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PHYSIOLOGICAL AND SANITARY QUALITY OF WHEAT CULTIVARS SEEDS SUBJECTED TO CHEMICAL AND BIOLOGICAL SEED TREATMENTS

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Abstract

Brazilian wheat farming is characterized by a high occurrence of seed-transmitted diseases that can cause significant damage to grain production. The objective of this study was to evaluate the influence of seed treatments on the physiological and sanitary quality of wheat cultivar seeds. Seeds from three commercial wheat cultivars (TBIO Sossego, TBIO Sinuelo, and TBIO Toruk) were used, along with four seed treatments (Control, Chemical, Biological, and Chemical + Biological). The physiological quality of the seeds was determined based on first germination count, germination percentage, quantification of abnormal seedlings, shoot and radicle length, whole seedling dry weight, and emergence velocity index (EVI). The sanitary quality was assessed using the Blotter test. Overall, the chemical treatment combined with the seeds of the TBIO Toruk cultivar provided better indices of physiological and sanitary quality.

Keywords: sanity; *Triticum aestivum*; viability; vigor.

QUALIDADE FISIOLÓGICA E SANITÁRIA DE SEMENTES DE CULTIVARES DE TRIGO SUBMETIDAS A TRATAMENTOS DE SEMENTES QUÍMICO E BIOLÓGICO

Resumo

A triticultura brasileira é marcada pela elevada ocorrência de doenças transmitidas via sementes, capazes de causar danos à produção de grãos. Objetivou-se avaliar a influência de tratamentos de sementes na qualidade fisiológica e sanitária de sementes de cultivares de trigo. Foram utilizadas sementes de três cultivares comerciais de trigo (TBIO Sossego, TBIO Sinuelo e TBIO Toruk) e quatro tratamentos de sementes (Controle, Químico, Biológico e Químico + Biológico). A qualidade fisiológica das sementes foi determinada com base nos testes de primeira contagem de germinação, germinação, quantificação número de plântulas anormais, comprimento de parte aérea e radícula, massa seca de plântula inteira e índice de velocidade de emergência (IVE). A qualidade sanitária foi determinada através do *Blotter test*. Em geral, o tratamento químico associado às sementes da cultivar TBIO Toruk proporcionou melhores índices de qualidade fisiológica e sanitária.

Palavras-chave: sanidade; *Triticum aestivum*; viabilidade; vigor.

Introduction

Among cereals in the annual and cool-season categories, wheat (*Triticum aestivum* L.) is the most economically significant crop due to its high grain production capacity and remarkable adaptability to diverse environmental conditions (MARINI *et al*., 2011). In the 2022 harvest, Brazil yielded 10.5 million tons over an area of 3.08 million hectares, achieving an average productivity of 3420 kg ha^{-1} (CONAB, 2023).

Currently, multiple studies are underway with the aim of developing cultivars that exhibit high productivity, commercial quality, and enhanced genetic resistance to pathogen infections. Wheat cultivation in Brazil is characterized by a high incidence of diseases capable of causing substantial damage to grain production, evident in the current production stagnation nationwide. The principal factors limiting cereal production are attributed to various diseases affecting the aerial parts, such as leaf rust (*Puccinia recondita f.* sp. *tritici*), powdery mildew (*Blumeria graminis f.* sp. *tritici*), leaf spots, gibberella (*Fusarium graminearum*), and wheat blast (*Magnaporthe oryzae*), particularly under climatic conditions favorable for epidemic development (MARINI *et al*., 2011).

Wheat sown in the southern region of Brazil has encountered significant issues regarding seed quality, primarily due to pre-harvest rains and mechanical damage during harvesting, drying, and storage (OHLSON *et al*., 2010). The utilization of protective products that aid in safeguarding seeds against pathogen infections during the period between sowing and seedling emergence becomes indispensable. Seed treatment provides additional assurance for crop establishment, primarily facilitating the germination of infected seeds while protecting them from fungi present in the soil (MERTZ *et al*., 2009). It is noteworthy that the phytosanitary protection of wheat seeds via seed treatment is of vital importance in reducing significant interference from phytopathogenic inoculum and ensuring the attainment of an adequate plant population.

