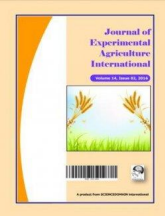

Journal of Experimental Agriculture International

34(1): 1-11, 2019; Article no.JEAI.48207
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Biocontrol Potential of *Trichoderma* and *Bacillus* Species on *Fusarium oxysporum* f. sp *vasinfectum*

Otília Ricardo de Farias[1*], Luciana Cordeiro de Nascimento[1], José Manoel Ferreira de Lima Cruz[1], Hiago Antônio Oliveira Silva[2], Mônica Danielly de Mello Oliveira[1], Riselane de Lucena Alcântara Bruno[1] and Nair Helena Castro Arriel[3]

[1]Universidade Federal da Paraíba (UFPB), Areia, PB, Brazil.
[2]Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil.
[3]Empresa Brasileira de Pesquisa e Agropecuária (EMBRAPA), Campina Grande, PB, Brazil.

Authors' contributions

This work was carried out in collaboration with all authors. Author ORF built the idea, carried out the experiment and writing of the article. Author HAOS collaborated in the conduction, evaluations, writing and translation of the article. The participation of the authors JMFLC, MDMO, RLAB and NHCA contributed to analysis of data, suggestions and materials used in the experiment. Author LCN worked as a research advisor for data analysis and writing of the article. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2019/v34i130166
Editor(s):
(1) Dr. Mirza Hasanuzzaman, Assistant Professor,Department of Agronomy, Sher-e-Bangla Agricultural University, Bangladesh.
Reviewers:
(1) Liamngee Kator, Benue State University, Makurdi, Nigeria.
(2) Dr. A. Borisade, Omotoso, Ekiti State University, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/48207

Original Research Article

Received 13 January 2019
Accepted 02 April 2019
Published 10 April 2019

ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum*, is one of the major diseases of cotton. Preventive methods to manage this disease should be adopted what includes the seed treatment with biocontrol agents as a good alternative. This work aimed to evaluate the efficiency of biological products based on *Trichoderma spp*. and *Bacillus subtilis* in the control of *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) applied 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 of 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

**Corresponding author: E-mail: otiliarfarias@gmail.com;*

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 (240 g / 100 kg of seeds) and inoculated with Fov. The pathogen incidence of the seeds was evaluated seven days after the inoculation (DAI). 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 pathogen conidia suspension; 2 - immersion of the seeds in the conidia suspension 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% of the seeds to the seedlings. Trichodel® reduced the incidence and severity of Fov in the cotton seedlings and was the most efficient product.

Keywords: Gossypium hirsutum; vascular wilt; Trichoderma sp.; Bacillus subtilis.

1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an annual plant, of well recognized commercial importance and cultivated throughout the world. Its fiber is one of the most used in the textile industry. The seed, which is rich in vegetable oil, after refined can have multiple uses, including human consumption. The linter consisting of a layer of short fibers adhered to the seed has a wide applicability in the industry. In addition, the seed bark can also be used for animal feeding [1].

The cotton cultivation stands out as one of the most important agricultural activities in the Brazilian agribusiness, mainly due to the advances in technology of seed production [2]. The use of seeds of low physical, physiological, genetic and sanitary quality are responsible for significant losses during the establishment of this crop, what can result in malformed, diseased, and low vigor seedlings, those factors has limited the yield increase and fiber quality in different producer regions [3].

Cotton is susceptible to *Fusarium oxysporum* f. sp. *vasinfectum* (Fov), which is mostly transmitted by the seeds [4]. The pathogen can be easily transported over long distances and when introduced in new areas, determine the initial cycle of the disease and finally spread in the field [5]. *Fusarium oxysporum* causes fusarium vascular wilt, also known as cotton fusariosis, is usually characterized by rotting of the root system, yellowing and wilting of the leaves and branches, dwarfism and plant death [6,7]. The inoculum of *F. oxysporum* in seeds, even at relatively low concentrations may result in quantitative and qualitative losses in production.

Use of resistant varieties has stood out as the main method for disease control. However, it shows little relevance in the management program for Fusarium wilt diseases since the pathogen breaks the resistance of the main commercially used cultivars, 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].

With exception of seed treatment, the use of fungicides is not recommended in the management of vascular wilt, 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., aiming to reduce the incidence of causal agents of vascular wilts, especially when applied in the seeds [11,12,13].

