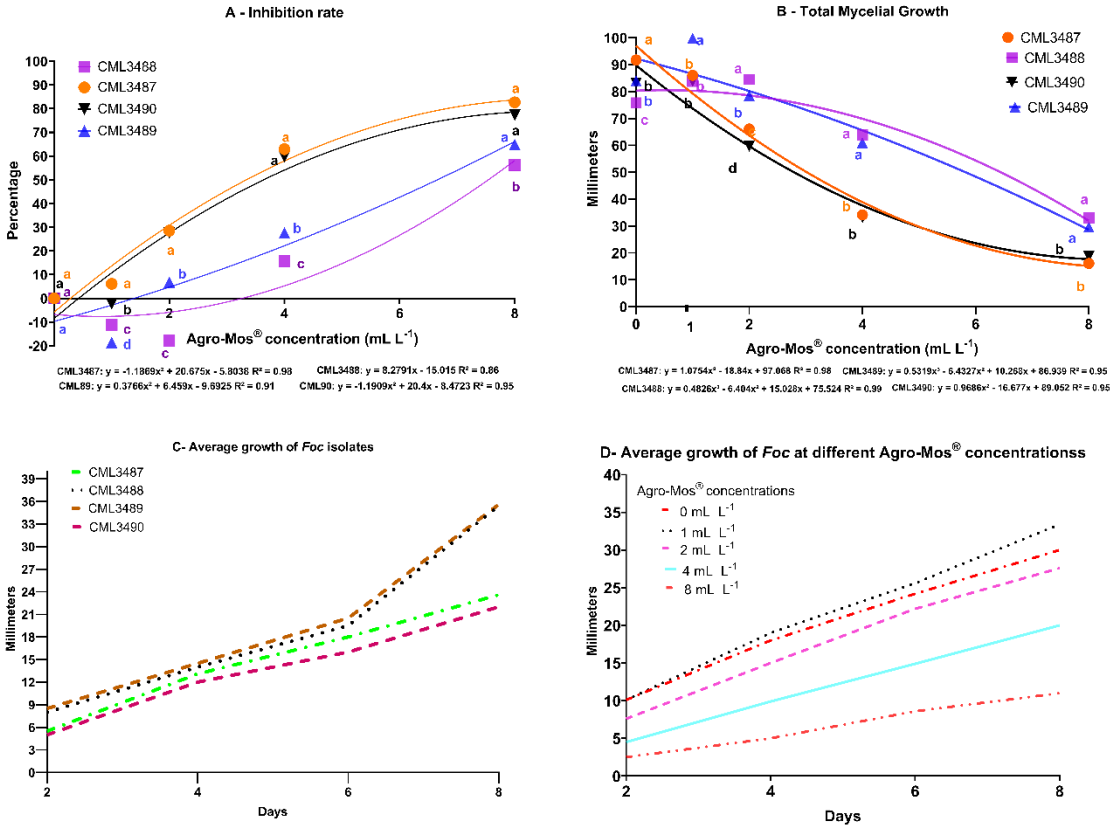

Figure 1. Regression equations for the mycelial inhibition rate (%) (A); total mycelial growth (mm) of *Foc* as affected by different isolates and Agro-Mos® concentrations (B); average growth behavior of the isolates (mm) (C); and growth of *Foc* at different Agro-Mos® concentrations over cultivation days (D). Means followed by the same letter in the columns belong to the same group according to the Scott-Knott test at the 5% probability level.

For the inhibition rate (Figure 2B), an increase in the inhibition rate of the isolates' growth is observed at concentrations of 0.5 and 1.0 mg L^{-1}, followed by a decrease and then another increase at the highest concentration (4.0 mg L^{-1}).

The isolates showed a decline in mycelial growth (Figure 2B) at the first two concentrations (0.5 and 1.0 mg L^{-1}). Then, a slight increase in mycelial growth was observed, which declined at the highest concentration (4.0 mg L^{-1}). At the first concentration studied (0.5 mg L^{-1}), there was a sharp reduction in the mycelial growth of the isolates, with no significant changes in growth at the successive concentrations of Compost-Aid®.

Figure 2C shows the behavior of the *Foc* isolates over time in response to Compost-Aid® treatment. Isolate CML3489 had the highest average growth over the days, followed by isolates CML3488 and CML3487, respectively. Isolate CML3490 exhibited the lowest mycelial growth. In Figure 2D, the average growth of *Foc* over time was reduced at all tested concentrations of the Compost-Aid® biofertilizer compared to the control.

