

Original Research Article

Biological Control of *Fusarium oxysporum* f. sp. *vasinfectum*

ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *vasinfectum*, is one of the major diseases of cotton. To manage this disease, preventive measures should be adopted, such as the treatment of seeds with biocontrol agents. The aim of this study was to evaluate the efficiency of biological products based on *Trichoderma* spp. and *Bacillus subtilis* in the control of *Fusarium oxysporum* f.sp. *vasinfectum* (Fov) in seeds and seedlings of cotton. The experiment was carried out at the Laboratório de Fitopatologia of the Centro de Ciências Agrárias, of the Universidade Federal de Paraíba (CCA-UFPB), located in the city of Areia, Paraíba - Brazil. The disease transmission from the seeds to the seedlings was evaluated. After the transmission test cotton seeds of the variety mocó (*Gossypium hirsutum* var. *Marie-gallante* (Watt) Hutch.), BRS 286 and Topázio cultivar (*Gossypium hirsutum* L.) were submitted to the treatments T1 - Control, T2 - Trichodel[®] (0,5 mL); T3-Trichodel[®] (1.0 mL); T4-Trichodel[®] (1.5 mL); T5-Trichodel[®] (2.0 mL); T6-Bactel[®] (2.0 mL); T7-Bactel[®] (2.5 mL); T8-Bactel[®] (3.0 mL); T9-Bactel[®] (3.5 mL) diluted in 100 mL SDW; T10 - Fungicide Captana (240g / 100 kg of seeds) and inoculated with Fov. The pathogen incidence in the seeds was evaluated seven days after the inoculation (DAI). In order to evaluate the biological control of Fov in the seedlings, the treated seeds were submitted to the following inoculation methods: 1 - inoculation of the substrate with a suspension of conidia of the pathogen; 2 - immersion of the seeds in the suspension of conidia and 3 - direct contact of the seeds with the pathogen mycelium. Twenty-one DAI the disease severity and percentage of seedlings with vascular darkening were evaluated. It was observed a transmission rate of 64.0 to 89.0% from the seeds to the seedlings. Trichodel[®] was the most efficient product, reducing the incidence and severity of Fov in the cotton seedlings.

Keywords: *Gossypium hirsutum*, vascular wilt, *Trichoderma* spp., *Bacillus subtilis*.

1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an annual plant, of recognized commercial importance and cultivated globally. Its fiber is one of the most used in the textile industry. ~~The and the seed is rich in vegetable oil (which is used to produce the cotton seed cake), the lint and bark, are also important to generate employment and income during the production and processing phases~~ [1].

Comment [B1]: Vegetable oil is not used to produce seed cake. Seed cake is what is left when the vegetable oil has been extracted. Re-frame the statement to reflect this and be clear about it.

Comment [B2]: Explain exactly what the lint and the bark is used for.

Cotton cultivation stands out as one of the most important agricultural activities for the Brazilian agribusiness, mainly due to the advances in seed production technology [2]. The use of seeds of low physical, physiological, genetic and sanitary ~~quality-qualities~~ are responsible for significant losses in the establishment of this crop, generating malformed, diseased, and low vigor seedlings, ~~which has with~~ limited the yield increase and ~~poor~~ fiber quality in the different ~~producer-production~~ regions [3].

~~This crop~~Cotton is susceptible to ~~a high number of pathogens,~~ *Fusarium oxysporum* f.sp. *vasinfectum* (Fov), which ~~are-is~~ mostly seed transmitted [4]. The pathogens can be easily transported over long distances and, when introduced in exempt areas, determine the initial cycle of the disease and finally ~~infest-spread in~~ the field [5]. *Fusarium oxysporum* causes ~~fusarium vascular wilt, also known as cotton fusariosis, which is usually characterized by rotting of the root system, yellowing and wilting of leaves and branches, dwarfism and death of infected plants [6, 7]. Occurrence of certain pathogens-F. oxysporum inoculum in the seeds, even at relatively low rates-concentrations may result in quantitative and qualitative losses in the production, as observed in infections of Fusarium oxysporum f.sp. vasinfectum (Fov). This pathogen causes the disease known as fusarium vascular wilt or also known as cotton fusariosis, which is usually characterized by rotting of the root system, yellowing and wilting of leaves and branches, dwarfism and death of infected plants [6, 7].~~

~~The use~~Use of resistant varieties has stood out as the main method of disease control. However, it shows little relevance in the management program for *Fusarium wilt disease* (Fov) since the pathogen breaks the resistance of the main commercially used ~~planted~~cultivars, ~~caused by the probably due to~~ high genetic variability and instability of the hosts [8]. In addition, the success of breeding programs to control the disease depends on the understanding of the population structure as well as the way of transmission of this pathogen [9].

