Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga

ISSN 0100-316X (impresso)
ISSN 1983-2125 (online)
http://dx.doi.org/10.1590/1983-21252021v34n408rc

SEED TREATMENT WITH *TRICHODERMA* AND CHEMICALS TO IMPROVE PHYSIOLOGICAL AND SANITARY QUALITY OF WHEAT CULTIVARS[1]

ANA PAULA SILVA COUTO[2]*, ALANA EMANOELE PEREIRA[3], JULIA ABATI[2], MAIRA LAÍZA CAMARGO FONTANELA[4], CLAUDIA REGINA DIAS-ARIEIRA[4], NÁDIA GRACIELE KROHN[5]

ABSTRACT – Seed treatment with fungi of the genus *Trichoderma* spp. provides several benefits, including plant growth promotion, stress tolerance, and pathogenic fungi control. Moreover, to avoid inadequate doses and unnecessary costs, these treatments must be applied in proper amounts. However, no study has evaluated their applicability in wheat seeds. This study aimed to determine the most efficient dose of *Trichoderma*-based products applied as a seed treatment for improving the physiological and sanitary quality of the wheat cultivars TBIO ‘Toruk’ and TBIO ‘Sossego’, besides comparing the performance of biological and chemical agents. Two biological treatments (*Trichoderma asperellum* SF 04 and *Trichoderma harzianum* IBLF006) were applied at 0 (control), 5×10^{11}, 1×10^{12}, 1.5×10^{12}, and 2×10^{12} colony-forming units (CFU) 100 kg^{-1} seed. Two chemical treatments (carboxin + thiram and pyraclostrobin + thiophanate-methyl + fipronil) were applied at the manufacturers’ recommended doses. Seed germination, shoot and root lengths, seedling dry matter, and sanitary quality were analyzed under laboratory conditions, while seedling emergence, shoot length, and shoot dry matter were analyzed under greenhouse conditions. The optimal dose for wheat seed treatment with *T. asperellum* SF 04 and *T. harzianum* IBLF006 was 2×10^{12} CFU 100 kg^{-1} seed. When comparing biological and chemical products, our findings indicate that both options are adequate for managing wheat diseases and providing seedling growth via seed treatment.

Keywords: *Triticum aestivum.* Biological control. Microbiolization. Fungicides.

TRATAMENTO DE SEMENTES COM *TRICODERMA* E QUÍMICOS PARA MELHORIA DA QUALIDADE FISIOLÓGICA E SANITÁRIA DE CULTIVARES DE TRIGO

RESUMO – O tratamento de sementes com fungos do gênero *Trichoderma* spp. proporciona diversos benefícios, incluindo promoção de crescimento das plantas, tolerância a estresses e controle de fungos patogênicos. Entretanto, para evitar o uso de doses inadequadas e custos desnecessários, é fundamental a utilização destes na quantidade correta, todavia não há estudos que avaliem a sua aplicabilidade em sementes de trigo. Objetivou-se encontrar a dose mais eficiente de produtos a base de *Trichoderma* no tratamento de sementes para melhorar a qualidade fisiológica e sanitária das cultivares de trigo TBIO 'Toruk' e TBIO 'Sossego', além de comparar o desempenho de agentes biológicos e químicos. Os tratamentos biológicos utilizados foram: *Trichoderma asperellum* SF 04 e *Trichoderma harzianum* IBLF006, aplicados nas doses de zero (testemunha); $5x10^{11}$; $1x10^{12}$; $1,5x10^{12}$ e $2x10^{12}$ UFC (unidades formadoras de colônias) 100 kg^{-1} de sementes, e os químicos foram: carboxina + tiram e piraclostrobina + tiofanato metílico + fipronil. Em laboratório, conduziu-se testes de germinação, comprimento e massa de matéria seca de plântulas, *blotter test* e, em casa de vegetação, emergência, comprimento e massa de matéria seca da parte aérea de plântulas. Os tratamentos biológicos *T. asperellum* SF 04 e *T. harzianum* IBLF006 apresentam melhor eficiência para aplicação no tratamento de sementes de trigo na dose $2x10^{12}$ UFC 100 kg^{-1} de sementes. Comparando os produtos biológicos com os químicos, os dados indicam que ambas as opções têm potencial para serem utilizadas no manejo de doenças do trigo, além de promoverem o crescimento de plântulas, por meio do tratamento de sementes.

Palavras-chave: *Triticum aestivum*. Controle biológico. Microbiolização. Fungicidas.

____

*Corresponding author
[1]Received for publication in 10/21/2020; accepted in 07/06/2021.
Paper extracted from the masters dissertation of the first author.
[2]Department of Agronomy, Center for Agricultural Sciences, Universidade Estadual de Londrina, Londrina, PR, Brazil; anapaula_couto@live.com - ORCID: 0000-0001-9754-8555, juliaabati@yahoo.com.br - ORCID: 0000-0002-6432-9126.
[3]Graduate Program in Agronomy, Universidade Estadual Paulista ‘Júlio de Mesquita Filho’, Botucatu, SP, Brazil; alana_emanoele@hotmail.com - ORCID: 0000-0002-2946-6651.
[4]Department of Agronomy, Center for Agricultural Sciences, Universidade Estadual de Maringá, Umuarama, PR, Brazil; mairafontanela@yahoo.com.br - ORCID: 0000-0002-9260-9934, crdarieira@uem.br - ORCID: 0000-0002-1567-816X.
[5]Agronomy Course, Universidade Tecnológica Federal do Paraná, Santa Helena, Paraná, Brazil; ngkrohn@utfpr.edu.br - ORCID: 0000-0001-9933-4163.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major cereal crops grown in Brazil. However, national yields are low, requiring significant import volumes to meet the domestic market demands (CONAB, 2021). Therefore, off-the-shelf management techniques should be used, as best as possible, to increase levels of wheat grain yield.

Among the techniques, seed treatment acts as an important tool in preserving seed quality. However, seeds themselves act as long-distance spreading mechanisms for pathogens. Therefore, wheat seed treatment with fungicides helps control causative agents of leaf spot, seed deterioration, common root rot, loose smut, and powdery mildew. Among the fungi transmitted via wheat seeds in the field are those of the genera *Bipolaris* and *Drechslera*, as well as *Fusarium* species and *Ustilago tritici* (KUHNEM et al., 2020). In short, fungi can cause several losses when present in seeds, including rotting, necrosis, and discoloration, as well as reducing seed vigor and germination (PESKE; ROSENTHAL; ROTA, 2012). Therefore, seed treatment helps maintain seedling stands and potential yields of wheat crops (FREIBERG et al., 2017).

Over recent years, demand for biological products has intensified, contributing to more sustainable cultivation systems with low dependence on chemical products. Off-the-shelf management techniques seed treatments for biological products include those based on fungi such as fungi *T. asperellum* and *T. harzianum* (ADAPAR, 2021).

