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CROP PROTECTION

Fungi associated with *Paspalum guenoarum* seeds: their impact on physiology and control

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ABSTRACT: *Of the natural pastures grown in southern Brazil, those of the genus Paspalum are the most important. One of the factors that hinder their cultivation is the availability of quality seeds, that are often compromised by the presence of fungi. This study determined the in vitro sensitivity of Paspalum guenoarum ecotype azulão seed-associated fungus to certain fungicides and to measure the efficiency of chemical treatments for fungal control and seed physiological performance. Bipolaris micropus, Epicoccum sorghinum, Curvularia geniculata and Fusarium incarnatum associated with seeds were tested in vitro against Carbendazim; Tiram, Fludioxonil; Metalaxil-M, Carboxina; Tiram and Tiofanato-metílico at 0, 1, 2.5, 5, 10 and 30 µg/mL. This experiment was conducted in a completely randomized design (factorial 4 × 4 × 6). Qualitative variables were compared using the Tukey test (P ≤ 0.05) and quantitative variables were subjected to regression analysis. Carbendazim; Tiram and Fludioxonil; Metalaxil-M had the best fungicidal performances, each inhibiting three of the four fungi with LD_{50}s below 1 µg/mL. Subsequently, these two fungicides were used alone or in combination in the in vivo test. A completely randomized design was used and the means were compared using the Tukey test (P < 0.05). The chemical treatment of the seeds resulted in improvement of five of the six evaluated physiological parameters. The identification of the primary fungi associated with Paspalum seeds reported in this research, as well as damage done to them, can be diminished using appropriate measures such as seed treatments.*
Key words: *seed fungus, health quality, germination, fungitoxicity.*

Fungos associados com sementes de *Paspalum guenoarum*: seus impactos na fisiologia e controle

RESUMO: *Das pastagens naturais cultivadas no sul do Brasil, as do gênero Paspalum são as mais importantes. Um dos fatores que dificultam o cultivo é a disponibilidade de sementes de qualidade, muitas vezes comprometidas pela presença de fungos. Os objetivos deste estudo foram determinar a sensibilidade in vitro de fungos associados a sementes de Paspalum guenoarum ecótipo azulão a determinados fungicidas, medir a eficiência de tratamentos químicos para controle desses fungos e o desempenho fisiológico das sementes. Os fungos associados às sementes, Bipolaris micropus, Epicoccum sorghinum, Curvularia geniculata e Fusarium incarnatum foram testados in vitro contra Carbendazim; Tiram, Fludioxonil; Metalaxil-M, Carboxina; Tiram e Tiofanato-metílico em 0, 1, 2,5, 5, 10 e 30 µg/mL. Este experimento foi conduzido em um delineamento inteiramente casualizado (fatorial 4 × 4 × 6). As variáveis qualitativas foram comparadas pelo teste de Tukey (P ≤ 0,05) e as variáveis quantitativas foram submetidas à análise de regressão. Carbendazim; Tiram e Fludioxonil; Metalaxil-M tiveram os melhores desempenhos fungicidas, cada um inibindo três dos quatro fungos com LD_{50} abaixo de 1 µg/mL. Posteriormente, esses dois fungicidas foram utilizados isoladamente ou em combinação no teste in vivo. O delineamento experimental foi inteiramente casualizado e as médias comparadas pelo teste de Tukey (P < 0,05). O tratamento químico das sementes resultou na melhoria de cinco dos seis parâmetros fisiológicos avaliados. A identificação dos fungos primários associados às sementes de Paspalum relatadas neste trabalho, bem como os danos causados por eles, podem ser diminuídos usando medidas apropriadas, como tratamento de sementes.*
Palavras-chave: *fungos de semente, qualidade sanitária, germinação, fungitoxicidade.*

INTRODUCTION

The genus *Paspalum* belongs to the Plicatula group, the most important group in Brazil and South America, particularly on account of its agronomic characteristics, being a source of food for wild and domesticated animals (MEIRELLES et al., 2013). Most important of these is *P. guenoarum* Arechav, ecotype *azulão*, which is notable for high total dry matter production (≅ 16 Mg ha^{-1}), leaf dry matter (≅ 11 Mg ha^{-1}), high digestibility and cold tolerance (PEREIRA et al., 2012; PEREIRA et al., 2015).

