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Full Length Article

Compatibility of Rhizobacteria that Potentially Biocontrol *Sclerotinia sclerotiorum* with the Main Agricultural Pesticides and Comercial Biologicals

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Abstract

The use of biological products based on fungi and bacteria in agriculture has increased. However, these microorganisms may be non-target species of agrochemicals that are usually employed in disease control. When used in mix or co-inoculated in cultures, the efficacy of biologicals may be modified. In this study, we tested the hypothesis that rhizobacteria are differentially affected by agricultural chemicals and their interaction with biological products that are currently on the market. In particular, we evaluated the interaction of three rhizobacteria that potentially biocontrol *Sclerotinia sclerotiorum* (BA123R - *Enterobacter asburiae*, BA81R – *Bacillus cereus* and BA88R – *B. cereus*) with pesticides commonly used in pest control and easily found in the agricultural market, and against commercial strains of *Trichoderma harzianum*, *Bradyrhizobium japonicum*, *Azospirillum brasilense* and *B. megaterium* + *B. subtilis*. Briefly, optical density (OD) tests, colony-forming unit (CFU) concentration, and paired cultures of the biologicals were employed in this study. The OD test revealed that the agrochemicals Avicta, Captan, Cropstar, Cruiser, Derosal, and Fortenza negatively affected the growth of the three rhizobacteria. In contrast, Imidacloprid, Imidagold, Maxim Advanced, Maxim, and Poncho had a beneficial effect on the growth of the strains. Based on the CFU counts, the insecticides, Cruiser and Fortenza, which are based on thiamethoxam and Cyantraniliprole, respectively, are highly incompatible with the tested strains. Further, co-cultures could not be established using combinations of the rhizobacteria with *B. japonicum* and *B. megaterium* + *B. subtilis*. The strains were compatible with *T. harzianum* and interacted with *A. brasilense*; however, bacteria of the genus *Bacillus*, formed large halos of inhibition. As the culture mix and multi-strain inoculants are stimulated, and agrochemicals are routinely applied in agriculture, we revealed the importance of compatibility tests for establishing strategies to obtain better efficiency of biologicals.

Keywords: Bioinputs; Insecticides; Fungicides; Rhizobacteria; Antibiosis

Introduction

Plant diseases lead to losses in agricultural production each year, thereby affecting the cost of planting and the expenses associated with commercial products used for phytopathogen control (Asad 2022). Soil fungi and intense attacks by insects significantly reduce the yield of crops, resulting in economic losses. Accordingly, agrochemicals are commonly applied pre and post-harvest to protect crops (Price *et al.* 2015); however, exposure to and the consumption of these agrochemicals can have detrimental effects on beneficial macroorganisms, such as bees, by affecting larval development (Mussen *et al.* 2004). Despite growing evidence regarding the detrimental effects of agrochemicals on macroorganisms, non-target microorganisms have received less attention. Nonetheless, many of these microorganisms have a beneficial effect on crop yield but see (Alvarez-Perez *et al.* 2016; Bartlewicz *et al.* 2016; Schaeffer *et al.* 2017).

Non-target microorganisms can either reside in the soil or be administered as bio-inputs to promote plant growth. Plant growth-promoting rhizobacteria (PGPR) affect the root through biofertilization, root growth stimulation, rhizoremediation, and plant stress control. The biological

control mechanisms by which rhizobacteria can indirectly promote plant growth (*i.e*., by reducing the disease level) include antibiosis, induction of systemic resistance, and competition for nutrients and niches (Lugtenberg and Kamilova 2009; Ahemad and Mulugeta 2014).

In recent decades, the use of microorganisms in biological control has gained global prominence in agriculture due to its greater safety to the environment relative to non-organic commercial products (Souza *et al.* 2021). The use of biologicals also aids in the recomposition of soil microbiota and stimulation of ecological interactions between plant and microorganisms.

For those that seek to adhere to and maintain more sustainable agriculture by using biological organisms, the compatibility of these biologicals with agrochemical products that are routinely used on farms and affect these non-target microorganisms has been identified as a critical factor (Yang *et al.* 2011). Noel *et al*. (2022) demonstrated that the application of fungicides with the active principles pyraclostrobin, prothioconazole and trifloxystrobin disturbs the phyllosphere of corn, reducing the abundance of important yeasts. Similarly, Andreolli *et al*. (2023) showed that growth-promoting bacteria present in grapevines can be inhibited by the application of seven fungicides commonly used in vineyards. In fact, these inorganic agents can directly affect the growth and development of symbiotic bacterial microorganisms, plant growth promoters, and even disease biocontrollers. Biological nitrogen fixation and the decomposition of organic matter are examples of microbial activities that can be impaired by pesticides. In addition, frantic and recurrent use of these chemicals can lead to the development of resistance among beneficial soil microorganisms (Shahid and Khan 2022).

Different microorganisms can interact ecologically during synergism or antagonism (Haggag and Faten 2001; Jambhulkar *et al.* 2018; Li *et al.* 2020). Thus, a combination of microorganisms in a single bioformulation or their simultaneous application may result in additive, synergistic, or antagonistic effects. Additive effects imply that the efficacy of the mixture is equal to the sum of the separate efficacies, synergistic effects suggest that the efficacy of the mixture is greater than the sum of the separate efficacies, and antagonistic effects imply conditions in which the efficacy of the mixture is less than the sum of the efficacies of the individual components (Guetsky *et al.* 2002). Thus, evaluating the interaction of potentially commercial biological products not only with agrochemicals, but also with other biological products already available on the market is important to define strategies for improving the performance of biologicals against more sustainable agricultural practices. In this study, we opted to test the hypothesis that rhizobacteria that potentially biocontrol *S. sclerotiorum* are differentially affected by agricultural chemicals and the interaction with biological products that are currently available on the market.