Fungi of the genus *Trichoderma* can reduce the incidence of several pathogenic fungi (AGÜERO et al., 2008; CARVALHO et al., 2011; PRABHAKARAN et al., 2015). Moreover, the interaction of these fungi with seeds promotes increases in germination speed and rate, shoot and root lengths, seedling dry weights, and tolerance to physiological, saline, osmotic, and water stresses (MASTOURI; BJÖRKMAN; HARMAN, 2010). Added together, these factors contribute to increasing crop yields (HASAN et al., 2012; HERMOSA et al., 2012; CHAGAS et al., 2017).

In this sense, to avoid inadequate doses and unnecessary costs, those fungi must be properly managed so that good results can be achieved. Studies have indicated that optimal doses do not depend on seed size but crop type (SINGH et al., 2016), strain efficiency, and product concentration range (RAMÍREZ; RAMELLI; REYNALDI, 2013).

Based on the above, this study aimed to assess the effects of *Trichoderma*-based seed treatments on the physiological and sanitary quality of two wheat cultivars, determine their optimal doses, and compare the efficiency of biological and chemical treatments.

MATERIAL AND METHODS

Two experiments were conducted at the Laboratory of Seed Production and Technology of the State University of Maringá (UEM), in Umuarama city, Paraná State (Brazil). Treatments were arranged in a fully randomized design, with four replications and seed numbers according to the test used.

Seeds: cultivars, storage, and treatment

Two wheat cultivars were used, namely TBIO Toruk and TBIO Sossego (hereafter referred to as ‘Toruk’ and ‘Sossego’, respectively). Seeds were obtained from seed-producing companies after being harvested in October 2018. The seeds were stored at 4 °C, before the beginning of the experiment in March 2019.

Treatment solution volumes for both biological and chemical treatments were 1000 mL 100 kg^{-1} seed, which was completed with distilled water when needed. Solution and seeds were placed in plastic bags, which then underwent vigorous stirring for homogenization of solutions on seeds. Afterwards, the seeds were left to dry at room temperature (25 °C) for 1 h.

Optimal doses for *Trichoderma*-based wheat seed treatments

Experiment I was designed to assess the effects of different doses of two *Trichoderma* spp.-based biological products on the physiological and sanitary quality of wheat seeds. The biological treatments were *T. asperellum* SF 04 (Quality®) and *T. harzianum* IBLF006 (Ecotrich®) at 0 (control), 5×10^{11}, 1×10^{12}, 1.5×10^{12}, and 2×10^{12} colony-forming units (CFUs) 100 kg^{-1} seed.

The doses used in this study followed the recommendations for seed treatment medium of cotton, common beans, and soybeans, as well as the package leaflets of both biological products used. Given the joint analysis of all significant data, the best dose for each biological treatment was the one that covered the largest number of benefits, according to analyses of physiological and sanitary quality of wheat seed, whose methodologies for both cultivars will be described below.

Comparing biological and chemical treatments efficiency

Experiment II was performed to compare statistically the efficiency of optimal doses of the biological agents (determined in Experiment I) to that of the two chemical agents, carboxin + thiram (Vitavax-Thiram® 200 SC, 300 mL 100 kg^{-1} seed) and pyraclostrobin + thiophanate-methyl + fipronil (Standak Top®, 200 mL 100 kg^{-1} seed). Chemical treatments were applied at the manufacturers' recommended doses and were evaluated for physiological and sanitary quality as described below.

Evaluations

Physiological and sanitary quality of wheat cultivars under laboratory conditions

Seed sanitary quality was evaluated by the Blotter test using four 50-seed repetitions per treatment (BRASIL, 2009a). The seeds were distributed on three sheets of special paper towels for germination placed inside clear acrylic boxes. These papers were moistened with saline solution (-0.6 MPa NaCl), at a ratio of 2.5 times the dry paperweight, to prevent seeds from germinating and facilitate microbial evaluation (FARIAS et al., 2003). The boxes were kept in a germination chamber at 20 ± 2 °C and 12-h photoperiod for eight days. Soon after, the seeds were examined under a stereomicroscope at 4 and 10 x magnifications for fungal fruiting bodies, according to Brasil (2009a). The results were expressed as a percentage of fungal structures.

As for the germination test, four 50-seed repetitions per treatment were uniformly distributed on three sheets of special paper towels for germination (two at the bottom and one on the top) and moistened with water at a ratio of 2.5 times the dry paperweight. The sheets were rolled and placed in a germination chamber at 20 °C and 12-h photoperiod. Seed vigor was assessed by counting geminated seeds on the first day (4 days after incubation). The percentages of germinated seeds (normal seedlings), abnormal seedlings, and dead seeds were determined 8 days after incubation (BRASIL, 2009b).

The total length of seedlings was determined on four 10-seed replicates for each treatment. Seeds were sown in a row on the upper third of moistened paper towel sheets. The sheets were rolled vertically and incubated for 8 days in a germination chamber at 25 °C and 12-h photoperiod. Afterwards, shoot and root lengths were measured. After the removal of reserve tissues, the seedlings were dried in an air-circulating oven at 65 °C for 48 h. Seedling dry matter was determined by dividing the sample dry matter by the number of normal seedlings (adapted from KRZYZANOWSKI et al., 2020).

Physiological quality of wheat cultivars under greenhouse conditions

Seedling emergence was recorded 8 days after sowing in plants grown under greenhouse conditions. Each treatment comprised four 50-seed repetitions. The seeds were sown at a depth of 3 to 4 cm in plastic trays placed 30 cm apart from each other. The substrate consisted of sandy dystrophic Red Latosol (SANTOS et al., 2018), previously sieved through 1.18-mm mesh (no. 14) sieves. The trays were irrigated every other day. 'Toruk' plants were cultivated in the second half of March 2019, while 'Sossego' plants in the first half of April 2019.

At 15 days after sowing, shoot lengths of seedlings grown in trays were measured with a millimeter ruler. As for shoot dry matter, the seedlings were harvested, and shoots were separated from roots and reserve tissues to be dried in an air-circulating oven at 65 °C for 48 h. The shoot dry matter was calculated by dividing the sample matter by the number of normal seedlings.

Statistical analysis

Data from both experiments were subjected to analysis of variance by the *F*-test. Dose-response data from Experiment I were subjected to regression analysis, using quadratic, linear and non-linear models (Brain - Cousins' and inverse quadratic). The most efficient dose of each biological product was determined based on their combined responses. In Experiment II, the best dose of each biological product was compared with those of chemicals by Tukey's test at $p < 0.05$. Both SISVAR version 5.3 (FERREIRA, 2011) and R software (R CORE TEAM, 2019) were used for data analysis.