The use of native forage species allows greater productive stability, conservation of natural

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resources and reduction of costs and risks in livestock. The ultimate result is sustainability of the overall system. In order to obtain sustainability, it is essential to offer viable seeds to consumers, as this is the most efficient method for the rapid dissemination of new materials. High-quality seeds are critical factors for the success of pasture establishment. According to CARVALHO & NAKAGAWA (2012), seed quality involves genetic, physical, physiological and health aspects that, when evaluated together, express the true potential of the seed.

Sanitary quality refers to the presence of pests and phytopathogens associated with seeds, all of which can impair their quality. Often, low germination potential of seeds is related to the presence of pathogens, also compromising the stand and the loss of seed lots; therefore, it is necessary to identify the agents and the consequences of their associations with seeds. (MARRONI et al., 2012)

The germination test is most-often used to determine the quality of a seed lot (BRASIL, 2009). Nevertheless, germination potential can be affected by the presence of microorganisms, because the germination pattern is measured in chambers that provide ideal conditions for the development not only of seeds but also for pathogens. According to OLIVEIRA et al. (2012), problems can occur related to the interpretation of results, causing the elimination of lots that do not reach minimum germination standards.

Chemical seed treatment is the most common method of controlling seed-borne pathogens (CARVALHO & NAKAGAWA, 2012). Seed treatment involves application processes and substances that preserve or improve seed performance, permitting maximum expression of the genetic potential of the species (LAMICHHANE et al., 2020).

Despite the fact that several authors have detected phytopathogenic fungi associated with forage seeds (LA VALE et al., 2017; MALLMANN et al., 2013; SANTOS et al., 2014), there is a relative lack of reports of the occurrence and control of fungal pathogens in *P. guenoarum* seeds. GASPARETTO et al. (2017) has been one of the first reports to describe fungi associated with *P. guenoarum* seeds; although no attempts to control them was done so far.

Therefore, the objectives of this study were to determine the sensitivities of the primary fungi associated with *P. guenoarum* seeds to a group of fungicides with potential use for seed treatment and to measure the impact of this control on physiological parameters.

MATERIALS AND METHODS

The experiments were conducted at the Winter Cereal Laboratory of the Plant Health Department and at the Seed Analysis Laboratory of the Forage Plants and Agrometeorology Department of the School of Agronomy of the Federal University of Rio Grande do Sul, RS.

We used seeds harvested in 2015 that were produced at the Agronomic Experimental Station of the Federal University of Rio Grande do Sul - EEA/UFRGS, in the municipality of Eldorado do Sul, RS (30º05'52" S, 51º39'08" W and elevation of 32 m). The average annual temperature was 19.3 °C, with a maximum average of 24.6 °C in January, and the minimum average of 13.8 °C in June.

The isolation, pathogenicity, molecular and morphological characterization of phytopathogenic fungi associated with *P. guenoarum* seeds from this batch were described by GASPARETTO et al. (2017). The most frequent seed-associated fungal species tested in this research were *Bipolaris micropus* (Drechsler) Shoemaker, *Curvularia geniculata* (Tracy & Earle) Boedijn, *Fusarium incarnatum* (Desm.) Sacc. and *Epicoccum sorghinum* (Sacc.) Aveskamp, Gruyter & Verkley, according to those reported by GASPARETTO et al. (2017). The fungicides used to verify their fungitoxicities to the observed fungi were Carbendazim; Thiram (150 + 350 g/L), formulated as Derosal Plus™, Fludioxonil; Metalaxyl-M (25 + 10 g/L), formulated as Maxim XL™, Carboxina; Tiram (200 + 200 g/L), formulated as Vitavax-Thiram™, and Tiofanato-metílico (700 g/Kg), formulated as Cercobin 700 WP™, where carbendazim, metalaxyl-M, carboxina and tiofanato-metílico are systemic fungicides. The fungicides were chosen because they have an excellent activity against a large number of fungal species (BRASIL, 2019. The first experiment initially tested the sensitivity of these fungal species to fungicides by determining the LD_{50}s in vitro. For this purpose, mycelial growth of the fungi was evaluated in disposable plastic Petri dishes containing potato-sucrose-agar and streptomycin culture medium, BSA + St (250 g potato broth, 10 g saccharose and 12 g agar in one liter of water plus 100 µg/mL streptomycin sulfate), supplemented with fungicides at various concentrations of their active ingredients under controlled environment conditions (25 °C and 12 h white fluorescent light/12 h dark) for seven days.

