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CROP PROTECTION

Creole bean seeds microbiolization with doses of *Trichoderma harzianum*

Laís Dalzotto[1] Brenda Tortelli[2] Francine Spitza Stefanski[3] Deivid Sacon[4] Vanessa Neumann Silva[5] Paola Mendes Milanesi[3*]

[1]Cooperativa de Produção e Consumo Familiar Nossa Terra, Erechim, RS, Brasil.
[2]Programa de Pós-graduação em Agronomia (PPGAgro), Bolsista Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PROSUC/CAPES), Universidade de Passo Fundo (UPF), Passo Fundo, RS, Brasil.
[3]Universidade Federal da Fronteira Sul (UFFS), Campus Erechim. Laboratório de Fitopatologia, 99700-970, Erechim, RS, Brasil. E-mail: paola.milanesi@uffs.edu.br. *Corresponding author.
[4]Programa de Pós-graduação em Fitopatologia, Mestrando, Bolsista Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Universidade Federal de Viçosa (UFV), Viçosa, MG, Brasil.
[5]Universidade Federal da Fronteira Sul (UFFS), Campus Chapecó, Laboratório de Sementes e Grãos, Chapecó, SC, Brasil.

ABSTRACT: *In the search for improved yields, seed treatment by microbiolization has been used as an alternative to chemical treatment. The objective was to verify the physiological and sanitary quality of creole bean seeds, var. Chumbinho, after microbiolization with doses of a commercial product (c.p.) with Trichoderma harzianum (strain ESALQ-1306). The treatments were: T1) 100 mL c.p./100 kg seeds; T2) 150 mL c.p.; T3) 200 mL c.p.; T4) 200 mL of chemical treatment (c.p., 250 g L^{-1} fipronil + 25 g L^{-1} pyraclostrobin + 225 g L^{-1} thiophanate-methyl); and T5) control (without coating of seeds). The tests were: sanitary test (blotter test); germination and first count; accelerated aging, cold germination without soil, speed of germination rate (SGR), seedling shoot and root lengths, and emergence of seedlings in a greenhouse. T. harzianum controlled Aspergillus spp., Penicillium spp. and Fusarium oxysporum. With 100 mL c.p. of T. harzianum dose had better results for the germination and vigor, and this dose it is an alternative to chemical treatment in creole bean seeds.*
Key words: *antagonism, biological control, Phaseolus vulgaris L., vigor.*

Microbiolização de sementes de feijão crioulo com doses de *Trichoderma harzianum*

RESUMO: *Na busca por melhores rendimentos, o tratamento de sementes por microbiolização tem sido utilizado como alternativa ao tratamento químico. Objetivou-se verificar a qualidade fisiológica e sanitária de sementes de feijão crioulo, var. Chumbinho, após microbiolização com doses de um produto comercial (p.c.) com Trichoderma harzianum (cepa ESALQ-1306). Os tratamentos foram: T1) 100 mL p.c./100 kg de sementes; T2) 150 mL p.c.; T3) 200 mL p.c.; T4) 200 mL tratamento químico (p.c., 250 g L^{-1} fipronil + 25 g L^{-1} piraclostrobina + 225 g L^{-1} tiofanato metílico); e T5) controle (sem revestimento de sementes). Os testes foram: sanidade (blotter test), germinação, primeira contagem, envelhecimento acelerado, teste de frio sem solo, índice de velocidade de germinação (IVG), comprimento de parte aérea e de raiz das plântulas e emergência em casa de vegetação. T. harzianum controlou Aspergillus spp., Penicillium spp. e Fusarium oxysporum. A dose 100 mL de p.c. teve melhores resultados para germinação e vigor, sendo que essa dose é uma alternativa ao tratamento químico em sementes de feijão crioulo.*
Palavras-chave: *antagonismo, controle biológico, Phaseolus vulgaris L., vigor.*

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.), belonging to the family Fabaceae, is a common food on the tables of Brazilians, being a source of vegetable protein. In Rio Grande do Sul, Brazil, in 2018/19 crop season, 67.7 thousand tons of common black beans were produced in the first harvest and 27.3 thousand tons in the second (CONAB, 2019).