RESULTS AND DISCUSSION

Optimal doses for *Trichoderma*-based wheat seed treatments

For physiological quality analysis under laboratory and greenhouse conditions, treatment with *T. asperellum* SF 04 from 'Toruk' influenced only root length (Figure 1 A). Yet, root growth was inhibited at the three lowest doses compared with the control. However, doses of 8.8×10^{11} CFU (minimal effective dose) and above increased root length. Beneficial effects were observed at doses of 1.76×10^{12} CFU or higher. Root length means were non-significant for 'Sossego' seeds treated with *T. asperellum* SF 04 (Figure 1 C), and for 'Toruk' (Figure 1 B) and 'Sossego' seeds treated with *T. harzianum* IBLF006 (Figures 1 D).

A first hypothesis would be an endophytic interaction between *Trichoderma* and plant roots. According to Hardoim et al. (2015), endophytic organisms can have negative effects on plants grown under unfavorable or stressful conditions. Therefore, in our study, the plants grown on special paper towels under laboratory conditions and treated with fungi may have undergone stress. This led to competition for space and nutrients between seeds and fungi, reducing root growth. In our study, since the test was carried out on substrate paper, no nutrients were available for *Trichoderma* during the establishment of an endophytic relationship, so the fungi may have consumed seedlings or seed reserves.

A second hypothesis for such a result is that plants express resistance in response to interactions with other organisms. This can be induced by a variety of microorganisms, including mycorrhizal individuals and biological nitrogen fixers. Therefore, negative effects were initially caused in response to inducers. After the interaction, this induction can reduce the biosynthesis of proteins required for the metabolism and growth of plants, hence reducing their development. However, more recent studies are still required to explain such a fact (HEIL; BALDWIN, 2002).

Within this context, in addition to the mentioned organisms, some authors have reported the systemic resistance induction capacity of *Trichoderma* spp. fungus. For instance, Yoshioka et al. (2011) found that *Trichoderma asperellum* SKT1 promoted systemic resistance induced in *Arabidopsis thaliana* plants against *Pseudomonas syringae* pv. tomato DC3000 pathogen.

In this regard, the effects of lower doses of seed treatment with *T. asperellum* SF 04 on the root growth of seedlings grown on a paper substrate may have been due to an energy imbalance and/or stress due to endophytic fungus and seed interaction. This, therefore, must have resulted in less root growth, as mentioned earlier.

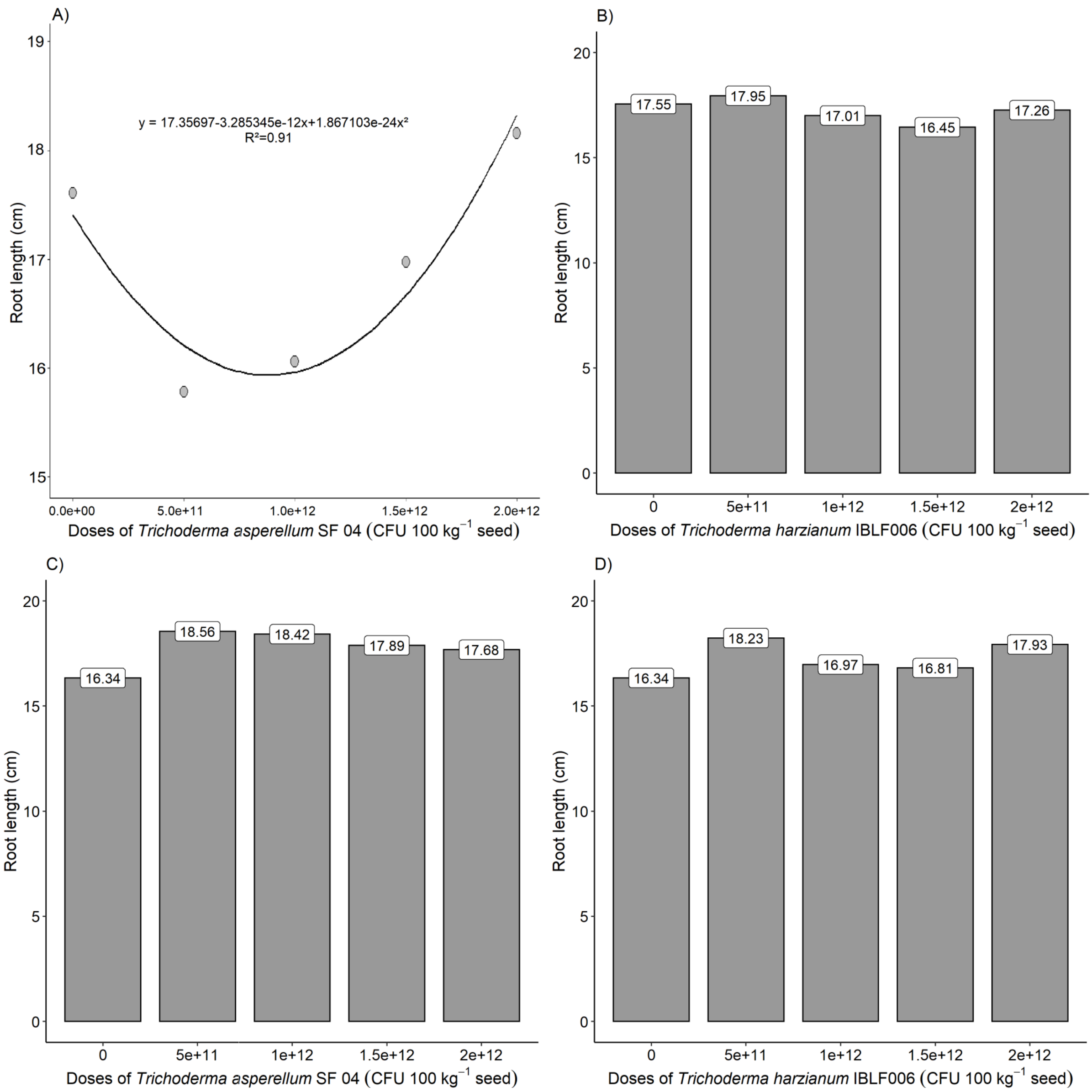

Figure 1. Root length of seedlings of the wheat cultivars ‘Toruk’ (A and B) and ‘Sossego’ (C and D) for seeds treated with *Trichoderma asperellum* SF 04 (A and C) and *Trichoderma harzianum* IBLF006 (B and D). ** Significant at $p < 0.01$ by the F-test.

Doses above 1.76 × 10^{12} CFU of *Trichoderma* had evident positive effects on plant growth. These higher doses of fungi may have stimulated greater production of phyto-growth hormones such as indole-3-acetic acid, as already reported by Carvalho Filho et al. (2008) in *Eucalyptus* seedlings. At higher doses, a greater production of phytohormones may have been enough to compensate for the effects of additional energy expenditure due to the endophytic interaction. Thus, increases in root length due to application of *Trichoderma* via seeds is one of the positive effects that this fungus provides, with results corroborated by Mastouri, Björkman and Harman (2010). These authors found that tomato seeds treated with *T. harzianum* T-22 at 2 × 10^{12} CFU 100 kg^{-1} showed increased root lengths, even under accelerated aging.

