BIOLOGICAL CONTROL OF ALTERNARIA BROWN SPOT IN TANGERINE TREES USING DIFFERENT BENEFICIAL MICROORGANISMS

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ABSTRACT

The use of beneficial microorganisms for biological control of pest and disease is an emerging and potential approach to promoting a more sustainable agriculture. In this context, this work aimed to evaluate the potential of bioinputs based on *Trichoderma* spp. and *Bacillus subtilis* in controlling Alternaria Brown Spot on tangerine leaves. To this end, *in vitro* and *in vivo* tests were carried out. In the *in vitro* tests, the antagonism of microorganisms against *Alternaria Alternata* in plaque was evaluated. The *in vivo* experiments were conducted in a completely randomized design (5 treatments and 4 replications each) with tangerine trees in a greenhouse. The efficiency of Alternaria Brown Spot control was determined by evaluating tangerine leaves according to a diagrammatic scale. Although fungicide was the most effective treatment, bioinputs showed promising control of the disease, especially treatments containing *Trichoderma* spp. Therefore, the individual or consortium use of the beneficial microorganisms tested is a promising alternative for controlling diseases that affect citrus farming, promoting more sustainable food production by reducing the dependence on the use of agrochemicals.

Keywords: Biocontrol. *Alternaria alternata*. *Bacillus subtilis*. *Trichoderma* spp. Sustainable agriculture.

1 INTRODUCTION

Citrus farming is of great importance worldwide, with around 144 million tons of citrus fruits produced in 2019, of which Brazil was responsible for almost 14% of production, making the country the second largest producer[1]. Citrus fruits, such as oranges, tangerines, lemons, among others, are affected by several types of pests, with *Alternaria alternata* being the fungus that most affects these crops worldwide, causing the disease called Alternaria Brown Spot, which can affect from young leaves to the final fruit[2-4].

The symptoms of Alternaria Brown Spot are due to the ACT toxin released by the fungus in its conidial phase, which acts on the plasma membrane of plant cells[2,3,5]. Mycotoxins produced by *Alternaria* spp. cause concern in food safety, as the consumption of contaminated food can lead to serious diseases in living beings[4,6]. In order to control Alternaria Brown Spot, the most commonly used method is the application of chemical fungicide, which needs to be applied 7 to 10 times during the fruit cycle[7]. However, the excessive use of agrochemicals is reported for its various adverse impacts on the environment and human health[8]. In this context, the use of beneficial microorganisms-based bioputs is an approach capable of increasing crop yields in a more sustainable way[9,10]. Among the promising microorganisms able to biocontrol pests and diseases, several fungi of the genus *Trichoderma* and bacteria of the genus *Bacillus* stand out[11,12]. Therefore, this work aimed to evaluate the potential of bioinputs based on *Trichoderma* spp. and *Bacillus subtilis* to control Alternaria Brown Spot on tangerine trees.

2 MATERIAL & METHODS

To evaluate the potential of bioinputs in controlling Alternaria Brown Spot, *in vivo* treatments were conducted on tangerine trees in a greenhouse. Prior to this, *in vitro* tests were performed to verify the pathogenicity of the fungus *Alternaria Alternata* on tangerine leaves. Subsequently, the antagonistic effects of two commercial products against the fungus *A. Alternata* was assessed, one of them based on *Trichoderma* spp. and another on *Bacillus subtilis,* and a consortia of both bioinputs. The greenhouse experiments were carried out in a completely randomized design, comprising 5 treatments and 4 replications (Table 1). Each replication consisted of 1 one-year-old tangerine trees, arranged individually in 5 L pots within the greenhouse.

Table 1 Treatments used in *in vivo* experiments on tangerine trees

Treatment	Composition	Active ingredient concentration	Dosage
T1	Control (water)	-	-
T2	Fungicide (triazol + estrobirulina)	100 mg/mL and 200 mg/mL, respectively	0,3 mL/L
T3	*Trichoderma* spp.	$1x10^9$ CFU/mL	1 mL/L
T4	*Bacillus subtilis*	$2x10^8$ CFU/mL	1 mL/L
T5	*Trichoderma* spp. and *Bacillus subtilis*	$1x10^9$ CFU/mL and $2x10^8$ CFU/mL, respectively	0,5 mL/L of each

The tangerine trees were inoculated using a manual sprayer with an aqueous suspension of *A. alternata* conidia, then covered with pre-moistened plastic packaging for protection. The treatments outlined in this study (Table 1) were applied shortly after the appearance of the first symptoms of brown spot on the leaves. Application took place immediately after the products were mixed in water, with two applications administered for each treatment. Foliar application was conducted using a manual sprayer until the liquid ran down the plant leaves. Plant evaluations were conducted 7 days after each application. Assessment was focused on the upper 20 cm of the plant, as this portion contains the youngest and most susceptible leaves to pathogen attack. Within this 20 cm interval, ten leaves were selected and marked for evaluation based on spots incidence and the percentage of the damaged area, employing the diagrammatic scale validated by Martelli et al.[13]. The values obtained from this scale were statistically evaluated using the Tukey test at a 95% confidence interval ($p<0.05$).