Comment [B3]: What is this?

With exception of seed treatment, the use of fungicides is not recommended in the management of vascular wilts, since they are not able to prevent subsequent infection and colonization of the pathogen in the phloem [10]. Researchers have sought out efficient methods of disease management based on biological control using *Trichoderma* spp. and *Bacillus* spp., which aims to reduce the incidence of causal agents of vascular wilts, especially when applied in the seeds [11, 12, 13].

Comment [B4]: There is need for more literature on biocontrol of Fusarium wilt and the level of success in Cotton or another crop that is susceptible to Fusarium wilt disease.



The ~~objective aim~~ of this study was to ~~determine-evaluate the biocontrol efficiency~~ efficacy of ~~biological products~~ of *Trichoderma* spp. and *Bacillus subtilis* in the control of *Fusarium oxysporum* f.sp. *vasinfectum* inoculated ~~in the on cotton~~ seeds and seedlings ~~of cotton~~.

Comment [B5]:

1. You need to improve on your Introduction Section. For example, as part of your introduction, there is need for you to strongly justify your experiment. This is not likely to be the first study in this field. You ought to review, report and cite previous studies in this area of research.

2. MATERIAL AND METHODS

Source of pathogen, cotton seed and Description of experimental site

The experiments were carried out in green house and at the Laboratório de Fitopatologia (LAFIT), of the Departamento de Fitotecnia e Ciências Ambientais (DFCA), of the Centro de Ciências Agrárias (CCA), Campus II, of the Universidade Federal da Paraíba (UFPB), Areia-PB. The isolate ~~of *Fusarium oxysporum* f.sp. *vasinfectum* (Fov)~~, CCMF-CNPA 0007 ~~of *Fusarium oxysporum* f.sp. *vasinfectum* (Fov)~~, which belongs to the collection of plant pathogenic microorganisms of Empresa Brasileira de Pesquisa e Agropecuária (EMBRAPA, Campina Grande, PB, Brazil) was used. The seeds of the cotton variety ~~_-moc6_~~ ~~Moc6~~ were collected in the municipality of Casserengue-PB and the cultivars BRS 286 (white fiber) and Topázio (colored fiber) were donated by the EMBRAPA.

Comment [B6]: Is Moco a variety of cotton?

Laboratory culture of *F. oxysporum* and inoculation of cotton plants

~~The pathogen isolate of *F. oxysporum* was grown on PDA medium in Petri dishes~~
~~containing PDA medium and incubated under controlled conditions (25 ° C + 2 °C) for 10~~
~~days. The inoculum was prepared by the addition of pouring 10 mL of sterile distilled water~~
~~(SDW) in on the culture in each Petri dish and the conidia was liberated using a soft bristle~~
~~brush (Model). The suspension was filtered through sterile gauze (.....) to separate~~
~~mycellial fragments. (...) The seeds were immersed in the suspension for 5 minutes,~~
~~manually shaken, incubated for 12 hours under the conditions previously described and~~
~~sown in pots containing commercial substrate Basaplant® and sterilized vermiculite (2:1~~
~~v/v).~~

Comment [B7]: What is the inoculum? If you have used the infective fungal conidia, you have to state this clearly.

Comment [B8]: What is the pore size?

Comment [B9]: Did you estimate the conidia number and standardized the concentration to a specific value, for example 1.0×10^6 conidia per mL? If the conidia concentration was not standardized, you have to clearly state this.

Comment [B10]: What conditions? Re-describe the condition please.

The determination of pathogen transmission to the plants was accessed by using 100 seeds of the cotton variety mocó and the cultivars BRS 286 and Topázio, which were inoculated by the spore suspension method.