Several studies have demonstrated the biocontrol efficiency in the management of fusarium wilts, such as *Trichoderma spp*. in the control of *Fusarium sp.*in soybean [14], six *T. harzianum* isolates against *F. oxysporum f. sp*. *phaseoli* in common bean [15], *Trichoderma sp*. and *Bacillus subtilis* in the control of *Fusarium sp*. in soybean [16], *Trichoderma spp*. and *Bacillus subtilis*in the control of *Fusarium sambucinum* in *Pinus elliottii* seedlings [17] and *Bacillus subtilis* in the control of *Fusarium graminearum* in wheat and barley [18]. However, there is lack of information regarding the treatment of seeds with biocontrol agents in cotton.

Therefore, this study aimed to evaluate biocontrol efficiency of *Trichoderma* sp. and

Bacillus subtilis in the control of *Fusarium oxysporum* f. sp. *vasinfectum* inoculated on cotton seeds, and provide information regarding strategies of management of cotton vascular wilt.

2. MATERIALS AND METHODS

The experiments were carried out under a greenhouse 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, Brazil.

2.1 Isolates of *Fusarium oxysporum* f. sp. *vasinfectum* and Cotton Seeds Used

The isolate of *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) CCMF-CNPA 0007 was used, belonging to the Collection of Plant pathogenic Microorganisms of Embrapa- Algodão (Campina Grande - PB).

The cottons seeds of the variety Mocó were harvested in municipality of Casserengue - PB and the seeds of the cultivars BRS 286 (white fiber) and Topázio (colored fiber) were donated by Embrapa Algodão.

2.2 Fov Seed Transmission to the Seedlings

To determine the transmission of the pathogen to the seedlings, 100 seeds of the variety Mocó and cultivars BRS 286 and Topázio were used and inoculated by the spore suspension method.

The isolate of Fov CCMF-CNPA 0007 was cultivated in PDA medium and incubated under controlled conditions (25ºC+ 2ºC) for 10 days.

For the inoculum preparation, 10 mL of sterile distilled water (SDW) were added in each Petri dish containing the pathogen and the spores were released with the aid of a soft bristle brush. The suspension was filtered through sterile double gauze to separate fragments from the mycelia. The number of spores was estimated in a Neubauer's chamber and standardized at the concentration of 1,0 x 10^5 spores/mL.

The cotton seeds of the Mocó variety and the cultivars Topázio and BRS 286 were immersed in the Fov spore suspension for 5 min, shaken manually, incubated for 12 hours under controlled conditions (25ºC + 2ºC) and sown in pots containing commercial Basaplant® substrate and vermiculite (2:1 v/v). The pots were kept in a greenhouse under the conditions of 30ºC + 2ºC and were daily watered.

The seedlings infection was evaluated at 15 days after sowing, based on the observation of the presence of internal and external symptoms in the roots and vascular bundle of the seedlings. The stem and roots were submitted to indirect isolation [19] to confirm the infection by the causal agent.

2.3 Efficiency of the Biocontrol Agents against *Fusarium oxysporum* f. sp. *Vasinfectum*

Two-hundred seeds per treatment were used with 10 replications of 20 seeds. The seeds of the variety Mocó and cultivars BRS 286 and Topázio were disinfested in 1% sodium hypochlorite for three minutes, washed with sterile distilled water, and set to dry. After dried, the seeds were immersed in the treatments with the biological products for 5 min and conditioned in a humid chamber for a period of 24 hours.

The following treatments were used: 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 (240 g of the product for 100 kg of seeds).

For the inoculation, after treated and conditioned for 24 hours, the seeds were immersed with Fov in a spore suspension at a concentration of 1,0 x 10^5 spores/mL for 5 minutes.

The seeds treated with the biocontrol agents and inoculated with Fov were distributed in Petri dishes containing PDA [20]. The petri dishes were conditioned at 25°C ± 2 for seven days, after that the pathogen was visualized and identified through microscopic observations and morphology descriptions by specialized literature [21]. The results were expressed as percentage of Fov incidence in the seeds.

2.4 Effect of the Biocontrol Agents in the Physiological Quality of the Cotton Seeds

A germination test [20] was performed to evaluated the effect of the biocontrol agents in

the physiological quality of the treated seeds. The previous mentioned treatments were used. One-hundred seeds, distributed in four replicates of 25 and placed in "Germitest" paper substrate were used, the paper was moistened with a volume of 2.5 times the dry paper weight and distributed in a germ cell type Biochemical Oxigen Demand (BOD), at 30°C in a photoperiod of eight hours. After placed in the paper, the seed rolls were packed in transparent plastic bags, in order to avoid the loss of water by evaporation. Germinated seed counts were performed daily from the $4^{th}14^{th}$ day, the normal, abnormal seedlings with primary infection, dead and hard seeds were counted.