Various concentrations of the fungicides were created by transferring aliquots from stock solutions to 1000 mL volume Erlenmeyer flasks

containing 500 mL of liquid culture medium at 45 to 47 °C, obtaining 1, 2.5, 5, 10 and 30 µg/mL of the fungicides. The criterion for using these concentrations was in accordance with Edington's classification (EDINGTON et al., 1971) for establishing fungitoxicity of fungicides. After homogenization, 20 mL of the culture medium was poured into each Petri dish. This was performed in a continuous laminar flow chamber to ensure complete asepsis. The control treatment (concentration 0) did not have the addition of fungicide to the culture medium.

When solidifying the culture medium, 4-mm diameter discs containing mycelium were cut from the edge of young fungal colonies, grown only in BSA + St medium to the center of the Petri dishes with the various fungicidal concentrations and the control treatment.

Mycelial growth readings were taken using a caliper when the fungi in the control treatment reached a mean diameter on the seventh day, near the edges of the plates (≅ 6.5 cm).

To test the efficacy of fungicides as chemical treatment of *Paspalum* seeds in controlling the associated fungi, as well as to evaluate their effects on physiological aspects of seeds such as germination (normal and abnormal seedlings, dormant, hard and dead seeds) and vigor (first germination count and germination speed index), we performed a second group of experiments. In the second experiment, only the fungicides that presented the highest fungitoxicities *in vitro* were used: Carboxina; Tiram (LD_{50} <1 µg/mL for *F. incarnatum*, *C. geniculata* and *B. micropus*) and Fludioxonil; Metalaxil-M, (LD_{50} <1 µg/mL for *E. sorghinum, C. geniculata* and *B. micropus*). These two fungicides were tested both pure (300 mL c.p./100 kg of seeds), and each mixture was also tested at 150 mL c.p./100 kg of seeds.

Fungicide treatment of seeds was carried out in 2-L Erlenmeyer flasks, adding volumes corresponding to the weight of 100 grams of seeds for each treatment. The container was shaken until fungicide completely covered the seeds. Then, the treated seeds were subjected to health and germination tests according to recommended methodology (BRASIL, 2009). The health test was performed using the seed plating method in potato-sucrose-agar medium with addition of streptomycin (BSA + St). We performed 40 replications of ten seeds per treatment, evaluated in Petri dishes and incubated for seven days in a growth chamber at 25 ºC and alternating light regime (12 h in the dark and 12 h with light). Seeds were individually examined under a stereomicroscope and when necessary under a compound microscope. Results obtained were expressed as percentage of seeds infected by phytopathogenic fungi.

The germination test was performed using 400 seeds per treatment, divided into four repetitions of 100 seeds each, on KNO_3 (0.2%) wetted Germitest sheets in an amount equivalent to 2.5 times the dry paper mass, in clear plastic boxes with lids (11 × 11 × 3.5 cm), followed by seven days of pre-cooling (5–10 °C). After pre-cooling, the boxes were placed in germination under alternating temperature and light regime (20 °C/16 h in the dark and 30 °C/8 h with light). Results were expressed as percentage of normal seedlings obtained on the twenty-first day (BRASIL, 2009). The first germination count (FGC) was performed considering the number of normal seedlings on the seventh day of the germination test (BRASIL, 2009). The germination speed index (GSI) was obtained by summing the daily readings of germinated seeds divided by the number of days of sowing.