In recent years, genetic improvement of plants to achieve resistance against key phytopathogens of the crop has been demonstrated as the most effective and economical approach to prevent and minimize disease-induced damage. However, when genetic resistance and chemical control are combined, not only does it enhance phytopathogen control efficiency, but it also contributes to their greater preservation (TORMEN *et al*., 2013).

The application of biocontrol agents, such as *Trichoderma harzianum*, in seed treatment has emerged as a highly relevant strategy, not only for disease transmission and infection control via seeds but also for enabling the reduction or substitution of pesticide use (CARVALHO *et al*., 2011; SCUDELER; VENEGAS, 2012). The bioprotective action of *T. harzian*um is attributable to its distinct mechanisms of action in the soil, including parasitism, antibiosis, and competition, which collectively contribute to preventive pathogen control. The primary pathogenic fungi controlled by this antagonist encompass *Rhizoctonia solani*, *Fusarium* spp., *Sclerotinia* spp., and *Pythium* spp. (SCUDELER; VENEGAS, 2012).

The objective of this study was to evaluate the influence of chemical, biological, and chemical + biological seed treatments on the physiological and sanitary quality of seeds from three commercial wheat cultivars.

Material and methods

The experiment was conducted at the Laboratory of Production and Technology of Seeds, located at the Federal University of Santa Maria Campus Frederico Westphalen.

Three commercial wheat cultivars (TBIO Sossego, TBIO Sinuelo, and TBIO Toruk) were used, along with four seed treatments: Control (no seed treatment), Chemical (Imidacloprid, Carboxin + Thiram), Biological [*T. harzianum,* strain CCT 7589, at a concentration of $1x10^9$ CFU mL^{-1} (CFU = Colony Forming Unit)], and Chemical + Biological (Imidacloprid, Carboxin, Thiram + *T. harzianum,* strain CCT 7589, at a concentration of $1x10^9$ CFU mL^{-1}).

For the chemical treatment, 3 mL of a commercial product formulated with Carboxin + Thiram and 1.3 mL of a commercial product formulated with Imidacloprid were added to 1000 g of seeds for each cultivar. For the biological treatment, 15 mL of a commercial product based on *T. harzianum* was added to 1000 g of seeds for each cultivar. For the chemical + biological treatment, 3 mL of a commercial product formulated with Carboxin + Thiram and 1.3 mL of a commercial product formulated with Imidacloprid were added to 1000 g of seeds for each cultivar, and 30 minutes later, 15 mL of a commercial product based on *T. harzianum* was added.

Aiming to assess the influence of seed treatments on the physiological quality of seeds, the following variables were evaluated: first germination count, germination, abnormal seedlings, non-germinated seeds, aerial part length, radicle length, dry mass of whole seedlings, and emergence velocity index (EVI). The germination test was conducted on germ paper rolls, moistened with distilled water, in an amount equivalent to 2.5 times the weight of the dry substrate, and placed in a BOD incubator at a temperature of 20 ºC ± 2 ºC and photoperiod of 12 hours. Four hundred seeds were used per treatment, divided into four repetitions, as specified by the Rules for Seed Analysis (BRASIL, 2009). The counts were made at four and eight days after sowing since the first germination count was conducted together with the germination test, which was evaluated and quantified in percentage at four days after the establishment of the test. At eight days after the establishment of the test, germination and abnormal seedlings were evaluated and quantified. In addition, the performance of the seedlings was determined in conjunction with the germination test; thus, 10 normal seedlings were randomly selected for evaluation, to measure the length of the aerial part and radicle. For aerial part length, the seedling was measured from the neck to the apex, and for radicle length, the main root length was measured, starting at the neck of the seedling. The measurements were performed with a ruler graduated in mm. For quantification of the dry mass of the whole seedling, the same 10 normal seedlings were used for the measurements of aboveground and radicle length. These seedlings were placed in paper bags and transferred to an oven with a controlled temperature of 35 ºC ± 2 ºC, where they remained until reaching a constant mass, to determine the dry mass. The weighing was performed on precision scales and the values were expressed in grams. To obtain the EVI, 200 seeds of each treatment were sown in pots, with four repetitions of 50 seeds each, where the number of emerged seedlings was counted daily until the stabilization of the stand. Calculated using the formula proposed by Maguire (1962):

$$EVI = E1/N1 + E2/N2 + ... + En/Nn$$

Where: *EVI* = Emergence Velocity Index. *E1, E2, ..., En* = Number of normal seedlings counted on the first count, on the second count, and so on until the nth (last) count. *N1, N2, ... Nn* = Number of days from sowing to the first, second, and so on up to the n-th (last) count.