~~The pathogen isolate was grown in Petri dishes containing PDA medium and incubated under controlled conditions (25 ° C + 2 °C) for 10 days. The inoculum was prepared by the addition of 10 mL of sterile distilled water (SDW) in each Petri dish and the conidia was liberated using a soft bristle brush, the suspension was filtered through sterile gauze. The seeds were immersed in the suspension for 5 minutes, manually shaken, incubated for 12 hours under the conditions previously described and sown in pots containing commercial substrate Basaplant® and sterilized vermiculite (2:1 v/v).~~

Evaluation of infectivity and pathogenicity

~~The transmission evaluation~~
~~Infection of the seedlings by the pathogen of the pathogen to the~~
~~seedlings was carried out as assessed at~~ 15 days after sowing, based on the observation of the presence of internal and external symptoms in the roots and vascular structures of the seedlings. The stem and roots were ~~submitted subjected~~ to indirect isolation [14] to confirm the causal agent.

The seeds of the variety mocó and the cultivars BRS 286 and Topázio were submitted to the treatments: T1 - Control, treated with SDW; T2-Trichodel[®] (0.5 mL); T3-Trichodel[®] (1.0 mL); T4-Trichodel[®] (1.5 mL); T5-Trichodel[®] (2.0 mL); T6-Bactel[®] (2.0 mL); T7-Bactel[®] (2.5 mL); T8-Bactel[®] (3.0 mL); T9-Bactel[®] (3.5 mL), diluted in 100 mL of SDW; T10 - Fungicide Captana (240g of the product for 100kg of seeds).

Two-hundred seeds were used per treatment with 10 replicates of 20 seeds. The seeds were previously disinfested in 1% sodium hypochlorite for three minutes, washed with sterile distilled water, dried out, immersed in the treatments for 5 minutes and conditioned in a humid chamber for a period of 24 hours. After this period, inoculation with Fov was performed by immersion of the seeds in a spore suspension for 5 minutes.

The seeds previously treated with the biological products and inoculated with Fov were distributed under aseptic conditions in Petri dishes containing PDA medium [16]. The Petri dishes were maintained at 25 ° C ± 2 for seven days, and then the visualization and identification process were performed through microscopic observations and morphology descriptions using specialized literature [15]. The results obtained were expressed as percentages of fungus incidence.

The previously mentioned treatments were used to evaluate the influence of the biological control on the physiological quality of the cotton seeds. The germination test [16] was carried out, in which 100 seeds were used, distributed in four replicates of 25 seeds and placed in a "Germitest" paper substrate, moistened with 2.5 times the dry paper weight and distributed in a germination chamber type Biochemical Oxygen Demand (BOD), regulated at 30 °C with photoperiod of eight hours. The seed rolls were packed in transparent plastic bags, to avoid water loss by evaporation. Germinated seed counts were performed daily from the fourth day to fourteenth, counting the normal, abnormal seedlings with primary infection, dead and hard seeds.

Comment [B11]: Not appropriate

The first germination counting was conducted concurrently with the germination test, where the germinated seeds were counted on the fourth day after the start of the test [16].

The germination speed index (GSI) was conducted concurrently with the germination test, where the number of germinated seeds were recorded daily. The index was determined according to the formula proposed by Maguire [17].

The seedling length and dry matter were evaluated after the germination test. The length of shoot and root of normal seedlings were measured with a ruler graduated in millimeters. The shoot and roots of the seedlings were placed in Kraft paper bags separately and taken to a stove with forced air circulation at a temperature of 65°C, until they reached a constant weight for 48 hours. After this period, they were weighed on an analytical scale with precision of 0.001g, the results were expressed in g. plantula⁻¹.

The experimental design used for the physiological and sanitary quality evaluation was completely randomized. The means were compared by the Scott-Knott test, at 5% of probability using the Sisvar 5.4 software [18].

The seeds of the variety mocó and the cultivars BRS 286 and Topázio were previously disinfested in 1% sodium hypochlorite for three minutes and immersed in the biological treatments for 5 minutes and manually shaken. They were then conditioned in a humid chamber for a period of 24 hours and submitted to the treatments described previously.

After this period, the inoculation with *Fov* was carried out and the seeds were dried at 25°C ± 2 on sterilized germitest paper and sown in polyethylene bags with 1.5 L capacity. The commercial substrate used was sterilized Basaplant[®] and vermiculite, in the ratio of 2:1 (substrate / vermiculite - v/v).