The experiment that determined LD_{50} in vitro was conducted according to a completely randomized design, in a 4 x 4 x 6 factorial arrangement (active ingredient x fungus x active ingredient concentration), with 10 replications. Data were subjected to analysis of variance using the F test ($P \leq 0.05$) and; subsequently, when a significant effect was reported, the qualitative variables were compared using the Tukey test ($P \leq 0.05$) and the quantitative variables were subjected to regression analysis, because the data met the assumptions for performing parametric analysis. The mathematical models were selected based on the significance of the regression coefficients using the "t" test ($P \leq 0.05$), the coefficient of determination ($r^2 > 0.60$) and the significance of the mathematical model ($P \leq 0.05$). Using the regression equation, the LD_{50} (dose required to inhibit fungal growth by 50%) was calculated for each treatment. Fungi were considered highly sensitive to fungicides when LD_{50} was <1 µg/mL, moderately sensitive when LD_{50} between 1 and 10 µg/mL, and insensitive when LD_{50} was 50 µg/mL or greater (EDGINGTON et al., 1971).

The second group of experiments was conducted according to a completely randomized design, with four and 40 replications to measure the physiological effects and efficacy of fungicide controlling the fungi in the seeds, respectively. Data were subjected to normality and homogeneity of variance tests. Values referring to physiological tests met the assumptions for the analysis of variance using the F test, with subsequent application of the Tukey test ($P \leq 0.05$). Data on fungicide efficacy were subjected to the Kruskal–Wallis test, followed by Dunn's test ($P \leq 0.05$).

RESULTS

For the experiment that determined the in vitro LD_{50} of fungicides, the analysis of data variance by the F test revealed a significant triple interaction between fungicides, fungi and concentrations, with $P < 0.0001$.

Data on mycelial growth inhibition of the four fungi tested under various fungicide concentrations are shown in table 1. The equations of the mycelial growth regressions as functions of the increasing fungicide concentrations are presented, all of them having highly significant p-values, where the decreasing exponential model presented the best fit to the experimental data. Figure 1 illustrates the mycelial growth inhibition behavior of each fungus when subjected to increasing fungicide concentrations. All models showed high correlations, with r^2 values above 0.95, except for *F. incarnatum* and the Fludioxonil; Metalaxil-M fungicide (r^2 = 0.85). From these regression models, fungicide dose values were calculated in µg/mL, capable of inhibiting 50% of mycelial growth, or LD_{50}. Of these values, fungicides with LD_{50} values lower than 1 µg/mL were considered highly fungitoxic. For control of *F. incarnatum*, Carboxina; Tiram and Carbendazim; Tiram stood out, with LD_{50} values of 0.38 and 0.78 µg/mL, respectively. *E. sorghinum* was only effectively controlled by Fludioxonil; Metalaxil-M (LD_{50} = 0.23 µg/mL). *C. geniculata* and *B. micropus* were efficiently controlled by Carboxina; Tiram and Fludioxonil; Metalaxil-M. For *C. geniculate*, LD_{50} values were 0.59 and 0.17 µg/mL for Carboxina; Tiram and Fludioxonil; Metalaxil-M, respectively, while for *B. micropus*, LD_{50} values were 0.51 and 0.28 µg/mL for the same fungicides, respectively (Table 1).

Carboxina; Tiram presented LD_{50} values below 1 µg/mL for three of the four fungi tested: *F. incarnatum*, *C. geniculata* and *B. micropus*. Similarly, the Fludioxonil; Metalaxil-M controlled three fungi with LD_{50} below 1 µg/mL: *E. sorghinum*, *C. geniculata* and *B. micropus* (Table 1). Because of the higher spectrum of fungicidal activity at low concentrations, these two products were considered the best among the four tested, and were chosen to evaluate their effectiveness in controlling *Paspalum* seed-associated fungi, as well as to evaluate physiological effects on them in subsequent experiments.

The highest fungal growth inhibition rate was obtained at the lowest fungicide concentrations, decreasing with increasing concentration. For the same concentration of fungicides, we observed varying reductions in the diameter of the growth halo, regardless of the fungus evaluated. Nevertheless, among the evaluated fungi, there were variations in

Table 1 - Concentrations of various fungicides (µg/mL) able to inhibit by 50% (LD_{50}) mycelial growth *in vitro* of phytopathogenic fungi associated with seeds of *Paspalum guenoarum*.