Aiming to evaluate the influence of seed treatments on sanitary quality, the Blotter test was performed with a germination restrictor, which was a saline solution with a concentration of 8% (8 g of NaCl for 100 mL of distilled water). We analyzed 200 seeds for each treatment, arranged in four repetitions of 50 seeds each. The gearboxes were closed and placed in a BOD incubation chamber at 25 ºC ± 2 °C, with photoperiod of 12 hours. After eight days of incubation, the seeds were individually evaluated for pathogen incidence with the aid of a magnifying glass. When necessary, a stereomicroscope was used to visualize and identify the generic group of pathogens. Pathogen incidence was expressed as a percentage.

Then, the average of each variable in each plot was calculated and variance analysis was performed according to a two-factor mathematical model in an completely randomized design given by:

$$Y_{ijk} = \mu + C_i + T_j + (CT)_{ij} + \varepsilon_{ijk}$$

In which Y_{ijk} is the observed mean value of the response variable in plot *ijk*, μ is the overall mean, C_i is the fixed effect of level *i* (*i* = TBIO Sossego, TBIO Sinuelo, TBIO Toruk) of the cultivar factor, T_j is the fixed effect of level *j* (*j* = Control, Chemical, Biological, Chemical + Biological) of the seed treatments factor, $(CT)_{ij}$ is the effect of the interaction of level *i* of the cultivar factor with level j of the seed treatment factor, and ε_{ijk} is the effect of the experimental error assumed to be normal and independently distributed with zero mean and common variance σ^2 (STORCK *et al*., 2016).

Subsequently, the experimental precision and the grouping of means were calculated using the Scott-Knott test, both for main effects and the unfolded results. The following criteria were used for interpretation: when interactions between the cultivars and seed treatments were significant ($p \leq 0.05$), the unfolding of the seed treatment factor within each cultivar was evaluated; when interactions were not significant ($p > 0.05$), only the main effects of the cultivars and seed treatments were evaluated separately. In all analyses, a 5% probability of error was established, and the analyses were performed using the Software Microsoft Office Excel and Sisvar (FERREIRA, 2019).

Results and discussion

Among the variables associated with seed physiological quality, only the first count (FC) and the number of abnormal seedlings (AS) showed a significant interaction between the cultivar and seed treatment factors. Conversely, for the variables considered in terms of sanitary quality, such as the incidence of the fungi *Fusarium graminearum*, *Aspergillus* spp., and *Colletotrichum graminicola*, significant interactions were observed (Table 1). Thus, the effect of seed treatment is influenced by the conditions in which the wheat cultivar seeds are found prior to their implementation.

Table 1. Source of variation (SV), degrees of freedom (DF) and mean squares of the analysis of variance, coefficient of variation (CV) and overall mean of the experiment for variables evaluated in the completely randomized design in a two-factor scheme with wheat cultivars and seed treatments in the 2017 crop.

		Mean squares of the evaluated variables (1)					
SV	**DF**	**FC**	**G**	**AS**	**APL**	**RC**	**DMWS**
Cultivar	2	48,25**	11,08**	85,15*	26,02**	6,83**	0,000111**
Treatment	3	8,47*	7,56*	271,80**	44,01**	2,56*	0,000004^{ns}
Interaction	6	8,39*	3,14^{ns}	364,51**	2,40^{ns}	0,43^{ns}	0,000005^{ns}
Residue	36	2,64	1,75	20,88	2,22	0,61	0,000004
CV (%)		1,68	1,35	20,87	14,04	7,19	8,67
Overall mean		96,88	97,83	21,90	10,62	10,90	0,02