In the search of high yields, seed treatments are frequently used, aiming to reduce losses caused by pathogens and improving the initial stand of the crop. Microbiolization has been used as an alternative to chemical treatment, consisting of the application of beneficial microorganisms (e.g. *Trichoderma* spp.) to seeds in order to control phytopathogens (MACHADO et al., 2012). This has been used in black oats (BARBIERI et al.,

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2013), beans (CARVALHO et al., 2011) and corn (LUZ, 2001).

Species of the genus *Trichoderma* are free-living fungi that interact with soil, roots and leaves. They are widely used in agricultural crops because of their high reproductive capacity and ability to survive under unfavorable conditions, contributing to the stimulation of defense mechanisms against pathogenic fungi (HARMAN, 2000).

The use of biological control agents, such as the fungus *Trichoderma harzianum*, is one of the alternatives for seed treatment, aiming greater sustainability in agriculture (XU et al., 2011). Although, this fungus is widely used in seed treatment, little is known about the possible interactions between *Trichoderma* and the early stages of seed germination (MASTOURI et al., 2010), as well as the dosage to be applied, according to antagonist does not impair seed germination and vigor.

SINGH et al. (2016) proposed that doses of *Trichoderma asperellum* (BHUT8) ranged from 10^2 to 10^8 spores mL^{-1} in the treatment of vegetable seeds. However, the authors reported that depending on the culture, there is a more assertive dose. When it comes to beans, as well as creole varieties of this crop, information about dose of *Trichoderma harzianum* its still quite scarce.

In this context, the objective of this study was to verify the physiological and sanitary quality of creole bean seeds, var. Chumbinho, after microbiolization with doses of a commercial product (c.p.) with *Trichoderma harzianum* (strain ESALQ-1306).

MATERIALS AND METHODS

The research was carried out in Erechim (*27° 37'50 "S, 52° 14'11" W*; 753 m above sea level), Rio Grande do Sul, Brazil. *Phaseolus vulgaris* beans of the "Chumbinho" creole variety, belonging to the black group, with a life-cycle of approximately 90 days were used. Seeds were obtained from a family estate.

At the beginning of their storage, bean seeds were characterized, showing 14.2% humidity and an electrical conductivity of 93.6 $\mu S\ cm^{-1}g^{-1}$. At the end of the study, the same seeds had 13.4% moisture and an electrical conductivity of 88.1 $\mu S\ cm^{-1}\ g^{-1}$.

The treatments evaluated were: T1) 100 mL c.p. containing *Trichoderma harzianum* (strain ESALQ-1306) at 2.0 x 10^9 viable conidia mL^{-1}/100 kg seeds; T2) 150 mL c.p./100 kg seeds; T3) 200 mL c.p./100 kg seeds; T4) 200 mL chemical treatment (250 g L^{-1} fipronil + 25 g L^{-1} pyraclostrobin + 225 g L^{-1} thiophanate-methyl)/100 kg seeds; and T5) control (without coating of seeds). The doses in T3 and T4 followed the recommendations of the manufacturers. The doses in T1 and T2 were below that recommended by the manufacturer. To evaluate these treatments, tests were performed in duplicate and in a randomized design:

1) Sanitary test (blotter test): eight replicates of 25 seeds were placed in plastic germination boxes – gerbox (11 x 11 x 3.5 cm) containing two sheets of sterilized and moistened germitest paper. The boxes were placed in an incubator at 20 ± 2 °C for 7 days, with a 12 hour photoperiod, when the fungi were identified (BRASIL, 2009a).

2) Germination test: for each treatment, 200 seeds were placed on sterilized and moistened germitest paper, and kept in an incubator chamber at 25 ± 1 °C with a 12 hour photoperiod. First and second counts of germinated seeds were performed at five and nine days after the start of incubation, respectively (BRASIL, 2009b).