Fungi	Fungicide[£]	Regression equation[β]	r^2	LD_{50} (µg/mL)
Fusarium incarnatum	CT	Y= (65.2907*0.3824)/(0.3824+x)	0.986	0.381157
	T	Y= 66.0282*exp(-0.5757x)	0.990	1.202503
	C	Y= 64.9957*exp(-0.8991x)	0.979	0.78356
	FM	Y= 6.9620+50.6925*exp(-0.1962x)	0.853	3.62094
Epicoccum sorghinum	CT	Y= 26.0757*exp(-1.0045x)+42.8650*exp(-0.0598x)	0.991	3.89789
	T	Y= 72.4592*exp(-0.2199x)	0.975	3.47067
	C	Y= 69.4167*exp(-0.2152x)	0.971	3.33067
	FM	Y= 7.9056+58.4622*exp(-3.5942x)-0.1969x	0.993	0.232775
Curvularia geniculata	CT	Y= (68.0131*0.5993)/(0.5993+x)	0.980	0.595245
	T	Y= 15.2086+47.9256*exp(-0.1036x)	0.962	9.5478
	C	Y= 9.5705+57.9978*exp(-0.0827x)	0.968	10.1412
	FM	Y= 2.5779+65.2561*exp(-4.3613x)-0.1059x	0.988	0.17548
Bipolaris micropus	CT	Y= (64.0643*0.5183)/(0.5183x)	0.978	0.513858
	T	Y= 13.0820+50.1661*exp(-0.0985x)	0.965	9.563705
	C	Y= 14.1910+51.1672*exp(-0.2361x)	0.952	4.5023
	FM	Y= 2.4208+60.0888*exp(-2.6100*X)-0.0993x	0.992	0.2809

[£] CT = Carboxina; Tiram; T = Tiofanato-metílico; C = Carbendazim; Tiram; FM = Fludioxonil; Metalaxil-M.
[β] All "p" values were smaller than 0.0001.

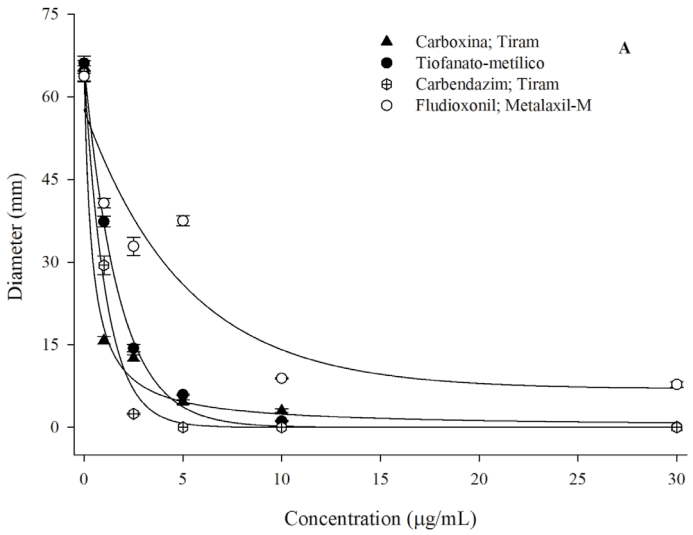

Figure 1 - Graphical representation of regressions illustrating inhibition of mycelial growth of *Fusarium incarnatum* (A), *Epicoccum sorghinum* (B), *Curvularia geniculata* (C) and *Bipolaris micropus* (D) when subjected to increasing fungicide concentrations. Bars illustrate standard deviation of means.

control efficiency, suggesting that there is specificity among the fungicides in terms of fungal control.

Carboxina; Tiram and Fludioxonil; Metalaxil-M efficiently controlled *B. micropus*, *C. geniculata*, *F. incarnatum* and *E. sorghinum* associated with *Paspalum* seeds ($P < 0.0001$). Incidences of untreated seeds of these four fungi occurred in 19.23%, 13.13%, 3.85% and 15.50%, respectively. All chemical treatments were similar and highly efficient, reducing the percentage of their incidences to 0% (Table 2).

The effect of seed treatment on physiological parameters was significant for the first germination count test, normal seedling germination, abnormal seedling growth, dead seeds and germination speed index. By contrast, for hard seeds, p-values were higher than 0.05 (Table 3).