Materials and Methods

Pesticide compatibility test

The tests were conducted using three bacterial strains isolated from the rhizosphere of Arecaceae *Butia archeri* (Silva *et al.* 2021). Based on previous tests, these strains were found to show potential for the biocontrol of *Sclerotinia sclerotiorum* (Vitorino *et al.* 2020), and were retained in stock cultures in the bacterioteca collection of the Laboratory of Agricultural Microbiology of IFGoiano campus Rio Verde. The strains were identified by sequencing the 16S rDNA and internal transcribed spacer (ITS) regions and stored with the codes: BA123R (*Enterobacter asburiae*), BA81R (*Bacillus cereus)*, and BA88R (*B. cereus*).

To perform the interaction tests with different pesticides, these bacteria were previously activated in nutrient broth (meat extract, 1.0 g; yeast extract, 2.0 g; peptone, 5.0 g; sodium chloride, 5.0 g; and H_2 O q.s., 1 L) under constant stirring at 90 rpm for 48 h at 28 ± 1°C. The optical density (OD) of the strains were then determined at 600 nm and adjusted to 0.3 using saline solution (0.85%).

Pesticides that are commonly used in pest control and are easily found on the agricultural market were evaluated (Table 1). The tested concentration of each pesticide was established based on the maximum dose of the commercial product recommended by the manufacturer; the amount of each pesticide necessary to obtain, per milliliter of culture medium, the same concentration of active ingredient used in the syrup applied in the field was calculated. The agrochemicals were added to the nutrient broth at a temperature between 45 and 50ºC to avoid possible changes in their properties. Further, the tests were conducted in 25 mL test tubes. Aliquots of 1 mL of each bacterium, obtained from the OD adjustment solution, were used as the inoculum. The cultures were maintained on an orbital shaking table under constant agitation at 90 rpm for 72 h at 28 ± 1°C. Tubes containing chemical-free nutrient broth were used as controls. Colony-forming units ($CFUs \cdot mL^{-1}$) were obtained at 72 h of exposure of rhizospheric strains to agrochemicals. For this, the serial dilution method was used, followed by the inoculation of 100 microliters of cultures in petri dishes containing nutrient broth. Colonies were counted after 48 h of incubation.

Biological compatibility test

Antibiosis assays of the bacterial strains BA123R (*E. asburiae*), BA81R (*B. cereus)*, and BA88R (*B. cereus*) against the commercial strains of *Trichoderma harzianum* Rifai – ESALQ-1306 (Trichodermil® SC 1306, Koppert), *Bradyrhizobium japonicum* – SEMIA 5079 and SEMIA 5080 (Bioma Brady®, Bioma), *Azospirillum brasilense* – Abv5 and Abv6 (Vitale Azzos®, Vitale Corp Agroscience), and *B. megaterium* + *B. subtilis* – BRM034840 and

BRM033112-B (Biomaphos®, Bioma) were conducted for the biological compatibility test.

The assays were conducted in two stages: against *T. harzianum* and then against commercial bacterial strains. *T. harzianum* was previously cultivated in Potato Dextrose Agar (PDA) (infusion of 200 g potato, 20 g dextrose and 15 g agar) for 7 days at 28°C in a microbiological incubator, while the strains BA123R (*E. asburiae*), BA81R (*B. cereus)*, and BA88R (*B. cereus*) were incubated in nutrient broth for 48 h at 28°C.

The tests were established based on the paired culture technique. In the first step, 6 mm diameter discs of *T. harzianum* colonies and a 6 cm streak of a bacterial colony obtained with a bacteriological loop were employed. The colonies were inoculated at opposite poles in Petri plates (4 cm apart) containing BDA medium. The tests were performed in triplicate and the cultures were paired and incubated at 28ºC for 7 days. In the first step, only *T. harzianum* was inoculated in one pole of the plate to serve as a control. Compatibility was evaluated for 7 days, starting at 24 h after inoculation.

The competitive interactions were analyzed according to the scale by Badalyan *et al.* (2002). *T. harzianum* colony diameter was measured using a digital pachymeter (cm) and the percentage of suppression of each bacterium was calculated using the relative inhibition index (RI):

$$RI\ (\%) = \frac{CR - XR}{CR} \times 100$$

Where CR = colony radius of *T. harzianum* in the control treatment; XR = radius of the *T. harzianum* colony paired with the test rhizospheric strains.

In the second step, the interaction was evaluated by inoculating the rhizosphere bacteria in established cultures of the commercial strains. Briefly, the commercial strains of *B. japonicum*, *A. brasilense*, and *B. megaterium* + *B. subtilis* were initially grown in nutrient broth for 48 h at 28°C with constant agitation. Aliquots of 1 mL of the cultures were plated on NA and incubated for 48 h at 28°C. There after, 20-μL drops of the culture of the strains BA123R (*E. asburiae*), BA81R (*B. cereus*), and BA88R (*B. cereus*), which were previously grown in nutrient broth for 48 h at 28°C with constant agitation, were aseptically placed in the center of the plates containing the commercial strains. The apparatus was returned to the microbiological incubator, and was incubated for 04 days at 28°C. The compatibility was assessed *via* daily monitoring of the size of the diameter + zone of inhibition produced by the colonies of the rhizospheric bacteria (cm).