3) Soilless cold test: seedless seedlings were incubated at 10 ± 2 °C for three days, without light. Afterwards, they were placed in an incubator at 25 ± 1 °C, with a 12 hour photoperiod for four days, at which point, normal seedlings were counted (BARROS et al., 1999).

4) Accelerated aging test: for each treatment, 200 seeds were distributed on aluminum screens, suspended in boxes containing 40 mL distilled water. The gerbox were kept in an incubator chamber for 72 hours at 42 ± 1 ºC (MARCOS FILHO et al., 1987), and then the germination test was carried out as described above (BRASIL, 2009b).

5) Speed of germination rate (SGR): for each treatment, 200 seeds were distributed on sterilized and moistened germitest paper. Samples were incubated at 25 ± 1 °C, with a 12 hour photoperiod, and germinated seeds were evaluated daily by counting normal seedlings until the fifth day, along with the germination test (MAGUIRE, 1962).

6) Shoot and root seedling length: four replicates of 20 seeds were seeded on the upper third of a sheet of germitest paper. Samples were kept in an incubator chamber at 25 ± 1 °C, with a 12 hour photoperiod and, on day 5, the shoots and roots of 10 normal seedlings per replicate were measured. The measurements were made with a ruler graduated in millimeters, mm (NAKAGAWA, 1999).

7) Emergence in a greenhouse: four replicates of 50 seeds were sown in 128 well trays filled with autoclaved commercial substrate (Dacko™). The trays were kept in a greenhouse, with

two daily irrigations by micro sprinkler system of 2 min and 5-10 min each, one at 10:00 hours and the other at 17:00 hours. A seedling count was performed on the tenth day after the start of the test (SENA et al., 2017).

The data obtained were submitted to analysis of variance (ANOVA) using the F test ($P \leq 0.05$) and, when significant, Tukey's test ($P \leq 0.05$). The analyses were performed with ASSISTAT, beta version 7.7 statistical software (SILVA & AZEVEDO, 2016).

RESULTS AND DISCUSSION

In the sanitary evaluation of bean seeds (Table 1), all treatments grew the grain storage fungi *Aspergillus* spp. and *Penicillium* spp. *Trichoderma harzianum*, derived from the commercial product used to treat the seeds, also grew, though it was less evident in the chemical treatment. *Fusarium oxysporum*, and *Macrophomina phaseolina*, etiological agents of wilt and stem rot in common bean (WENDLAND et al., 2016), were also identified in the samples.

Treatments with *Trichoderma harzianum* (100, 150 and 200 mL) and the chemical treatment did not differ statistically from the control, though they presented with a lower incidence of *Aspergillus* spp. and *Macrophomina phaseolina* on the creole bean seeds (Table 1), or because the isolate of *Trichoderma* spp. probably there was no antagonistic control of this fungi (MIGLIORINI et al., 2012).

Evaluating *Trichoderma* isolates as biological agents for the control of *M. phaseolina* in soybean, KHALILI et al. (2016) observed an incidence of only 5.5% after seed treatment, while that of the control reached 49%. This response might be due to the release of volatile compounds capable of inhibiting phytopathogenic growth. These authors also highlighted the importance of selection by isolates of *Trichoderma* spp. obtained in the locality or region where *M. phaseolina* occurs, because taking into consideration the evolution of the antagonist, such isolates would be more competent in relation to the phytopathogens present in a given location.

All doses of *T. harzianum* significantly reduced the incidence of *Fusarium* spp., Similar to the chemical fungicide (Table 1). However, this efficiency varies according to the isolate of *Trichoderma* spp. and the bean cultivar (CARVALHO et al., 2011), and also depends on the plant species to which the antagonist will apply (MIGLIORINI et al., 2012).

ZHANG et al. (2017) reported that soybean plants using *Trichoderma* to control rot caused by *F. oxysporum* had increased O_2 and H_2O_2 levels compared to control plants. These results suggested that accumulation of reactive oxygen species (ROS) could be one of the mechanisms by which *Trichoderma*-treated soybeans induce resistance to plant diseases.