Regarding the inhibition rate in the presence of Nem-Out® (Figure 3A), an increase in the growth of the isolates was observed up to a concentration of 5.0 mg L^{-1}, after which the growth inhibition rate decreased (at a concentration of 10.0 mg L^{-1}) and increased again at the highest concentration (20 mg L^{-1}).

The isolates showed a decline in mycelial growth (Figure 3B) at the first two concentrations (2.5 and 5.0 mg L^{-1}), after which the isolates grew at successive concentrations and decreased again at the highest concentration of 20.0 mg L^{-1}.

Figure 3C shows the average growth behavior over the days for each isolate in the presence of Nem-Out® is shown. The highest average growth over the days was observed for isolate CML3489, followed by isolates CML3488, CML3487, and CML3490, respectively. Figure 3D shows that all concentrations promoted inhibition of average growth over the days compared to the control (zero concentration). However, the concentration of 20.0 mg L^{-1} resulted in the lowest average growth of *Foc* over the days.

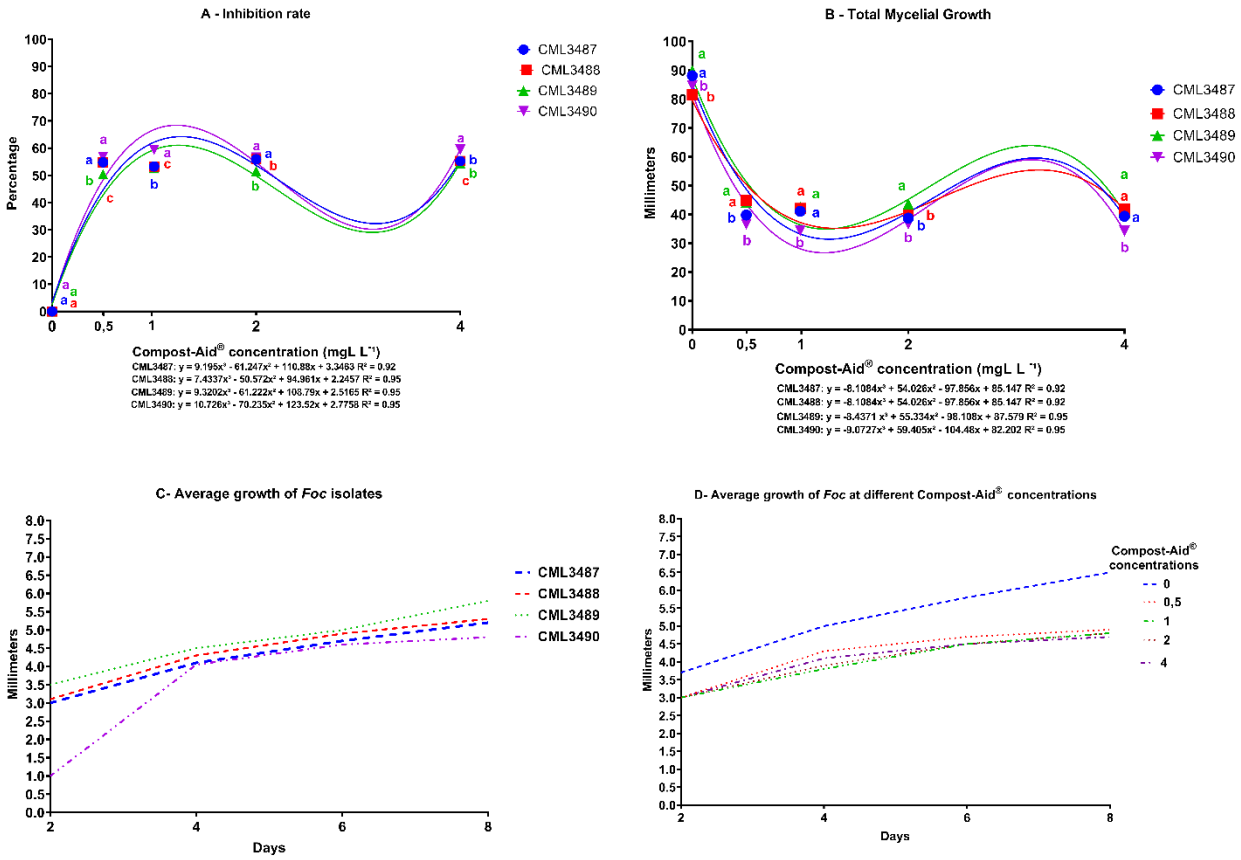

Figure 2. Regression equations for the mycelial inhibition rate (%) (A); total mycelial growth (mm) of *Foc* as a function of different isolates and Compost-Aid® concentrations (B); average growth behavior of the isolates (mm) (C); and growth of *Foc* at different Compost-Aid® concentrations over the cultivation period (D). Means followed by the same letter in the columns belong to the same group according to the Scott-Knott test at the 5% probability level.