SV	DF	EVI	Healthy (%)	*Fusarium graminearum*	*Phoma* spp.	*Aspergillus* spp.	*Colletotrichum graminicola*
Cultivar	2	436,53**	754,75*	61,58^{ns}	752,33^{ns}	39,08*	105,58**
Treatment	3	95,29^{ns}	14090,97**	1599,86**	9070,33**	26,08^{ns}	52,67*
Interaction	6	19,59^{ns}	315,31^{ns}	1202,03**	257,33^{ns}	43,08**	37,92*
Residue	36	71,89	209,81	274,81	380,33	10,08	15,28
CV (%)		20,89	25,92	95,87	76,23	129,17	234,52
Overall mean		40,59	55,88	17,29	25,58	2,46	1,67

(1) FC = First count, in %; G = Germination, in %; AS = Abnormal seedlings, in %; APL = Aerial part length, in cm; RC = Radicle length, in cm; DMWS = Whole seedling dry mass, in g; EVI = Emergence velocity index. * and ** indicate significant effect by F test at 5% and 1% probability, respectively. ^{ns} indicates non-significant effect.

A significant effect of cultivars was observed through the F test ($p \leq 0.05$) for all variables for physiological quality. In terms of sanitary quality, there was a significant effect of cultivars solely on the percentage of healthy seeds, as well as on the incidence of the fungi *Aspergillus* spp. and *C. graminicola*. Regarding the utilized seed treatments, a significant effect was observed through the F test for all variables considered in the assessment of seed physiological quality, except for the emergence speed index and the dry mass of the entire seedling. Furthermore, a significant effect was detected through the F test for all variables related to seed sanitary quality, except for the percentage of colonies of the fungus *Aspergillus* spp. (Table 1).

The experimental precision in the physiological quality variables ranged from high to very low (PIMENTEL-GOMES, 2009), depending on the variable evaluated (1.35% ≤ CV ≤ 61.06%) (Table 1). In the sanitary quality variables, the coefficient of variation was high or very high for all variables evaluated (CV ≥ 25.92%).

Procedures such as seed treatment are used to improve seed performance and delay losses of vigor and viability during the storage period (FREIBERG *et al*., 2017). The protection of seeds against pathogens can be achieved efficiently with the incorporation of fungicides, however, it can also be obtained through the use of biological agents, since these can promote better seed and seedling performance, and thus reduce the potential for transmission of pathogens present both in the seeds and in the soil. According to Almeida *et al*. (2009), seed treatment activates several physiological reactions in the seed, such as the expression of proteins, where they interact with defense mechanisms, thus allowing the plant to better withstand adverse environmental conditions after germination and emergence. Based on this principle, we evaluated and discussed some of the main characteristics that allow us to quantify the physiological quality of seeds.

In the first count, the significant interaction indicates that the different seed treatments had varying effects among the cultivars. In this regard, it can be observed that the cultivar TBIO Sinuelo, when associated with the chemical + biological treatment, exhibited a lower percentage of germinated seedlings (91.75%), indicating a reduction in vigor compared to the control treatment and the other treatments. No significant difference was found between the seed treatments used for the other cultivars (TBIO Sossego and TBIO Toruk). Overall, regardless of the treatment employed, the lowest percentage was observed in the TBIO Sinuelo (94.88%), while the highest percentage was achieved with the treatment chemical of seeds (98.00%) (Table 2).

When evaluating seed treatments for castor oil plants, Tropaldi *et al*. (2010) found a significant difference for the first count variable, with the majority of chemical treatments yielding the best results. Similarly, Rosa *et al*. (2015) conducted a study on the effect of chemical seed treatment on the physiological and sanitary quality of canola cultivars and reported that most of the treatments used were effective in enhancing the first count and germination variables.

Table 2. Comparison of means for physiological quality variables evaluated in the completely randomized design in a two-factor scheme with cultivars and seed treatments in wheat in the 2017 crop.