In the sanitary quality test, all control seeds were contaminated by pathogenic fungi. *Alternaria* spp., *Bipolaris* spp., and *Fusarium* spp. were found in seeds of both genotypes and their frequency rates are shown in Figure 2 (A to H) for ‘Toruk’ and in Figure 2 (I to P) for ‘Sossego’. *Aspergillus flavus* was found only in ‘Toruk’ seeds (Figure 4).

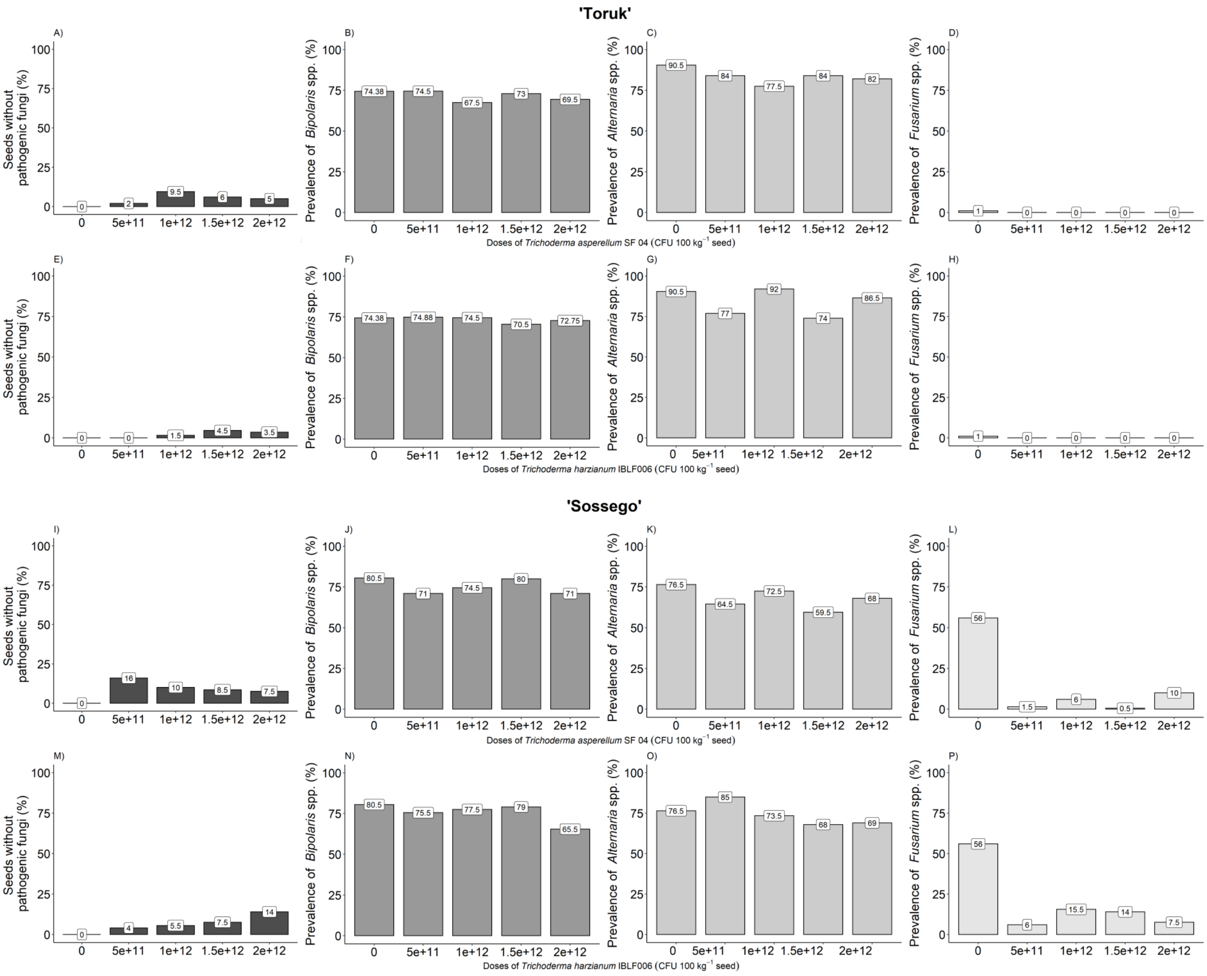

Figure 2. Percentage of seeds of the wheat cultivars ‘Toruk’ (A and H) and ‘Sossego’ (I and P) treated with doses of *Trichoderma asperellum* SF 04 and *Trichoderma harzianum* IBLF006, and evaluated by the Blotter test: Percentage of seeds without pathogenic fungi (A, E, I, and M), and prevalence percentages (%) of *Bipolaris* spp. (B, F, J, and N), *Alternaria* spp. (C, G, K, and O), and *Fusarium* spp. (D, H, L, and P). CFUs, colony-forming units 100 kg^{-1} seed.

T. asperellum SF 04 treatment influenced the percentage of seeds without pathogenic fungi in both cultivars (Figure 3 A and B), whereas *T. harzianum* IBLF006 influenced this parameter only in ‘Sossego’ (Figure 3 C). According to regression analysis, maximum control efficiency of *T. asperellum* SF 04 is obtained at 1.28 x 10^{12} and 1.58 x 10^{11} CFUs for ‘Toruk’ and ‘Sossego’, respectively (Figure 3 A and B). The percentage of seeds without pathogenic fungi increased linearly with *T. harzianum* IBLF006 doses, with the most efficient being 2 × 10^{12} CFU for ‘Sossego’ (Figure 3 C).