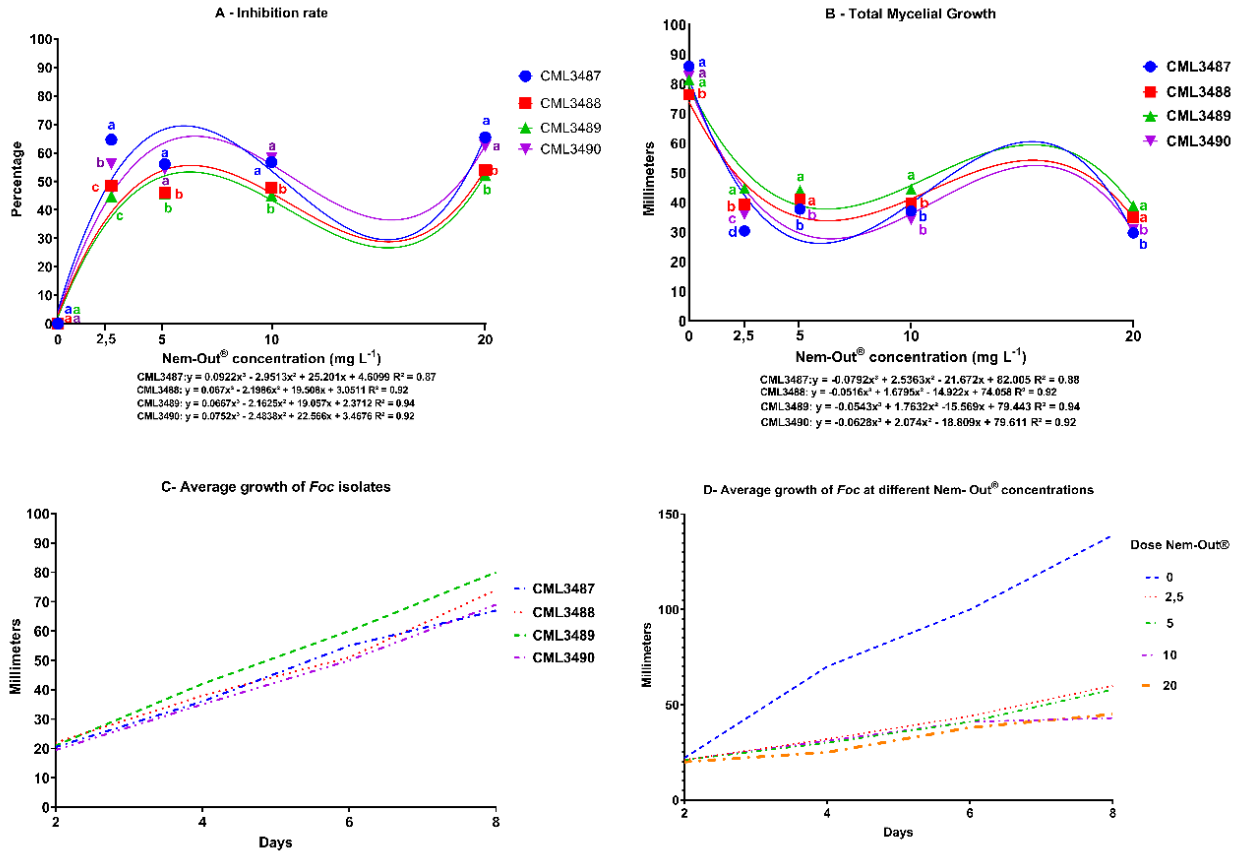

Figure 3. Regression equations for the mycelial inhibition rate (%) (A); total mycelial growth (mm) of *Foc* as a function of different isolates and Compost-Aid® concentrations (B); average growth behavior of the isolates (mm) (C); and growth of *Foc* at different Nem-Out® concentrations over the cultivation period (D). Means followed by the same letter in the columns belong to the same group according to the Scott-Knott test at the 5% probability level.