To evaluate the efficiency of the biological products, three types of inoculation were performed: 1) Inoculation of the substrate with suspension of conidia: the substrate was

previously moistened, then perforations with a depth of 5 cm were made, and 20 mL of the conidia suspension were added. Seeds treated with the biological products were sown on the substrate; 2) Immersion of the seeds in the conidia suspension: the seeds previously treated with the biological products were immersed in the conidia suspension of the pathogen for 5 minutes, manually shaken and placed to dry in plastic trays with two sheets of sterile filter paper. The seeds were sown in the already mentioned substrate, the control was composed of seeds treated with fungicide Captana and immersed in SDW and 3) Direct contact of the seeds with the pathogen mycelium: after the mycelial growth of the fungi in Petri dishes containing PDA medium, incubated under controlled conditions of $25^{\circ}\text{C} \pm 2$, for a period of 10 days, the seeds treated with the biological products were conditioned on these colonies and maintained in this condition for 24 hours. Seeds placed in contact with inoculum-free substrate (pure PDA medium) were used as control.

Twenty-one days after the inoculation, the seedlings were removed from the substrate, sectioned in the basal region using a previously sterilized scalpel, and by visual inspection the incidence of the disease was assessed by the percentage of wilted seedlings and vascular darkening. The quantification of shoot length was performed using a millimeter ruler. In order to evaluate the severity of the disease, a severity scale proposed by Wickens (1964) was used. To evaluate the severity of vascular darkening a scale proposed by Becerra Lopez-Luvalle et al [19] was used.

The experimental design was a complete randomized block using a 3x3 factorial scheme (3 cotton varieties x 3 inoculation methods). Data was submitted to analysis of variance and the means were compared by the Scott-Knott test at 5% of probability, using the statistical software Sisvar 5.4 [18].

3. RESULTS AND DISCUSSION

In the *Fusarium oxysporum* f.sp. *vasinfectum* (Fov) transmission evaluation there was a high incidence of seedlings with typical symptoms caused by Fov, such as wilt of cotyledons

Comment [B12]: Your Materials and Methods are rather confusing and incomprehensible. Create sub-headings and let your methods be arranged under the relevant sub-heading. I have done some examples for you. You may create the following subheadings, copy and paste the relevant Sections of your write-up under the subheadings.

YOU MAY CREATE THE FOLLOWING SUB-HEADINGS:

1. Source of pathogen, cotton seed and description of experimental site
2. Laboratory culture of *Fusarium oxysporum*
3. Inoculation of cotton seeds/plants
4. Evaluation of biocontrol agents
5. Assessment of disease incidence and crop performance

Comment [B13]: While it is good sometimes to Join Results and Discussion, this work would be more interesting if you can separate your results from the discussion. You are likely going to be able to do more justice to your data and discuss them in a more interesting manner. This is just a suggestion please.

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(Figure 1A), vascular darkening (Figure 1B) and seedling death (Figure 1C). In addition, the pathogen also caused seed rot right after the radicle emission (Figure 1D).

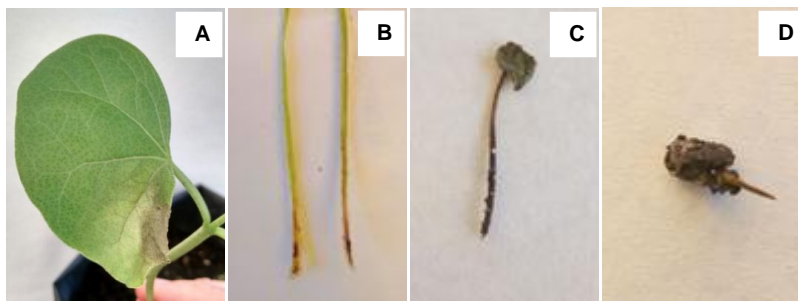


Figure 1. Cotton seedlings infected with *Fusarium oxysporum* f.sp. *vasinfectum* (Fov), after the transmission test: (Aa) wilt of cotyledonary leaf; (Bb) Darkening of the vascular tissues; (Cc) Dead seedlings; (Dd) Dead seedling, right after radicle emission.

The maximum Fov transmission rate from the seed to the plants were of 89.0, 71.0 and 64.0% for BRS 286, Topázio cultivars and mocó variety, respectively (Figure 2). In a similar study, Araújo et al. [7] verified a maximum transmission rate of different Fov isolates, around 50.0% in the cotton cultivars FM 966 and IAC 20-233.