Statistical analyses

Tests were conducted under a completely randomized design. The optical density (OD) data collected over the time of bacteria exposure to the different pesticides were compared by analysis of variance (ANOVA). Significant differences were determined based on regression analysis, and the trend of the effects was evaluated using the slope β of x obtained from the linear regression models. The mean number of CFU observed at 72 h of exposure of the strains to the pesticides, the mean percentages of the IR, and the measurements of the inhibition halos observed in the interaction of the test strains with *T. harzianum* and the commercial bacteria were subjected to ANOVA. Finally, means were compared using the Tukey test at a probability level of 5%.

Results

Agricultural pesticides on BA123R (*E. asburiae*)

The chemicals, Avicta, Captan, Cropstar, Cruiser, Derosal, Fortenza, and Vitavax, were found to negatively affect the growth of the BA123R strain throughout the evaluation period; however, the highest negative values for the slope β of x were obtained with the chemical, Cropstar (β_1 = -0.0153) (Fig. 1A, B and D). Some chemicals positively stimulated the growth of the rhizobacterium, BA123R, over time, even exceeding the growth pattern presented by this bacterium in the control treatment. Chemicals with a positive effect included Fipronil, Imidacloprid, Imidagold, Maxim Advanced, Maxim, Poncho, and Standark, which had positive values for the slope of x. The highest values of β_1 were verified based on the behavioral line for Maxim (β_1 = 0.0043) (Fig. 1C).

The agrochemical, Certeza, did not affect crop growth over time as the average OD was approximately 0.89 throughout the evaluation period (Fig. 1A).

Agricultural pesticides on BA81R (*B. cereus*)

The pesticides, Avicta, Captan, Cropstar, Cruiser, Derosal, Fortenza, and Standark, negatively affected the cell growth of the BA81R strain over the evaluation period; however, the highest negative values for the slope β of x were obtained using the chemical, Cropstar (β_1 = -0.0125) (Fig. 2A, B and D). Some of the chemicals had a positive effect on the growth of the rhizobacterium, BA81R, over time, even exceeding the growth pattern presented by this bacterium in the control treatment. The agrochemicals, Imidacloprid, Imidagold, Maxim Advanced, Maxim, and Poncho, had positive values for the slope of x. Further, the highest values of β_1 were confirmed by the behavioral straight line of Maxim (β_1 = 0.0043) (Fig. 2C).

The agrochemicals, Certeza, Fipronil, and Vitavax, did not affect crop growth over time, with average ODs of approximately 0.811, 0.597 and 0.025, respectively, throughout the evaluation period (Fig. 2A, B and D).

Table 1: Description of the chemicals evaluated to elucidate their compatibility with the rhizospheric bacterial strains, BA123R (*E. asburiae*), BA81R (*B. cereus*), and BA88R (*B. cereus*) isolated from *Butia archeri*

Avicta® 500 FS PRO	Insecticide and nematicide	ABAMECTIN	AVERMECTINS	75 mL·ha^{-1}	600 mL	0.125 mL·mL^{-1}
Captan® 200 SC	Fungicide	CAPTANA	Dicarboximide	350 mL/100 kg sedes	-	0.0035 mL·mL^{-1}
Certeza®	Fungicide	Fluazinam + Thiophanate Methyl	Phenylpyrimidamine + Benzimidazole	570 mL/100 kg sedes	-	0.0057 mL·mL^{-1}
Cropstar	Insecticide	IMIDACLOPRID + THIODICARB	Neonicotinoid + oxime methylcarbamate	2400 mL/100 kg seeds	-	0.024 mL·mL^{-1}
Cruiser® 350 FS	Insecticide	TIAMETHOXAM	Neonicotinoid	600 mL/100 kg seeds	-	0.006 mL·mL^{-1}
Derosal® Plus	Fungicide	CARBENDAZIM + Thiram	Benzimidazole and Dimethyldithiocarbamate	600 mL /100 kg seeds	-	0.006 mL·mL^{-1}
Fipronil Nortox®	Insecticide and terminicide	FIPRONIL	Pirazol	412.5 g·ha^{-1}	200 mL	2062.5 mg·mL^{-1}
Fortenza® 600 FS	Insecticide	Cyantraniliprole	ANTRANILAMIDE	100 mL·ha^{-1}	750 mL	0.133 mL·mL^{-1}
Imidacloprid® 350 SC	Insecticide	IMIDACLOPRID	Neonicotinoid	1370 mL·ha^{-1}	45000 mL	0.30 mL·mL^{-1}
Imida gold® 700 WG	Insecticide	IMIDACLOPRID	Neonicotinoid	400 g·ha^{-1}	410 L	0.975 mg·mL^{-1}
Maxim Advanced	Fungicide	METALAXYL-M + THIABENDAZOLE + FLUDIOXONIL	ACYLALANINATE + BENZIMIDAZOLE + FLUDIOXONIL	150 mL /100 kg seeds	1000 ml	0.15 mL·mL^{-1}
Maxim® XL	Fungicide	METALAXYL-M + FLUDIOXONIL	ACYLALANINATE + FLUDIOXONIL	300 mL/100 kg seeds	500 mL	0.6 mL·mL^{-1}
Poncho®	Insecticide	CLOTHIANIDIN	Neonicotinoid	450 mL/100 kg seeds	-	0.0045 mL·mL^{-1}
Standark® top	Fungicide and insecticide	PIRACLOSTROBIN + METHYL THIOFANATE + Fipronil	Strobilurins + Benzimidazole + Pyrazole	200 mL·ha^{-1}	750 ml	0.27 mL·mL^{-1}
Vitavax®-thiram 200 SC	Fungicide	Carboxin + Thiram	Carboxanilide + Dimethyldithiocarbamate	800 mL/100 kg	1300 mL	0.615 mL·mL^{-1}