Cultivar	Control	Chemical	Biological	Chemical+Biological	General/Cultivar
	FC = First Count (%)				
TBIO Sinuelo	95,25a	96,75a	95,75a	91,75b	94,88B
TBIO Sossego	96,50a	98,50a	98,25a	97,75a	97,75A
TBIO Toruk	98,00a	98,75a	96,75a	98,50a	98,00A
General	96,58b	98,00a	96,92b	96,00b	96,88
	G = Germination (%)				
TBIO Sinuelo	96,75a	98,50a	97,00a	95,25b	96,88B
TBIO Sossego	98,25a	99,25a	98,25a	97,75a	98,38A
TBIO Toruk	98,00a	99,25a	96,75a	99,00a	98,25A
General	97,67b	99,00a	97,33b	97,33b	97,83
	AS = Abnormal seedlings (%)				
TBIO Sinuelo	15,25b	19,25b	26,50a	18,25b	19,81B
TBIO Sossego	21,25b	17,25b	44,75a	14,25b	24,38A
TBIO Toruk	18,25b	27,75a	15,00b	25,00a	21,50B
General	18,25b	21,42b	28,75a	19,17b	21,90
	APL = Aerial part length (cm)				

TBIO Sinuelo	13,15a	9,53b	9,54b	6,87c	9,77B
TBIO Sossego	14,20a	11,06b	12,77a	10,30b	12,08A
TBIO Toruk	11,84a	8,83b	10,77a	8,54b	9,99B
General	13,06a	9,81c	11,02b	8,57c	10,62
			RC = Radicle length (cm)		
TBIO Sinuelo	10,78a	10,65a	10,00a	10,91a	10,58B
TBIO Sossego	10,09a	11,20a	9,93a	10,68a	10,47B
TBIO Toruk	11,64a	11,69a	10,92a	12,38a	11,66A
General	10,83a	11,18a	10,28b	11,32a	10,90
			DMWS = Whole seedling dry mass (g)		
TBIO Sinuelo	0,024a	0,022a	0,021a	0,021a	0,022B
TBIO Sossego	0,019a	0,020a	0,020a	0,021a	0,020C
TBIO Toruk	0,025a	0,026a	0,024a	0,025a	0,025A
General	0,023a	0,023a	0,021a	0,022a	0,022
			EVI = Emergence velocity index		
TBIO Sinuelo	34,24a	41,00a	33,86a	32,69a	35,45B
TBIO Sossego	40,91a	42,40a	36,78a	41,63a	40,43B
TBIO Toruk	42,25a	50,91a	45,51a	44,88a	45,89A
General	39,13a	44,77a	38,72a	39,73a	40,59

(1) Cultivar means not followed by the same capital letter in the column, and seed treatment means not followed by the same small letter in the row differ by the Scott-Knott test at 5% probability.

As for the effect of the treatments on germination, the cultivar TBIO Sinuelo showed the lowest percentage (95.25%) when the seeds received the treatment chemical + biological, while the other cultivars showed no significant difference regarding the treatment used. In general, the treatment chemical provided the highest percentage of germination (99.00%), and the cultivar TBIO Sinuelo showed the lowest percentage of germination (96.88%) (Table 2). When evaluating the effect of treatment chemical and biological on canola seeds, Migliorini *et al*. (2012) found that the chemical treatment resulted in increased germination percentage, compared to the other treatments used. This reaffirms that chemical treatment is more efficient than biological treatment in seeds. Tavares *et al*. (2014) emphasize that chemical treatments with fungicides promote the conservation of commercial germination standards for a certain period of storage. Hossen *et al*. (2014) suggest that seed treatment, in general, can be an interesting tool for obtaining more vigorous plants, as it provides a higher germination percentage.

As for the variable abnormal seedlings, the biological treatment stood out, in general, presenting the highest percentage of these (28.75%), concerning the others. As for the cultivars, in general, TBIO Sossego showed the highest percentage among the cultivars (24.38%). Also, the association of the cultivar and treatment in question provided the highest percentage of abnormal seedlings in the experiment (44.75%) (Table 2).

When analyzing the influence of treatments on the length of the aerial part of seedlings, it was found that, in general, in both cultivars, the control treatment stood out compared to the others, followed by the biological treatment (Table 2). According to Bécquer *et al*. (2015), seedlings from wheat seeds inoculated with Trichoderma may present a brief increase in the length of the aerial part. Thus, justifying the better result of the biological treatment compared to the chemical treatment for this variable. The same did not occur for the variable radicle length, since the treatment resulted in the lowest value (10.28 cm). Overall, among the cultivars, TBIO Toruk stood out (11.66 cm) in radicle length (Table 2).