The first germination count was conducted in conjunction with the germination test, where germinated seeds were counted on the fourth day after sowing [20].

The germination speed index (GSI) was conducted concurrently with the germination test, where the number of germinated seeds was recorded daily. The index was determined according to the formula proposed by Maguire [22]:

$$\mathrm{GSI} = \frac{\mathrm{G1}}{\mathrm{N1}} + \frac{\mathrm{G2}}{\mathrm{N2}} \ldots \frac{\mathrm{Gn}}{\mathrm{Nn}}$$

Where, G1, G2, Gn = number of germinated seeds in the first, second… until last counting and N1, N2, Nn = number of weeks from the first, second, … until the last counting.

After the germination test the seedling length and dry matter were evaluated. The shoot and root length 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 65ºC for 48 hours until constant weightwas reached. After this period, they were weighed on an analytical scale with precision of 0.001 g, the results were expressed in g. plantula^{-1}.

A completely randomized experimental design was used. The means were compared by the Scott-Knott test at 5% probability. The statistical software Sisvar version 5.4 [23] was used.

2.5 Evaluation of Disease Incidence and Severity

Seeds of the variety Mocó and cultivars BRS 286 and Topázio were previously disinfested in 1% sodium hypochlorite for three minutes and washed with sterile distilled water and placed to dry. After dried, they were immersed in the biological treatments for 5 minutes, with manual agitation and conditioned in a humid chamber for a period of 24 hours.

The following treatments were used: 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 100 kg of seeds).

After treated with the biological products, the seeds were inoculated with Fov. To evaluated the efficiency of the products three types of inoculation were performed:

Inoculation 1 –Inoculation of the substrate with the spore suspension; the substrate was previously moistened, then perforations with a depth of 5 cm were made, and 20 mL of the spore suspension at the concentration of $1.0x10^5$ spores/mL were added. The seeds treated with the biological products were sown in the substrate;

Inoculation2 – Immersion of the seeds in the spore suspension; the seeds treated with the biological products were immersed in the spore suspension for 5 minutes, manually shaken and placed to dry in plastic trays with two sheets of sterile filter paper. After dried, the seeds were sown in the substrate, the controls were composed of seeds treated with fungicide Captana and immersed in SDW.

Inoculation3 –Direct contact of the seeds with the pathogen mycelium; the seeds treated with the biological products were conditioned in direct contact with the pathogen colony and maintained in this condition for 24 hours. The pathogen was cultivated in Petri dishes containing PDA medium, incubated under controlled at 25ºC ± 2, for a period of 10 days. Seeds placed in contact with inoculum-free substrate (pure PDA medium) were used as control.

The seeds were sown in polyethylene bags of 1.5 L capacity containing the commercial substrate Basaplant® and sterilized vermiculite, at the proportion of 2:1 (substrate/vermiculite – v/v).

Twenty-one days after the inoculation the disease incidence was assessed by the

percentage of wilted seedlings and vascular darkening. To perform the evaluation, the seedlings were removed from the substrate, sectioned in the basal region using a previously sterilized scalpel, and visualized the symptoms.

To evaluate the severity of the disease, a severity scale proposed by Wickens [24] was used. To evaluate the severity of vascular darkening a scale proposed by Becerra Lopez-Luvalle et al. [25] was used, where the following grades were given: 1 - Healthy plant; 2- Plants with 25% of vascular darkening; 3- Plants with 50% of vascular darkening; 4- plants with 75% of vascular darkening; 5- Dead plant.

A complete randomized block experimental design was used, composed of a 3x3 factorial scheme (1 cotton variety and 2 cultivars 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. The statistical software Sisvar version 5.4 [23] was used.

3. RESULTS AND DISCUSSION

3.1 Fov Seed Transmission to the Seedlings

For *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) transmission evaluation, a high incidence of seedlings with typical symptoms caused by Fov was observed, such as wilt of cotyledons, vascular darkening and seedlings death. In addition, the pathogen also caused seed rot right after the radicle emission.

The maximum Fov transmission rate from the seed to the seedlings were of 89.0, 71.0 and 64.0% for BRS 286, Topázio cultivars and Mocó variety, respectively (Fig. 2).The high percentage of Fov transmission in the cultivar BRS 286 occurred due to its susceptibility to the *Fusarium oxysporum f. sp. vasinfectum* complex. In a similar study, Araújo et al. [7] verified the maximum transmission rate of different Fov isolates, of approximately 50.0% in the cotton cultivars FM 966 and IAC 20-233.