*Maximum dose of the commercial product recommended by the manufacturer. ** Average volume between the maximum and minimum volumes recommended by the manufacturer

The effect of the agrochemicals on the growth of the cultures was determined by monitoring the optical densities measured at 0, 24, 48, and 72 h, and the number of Colony-Forming Units (CFUs) obtained by plating the solutions 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} with the cultures after 72 h of growth

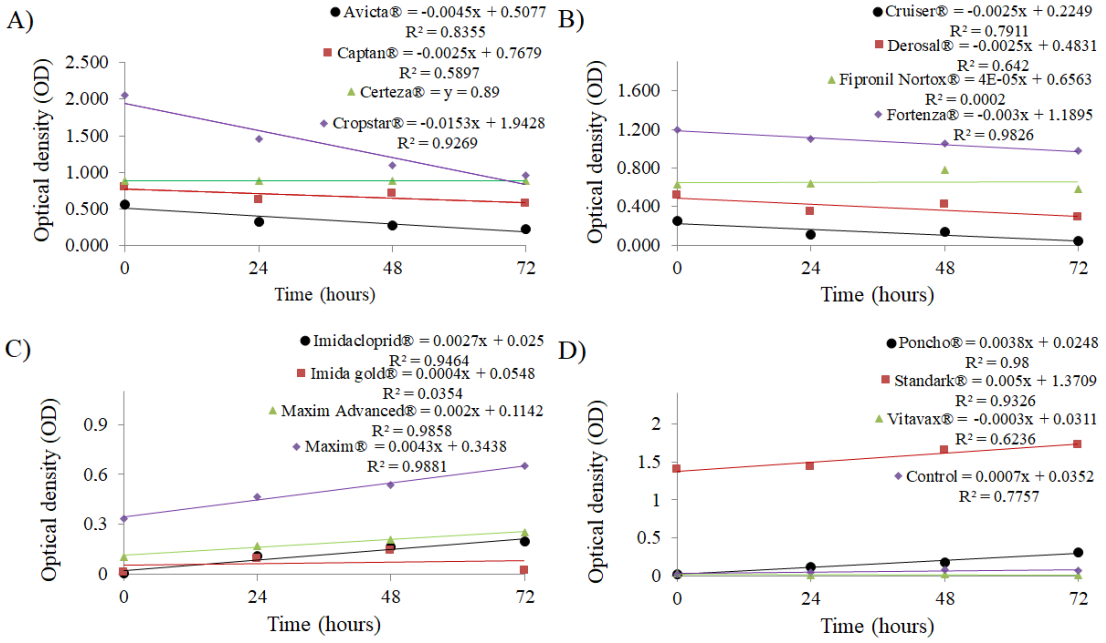

Fig. 1: Mean optical densities for cultures of the rhizospheric strain, BA123R (*E. asburiae*), as a function of exposure time to different pesticides. **A**) Avicta, Captan, Certeza, and Cropstar; **B**) Cruise, Derosol, Fipronil, and Fortenza; **C**) Imidacloprid, Imidagold, Maxim Advanced, and Maxim; and **D**) Poncho, Standark, Vitavax, and Control (no chemical)

Agricultural pesticides on BA88R (*B. cereus*)

The chemicals, Avicta, Captan, Cropstar, Cruiser, Derosal, and Fortenza, negatively affected the growth of the strain, BA88R. Further, the highest negative values for the slope β_1 were found for Cropstar (-0.0093) (Fig. 3A and B). When exposed to the chemicals, Fipronil, Imidacloprid, Imidagold, Maxim Advanced, Maxim, and Poncho, the crop ODs increased over time, indicating that these chemicals enhanced the performance of this bacterium. The highest positive values for the slope β_1 were obtained using the Maxim Advanced treatment (0.0049) (Fig. 3C and D).

The agrochemicals, Certeza, Standark, and Vitavax, did not have a negative or positive effect on the growth of

Table 2: Colony-forming units (CFUs·mL^{-1}) were obtained at 72 h of exposure of the rhizospheric strains, BA123R (*E. asburiae*), BA81R (*B. cereus)*, and BA88R (*B. cereus*), which were subjected to compatibility testing with the pesticides, Avicta, Captan, Certeza, Cropstar, Cruiser, Derosol, Fipronil, Fortenza, Imidacloprid, Imidagold, Maxim Advanced, Maxim, Poncho, Standark and Vitavax