The cultivar TBIO Toruk stood out among the other cultivars about the dry mass of the whole seedling (0.025 g), even though there was no significant difference between the treatments. Evaluating different chemical treatments for wheat seeds, Hossen *et al*. (2014) found that the mixture of fungicides Carboxin + Thiram promoted greater accumulation of dry mass for the wheat cultivar Pampeano, among the cultivars evaluated. This suggests that the mixture of these fungicides acts differently among wheat cultivars, thus justifying the non-occurrence of dry mass increase in the seedlings evaluated in this study.

The EVI indicates the speed of stabilization of seedling emergence in the field, and the higher the EVI, the faster the stabilization of the seedling stand. It was found that there was no significant difference for the treatments used, corroborating Tropaldi *et al*. (2010) and Marroni *et al*. (2012), who observed in castor oil plant seeds, that among the treatments used, there was no significant differentiation in the EVI. However, it is noted that numerically the fastest stabilization of emergence occurred with the chemical treatment (44.77), and the slowest occurred with the biological treatment (38.72). Corroborating with data from Abati *et al*. (2014), where the best results of seedling emergence in the field found, compared to the control, was for seed treatments with the fungicides Carboxin, Thiram and Imidacloprid. In general, the cultivar TBIO Toruk stood out from the others, regarding the EVI (45.89). These results prove the importance of seed treatment for a rapid stabilization of the plant stand.

According to Ishizuca *et al*. (2018), the presence of fungi negatively affects the sanitary quality of seeds, as well as hinders the quantification of their physiological quality, since the pathogens promote a reduction in germination power. According to Silva *et al*. (2011), the occurrence of diseases is one of the factors that most affects agricultural production, where the seed treatment behaves as a valuable tool for integrated control of plant diseases. This makes the sanitary evaluation of seeds a very important aspect since it facilitates and increases the efficiency of the choice of the most appropriate seed treatment for each cultivation area. Nobre *et al*. (2015) emphasize that it is necessary to bring together all the genetic, physical, physiological, and health attributes that contribute to the formation of vigorous seedlings and ensure the rapid establishment of the desired plant stand.

In general, the control treatment showed 7.00% healthy seeds, and, among the cultivars, TBIO Sinuelo did not show healthy seeds, differing from the others, characterizing the seed sample of this cultivar as of low sanitary quality. The biological treatment showed reasonable efficiency (57.17%) among the others (Table 3). Corroborating with Brand *et al*. (2009), who observed that seed treatment with a product based on *Trichoderma* spp. showed about 60% control of phytopathogenic fungi. As well as with Migliorini *et al*. (2012), who observed and concluded that the seed treatment with the bioprotector *Trichoderma* spp. showed an antagonistic effect to fungi associated with canola seeds, however, except *Penicillium* spp. and *Aspergillus* spp. In the present study, the best results were obtained in the chemical and chemical + biological treatments, and they presented efficiency of 79.33% and 80.00%, respectively, not differing from each other (Table 3). Conceição *et al*. (2016) concluded that among the seed treatments used, the mixture of fungicides Carbendazim + Thiram promoted the highest efficiency in fungal control during certain periods of seed storage. Reinforcing the efficiency of Thiram fungicide in the control of pathogens through seed treatment, Migliorini *et al.* (2012) point out that the chemical treatment of seeds shows to be efficient in reducing the influence of the main fungi associated with canola seeds. Corroborating with Abati *et al*. (2014), who points out that chemical seed treatment is considered one of the most effective methods to control fungi.

Table 3. Percentage of healthy seeds and fungus incidence in wheat seeds treated with bioprotector and/or fungicides in the 2017 crop.

Cultivar	Control	Chemical	Biological	Chemical+Biological	General/Cultivar
	Healthy seeds				
TBIO Sinuelo	0,00c	80,00a	36,50b	75,50a	48,00B
TBIO Sossego	11,00b	82,50a	66,50a	76,00a	59,00A
TBIO Toruk	10,00b	75,50a	68,50a	88,50a	60,63A
General	7,00c	79,33a	57,17b	80,00a	55,88
	Fusarium graminearum				
TBIO Sinuelo	7,50a	12,00a	33,50a	10,00a	15,75A
TBIO Sossego	34,50a	12,50a	13,50a	6,00a	16,63A