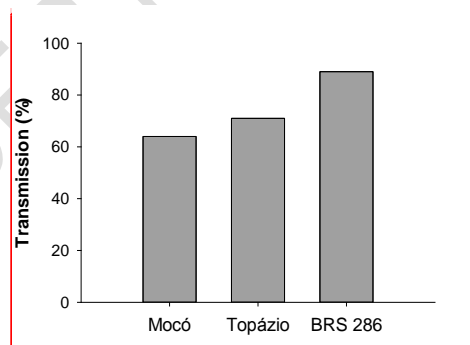


Fig. 2. *Fusarium oxysporum* f. sp. *vasinfectum* transmission (%) in cotton seedlings inoculated 21 days after sowing.

As observed in Table 1, a significant decrease of Fov development in all treatments was observed when compared to the control, with the exception of the cultivar BRS 286 treated

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Comment [B14]: Before you make a reference to a similar study, you ought to discuss your own result. For example, why do you have the variabilities you observed in your own result? Having justified what you observe, you can proceed to make reference to another study.

Comment [B15]: Please, include the standard error bars in your graph. It may be interesting if you can indicate the LSD values too.

Comment [B16]: Here, you are talking about significant increase but you did not perform any statistical analysis. There is no section in your materials and methods where you explained the statistical analysis methods used. Please create Statistical Analysis Section in your materials and Methods.

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with 0.5% Trichodel[®]. The use of the Captana fungicide provided the lowest incidence of this pathogen, with reduction of 76.0, 70.5 and 68.0%, for BRS 286, mocó and Topázio, respectively, differing from the control and the biological treatments.

Table 1. Incidence of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton seedlings treated with *Trichoderma* spp. and *Bacillus subtilis*.

Treatments	Incidence (%)		
	Mocó	Topázio	BRS 286
Control	94.5a	98.5a	97.0a
Trichodel [®] (0.5%)	51.1b	53.5b	74.0a
Trichodel [®] (1.0%)	46.0b	56.5b	44.5b
Trichodel [®] (1.5%)	43.5b	44.0b	50.0b
Trichodel [®] (2.0%)	38.0b	54.5b	46.5b
Bactel [®] (2.0%)	40.0b	43.0b	45.0b
Bactel [®] (2.5%)	39.5b	49.0b	42.0b
Bactel [®] (3.0%)	44.5b	59.0b	41.0b
Bactel [®] (3.5%)	40.0b	53.5b	42.5b
Fungicide	29.5c	32.0c	24.0c
CV (%)	24.79	28.12	28.36

Means followed by the same letter do not differ from using the Scott-Knott test at 5% of probability.

Studies with biological control in seed treatment have been carried out and promising results have been obtained. Carvalho et al. [10] found that *T. harzianum*, isolated from commercial products, is able to efficiently colonize common bean (*Phaseolus vulgaris* L.) cv. Jalo and control *Aspergillus* spp., *Cladosporium* sp. and *S. sclerotiorum* that were associated with the treated seeds. Carvalho et al. [11] found that *T. harzianum* was able to reduce the incidence of *F. oxysporum* f. sp. *phaseoli* by 35.0 to 51.0% in common bean seeds. Those results were very similar to those found in the present study. Mura et al. [20] also found that *Bacillus* spp. inoculated on rice seeds (*Oryza sativa* L.) was efficient in the control of *Gerlachia oryzae*, causal agent of scald spot and Silva et al. [5] confirmed the potential of *B. cereus* and *Bacillus* sp. in reducing the incidence of *Curvularia lunata* in rice seeds.

Comment [B17]: What is the meaning of this abbreviation? Write it in full at the first time and enclose the abbreviation in a bracket. Subsequently, you can freely use the abbreviation.

Comment [B18]: Scott-Knott Test suggests you have used R-Programming. Please indicate this under your Statistical Analysis Section.

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Comment [B19]: Discuss the results of the present study before making references to other studies.

For the germination, with the exception of the treatments with Trichodel[®] in the cultivar Topázio, there was no significant difference in the percentage of germination, first germination counting and germination speed index, among the other treatments when compared to the control (Table 2).

Table 2. Germination (G), first counting of germination (FCG) and germination speed index (GSI) of cotton seeds (*Gossypium hirsutum* L.) treated with *Trichoderma* spp. and *Bacillus subtilis*.