Chemicals	123a	81a	88a	Error	C.V
Avicta	1.04 x10^{8} Aa	1.04 x10^{8} Aa	1.04 x10^{8} Aa	± 0.00	0
Captan	1.04 x10^{8} Aa	1.04 x10^{8} Aa	1.04 x10^{8} Aa	± 0.00	0
Certeza	1.04 x10^{8} Aa	1.04 x10^{8} Aa	1.04 x10^{8} Aa	± 0.00	0
Cropstar	1.04 x10^{8} Aa	1.04 x10^{8} Aa	1.04 x10^{8} Aa	± 0.00	0
Cruiser	0.00 Da	0.00 Ea	0.00 Da	± 0.00	0
Derosal	6.00 x10^{7} Bab	1.04 x10^{8} Aa	0.00 Db	± 1.45 x10^{-7}	46.04
Fipronil	1.17 x10^{6} Ca	1.17 x10^{6} DEa	1.20 x10^{6} CDa	± 9.23 x10^{-4}	13.57
Fortenza	0.00 Da	0.00 Ea	0.00 Da	± 0.00	0
Imidacloprid	1.60 x10^{6} Cb	2.00 x10^{6} Db	2.67 x10^{6} Ca	± 1.26 x10^{5}	10.46
Imidagold	2.40 x10^{6} Cb	4.60 x10^{6} Cb	4.63 x10^{6} Ca	± 3.04 x10^{5}	13.59
Maxim Advanced	6.70 x10^{6} Ca	1.23 x10^{6} DEb	5.37 x10^{6} Ba	± 5.94 x10^{5}	23.21
Maxim	1.04 x10^{8} Aa	1.04 x10^{8} Aa	1.04 x10^{8} Aa	± 0.00	0
Poncho	3.03 x10^{6} Cb	2.10x10^{6} Bb	8.17 x10^{6} Ca	± 1.12 x10^{6}	43.58
Standak	1.04 x10^{8} Aa	1.04 x10^{8} Aa	1.04 x10^{8} Aa	± 0.00	0
Vitavax	1.04 x10^{8} Aa	1.04 x10^{8} Aa	1.04 x10^{8} Aa	± 0.00	0
Control	2.17 x10^{6} Ca	1.45x10^{6} DEb	1.98 x10^{6} Ca	± 9.40 x10^{44}	8.73

Means followed by the same letter in the column or row do not differ based on Tukey test (5%). Upper case letters indicate differences between chemicals and lowercase letters indicate difference between microorganisms

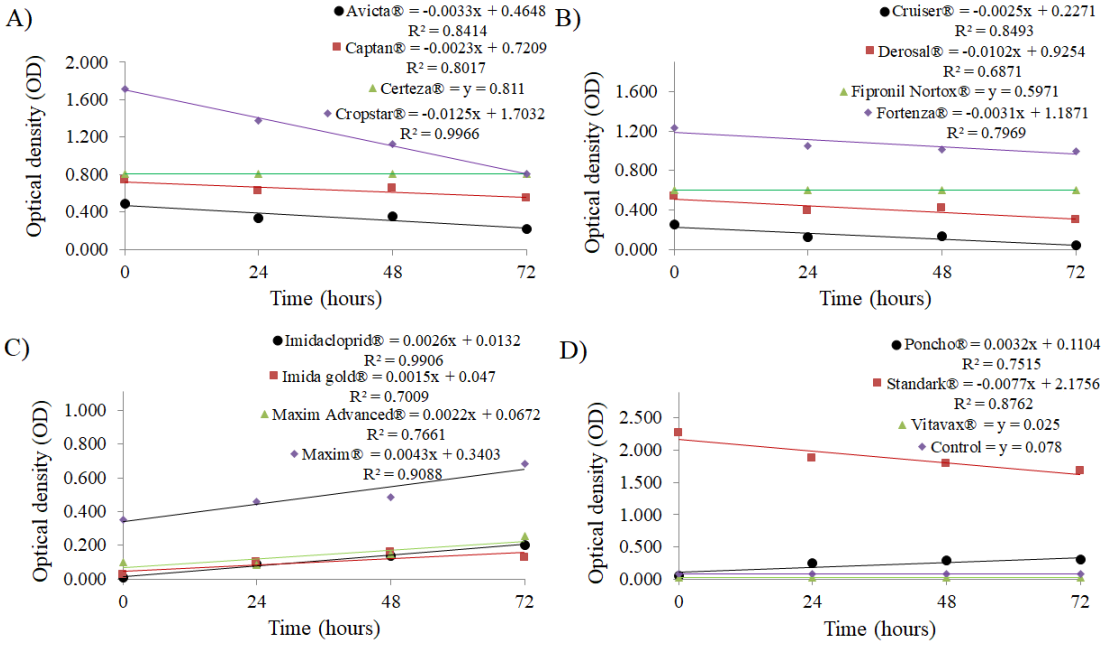

Fig. 2: Mean optical densities for cultures of the rhizospheric strain, BA81R (*Bacillus cereus*), as a function of exposure time to different pesticides. **A**) Avicta, Captan, Certeza, and Cropstar; **B**) Cruise, Derosol, Fipronil, and Fortenza; **C**) Imidacloprid, Imidagold, Maxim Advance, and Maxim; and **D**) Poncho, Standark, Vitavax, and Control (no chemical)

this rhizospheric bacterium. In fact, their average OD values were approximately 0.824, 1.924 and 0.029, respectively, throughout the evaluation period (Fig. 3A and D).

When the growth response of bacteria was evaluated based on the CFUs, BA123R proved to be compatible with Avicta, Captan, Certeza, Cropstar, Maxim, Standak, and Vitavax, with values in the order of 1.04 × 10^{8} CFU·mL^{-1}. Notably, the values were higher than those obtained with the control treatment (2.17 × 10^{6} CFU·mL^{-1}). The presence of Cruiser and Fortenza was found to markedly limit the development of BA123R (Table 2).