3 RESULTS & DISCUSSION

Figure 1 presents the diagrammatic scale relating to the incidence of Alternaria Brown Spot on tangerine leaves according to the treatments carried out in this work.

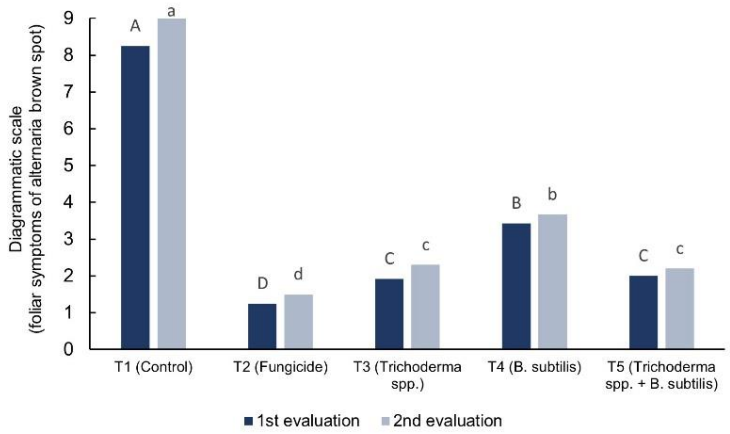

Figure 1 Diagrammatic scale for assessing control of alternaria brown spot in tangerine leaves

The control plants exhibited the highest scores on the diagrammatic scale, indicating a greater presence of disease symptoms on the leaves (90 to 97% of spots on the leaf area). After 14 days, symptoms extended to the branches, resulting in defoliation and lesions practically covering the entire leaf area due to the high aggressiveness of the pathogen. The lowest incidence of brown spot on the leaves was observed in the treatment with fungicide (with average scores of 1.25 and 1.50 in the first and second evaluation, respectively). Subsequently, biological treatments with *Trichoderma* spp. (T3) and *Trichoderma* + *B. subtilis* (T5) achieved average scores on the diagrammatic scale of 1.92 and 2.0, respectively, in the first evaluation (Figure 1). There are several microbial biological control agents (MBCAs) which can act through direct or indirect mechanisms, either by antagonizing the phytopathogen or by inducing resistance in the plant[14]. The majority of bacterial MBCAs belong to *Bacillus* spp., particularly *B. thuringiensis*, known for their antagonistic action against several phytopathogens[9]. Although co-inoculation of MBCAs is reported for its synergistic effects[9], such behavior was not observed in this study, as there was no statistical difference between T3 and T5 ($p>0.05$).

In both the initial and subsequent evaluation, *Trichoderma* spp. exhibited superior control compared to treatment with *Bacillus subtilis* ($p<0.05$), demonstrating higher efficiency in *in vivo* tests for the biological control of Brown Spot on tangerine leaves caused by the phytopathogen. *Trichoderma* spp. possess the ability to control phytopathogenic fungi through mycoparasitism, competition, or antibiosis, in addition to stimulating the plant's defense mechanisms[12]. When comparing the disease control results with the control experiments (T1), the antagonistic action of the beneficial microorganisms tested ($p<0.05$) against the fungus *A. Alternata*, particularly, *Trichoderma* spp., is evident. Therefore, the evaluated microorganisms shown great performance for biological control of Alternaria Brown Spot, serving as a potential tool to promote sustainability in agriculture and positively impact on several Sustainable Development Goals of the United Nations.

4 CONCLUSION

In vitro tests revealed the antagonistic effects of *Trichoderma* spp. and *Bacillus subtilis* on the phytopathogen *Alternaria alternata*. Bioinputs based on these beneficial microorganisms are effective in controlling Alternaria Brown Spot in tangerine leaves, with superior efficacy observed in treatments containing *Trichoderma* spp. While chemical treatment demonstrated the highest efficacy in disease control, bioinput-based treatments showed promising efficiencies in the biocontrol of Alternaria Brown Spot, representing a potential approach to control pests and diseases that affect citrus production in a more sustainable way.

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