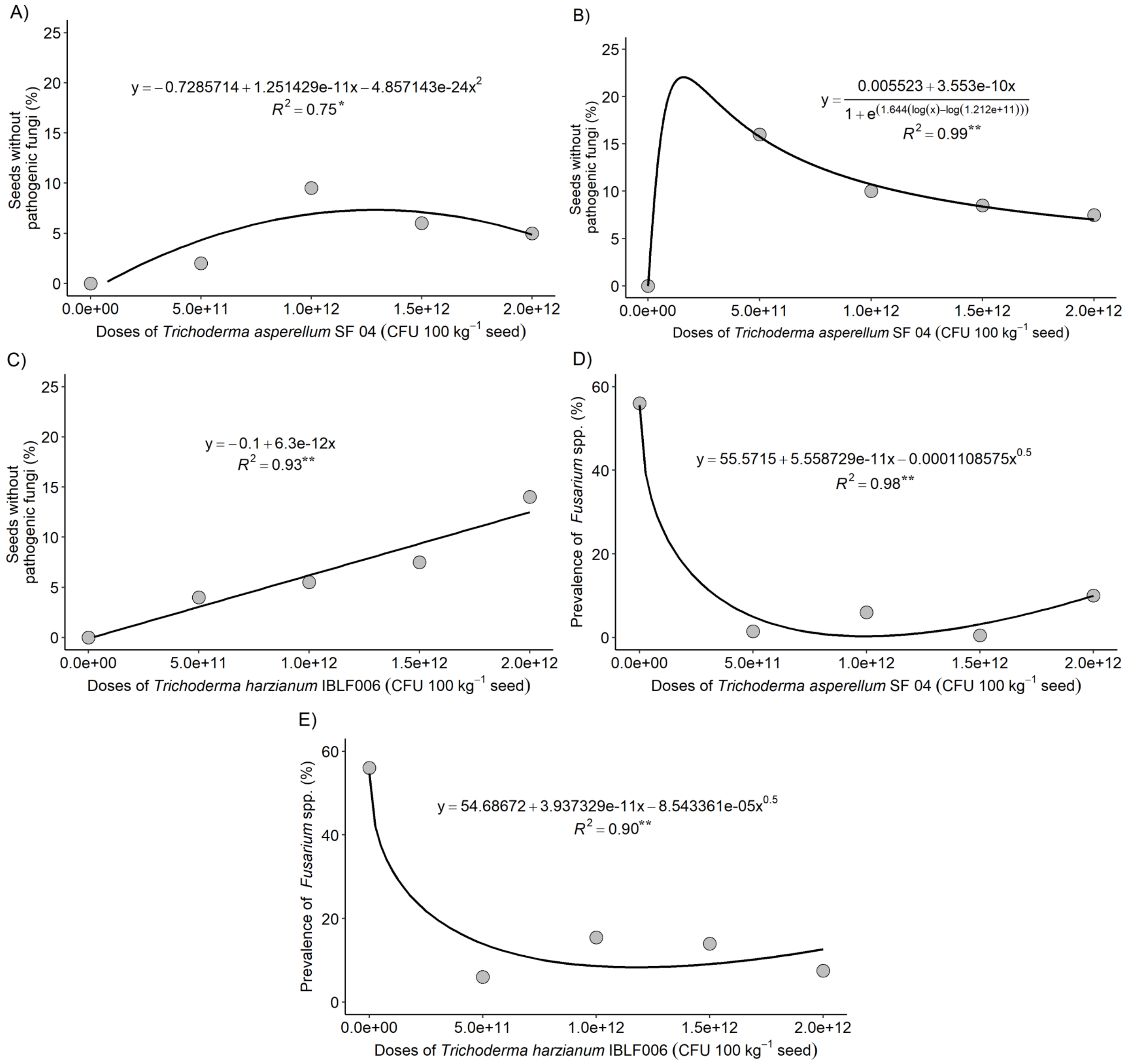

Figure 3. Percentage of seeds of the wheat cultivars ‘Toruk’ (A) and ‘Sossego’ (B, C, D, and E) treated with doses of *Trichoderma asperellum* SF 04 and *Trichoderma harzianum* IBLF006, and evaluated by the Blotter test: percentage of seeds without pathogenic fungi after treatment with *T. asperellum* SF 04, ‘Toruk’ (A) and ‘Sossego’ (B); percentage of seeds without pathogenic fungi after treatment with *T. harzianum* IBLF006, ‘Sossego’ (C); prevalence (%) of *Fusarium* spp. in ‘Sossego’ seeds treated with *T. asperellum* SF 04 (D) and *T. harzianum* IBLF006 (E). *Significant at $p < 0.05$. **Significant at $p < 0.01$ by the F-test.

For Vinale et al. (2009), *in vitro* antibiotic activity of *T. harzianum* strains against phytopathogens depends on their type and viability, produced secondary metabolites, and balance between their productions and biotransformation rates by phytopathogens. According to these authors, fungi can biotransform toxic substances produced by plants, helping them to survive in the environment. Studies have shown that the efficacy of *Trichoderma* to control pathogens may vary with parameter. In our study, both biological treatments were able to reduce the prevalence of phytopathogens in wheat seeds.

Alternaria spp. and *Bipolaris* spp. were detected in seeds treated with biological treatments, but no differences were observed between treatments. *Fusarium* spp. was detected in both cultivars, with differences between treatments but only in ‘Sossego’ seeds. The prevalence of *Fusarium* spp. was higher in ‘Sossego’ seeds of the control treatment than in seeds treated with doses of the biological treatments. Regression analysis revealed that the maximum control of *Fusarium* spp. was achieved using 9.96 x 10^{11} CFU *T. asperellum* SF 04 or 1.17 x 10^{12} CFU *T. harzianum* IBLF006 (Figure 3 D and E).

Trichoderma acts against phytopathogens through different mechanisms. Some reports have shown that *T. harzianum* decreases the *in vitro* mycelial growth of *Fusarium* (HASAN et al., 2012). Furthermore, *Trichoderma* produces antibiotic compounds that inhibit the production of mycotoxins by *Fusarium graminearum* (COONEY; LAUREN; DI MENNA, 2001). Mycotoxins are produced by many fungi, including those of the genera *Fusarium* and *Aspergillus*, and are commonly found in wheat, maize, and rice crops. These chemical compounds are toxic to humans. Plants contaminated by mycotoxigenic fungi often produce small-sized, soft, and wrinkled seeds (MORI et al., 2016). Therefore, the ability of *Trichoderma* to control *Fusarium* spp. in wheat seeds is particularly important.

Aspergillus flavus was detected in seeds of ‘Toruk’ treated with biological treatments, and doses were significantly different from each other (Figure 4). The doses of *T. asperellum* SF 04 treatment from 5×10^{11} CFUs completely reduced *A. flavus* prevalence. For *T. harzianum* IBLF006, the most efficient doses were from 8.4×10^{11} CFUs onwards (Figure 4 A and B).

The decrease in *A. flavus* prevalence may have occurred because of the ability of *Trichoderma* to produce antagonistic volatile compounds. Agüero et al. (2008) found that *T. harzianum* not only reduced *A. flavus* prevalence in maize seeds but also decreased the production of mycotoxins such as aflatoxin B1. In a study by Bagwan (2011), *Trichoderma* spp. reduced the *in vitro* mycelial growth of *A. flavus* and *Aspergillus niger*.