Treatments	Mocó			Topázio			BRS 286		
	G	FCG	GSI	G	FCG	GSI	G	FCG	GSI
Control	65.0a	63.0a	4.00a	83.0b	81.0a	5.16a	84.0a	82.0a	5.22a
Trichodel [®] (0.5%)	71.0a	64.0a	4.35a	89.0a	84.0a	5.55a	89.0a	85.0a	5.51a
Trichodel [®] (1.0%)	70.0a	64.0a	4.10a	89.0a	85.0a	5.50a	88.0a	85.0a	5.46a
Trichodel [®] (1.5%)	76.0a	69.0a	4.66a	90.0a	83.0a	5.53a	91.0a	87.0a	5.64a
Trichodel [®] (2.0%)	72.0a	64.0a	4.40a	91.0a	83.0a	5.58a	90.0a	86.0a	5.57a
Bactel [®] (2.0%)	66.0a	63.0a	4.10a	82.0b	80.0a	5.10a	84.0a	82.0a	5.37a
Bactel [®] (2.5%)	70.0a	67.0a	4.28a	84.0b	82.0a	5.22a	87.0a	83.0a	5.34a
Bactel [®] (3.0%)	71.0a	67.0a	4.38a	84.0b	82.0a	5.22a	89.0a	85.0a	5.51a
Bactel [®] (3.5%)	69.0a	64.0a	4.20a	83.0b	83.0a	5.19a	87.0a	85.0a	5.20a
Fungicide	68.0a	65.0a	4.20a	84.0b	80.0a	5.10a	83.0a	80.0a	5.15a
CV (%)	12.56	12.86	10.85	4.64	5.26	3.89	5.13	4.21	4.83

Means followed by the same letter do not differ from the Scott-Knott test at 5% of probability.

Similarly, Martini et al. [21] found that secondary metabolites released by *Trichoderma* spp. isolates, used to inhibit the development of *Fusarium* sp. and *Bipolaris oryzae*, propagated by rice seeds (*Oryza sativa* L.) did not interfere in their germination. Carvalho et al. [22] found a significant reduction in the incidence of *Fusarium oxysporum* f. sp. *phaseoli* and increased germination rate in seeds of common bean (*Phaseolus vulgaris* L.) inoculated with *T. harzianum*, with colonization of the seed surface and hypocotyl, which, according to the authors, is the main characteristic for the selection of potential biocontrol agents, also observed in the present study.

The treatments with Trichodel[®] provided an increase in shoot and root length, differing from Bactel[®], fungicide and the control treatments, which did not differ from each other (Table 3).

Table 3. Length of aerial part (LAP) and roots (LR) of cotton seedlings (*Gossypium hirsutum* L.) treated with *Trichoderma* spp. and *Bacillus subtilis*.

Comment [B20]: You did not work on metabolites! Why are you discussing what is not a part of your study?

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Treatments	Mocó		Topázio		BRS 286	
	LAP	LR	LAP	LR	LAP	LR
Control	5.00a	7.03b	6.00b	8.28b	6.57b	9.02b
Trichodel [®] (0.5%)	5.45a	6.95b	6.45a	8.78b	7.02b	9.55b
Trichodel [®] (1.0%)	5.15a	7.62a	6.97a	9.13b	7.42a	10.25a
Trichodel [®] (1.5%)	5.10a	7.61a	6.85a	9.63a	7.35a	10.35a
Trichodel [®] (2.0%)	5.22a	7.30a	6.72a	9.58a	7.04a	10.50a
Bactel [®] (2.0%)	5.05a	7.00b	6.23b	8.25b	6.52b	9.30b
Bactel [®] (2.5%)	5.10a	6.90b	6.10b	9.15b	6.50b	9.40b
Bactel [®] (3.0%)	5.23a	6.90b	6.10b	9.15b	6.32b	9.63b
Bactel [®] (3.5%)	5.10a	7.00b	6.27b	9.18b	6.35b	9.50b
Fungicide	5.20a	7.35b	6.15b	9.07b	6.40b	9.65b
CV (%)	4.28	6.01	7.25	16.60	5.90	4.17

Means followed by the same letter do not differ from the Scott-Knott test at 5% of probability.

Pereira et al. [13] observed an antagonistic effect of *T. harzianum* *R. solani* and *F. solani*, and also verified an increase in the shoot and root length of common bean (*Phaseolus vulgaris* L.) plants originated of treated seeds with this microorganism, corroborating with the results obtained in this work. A significant increase of the length and dry matter of seedlings were verified in some studies when treated with *Trichoderma* spp. [10, 11, 20, 23, 24].