In the study evaluating the sensitivity of hyperparasitic fungi to alternative products for use in controlling *Asperisporium caricae* in papaya crops (Vivas et al., 2020), the authors did not obtain results for control under in vitro conditions using Agro-Mos®. This is contrary to the results found in the present study, where it can be inferred that Agro-Mos® had a toxic effect on *Foc*, with an observed inhibition rate of up to 82% in isolate growth.

Gomes et al. (2016), also observed inhibition of mycelial growth under *in vitro* conditions using Agro-Mos® in controlling *Colletotrichum gloeosporioides* in guava. When inducing resistance to control black In Costa Rica, when resistance was induced to control black Sigatoka using biological fungicides, Agro-Mos® proved effective in inhibiting mycelial growth and may serve as an alternative to replace up to 25% of the chemical fungicides traditionally applied.

Studies by Demartelaere et al. (2017) demonstrated the positive effects of Agro-Mos® in controlling phytopathogens *Colletotrichum gloeosporioides* in papaya fruits.

According to Rodrigues and Silva et al. (2020), the toxicity of Agro-Mos® can be attributed to the presence of copper in its composition. Being a product based on phosphorylated mannan oligosaccharide derived from the yeast cell wall of *Saccharomyces cerevisiae* (Desm.) Meyen and a Copper-Zinc biocomplex, it aids in plant disease protection, but can also have toxic effects on different fungi, as some metals like copper play a fundamental role in fungal metabolism and can become toxic when in excess (Robinson et al., 2021).

The biofertilizer Compost-Aid® results from a mixture of enzymes and bacteria (*Lactobacillus plantarum*, Bacillus subtilis, and *Enterococcus faecium*) that naturally accelerate the composting

process, converting organic materials into a compound with a low C/N ratio and helping reduce the severity of soil pathogen diseases through microorganism competition (Miamoto et al., 2017).

Nascimento et al. (2016), studied the effect of the biofertilizer Compost-Aid® on soil pathogen survival and observed 100% inhibition of the fungus *Macrophomina phaseolina* and 98.57% of *Sclerotium rolfsii* using a concentration of 25g L^{-1}. In the present study, the highest inhibition rate of *Foc* growth was 54.9% at a concentration of 4 mg L^{-1}, but it is noted that the concentration used by Nascimento et al. (2016) was 6000 times higher than the highest concentration used in the present study. Therefore, the biofertilizer Compost-Aid® shows potential for controlling *Foc*, and other concentrations should be tested.

Nem-Out® has been mainly studied to control phytonematodes, but due to its composition, this product also shows promise in controlling pathogens (Hu et al., 2017).

One justification for this fact is that the product contains *Bacillus licheniformis* and *Bacillus subtilis* species, which are considered biocontrol agents of nematodes and soil pathogens (Migunova; Sasanelli, 2021). These bacteria can compete for ecological niches or substrates and produce toxins and various metabolites, directly affecting the pathogenic agent and/or inducing plant resistance (Das et al., 2017; Khalil; Hassouna, 2022).

In a study conducted by Wang et al. (2023), evaluating the efficacy of biological control with *Bacillus licheniformis* against peach soft rot, the authors observed a positive effect on disease control by improving free radical elimination capacity and disease resistance.

4. Conclusions

Foc isolates CML3487 and CML3490 for all studied biofertilizers had higher inhibition rates than isolates CML3488 and CML3489. Regarding average growth over time, the isolates showed the same behavior, with the highest growth observed in isolate CML3489 and the lowest in isolate CML3490. Except for the 1.0 ml L^{-1} concentration of Agro-Mos®, all studied concentrations of biofertilizers inhibited the mycelial growth of *Foc*.

Authors' Contribution

All authors contributed equally to this manuscript. Flávia Aparecida da Silva, Leila Aparecida Salles Pio, Carlos Henrique Milagres Ribeiro, and Paulo Henrique Sales Guimarães performed data analysis, writing, and revision. Carlos Henrique Milagres Ribeiro, Flávia Aparecida da Silva, and Leila Aparecida Salles Pio conducted text editing. Flávia Aparecida da Silva, Larissa Bitencourt Gomes, Neilton Antônio Fiusa Araújo, and Mariane Aparecida Rodrigues conducted the experiment and data collection.

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