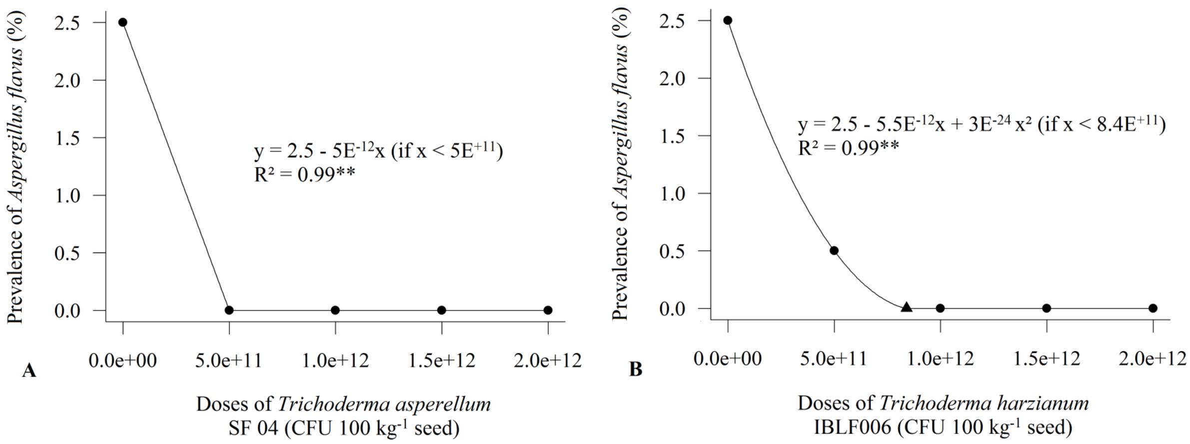

Figure 4. Prevalence (%) of *Aspergillus flavus* in seeds of the wheat cultivar ‘Toruk’ treated with *Trichoderma asperellum* SF 04 (A) and *Trichoderma harzianum* IBLF006 (B), as determined by the Blotter test. CFUs, colony-forming units. **Significant at $p < 0.01$.

In our study, the optimal dose was that which mostly contributed to the physiological and sanitary quality of wheat plants. Therefore, the most efficient dose of *T. asperellum* SF 04 and *T. harzianum* IBLF006 was 2×10^{12} CFUs. The most efficient doses of biological treatments were compared to those of chemical fungicides carboxin + thiram and pyraclostrobin + thiophanate-methyl + fipronil.

Comparing the efficacy of biological and chemical treatments

Under laboratory conditions, no differences in seed vigor (first germination count), germination, or the number of dead seeds were observed between biological and chemical treatments for both ‘Toruk’ and ‘Sossego’ (Table 1). All seeds showed high physiological quality. Germination rates were higher than those recommended for certified seeds (C_1 and C_2) and category S_1 and S_2 seeds, which should be equal to or greater than 80% (MAPA, 2013). The effects of seed treatment on high-quality seeds are less pronounced because of their high physiological quality (CARVALHO; NAKAGAWA, 2012), explaining the lack of positive results found in the present study.

Carboxin + thiram treatment increased the percentage of abnormal ‘Sossego’ seedlings compared to the control, but no differences were observed for the other treatments (Table 1). These results indicate that carboxin + thiram was phytotoxic to ‘Sossego’ seedlings, even if this is recommended for wheat seeds treatments (ADAPAR, 2021). Chemical fungicides may occasionally impair seedling development, depending on plant cultivar, product, and treatment dose, as observed by Marini et al. (2011) and us for wheat seeds treatment with carboxin + thiram.

T. harzianum IBLF006 treatment provided ‘Toruk’ seedlings with increased shoot length compared to pyraclostrobin + thiophanate-methyl + fipronil treatment, but no differences were found among the other treatments (Table 1). In another study, *T. harzianum* T-22 (2×10^{12} CFUs 100 kg^{-1} seed) promoted seedling growth in tomatoes, even when plants were subjected to physiological stress conditions (MASTOURI; BJÖRKMAN; HARMAN, 2010). These effects may be due to the ability of the beneficial fungus to stimulate growth hormone production (CARVALHO FILHO et al., 2008) and control pathogens (CARVALHO et al., 2011).

Table 1. Seed vigor (first germination count), germination percentage, abnormal seedlings, dead seeds, shoot length, root length, and seedling dry matter of the wheat cultivars ‘Toruk’ and ‘Sossego’ with seeds treated with *Trichoderma* spp. and chemical agents and grown under laboratory conditions.

Treatment	Seed vigor (%)		Germination percentage (%)		Abnormal seedling (%)		Dead seed (%)	
	‘Toruk’	‘Sossego’	‘Toruk’	‘Sossego’	‘Toruk’	‘Sossego’	‘Toruk’	‘Sossego’
Control	95	91	96	94	0	2b	4	4
T1	94	89	95	91	1	4ab	4	5
T2	95	84	97	90	0	5ab	3	5
T3	94	86	95	87	0	9 a	5	4
T4	94	91	96	91	1	6ab	3	3
MSD	7	9	6	8	2	6	5	6
F-test	ns	ns	ns	ns	ns	*	ns	ns

Treatment	Shoot length (cm)		Root length (cm)		Seedling dry matter (mg)	
	‘Toruk’	‘Sossego’	‘Toruk’	‘Sossego’	‘Toruk’	‘Sossego’
Control	12.09 ab	11.46	17.55	16.33	21.69	13.57
T1	12.39 ab	11.21	18.09	17.68	20.92	13.81
T2	12.94 a	11.72	17.26	17.92	20.30	13.80
T3	12.09 ab	10.58	17.65	17.18	22.69	12.47
T4	11.54 b	10.57	17.37	17.20	22.20	13.08
MSD	1.17	2.84	2.16	2.72	4.37	4.68
F-test	*	ns	ns	ns	ns	ns

T1, *Trichoderma asperellum* SF 04 (2×10^{12} colony-forming units, CFUs, 100 kg^{-1} seed); T2, *Trichoderma harzianum* IBLF006 (2×10^{12} CFUs 100 kg^{-1} seed); T3, carboxin + thiram (300 mL 100 kg^{-1} seed); T4, pyraclostrobin + thiophanate-methyl + fipronil (200 mL 100 kg^{-1} seed); MSD, minimum significant difference; ns, non-significant; * significant at $p < 0.05$. Means within columns followed by the same letter do not differ significantly by the Tukey’s test ($p < 0.05$).

Under laboratory conditions, root length and seedling dry matter did not differ among treatments for both cultivars (Table 1). While under greenhouse conditions, no differences were observed in seedling emergence and shoot dry matter among treatments for both cultivars (Table 2). Our findings corroborate those of Pereira et al. (2019), who observed no differences among control, *T. asperellum* (1×10^{12} CFU 100 kg^{-1} seed), and pyraclostrobin + thiophanate-methyl + fipronil (200 mL 100 kg^{-1} seed) treatments for seedling emergence of the wheat cultivars ‘Toruk’ and BRS Guamirim and for shoot fresh matter of the cultivar BRS Guamirim.

Table 2. Seedling emergence, shoot length, and seedling dry matter of the wheat cultivars ‘Toruk’ and ‘Sossego’ with seeds treated with *Trichoderma* spp. and chemical agents and grown under greenhouse conditions.