In Table 4, it was observed that, except for the dry matter of the root for the variety mocó, the use of Trichodel[®] (concentration 1.0%) and Bactel[®] (concentrations 2.0, 2.5 and 3.0%), had no significant effect on the shoot dry matter of the mocó variety and for the shoot and root dry matter of Topázio and BRS 286, when compared to the control.

Table 4. Dry matter of the aerial part (DMAP) and roots (DMR) of cotton seedlings (*Gossypium hirsutum* L.) treated with *Trichoderma* spp. and *Bacillus subtilis*.

Treatments	Mocó		Topázio		BRS 286	
	MSPA	MSRA	MSPA	MSRA	MSPA	MSRA
Control	0.82a	0.36b	1.13a	0.48 ^a	1.24a	0.56a
Trichodel [®] (0.5%)	0.90a	0.38b	1.26a	0.55 ^a	1.33a	0.62a
Trichodel [®] (1.0%)	0.88a	0.40a	1.12a	0.56 ^a	1.28a	0.58a
Trichodel [®] (1.5%)	0.85a	0.37b	1.18a	0.53 ^a	1.29a	0.60a
Trichodel [®] (2.0%)	0.89a	0.40a	1.18a	0.58a	1.28a	0.65a
Bactel [®] (2.0%)	0.80a	0.38b	1.10a	0.53a	1.13a	0.55a
Bactel [®] (2.5%)	0.85a	0.36b	1.12a	0.55a	1.30a	0.60a
Bactel [®] (3.0%)	0.84a	0.35b	1.20a	0.51a	1.20a	0.55a
Bactel [®] (3.5%)	0.83a	0.40a	1.25a	0.54a	1.25a	0.57a
Fungicide	0.86a	0.40a	1.27a	0.54a	1.12a	0.55a
CV (%)	9.48	6.96	8.35	6.59	9.23	8.55

Means followed by the same letter do not differ from the Scott-Knott test at 5% of probability.

Regarding the disease severity, for the mocó variety and the cultivars Topázio and BRS 286, a significant reduction in all of the seedlings treated with Trichodel[®] and Bactel[®] was verified,

except in the concentrations 2.0; 2.5 and 3.0%, of these products in the mocó variety using the inoculation method 1. No significant differences among the treatments were observed in the other inoculation methods (Table 5).

Table 5. Severity of symptoms of cotton seedlings (*Gossypium hirsutum* L.) from seeds treated with *Trichoderma* spp. and *Bacillus subtilis* and inoculated with *Fusarium oxysporum* f. sp. *vasinfectum*.

Treatments	Severity								
	Mocó			Topázio			BRS 286		
	I1	I2	I3	I1	I2	I3	I1	I2	I3
Control	3.2a	2.8a	3.5a	4.0a	3.4a	3.4a	4.6a	2.9a	3.4a
Trichodel [®] (0.5%)	2.5b	2.8a	2.9a	3.1b	2.0b	2.8a	3.4b	2.2a	3.1a
Trichodel [®] (1.0%)	2.5b	2.7a	2.9a	2.8b	2.4b	3.1a	3.1b	2.3a	2.8a
Trichodel [®] (1.5%)	2.3b	2.4a	2.8a	2.7b	1.8b	2.8a	2.2c	1.9ab	2.4a
Trichodel [®] (2.0%)	1.9b	2.4a	3.1a	2.0b	1.7b	2.9a	2.0c	1.9ab	2.4a
Bactel [®] (2.0%)	3.4a	2.5a	2.8a	3.0b	2.4b	3.0a	2.9b	2.8a	2.5a
Bactel [®] (2.5%)	3.0a	2.5a	3.1a	3.0b	2.6b	2.8a	2.5c	1.8ab	2.9a
Bactel [®] (3.0%)	3.1a	2.2a	2.9a	2.4b	2.5b	3.0a	2.4c	2.8a	2.8a
Bactel [®] (3.5%)	2.5b	2.4a	2.8a	2.4b	2.5b	2.9a	2.0c	1.8ab	2.8a
Fungicide	2.4b	2.5a	2.1a	2.2b	1.9b	2.4a	1.8c	1.2b	2.4a
CV (%)	16.76								

Means followed by the same letter do not differ from the Scott-Knott test at 5% of probability.