Similar to the results with BA123R, the agrochemicals, Avicta, Captan, Certeza, Maxim, Standak, and Vitavax, with Derosal stimulated the development of the highest number of CFU by the BA81R strain (1.04 × 10^{8} CFU·mL^{-1}). Further, Cruiser and Fortenza completely inhibited the colonial development of the BA81R bacteria after 72 h of exposure. The rhizospheric strain, BA88R, proved to be highly compatible with Avicta, Captan, Certeza, Maxim, Standak, and Vitavax, with values in the

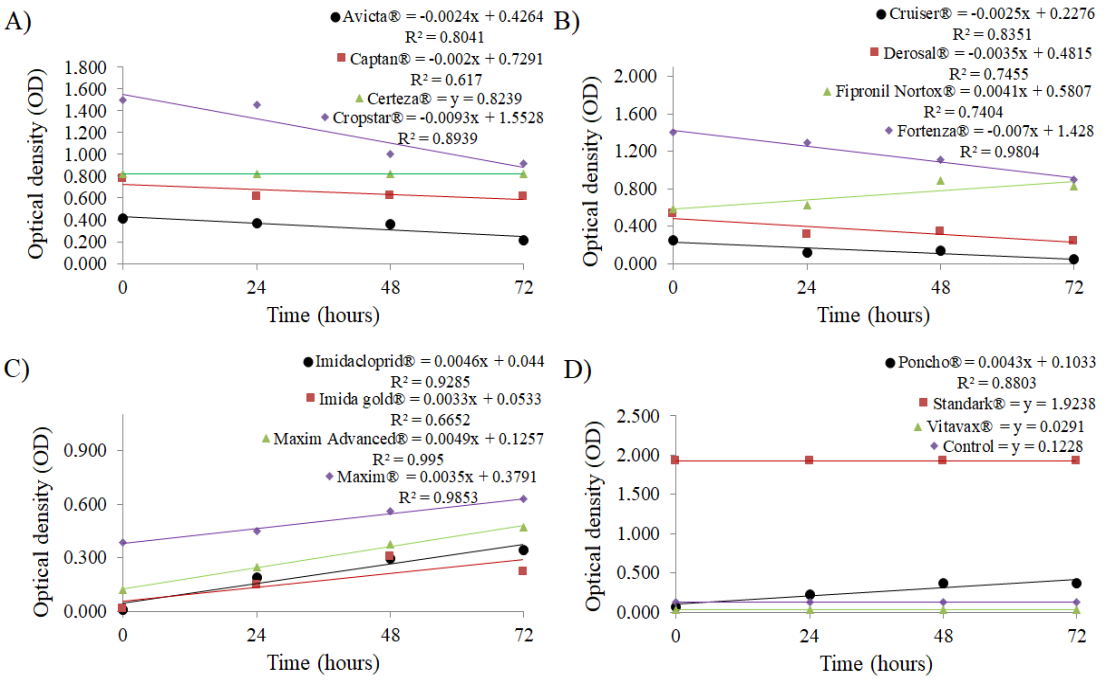

Fig. 3: Mean optical densities for cultures of the rhizospheric strain, BA88R (*B. cereus*), as a function of exposure time to different pesticides. **A**) Avicta, Captan, Certeza, and Cropstar; **B**) Cruiser, Derosol, Fipronil, and Fortenza; **C**) Imidacloprid, Imidagold, Maxim Advanced, and Maxim; and **D**) Poncho, Standark, Vitavax, and Control (no chemical)

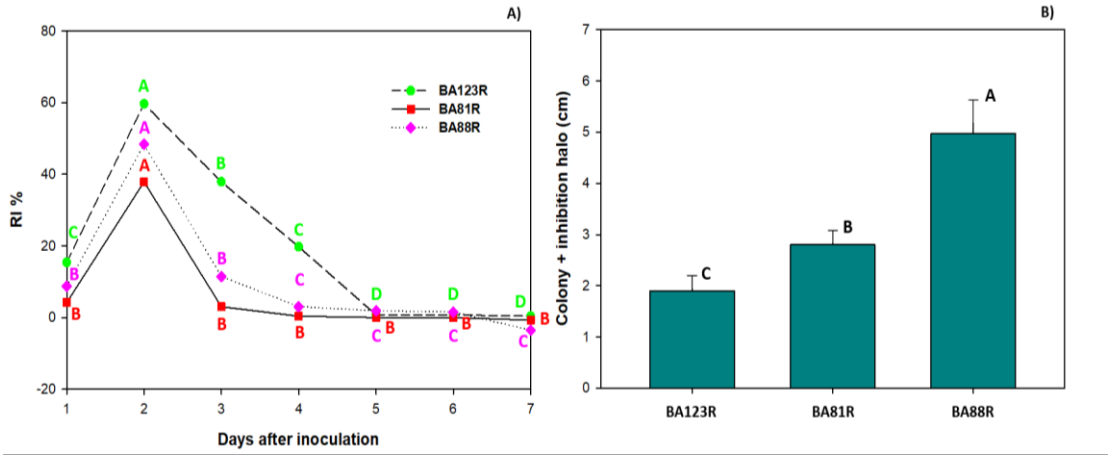

Fig. 4: Relative inhibition index (RI) for the paired cultures of *T. harzianum* and three strains of rhizospheric bacteria isolated from *Butia archeri*: BA123R (*E. asburiae*), BA81R (*B. cereus)* and BA88R (*B. cereus*), as a function of days, after inoculation (**A**), and the compatibility response of these three bacterial strains against *Azospirillum brasilense* (**B**)

order of 1.04×10^8 CFU mL^{-1} obtained at 72 h of treatment with these chemicals. Of note, the presence of Cruiser, Derosal, Fortenza, and Fipronil had a very negative effect on bacterial development (Table 2).