Treatment	Seedling emergence (%)		Shoot length (cm)		Seedling dry matter (mg)	
	‘Toruk’	‘Sossego’	‘Toruk’	‘Sossego’	‘Toruk’	‘Sossego’
Control	80	92	11.13 ab	13.82 ab	18.48	22.94
T1	84	91	10.78 b	13.64 ab	17.29	23.99
T2	84	92	10.92 ab	13.20 b	17.15	21.98
T3	78	89	11.60 a	15.27 a	18.67	24.82
T4	75	91	11.00 ab	14.75 ab	22.19	26.44
MSD	21	9	0.81	1.68	8.50	6.20
F-test	ns	ns	*	*	ns	ns

T1, *Trichoderma asperellum* SF 04 (2×10^{12} colony-forming units, CFUs, 100 kg^{-1} seed); T2, *Trichoderma harzianum* IBLF006 (2×10^{12} CFUs kg^{-1} 100 seed); T3, carboxin + thiram (300 mL 100 kg^{-1} seed); T4, pyraclostrobin + thiophanate-methyl + fipronil (200 mL 100 kg^{-1} seed); MSD, minimum significant difference; ns, non-significant; *significant at $p < 0.05$. Means within columns followed by the same letter do not differ significantly by the Tukey’s test ($p < 0.05$).

Carboxin + thiram treatment promoted the highest shoot length in both cultivars, differing only from *T. asperellum* SF 04 treatment in 'Toruk' seedlings and *T. harzianum* IBLF006 treatment in 'Sossego' seedlings (Table 2). In short, these results show that carboxin + thiram promoted seedling growth.

In the sanitary quality test, *T. asperellum* SF 04 provided the highest percentage of seeds without pathogenic fungi in 'Toruk' seeds, not differing from *T. harzianum* IBLF006 and chemical fungicides. For 'Sossego' seeds, carboxin + thiram treatment produced the highest percentage of seeds without pathogenic fungi, not differing from *T. harzianum* IBLF006, which, in turn, did not differ from *T. asperellum* SF 04. The prevalence of pathogenic fungi was 100% in both controls, not differing from the other treatments, except for *T. asperellum* SF 04 in 'Toruk' and *T. harzianum* IBLF006 and carboxin + thiram in 'Sossego' (Table 3).

For each cultivar, the efficacy of biological and chemical treatments may have ranged due to the different environments where seeds were produced. Accordingly, different incidence levels of *Alternaria* spp., *Bipolaris* spp. and *Fusarium* spp. fungi in seeds of 'Toruk' and 'Sossego', as well as their different inoculum levels, generated differentiated interactions between pathogenic fungi and treatments. However, the lack of similar studies in wheat crops prevents the comparison of results. In soybeans, Brand et al. (2009) observed that carboxin + thiram (300 mL 100 kg^{-1} seed) and *Trichoderma* spp. (250 g ha^{-1}) increased percentages of seeds without pathogenic fungi compared to control.

Table 3. Percentage of seeds without pathogenic fungi and prevalence of *Fusarium* spp., *Alternaria* spp., *Bipolaris* spp., *Rhizopus* spp., *Pyricularia* spp., and *Aspergillus flavus* in the wheat cultivars 'Toruk' and 'Sossego' treated with *Trichoderma* spp. and chemical agents and grown under laboratory conditions.

Treatment	Seeds without pathogenic fungi (%)		*Alternaria* spp. (%)		*Bipolaris* spp. (%)	
	'Toruk'	'Sossego'	'Toruk'	'Sossego'	'Toruk'	'Sossego'
Control	0.0 b	0.0 c	90.5	76.5 a	74.4	80.5 a
T1	5.0 a	7.5 bc	82.0	68.0 ab	69.5	71.0 a
T2	3.5 ab	14.0 ab	86.5	69.0 ab	72.7	65.5 ab
T3	1.0 ab	16.0 a	81.0	61.0 b	73.5	38.5 c
T4	2.0 ab	3.0 c	86.5	69.0 ab	72.5	46.0 bc
MSD	4.7	7.9	14.8	13.6	5.6	21.1
F-test	*	**	ns	*	ns	**

Treatment	*Fusarium* spp. (%)		*Aspergillus flavus* (%)	
	'Toruk'	'Sossego'	'Toruk'	'Sossego'
Control	1.0	56.0 a	2.5 a	n.d.
T1	0.0	10.0 b	0.0 b	n.d.
T2	0.0	7.5 b	0.0 b	n.d.
T3	1.0	11.0 b	0.5 ab	n.d.
T4	0.0	0.5 b	0.0 b	n.d.
MSD	2.2	17.7	2.1	n.d.
F-test	ns	**	**	-

T1, *Trichoderma asperellum* SF 04 (2 × 10^{12} colony-forming units, CFUs, 100 kg^{-1} seed); T2, *Trichoderma harzianum* IBLF006 (2 × 10^{12} CFUs 100 kg^{-1} seed); T3, carboxin + thiram (300 mL 100 kg^{-1} seed); T4, pyraclostrobin + thiophanate-methyl + fipronil (200 mL 100 kg^{-1} seed); MSD, minimum significant difference; ns, non-significant; *Significant at $p < 0.05$. **Significant at $p < 0.01$. n.d., not detected. Means within columns followed by the same letter do not differ significantly by the Tukey's test ($p < 0.05$).

The lowest prevalence of *Alternaria* spp. was found in 'Sossego' seeds treated with carboxin + thiram, while the other treatments had a high prevalence (61–77%) thereof (Table 3). Likewise, the lowest prevalence of *Bipolaris* spp. was observed in 'Sossego' seeds treated with carboxin + thiram, but not differing from that of seeds treated with pyraclostrobin + thiophanate-methyl + fipronil. The latter, however, did not differ from that of seeds treated with *T. harzianum* IBLF006 (Table 3). Pathogen control must have contributed to seedling growth, as evidenced by the increased shoot of plants treated with carboxin + thiram (Table 3).

The highest prevalence of *Fusarium* spp. was seen in 'Sossego' seeds of the control (Table 3), but no differences were observed between chemical and biological treatments; therefore, both options can be used for the management of wheat diseases by seed treatment. *A. flavus* had a low prevalence and was detected only in 'Toruk' seeds (Table 3). This fungus had the highest prevalence in the control, which did not differ from carboxin + thiram. The other treatments were able to completely control this pathogenic fungus. In assessing *Aspergillus* spp.

occurrence in seeds of the common bean cultivar 'Jalo Precoce,' Carvalho et al. (2011) found a high prevalence in the control (49.5%). These authors also observed that carboxin + thiram treatment (300 mL 100 kg^{-1} seed) completely inhibited pathogen growth, and the most effective biological treatments were *T. harzianum* CEN287 and CEN289 (2.5 × 10^{11} CFU 100 kg^{-1} seed).

Our results showed that seed treatment with *Trichoderma* spp. can be used to promote seedling growth and control pathogenic fungi in wheat plants, with efficiency comparable to the well-known chemical fungicides. Biological treatment is a sustainable alternative for plant growth promotion and fungal disease management in wheat crops.

CONCLUSIONS

The optimal dose for wheat seed treatment with *T. asperellum* SF 04 and *T. harzianum* IBLF006 was 2 × 10^{12} CFU 100 kg^{-1} seed.

Both biological and chemical products have the potential to prevent and control *A. flavus* and *Fusarium* spp., increase the number of seeds without pathogenic fungi, and promote seedling growth.