For seed germination, 100 mL c.p./100 kg seeds showed higher levels (84%) than 200 mL c.p./100 kg seeds (74%), being 4% higher than the germination obtained in the control (80%) (Table 2). The use of *Trichoderma* spp. can promote germination of seeds by the production of hormones. According to BUCIO et al. (2015), *T. harzianum* produces harzianic acid and isoharzianic acid, which promote plant growth.

Conversely, excessive production of indole acetic acid (IAA), ethylene (TAIZ & ZEIGER, 2009), auxins and cytokinins hormones (BROTMAN et al., 2010) inhibit cell division and elongation, impairing germination and the development of seedling. In this

Table 1 - Incidence (%) of fungi *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp., *Fusarium oxysporum* and *Macrophomina phaseolina* in seeds of *P. vulgaris* var. Chumbinho, treated with doses of the commercial product based on *Trichoderma harzianum* and with fungicide.

Treatment	----------Incidence (%)----------				
	Aspergillus spp.	*Penicillium* spp.	*Fusarium oxysporum*	*Trichoderma* spp.	*Macrophomina phaseolina*
100 mL c.p.[1]	0.06[ns]	0.18 a[3]	0.56 a	11.68 c	0.12[ns]
150 mL c.p.	0.12	0.06 a	0.18 a	12.43 c	0.06
200 mL c.p.	0.25	0.12 a	0.31 a	11.56 c	0.06
Fungicide[2]	0.12	0.12 a	1.00 a	0.18 a	0.06
Control	0.50	1.93 b	1.68 b	1.62 b	0.00
CV (%)[4]	17.95	11.22	17.44	7.18	7.56

[1]Based on *T. harzianum* (strain ESALQ-1306) at 2.0 x 10^9 viable conidia mL^{-1}; dose for 100 kg seeds; [2]250 g L^{-1} fipronil + 25 g L^{-1} pyraclostrobin + 225 g L^{-1} thiophanate-methyl/100 kg seeds; [3]Means followed by the same letter do not differ by Tukey's test ($P \leq 0.05$); transformed data [(x + 1) ^ 0.5]. [4]Coefficient of variation. [ns]not significant.

Table 2 - Percentages of germination, first count, cold test, accelerated aging and speed of germination rate of seeds of *P. vulgaris* var. Chumbinho treated with doses of the commercial product based on *Trichoderma harzianum* and with fungicide.

Treatment	Germination	First count	Cold test	Accelerated aging	Speed of germination rate
	----------(%)----------				
100 mL c.p.[1]	84 a[3]	78 a	69 b	73 a	10.6 a
150 mL c.p.	78 ab	56 b	80 a	66 ab	8.5 ab
200 mL c.p.	74 b	58 b	67 b	57 b	8.2 b
Fungicide[2]	77 ab	57 b	74 ab	66 ab	8.1 b
Control	80 ab	63 ab	73 ab	71 a	8.5 ab
CV (%)[4]	6.3	19.6	9.0	13.9	16.3

[1]Based on *T. harzianum* (strain ESALQ-1306) at 2.0 x 10^9 viable conidia mL^{-1}; dose for 100 kg seeds; [2]250 g L^{-1} fipronil + 25 g L^{-1} pyraclostrobin + 225 g L^{-1} thiophanate-methyl/100 kg seeds; [3]Means followed by the same letter do not differ by Tukey's test ($P \leq 0.05$); transformed data [(x + 1) ^ 0.5]. [4]Coefficient of variation. [ns]not significant.

context, REIS et al. (2019) reported that the dose of *Trichoderma* spp. on cowpea seeds treatment were effective up to 4.8 x 10^8 CFU g^{-1} ensuring better seed germination and root development.

Treatment with 100 mL c.p./100 kg seeds resulted in a first germination count of 78%, a 15% increase over the control treatment (63%), though not statistically different (Table 2). In the first count, treatments with 150 and 200 mL c.p./100 kg seeds and the chemical treatment caused a reduction in normal seedlings of 56%, 58% and 57%, respectively.