I1: Inoculation of the substrate with pathogen conidia suspension; I2: Immersion of the seeds in the pathogen conidia suspension; I3: Direct contact of the seeds with the pathogen mycelium.

Usually, plants infected with this pathogen had wilt symptoms ten days after seed inoculation. Ludwig et al. [25] when treating rice seeds (*Oryza sativa* L.) with *Bacillus* sp. verified a significant decrease in the severity of the rice scald spot caused by *Gerlachiaoryzae*. According to these authors, the plants treated with these microorganisms showed higher values of grain mass.

Sousa et al. [4] evaluated the severity of fusarium vascular wilt using three methods of inoculation in cotton seeds (*Gossypium hirsutum* L.) and observed that the inoculation through spore suspension and direct contact in the seeds with mycelium of the pathogen presented a higher incidence of Fov and disease severity, since it provides greater concentration and adhesion of the conidia in the inoculated seeds, corroborating with the results verified in the present study.

A significant decrease of plants of the cultivar BRS 286 with vascular darkening were observed using Trichodel[®] and Bactel[®] (except Bactel[®] in the concentrations 2.0, 2.5 and 3.0% using the inoculation method 3) (Table 6). The methods of inoculation did not differ from the treatment with the fungicide Captana regarding the incidence of the disease in the seedlings. For the cultivar Topázio, using the inoculation method 1, with Trichodel[®] at 0.5 and 1.0% and Bactel[®] at 2.5%, there were higher values of disease severity, differing from the other treatments, and they did not differ from each other. For the mocó variety all of the treatments were similar in all inoculation methods used (Table 6).

Table 6. Severity of vascular symptoms of the cotton seedlings (*Gossypium hirsutum* L.) treated via seeds with *Trichoderma* spp. and *Bacillus subtilis* and inoculated with *Fusarium oxysporum* f. sp. *vasinfectum*.

Treatments	Percentage of vascular browning (%)								
	Moco			Topázio			BRS 286		
	I1	I2	I3	I1	I2	I3	I1	I2	I3
Control	60.0a	66.7a	50.0a	23.3b	45.0a	46.6a	80.0a	78.3a	56.7a
Trichodel [®] (0,5%)	53.0a	46.7ab	48.3a	31.7a	30.0ab	45.0a	60.0b	51.7b	38.3b
Trichodel [®] (1,0%)	45.0a	53.3a	46.7a	33.3a	36.7a	43.3a	53.3b	45.0b	39.3b
Trichodel [®] (1,5%)	36.7a	45.0ab	45.0a	25.0b	23.3b	36.7a	30.0c	43.3b	30.3b
Trichodel [®] (2,0%)	36.7a	48.3ab	51.7a	15.6b	20.0b	36.7a	26.7c	25.0b	23.0b
Bactel [®] (2,0%)	38.3a	48.3ab	45.0a	20.0b	36.7a	38.7a	48.3b	50.0b	60.0a
Bactel [®] (2,5%)	48.3a	46.7ab	56.7a	46.7a	40.0a	41.7a	38.3c	50.0b	50.0a
Bactel [®] (3,0%)	46.7a	50.0a	48.2a	20.0b	38.3a	31.7a	36.7c	35.0b	51.7a
Bactel [®] (3,5%)	45.0a	48.3ab	46.7a	26.7b	40.0a	38.3a	25.0c	36.0b	38.7b
Fungicide	36.7a	35.0b	28.0b	16.7b	23.3b	40.0a	21.7c	30.0b	35.0b
CV (%)	45.54								

Means followed by the same letter do not differ from the Scott-Knott test at 5% of probability.
 I1: Inoculation of the substrate with pathogen conidia suspension; I2: Immersion of the seeds in the pathogen conidia suspension; I3: Direct contact of the seeds with the pathogen mycelium.

The biological products presented a varied efficiency in relation to the inoculation methods. According to Jung et al. [26], environmental conditions such as temperature and humidity influence the efficiency of biocontrol agents and products based on these microorganisms, having an instability in the suppression of diseases.

4. CONCLUSION

The product Trichodel[®], based on *Trichoderma* spp. was the most efficient in reducing the incidence of Fov in treated cotton seeds and the severity of fusarium vascular wilt in the seedlings.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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