ACKNOWLEDGEMENTS

To the Araucária Foundation (Paraná, Brazil) for the scholarship granted to the first author, the companies LALLEMAND, BALLAGRO, UPL, and BASF for providing the products, and the company BIOTRIGO for providing the seeds.

REFERENCES

ADAPAR - Agência de Defesa Agropecuária do Paraná. **Agrotóxicos no Paraná**. 2021. Disponível em: <http://celepar07web.pr.gov.br/agrotoxicos/pesquisar.asp>. Acesso em: 14 Jun. 2021.

AGÜERO, L. E. M. et al. Inhibition of *Aspergillus flavus* growth and aflatoxin b1 production in stored maize grains exposed to volatile compounds of *Trichoderma harzianum* Rifai. **Interciência**, 33: 219 -222, 2008.

BAGWAN, N. B. Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotiumrolfsii, Aspergillus niger* and *Aspergillus flavus*. **International Journal of Plant Protection**, 4: 107-111, 2011.

BRAND, S. C. et al. Qualidade sanitária e fisiológica de sementes de soja submetidas a tratamento com bioprotetor e fungicida. **Revista Brasileira de Sementes**, 31: 87-94, 2009.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Manual de análise sanitária de sementes**. Brasília, DF: MAPA-ACS, 2009a. 200 p.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Brasília, DF: MAPA-ACS, 2009b. 399 p.

CARVALHO, D. D. C. et al. Biocontrol of seed pathogens and growth promotion of common bean seedlings by *Trichoderma harzianum*. **Pesquisa Agropecuária Brasileira,** 46: 822-828, 2011.

CARVALHO FILHO, M. R. et al. **Avaliação de isolados de *Trichoderma* na promoção de crescimento, produção de ácido indolacético in vitro e colonização endofítica de mudas de eucalipto**. Brasília, DF: Embrapa Recursos Genéticos e Biotecnologia, 2008. 16 p. (Boletim de pesquisa e desenvolvimento, 226).

CARVALHO, N. M., NAKAGAWA, J. **Sementes: ciência, tecnologia e produção**. 5ed. Jaboticabal, SP: Editora Funep, 2012. 590 p.

CHAGAS, L. F. B. et al. *Trichoderma asperellum* efficiency in soybean yield components. **Comunicata Scientiae**, 8: 165-169, 2017.

CONAB - Companhia Nacional de Abastecimento. **Acompanhamento da safra brasileira de grãos**: Nono levantamento, Brasília, 8: 121 p. 2021. Disponível em: < https://www.conab.gov.br >. Acesso em: 2 Jul. 2021.

COONEY, J. M., LAUREN, D. R., DI MENNA, M. E. Impact of competitive fungi on Trichothecene production by *Fusarium graminareum.* **Journal of Agricultural and Food Chemistry**, 49: 522-526. 2001.

FARIAS, C. R. J. et al. Inibição de germinação de sementes de trigo e milho em teste de sanidade em substrato de papel. **Revista Brasileira Agrociência**, 9: 11-144, 2003.

FERREIRA, D.F. SISVAR: A computer statistical analysis system. **Ciência e Agrotecnologia**, 35: 1039-1042, 2011.

FREIBERG, J. A. et al. Seed treatment and its impact on wheat crop yield potential. **Journal of Seed Science**, 39: 280-287, 2017.

HARDOIM, P. R. et al. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. **Microbiology and Molecular Biology Reviews**, 79:

293-320, 2015.

HASAN, M. M. et al. Antagonistic potentiality of *Trichoderma harzianum* towards seed-borne fungal pathogens of winter wheat cv. Protiva *in vitro* and *in vivo*. **Journal of Microbiology and Biotechnology**, 22: 585-591, 2012.

HEIL, M.; BALDWIN, I. T. Fitness costs of induced resistance: emerging experimental support for a slippery concept. **Trends in Plant Science**, 7: 61-67, 2002.

HERMOSA, R. et al. Plant-beneficial effects of *Trichoderma* and its genes. **BMC Microbiology**, 158: 17-25, 2012.

KRZYZANOWSKI, F. C. et al. Testes de vigor baseados em desempenho de plântulas. In: KRZYZANOWSKI, F.C. et al. **Vigor de sementes: conceitos e testes**. 2 ed. Londrina, PR: ABRATES, 2020. cap. 2, p. 93-104.

KUHNEM, P. et al. **Informações técnicas para trigo e triticale: Safra 2020, XIII Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale**. 1. ed. Passo Fundo, RS: Biotrigo Genética, 2020. 255 p.

MARINI, N. et al. Carboxim Tiram fungicide effect in wheat seeds physiological quality (*Triticum aestivum* L.). **Revista Brasileira de Ciências Agrárias,** 6: 17-22, 2011.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa n° 45, de 17 de setembro de 2013:** Anexo XXVII. Brasília: D.O.U, 34 p. 2013.

MASTOURI, F., BJÖRKMAN, T., HARMAN, G. E. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. **Phytopathology**, 100: 1213-1221, 2010.

MORI, C. et al. **Trigo: o produtor pergunta, a Embrapa responde**. Brasília, DF: Embrapa, 2016. 309 p.

PEREIRA, F. S. et al. Tratamento de sementes sobre a germinação, o vigor e o desenvolvimento do trigo. **Ciências Agroveterinárias**, 18: 395-399. 2019.

PESKE, S. T., ROSENTHAL, M. D., ROTA, G. R. M. **Sementes: fundamentos científicos e tecnológicos.** 3. ed. Pelotas, RS: Ed. Universitária/ UFPel, 2012. 573 p.

PRABHAKARAN, N. et al. Screening of difference *Trichoderma* species against agriculturally important foliar plant pathogens. **Journal of Environmental Biology**, 36: 191-198. 2015.

R CORE TEAM (2019). **R: A language and environment for statistical computing**. R Foundation for Statistical Computing. 2019.

RAMÍREZ, S. E. G., RAMELLI, E. G., REYNALDI, S. Importancia del aislamiento y del rango de concentración de conidias en el efecto de *Trichoderma asperellum* sobre el crecimiento de plántulas de *Solanum lycopersicum* L. **Revista Colombiana de Biotecnología**, 15: 118-125, 2013.

SANTOS, H. G. et al. **Sistema brasileiro de classificação de solos.** 5. ed. Brasília, DF: Embrapa Solos, 2018. 356 p.

SINGH, V. et al. *Trichoderma asperellum* spore dose depended on modulation of plant growth in vegetable crops**. Microbiological Research,** 193: 74 -86, 2016.

VINALE, F. et al. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. **Letters in Applied Microbiology**, 48: 705-711, 2009.

YOSHIOKA, Y. et al. Systemic resistance induced in Arabidopsis thaliana by *Trichoderma asperellum* SKT-1, a microbial pesticide of seed-borne diseases of rice. **Pest Management Science**, 68: 60-66, 2011.