In the cold test, treatment with 150 mL c.p./100 kg seeds resulted in the highest germination percentage (80%), though it did not differ statistically from the chemical (74%) and control treatments (73%) (Table 2). Treatments of 100 and 200 mL c.p./100 kg seeds produced 69% and 67% germination, respectively, demonstrating that these doses were harmful to seed germination in the cold test.

According to BARROS et al. (1999), it is normal for germination results in soil-free cold tests to be similar to those of the standard germination test, which occurred in the current study with the treatment 150 mL c.p./100 kg seeds and the control (without coating).

In the accelerated aging test (Table 2), treatment with 100 mL c.p./100 kg seeds and the control had the highest germination levels of 73% and 71%, respectively, compared to 57% for the 200 mL c.p./100 kg seeds dose. SINGH et al. (2014) reported that the incubation temperature of several fungal isolates, such as *Fusarium* sp., *Penicillium* sp. and *Trichoderma* was 30 °C, demonstrating that none of the *Trichoderma* species grew above 40 °C. This might explain why increased temperatures did not benefit the vigor of the seeds.

In the speed of germination rate test (Table 2), treatment with 100 mL c.p./100 kg seed showed a better result (10.6) than with 200 mL c.p./100 kg seeds (8.2) and the chemical treatment (8.1). An increase in germination speed is an advantage in grain production, because it reduces the time needed to establish a crop, giving it a competitive advantage against other plants, which can reduce productivity by 60-70% (SALGADO et al., 2007).

With regard to seedling length, treatment with 150 mL c.p./100 kg seeds was superior to 200 mL c.p./100 kg seeds and the chemical treatment (Table 3). In addition, treatments with *T. harzianum* at different doses showed a longer root length than the chemical treatment, though not differing from the control (without coating).

The chemical treatment resulted in both lower shoot and root lengths. For greenhouse plant emergence, treatment with 200 mL c.p./100 kg seeds showed a higher level of emerged seedlings (83.3%) than the chemical treatment (62.7%; Table 3).

The performance of *Trichoderma* spp. for biological control of diseases can be quite variable, depending on the species used and the edaphoclimatic conditions under which the tests are conducted. According to the current study and that of ZHANG et al. (2017), plants colonized by *Trichoderma* showed a great capacity for combatting attacks by pathogenic fungi, improving seed potential.

CONCLUSION

The dose of 100 mL c.p. of *Trichoderma harzianum* (strain ESALQ-1306 at 2.0 x 10^9 viable conidia mL^{-1}) per 100 kg seeds reduces the incidence of *Penicillium* spp. and *Fusarium oxysporum*,

Table 3 - Length of aerial part (cm), root length (cm) and seedling emergence (%), of seeds of *P. vulgaris* var. Chumbinho treated with doses of the commercial product based on *Trichoderma harzianum* and with fungicide.

Treatment	Length of aerial part	Root length	Seedling emergence (%)
	----------(cm)----------		
100 mL c.p.[1]	9.18 ab[3]	16.85 a	80.7 ab
150 mL c.p.	10.32 a	16.98 a	79.3 ab
200 mL c.p.	9.95 ab	16.55 a	83.3 a
Fungicide[2]	8.95 b	14.88 b	62.7 b
Control	9.08 b	15.78 ab	65.3 ab
CV (%)[4]	8.67	5.99	9.22

[1]Based on *T. harzianum* (strain ESALQ-1306) at 2.0 x 10^9 viable conidia mL^{-1}; dose for 100 kg seeds; [2]250 g L^{-1} fipronil + 25 g L^{-1} pyraclostrobin + 225 g L^{-1} thiophanate-methyl/100 kg seeds; [3]Means followed by the same letter do not differ by Tukey's test ($P \leq 0.05$); [4]Coefficient of variation. [ns]not significant.

improving the sanitary aspect. This dose does not affect the physiological quality of the creole bean seeds, var. Chumbinho.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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