Based on a comparison of the response of the three tested strains to each agrochemical separately, the strains were found to respond similarly to the presence of Avicta, Captan, Certeza, Cruiser, Cropstar, Fipronil, Maxim, Standak, and Vitavax (Table 2). However, their responses were found to differ for Derosal, which was found to be compatible with BA123R and BA81R (1.04×10^8 and 6.00×10^7 CFU·mL^{-1}, respectively), and more incompatible with BA88R. Meanwhile, Imidacloprid was more compatible with BA88R (2.67×10^6 CFU·mL^{-1}) than BA123R and BA81R (1.60×10^6 and 2.00×10^6 CFU·mL^{-1}, respectively). A similar behavior was found for Imidagold, which was more incompatible with BA88R (4.63×10^6 CFU·mL^{-1}) than BA123R and BA81R (2.40×10^6 and 4.60×10^6

CFU·mL^{-1}, respectively). Notably, Poncho followed this interaction trend as it was found to be more compatible with BA88R (8.17×10^6 CFU·mL^{-1}) than BA123R and BA81R (3.03×10^6 and 2.10×10^6 CFU·mL^{-1}, respectively). The agrochemical, Maxim Advanced, had a differential effect on the strains as was more compatible with BA123R and BA88R (6.70×10^6 and 5.37×10^6 CFU·mL^{-1}, respectively) and less compatible with BA81R (1.23×10^6 CFU·mL^{-1}).

T. harzianum on rhizospheric bacteria

When the compatibility of *T. harzianum* with rhizospheric bacteria was evaluated, significant differences were found in the percentages of inhibition induced by the three bacterial strains throughout the evaluation times. In general, the presence of bacteria significantly affected the mycelial growth of fungus relative to the control treatment in the initial phases of paired growth. Further, from day 5 of the evaluation, this growth was already similar to the rates observed with the control treatment (Fig. 4A). The highest rates of relative inhibition were observed on the second day after inoculation, with mean percentages of 59.6, 48.3, and 37.8, respectively, for the bacteria, Ba123R, BA88R, and BA81R. Accordingly, the greatest incompatibility between these biologicals was due to the action of the bacteria, BA123R, with significant reductions in mycelial growth observed until the fourth day of paired growth. Based on our results, *T. harzianum* demonstrated a remarkable ability to recover from antibiotic effects possibly induced by the bacterial activity.

The rhizospheric bacteria evaluated did not grow in the presence of the biological, *B. japonicum*, and the mixture, *B. megaterium* + *B. subtilis*, revealing high incompatibility between these biologicals. However, the strains were found to interact with *A. brasilense*. As a result, bacteria of the genus, *Bacillus*, formed large halos of inhibition beyond colonial growth. The largest halos were observed for the interaction of *A. brasilense* with strain BA88R (Fig. 4B).

Discussion

In general, the OD test revealed that the agrochemicals, Avicta, Captan, Cropstar, Cruiser, Derosal, and Fortenza, negatively affected the growth of the three rhizobacteria. However, Imidacloprid, Imidagold, Maxim Advanced, Maxim, and Poncho seemed to benefit from the growth of the strains and did not affect the crops. The similar behavior between Imidacloprid and Imidagold is because they share the same active ingredient in their formulation. This notion can also be applied to Maxim Advanced and Maxim as both contain Metalaxyl-M as a component. Prior studies revealed that bacteria, such as *Pseudomonas* spp. and *B. aerophilus*, can biodegrade imidacloprid-based neonicotinoid insecticides (Pandey *et al.* 2009; Sharma *et al.* 2016). Thus, the strains evaluated in this study may have acted by biodegrading these agrochemicals and utilizing the products of this degradation in their primary metabolism. Of note, Maxim Advanced and Maxim did not appear to affect PGPRs. Fernandez *et al.* (2021) demonstrated that the combination of Metalaxyl-M + Thiabendazole + Fludioxonil with other active ingredients does not harm *Pseudomonas fluorescens* cultures, even at high concentrations. Kintschev *et al.* (2014) also revealed that the use of Metalaxyl-M + Fludioxonil or Metalaxyl-M + Thiabendazole + Fludioxonil does not affect the productivity of cowpea, despite a reduction in the dry mass of nodules produced by *Rhizobium tropici*. Nonetheless, for one of the active ingredients used in the composition of the fungicide, Certeza, Zhang *et al.* (2019) demonstrated that the inoculation of an endophytic strain of *Paenibacillus polymyxa* can be effective in the degradation of fluazinam.

When CFUs were monitored, the most drastic antibiosis effects were observed with the insecticides, Cruiser and Fortenza. Wu *et al.* (2021) showed that treatment with thiamethoxam significantly affects soil bacterial abundance, reducing microbial diversity and altering the bacterial community structure in the short term. However, the results indicate that the structure can be recovered to a steady state in a short time. Filimon *et al.* (2015) evaluated the effect of thiamethoxam on soil microorganisms based on enzymatic and bacteriological analyses and concluded that this chemical inhibits metabolic processes in soil, reducing the values recorded for dehydrogenase, urease, catalase, and phosphatase enzyme activity. The ecophysiological groups of bacteria (ammonifying bacteria, nitrifying bacteria and denitrifying bacteria) showed statistically significant decreases in the experimental variants treated with thiamethoxam.

Unlike the present study, Cavalcanti *et al.* (2002) demonstrated the compatibility between thiamethoxam and the fungus that controls phytopathogens, *Beauveria bassiana*. Filho *et al.* (2001) also revealed the compatibility between thiamethoxam and inoculums of *B. thuringiensis*, *B. bassiana*, and *Metarhizium anisopliae* when applied to bean (*Phaseolus vulgaris*) crops. Thus, fungi seem to be less sensitive to this active ingredient. Furthermore, the inhibitory effect might be a function of the specific characteristics of the microbiota, as some bacterial strains have been demonstrated to be biodegraders of thiamethoxam (Rana *et al.* 2015).