Fig. 1. Cotton seedlings infected with *Fusarium oxysporum* f. sp. *vasinfectum* after the transmission test: (a) wilt of cotyledonary leaf (b) Darkening of the vascular tissues(c) Dead seedlings(d) Dead seedling, right after radicle emission

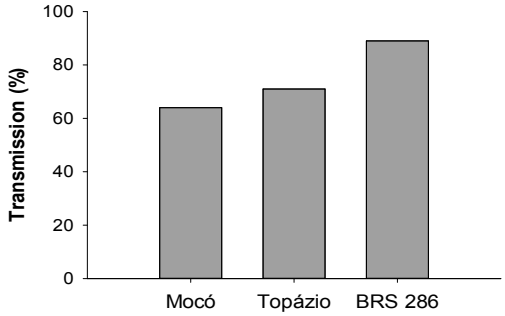

Fig. 2. *Fusarium oxysporum* f. sp. *vasinfectum* transmission (%) in cotton seedlings inoculated 21 days after sowing

Table 1. Incidence of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton seedlings treated with *Trichoderma* sp. 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 using the Scott-Knott test at 5% of probability.*CV= Coefficient of variance*

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

Treatments	Mocó			Topázio			BRS 286		
	G	FGC	GSI	G	FGC	GSI	G	FGC	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 by the Scott-Knott test at 5% of probability.*CV= Coefficient of variance*

3.2 Efficiency of the Biocontrol Agents against *Fusarium oxysporum* f. sp. *vasinfectum*

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

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. Moura et al. [26] 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.

Table 3. Shoot (SL) and root (RL) length of cotton seedlings (*Gossypium hirsutum* L.*)* treated with *Trichoderma* sp. and *Bacillus subtilis*

Treatments	Mocó		Topázio		BRS 286	
	SL	RL	SL	RL	SL	RL
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 by the Scott-Knott test at 5% of probability.*CV= Coefficient of variance*

Table 4. Shoot (SDM) and root (RDM) dry matter of cotton seedlings (*Gossypium hirsutum* L.) treated with *Trichoderma* sp. and *Bacillus subtilis*

Treatments	Mocó		Topázio		BRS 286	
	SDM	RDM	SDM	RDM	SDM	RDM
Control	0.82a	0.36b	1.13a	0.48a	1.24a	0.56a
Trichodel® (0.5%)	0.90a	0.38b	1.26a	0.55a	1.33a	0.62a
Trichodel® (1.0%)	0.88a	0.40a	1.12a	0.56a	1.28a	0.58a
Trichodel® (1.5%)	0.85a	0.37b	1.18a	0.53a	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 by the Scott-Knott test at 5% of probability.*CV= Coefficient of variance*

3.3 Effect of the Biocontrol Agents in the Physiological Quality of the Cotton Seeds

For the germination, with 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 treatments when compared to the control (Table 2).

Carvalho et al. [15] found a significant decrease in the incidence of *Fusarium oxysporum* f. sp. *phaseoli* and anincreased 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® promoted an increase in shoot and root length, and differed from Bactel®, fungicide and the control treatments, which did not differ from each other (Table 3).

Pereira et al. [13] observed an antagonistic effect of *T. harzianum* on *R. solani* and *F. solani*, and also verified an increase in the shoot and root length of common bean (*Phaseolus vulgaris* L.)

Table 5. Severity of symptoms of cotton seedlings (*Gossypium hirsutum* L.) in seeds treated with *Trichoderma* sp. 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 by the Scott-Knott test at 5% of probability. 1: Inoculation of the substrate with pathogen spore suspension; I2: Immersion of the seeds in the pathogen spore suspension; I3: Direct contact of the seeds with the pathogen mycelium. *CV= Coefficient of variance*

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

Treatments	Percentage of vascular darkening (%)								
	Mocó			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 using the Scott-Knott test at 5% of probability. 1: 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.*CV= Coefficient of variance*

in plants originated of treated seeds with these microorganisms, corroborating with the results obtained in this work. A significant increase of length and dry matter of seedlings were verified in some studies of seeds treated with *Trichoderma* spp. [10,11,26,27,28].

In Table 4, it was observed that, except for the dry matter of the root in the variety Mocó, the use of Trichodel® (concentration of 1.0%) and Bactel® (concentrations of 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.