TBIO Toruk	58,50a	14,00b	2,00b	3,50b	19,50A
General	33,50a	12,83b	16,33b	6,50b	17,29
			Phoma spp.		
TBIO Sinuelo	82,50a	2,00c	37,00b	12,50c	33,50A
TBIO Sossego	57,00a	1,00b	20,50b	7,50b	21,50A
TBIO Toruk	49,50a	2,50b	29,50a	5,50b	21,75A
General	63,00a	1,83c	29,00b	8,50c	25,58
			Aspergillus spp.		
TBIO Sinuelo	1,50b	0,00b	9,00a	5,50a	4,00A
TBIO Sossego	7,00a	0,00b	0,50b	2,50b	2,50A
TBIO Toruk	2,50a	1,00a	0,00a	0,00a	0,88B
General	3,67a	0,33b	3,17a	2,67a	2,46
			Colletotrichum graminicola		
TBIO Sinuelo	12,00a	0,00c	6,00b	0,50c	4,63A
TBIO Sossego	1,50a	0,00a	0,00a	0,00a	0,38B
TBIO Toruk	0,00a	0,00a	0,00a	0,00a	0,00B
General	4,50a	0,00b	2,00b	0,17b	1,67

(1) Cultivar means not followed by the same capital letter in the column and seed treatment means not followed by the same small letter in the row differ by the Scott-Knott test at 5% probability.

Regardless of the treatment used, the microorganisms detected in significant percentages in the seeds were *F. graminerarum*, *Phoma* spp., *Aspergillus* spp., and *C. graminicola*. Besides these, at low incidence percentages, *Penicillium* spp., *Botrytis* spp., and *Rhizopus stolonifer* were detected in the seeds.

Regarding the incidence of the pathogen *F. graminearum*, there was an interaction between seed treatments and cultivars (Table 1), where the lowest incidence of the pathogen was in the cultivar TBIO Toruk, associated with the biological treatment (2.00%), and the chemical + biological treatment (3.50%) (Table 3). In general, the treatments did not differ, however, the chemical + biological treatment was more efficient than the others in controlling *F. graminearum*. In general, the cultivars did not differ regarding the incidence of this pathogen. This corroborates Agostinetto *et al*. (2018), who found that there was an incidence of the fungus in all seed treatments tested for barley, demonstrating no eradicating effect by fungicides against this pathogen. However, they still observed a greater control of this fungus in the treatments that contained the fungicides Carboxin + Thiram. This agrees with what was found in this study when comparing the chemical treatment concerning the control and biological treatment.

For the control of *Phoma* spp, the chemical treatment was more efficient, providing a low incidence of the pathogen (01.83%), not differing, and being followed by the chemical + biological treatment (08.50%) (Table 3). Among the cultivars, the highest incidence of the pathogen was quantified in TBIO Sinuelo (33.50%), without being statistically different from the others.

Regarding the incidence of *Aspergillus* spp. there was an interaction between treatments and cultivars, indicating a change in behavior among cultivars. TBIO Sinuelo did not show the incidence of *Aspergillus* spp. in association with chemical treatment but did not differ statistically from the control (Table 3). For TBIO Sossego, the control showed the highest incidence of the pathogen, but the other treatments did not differ. TBIO Toruk showed no statistical difference between the treatments. The association of the cultivar TBIO Toruk with the chemical treatment was superior, showing the lowest percentage of *Aspergillus* spp. incidence.

As for the incidence of *C. graminicola*, there was an interaction between treatments and cultivars, indicating variation in behavior among cultivars. For TBIO Sinuelo, there was no incidence of *C. graminicola* in the chemical treatment, not statistically different from the chemical + biological treatment (Table 3). In the cultivar TBIO Sossego, it was found that all treatments were efficient, eradicating the pathogen from the seeds since the control treatment had a very low presence of this pathogen. For TBIO Toruk there was no incidence of the pathogen. Overall, the cultivar TBIO Sinuelo and the control treatment showed a higher incidence of *C. graminicola*.

Conclusions

The chemical treatment and the use of the cultivar TBIO Toruk guaranteed higher physiological and sanitary quality of the seeds. It is also possible to conclude that the use of the cultivar TBIO Sinuelo resulted in the worst indicators of evaluation of the physiological quality of seeds.

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