Regarding the insecticide, Fortenza, which has cyantraniliprole as its active ingredient, studies have shown that the carbon of the microbial biomass and the microbial activity index positively correlate with the degradation of this ingredient, attesting to its impact on the soil microbial community (Kumar and Gupta 2020).

In the present study, the rhizobacteria displayed differential responses to the chemicals, Derosal, Imidacloprid, Imidagold, Poncho, and Maxim Advanced, indicating that tests for compatibility between chemicals and biologicals should be performed in isolation for microbial

species and strains owing to the complexity of the responses. The mechanisms that cause different sensitivities to fungicides and insecticides have not been fully elucidated; however, some studies revealed that ATP binding and active transport, by which endogenous and exogenous substances can be separated, would be among the mechanisms (Widmer 2019). Yang *et al.* (2011) showed that fungicides can target cell membrane components, protein synthesis, signal transduction, respiration, cell mitosis, and nucleic acid synthesis.

In this study, some agrochemicals were found to be toxic to rhizobacteria. Similar results were observed in another study that sought to determine the compatibility of *Pseudomonas fluorescens* with some insecticides and fungicides. This prior study revealed that products, such as chlorpyrifos, acetamiprid, and carbosulfan; fungicides, such as aluminum fosetyl, copper hydroxide, COC, cymoxanil + mancozeb mixture, and micronutrient mixture significantly inhibited the growth of *P. fluorescens* (Dhanya *et al.* 2017). The compatibility of biological agents in the presence of chemical products of commercial importance must be known. Such information will enable the selection of appropriate fungicides, insecticides, and nematicides with commercial dosages, and microorganisms that promote plant growth through BNF, production of phytohormones, and phosphate solubilization, and disease biocontrol.

Combining the biological agent with fungicides/insecticides or non-toxic concentrations of these chemicals is becoming an important approach for obtaining a more sustainable and less aggressive agricultural system (Coca and Gakegne 2020). Our results support the notion that some commercial insecticides can be used in conjunction with the bacterial isolates, 81R, 88R and 123R; however, others affect colony growth. The correct combination of fungicides and biologicals will effectively contribute to the sustainability of the agricultural production system. The modes of action and potential non-target effects on soil microorganisms should be considered during the selection of insecticides and fungicides to protect soil biological functions and optimize the benefits derived from fungicide use in agricultural systems.

For the compatibility of rhizobacteria with *T. harzianum*, our results demonstrate a more expressive incompatibility in the first phases of contact, especially by 123R, with a high ability of *T. harzianum* to recover from the antibiotic effects of bacterial activity. Many studies revealed the synergistic effect of the combination of *Trichoderma* and PGPRs. The combined application of the *Trichoderma* isolate Tr6 and *Pseudomonas* isolate Ps14 was reported to induce systemic resistance against *F. oxysporum* f. spp. *radicis cucumerium* in cucumber and *Botrytis cinerea* in *Arabidopsis* (Alizadeh *et al.* 2013). Chemeltorit *et al.* (2017) reported that the combination of *T. hamatum* THSW13 and *P. aeruginosa* BJ10-86 synergistically reduced the number of pepper deaths caused by *Phytophthora capsici*. Recently, the efficacy of a combined application of Pusa 5SD seed cover formulation developed from *T. harzianum*, P. *fluorescens* (Pf 80), *Mesorhizobium cicero*, and Vitavax Power® (Dhanuka Agritech ltd., Gurgaon, HR, India) was revealed against chickpea wilt caused by *F. oxysporum* f. spp. *ciceris* (Dubey *et al.* 2015). Our results suggest that the initial contact between *Trichoderma* and the rhizobacteria induces a state of biotic stress, which is subsequently resolved, enabling the co-inoculation of *T. harzianum* with the strains in question.

In the present study, the rhizospheric bacteria did not grow in the presence of the biological, *B. japonicum*, and the mixture, *B. megaterium* + *B. subtilis*, demonstrating their high incompatibility. However, the strains interacted with *A. brasilense*. As a result, the bacteria of the genus, *Bacillus*, formed large halos of inhibition, especially the BA88R strain. These results differed from those expected as some studies revealed the co-inoculation efficiency between bacteria of the genus, *Bacillus* and *Bradyrhizobium* (Atieno *et al.* 2012; Figueredo *et al.* 2014; Subramanian *et al.* 2015). In general, the use of culture mixes and multistrain inoculants is encouraged (Thomloudi *et al.* 2019). However, compatibility tests should first be performed between the biologicals to be co-inoculated to evaluate the occurrence of symbiosis or antibiosis.

Conclusion

Based on the findings of this study, we confirmed the hypothesis that rhizobacteria that potentially biocontrol *S. sclerotiorum* are differentially affected by agricultural chemicals. The agrochemicals, Cruiser and Fortenza, should not be used in conjunction with the *rhizobacteria*, BA123R (*E. asburiae*), BA81R (*B. cereus)*, and BA88R (*B. cereus*). These rhizobacteria were also found to be incompatible with *B. japonicum* and the mix, *B. megaterium* + *B. subtilis*; however, they could be co-inoculated with *T. harzianum*. Knowing the effects of the interaction between biologicals and agrochemicals is fundamental for carrying out conscious management and adoption of more sustainable agricultural practices.

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Author Contributions

LAB designed and performed the study; LMSM and LSSS performed the experiments; LCV analyzed the data; LAB

wrote the manuscript; LCV supervised the process and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

We have no conflict of interest to declare.

Data Availability

All datasets presented in this study will be available on a fair request to the corresponding authors.

Ethics Approval

This research does not involve the ethical approval.

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