3.4 Evaluation of Disease Incidence and Severity

Regarding the disease severity, for the Mocó variety and the cultivars Topázio and BRS 286, a significant decrease was verified in all of the

seedlings treated with Trichodel® and Bactel®, except for the concentrations of 2.0; 2.5 and 3.0% of these products in the Mocó variety, using the inoculation 1 method. No significant differences among the treatments were observed in the other inoculation methods (Table 5).

Plants infected with this pathogen generally had wilt symptoms ten days after seed inoculation. Ludwig et al. [29] when treating rice seeds (*Oryza sativa* L.) with *Bacillus sp*. verified a significant decrease in the severity of the rice scald spot caused by *Gerlachia oryzae*. 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 of 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 spores in the 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 for Bactel® in the concentrations of 2.0, 2.5 and 3.0%, using the inoculation method 3 (Table 6). The inoculation methods 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%, higher values of disease severity were observed, 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).

The biological products presented a diversified efficiency in according to the inoculation method. According to Jung et al. [30], environmental conditions such as temperature and humidity influence the efficiency of biocontrol agents and products based on these microorganisms, causing an instability in the suppression of diseases.

4. CONCLUSION

The product Trichodel®, based on *Trichoderma spp*. was the most efficient product and reduced the incidence and severity of Fov, causal agent of fusarium vascular wilt of cotton in the treated seeds.

ACKNOWLEDGEMENTS

We wish to thank Dr. Breno Oliveira Sousa (in memoriam) for his relevant contribution to this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used in this research are commonly and predominantly used products in our area of research in our country. There is absolutely no conflict of interest between the authors and producers of the products.

REFERENCES

1. Silva RA, Rack VM, Vigolo F. Correlação entre densidade populacional de nematoides e produtividade de algodoeiro. Bioscience Journal. 2014;30(1):210-218.
2. Lauxen LR, Villela FA, Soares RC. Physiological performance of cotton seeds treated with thiametoxam. Revista Brasileira de Sementes. 2010;32(3):061-068.
3. Nunes JRG, Menezes NL, CargneluttiFilho A. Physiological quality of silage sorghum seeds submitted to different sequence of conditioning. Revista Pesquisa Agropecuária Gaúcha. 2009;15:21-28.
4. Sousa MV, Machado JC, Pfenning LH, Kawasaki VH, Araújo DV, Silva AA, Martini Neto A. Methods of inoculation and effects of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton seeds. Tropical Plant Pathology. 2008;33:041-049.
5. Silva MSBS, Rodrigues AAC, Oliveira LJMG, Silva EKC, Pereira TS. Sanity of rice seed, biocontrol, characterization and transmission of *Curvularia lunata* on rice seed-seedlings. Ceres. 2014;61(4):511-517.
6. Davis RM, Colyer PD, Rothrock CS, Kochman JK. Fusarium wilt of cotton: Population diversity and implications for

management. Plant Disease. 2006;90(6): 692-703.

7. Araújo DV, Machado JC, Pedrozo R, Pfenning LH, Kawasaki VH, Neto AM, Pizatto JA. Transmission and effects of *Fusarium oxysporum* f. sp. *vasinfectum* on cotton seeds. African Journal of Agriculture. 2016;11(20):1815-1823.
8. Egamberdiev SS, Ulloa M, Saha S, Salakhutdinov IB, Abdullaev A, Glukhova LA, Abdurakhmonov IY. Molecular characterization of Uzbekistan isolates of *Fusarium oxysporum* f. sp. *vasinfectum*. Journal Plant Science & Molecular Breed. 2013;2(1):3.
9. Wang B, Brubaker CL, Tate W, Woods MJ, Matheson BA, Burdon JJ. Genetic variation and population structure of *Fusarium oxysporum* f. sp. *vasinfectum* in Australia. Plant Pathology. 2006;55(6):746-755.
10. Carvalho DDC, Mello SCM, Lobo Júnior M, Geraldine AM. Biocontrol of seed pathogens and growth promotion of common bean seedlings by *Trichoderma harzianum*. Pesquisa Agropecuária Brasileira. 2011;46(8):822-828.
11. Carvalho DDC, Mello SCM, Lobo Júnior M, Silva MC. Control of *Fusarium oxysporum* f.sp *phaseoli in vitro* and on seeds and growth promotion of common bean in early stages by *Trichoderma harzianum*. Tropical Plant Pathology. 2011;36:028-034.
12. Lazarotto M, Muniz MFB, Beltrame R, Santos AF, Jucéli Müller J, Araújo MM. Biological and chemical treatment of *Cedrela fissilis* seeds for controlling Rhizoctonia sp. Cerne. 2013;19:169-175.
13. Pereira JL, Queiroz RML, Charneau SO, Felix CR, Ricart CAO, Silva FL, Steindorff AS, Ulhoa CJ, Noronha EF. Analysis of *Phaseolus vulgaris* response to its association with *Trichoderma harzianum* (ALL-42) in the presence or absence of the phytopathogenic fungi *Rhizoctonia solani* and *Fusarium solani*. Plos One. 2014;9:1-23.
14. Milanesi PM, Blume E, Antonioli ZI, Muniz MFB, Santos RF, Finger G, Durigon MR. Biocontrol of *Fusarium* spp. with *Trichoderma* spp. and growth promotion in soybean seedlings. Revista de Ciências Agrárias. 2013;36(3):347-356.
15. Carvalho DDC, Lobo Júnior M, Martins I, Inglis PW, Mello SCM. Biological control of *Fusarium oxysporum* f. sp. phaseoli by *Trichoderma harzianum* and its use for common bean seed treatment. Tropical Plant Pathology. 2014;39(5):384-391.
16. Rodrigues UL, Ribeiro RF, Tavares TCO. Silício e microrganismos antagonistas no controle de Fusarium sp. na soja. Revista Brasileira de Tecnologia Aplicada nas Ciências Agrárias. 2017;10(3).
17. Maciel CG, Walker C, Muniz MFB, Araújo MM. Antagonism of *Trichoderma* spp. and *Bacillus subtilis* (UFV3918) to *Fusarium sambucinum* in *Pinus elliottii* Engelm. Revista árvore. 2014;38(3):505-512.
18. Zhao Y, Selvaraj JN, Xing F, Zhou L, Wang Y, Song H, Tan X, Sun L, Sangare L, Folly YME, Liu Y. Antagonistic action of *Bacillus subtilis* strain SG6 on *Fusarium graminearum*. PloS One. 2014;9(3): e92486.
19. Alfenas AC, Mafia RG. Métodos em Fitopatologia. Viçosa: UFV. 2016;382.
20. Brazil. Ministério da Agricultura Pecuária e Abastecimento. Regras para análise de sementes. Secretaria Nacional de Defesa Agropecuária. Brasília: MAPA/ACS. 2009;395.
21. Seifert K, Morgan-Jones G, Gams W, Kendrick B. The genera of Hyphomycetes. CBS-KNAW Fungal Biodiversity Centre, Utrecht. 2011;866.
22. Maguire JD. Speed of germination aid in selection and evaluation of seedling emergence and vigor. Crop Science. 1962;2:176-177.
23. Ferreira DF. Programa computacional Sisvar – UFLA, 5.4; 2010.
24. Wickens GM. Methods for detection and selection of heritable resistance to *Fusarium* wilt of cotton. Emp Cotton Grow Rev. 1964;41:172-193.
25. Becerra Lopez-Lavalle LA, Potter N, Brubaker CL. Development of a rapid, accurate glasshouse bioassay for assessing *Fusarium* wilt disease responses in cultivated *Gossypium* species. Plant Pathology. 2012;61:1112-1120.
26. Moura AB, Ludwig J, Santos AG, Schafer JT, Soares VN, Corrêa BO. Biocontrol and seed transmission of *Bipolaris oryzae* and *Gerlachia oryzae* torice seedlings. Journal of Seed Science. 2014;36(4):407-412.
27. Martini LB, Ethur LZ, Dorneles KR. Influence of secondary metabolites of *Trichoderma* spp. development of fungi

transmitted by seeds and germination of Rice. Ciência e Natureza. 2014;36:86–91.

28. Silva VN, Guzzo SD, Lucon CMM, Harakava R. Growth promotion and resistance induction against anthracnose in cucumber using *Trichoderma* spp. Pesquisa Agropecuária Brasileira. 2011; 46(12):1609-1618.
29. Ludwig J, Moura AB, Santos AS, Ribeiro AS. Seed microbiolization for the control of rice brown spot and leaf scald. Tropical Plant Pathology. 2009;34(5):322-328.
30. Jung WJ, Mabood F, Souleimanov A, Zhou XM, Jaoua S, Kamoun F, Smith DL. Stability and antibacterial activity of bacteriocins produced by *Bacillus thuringiensis* and *Bacillus thuringiensis* kurstaki. Journal Microbiol Biotechnol. 2008;18(11):1836-1840.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/48207