The treatment of *Paspalum* seeds with Carboxina; Tiram, Fludioxonil; Metalaxil-M or the mixture of these two significantly improved five of the six evaluated physiological parameters (Table 3). The first germination count, normal seedling germination

Table 2 - Incidence (%) of phytopathogenic fungi in seeds of *Paspalum guenoarum* treated with fungicides, after incubation in potato-sucrose-agar and streptomycin culture medium.

Treatments	Dose†	----------Fungi----------			
		Fusarium incarnatum	*Epicoccum sorghinum*	*Curvularia geniculata*	*Bipolaris micropus*
Control	0	3.85 a	15.50 a	13.13 a	19.23 a
CT	300	0.00 b	0.00 b	0.00 b	0.00 b
FM	300	0.00 b	0.00 b	0.00 b	0.00 b
CT + FM	150 + 150	0.00 b	0.00 b	0.00 b	0.00 b

Values followed by the same letter in columns do not differ ($P > 0.05$) by the Dunn´s test. † mL of c.p./100 kg of seeds. CT = Carboxina; Tiram; FM = Fludioxonil; Metalaxil-M; CT + FM = Mixture of Carboxina; Tiram with Fludioxonil; Metalaxil-M.

Table 3 - Effect of chemical treatment of *Paspalum guenoarum* seeds on first germination count (FGC), germination (G), growth of abnormal seedlings (GAS), seed death (SD), hard seeds (HS) and germination speed index (GSI).

Treatment	Dose†	FGC (%)	G(%)	GAS (%)	SD (%)	HS (%)	GSI
Control	0	60.7 b	74.7 b	11.0 a	7.8 a	6.5 a	14.5 b
CT	300	71.3 a	90.2 a	1.7 b	2.8 b	5.3 a	16.3 a
FM	300	61.3 b	85.0 a	3.7 b	4.0 b	7.3 a	15.0 b
CT + FM	150 + 150	66.8 ab	89.0 a	1.5 b	3.5 b	6.0 a	15.2 b
CV (%)	–	6.8	4.5	33.0	37.7	43.9	3.19

Values followed by the same letter in the same column do not differ (P > 0.05) by the Tukey´s test. †mL of c.p./100 kg of seeds. CT = Carboxina; Tiram; FM = Fludioxonil; Metalaxil-M; VT + M = Mixture of Carboxina; Tiram with Fludioxonil; Metalaxil-M.

and the germination rate increased, improving in relation to the untreated control, just as the abnormal seedling growth and seed death decreased in relation to the control. The first germination count improved only when treated with Carboxina; Tiram, from 60.7% in the control to 71.3%. The same positive effect was observed for the same fungicide in the germination speed index parameter, which increased from 14.5% in the control to 16.3%. Regarding normal seedling germination, all fungicides had a positive effect, increasing from 74.7% in the control to above 85.0% in the fungicide treatments (Table 3). The number of abnormal seedlings decreased in relation to the control for all fungicidal treatments, declining from 11.0% to values below 3.8% (Table 3). Similarly, dead seed values were reduced as a function of seed treatment, from 7.8% in the control to below 4.0% in the fungicide treatments.

Figure 2 illustrates the growth in BSA + St culture medium of the four fungi tested in the presence of various concentrations of the four fungicides, from which regression equations were generated and we calculated LD_{50}s for mycelial growth in culture medium. In this figure 2, we can clearly see the significant reduction in the diameter of the fungal colonies as the fungicidal concentrations increase in the culture medium and, depending on the fungicide used, complete inhibition of mycelial growth at 30 µg/mL.

DISCUSSION

There was a significant triple interaction between fungicidal factors, fungi and concentrations in the experiment that determined the *in vitro* LD_{50} of fungal species to fungicides, suggesting the interdependence of these factors. The effect of various fungicide concentrations was expected because of the large gradient in which the fungi grew. Regarding the fungal species and the fungicides tested, they showed variations in terms of sensitivity to fungicides, and showed variations in the inherent fungitoxicity of each fungicide. According to BAMPI et al. (2013), the differences in fungitoxicity of the same product on mycelial growth inhibition of various fungi may be related to the genetic variability, with subsequent greater or lesser sensitivity to a given fungicide.

Mycelial growth of *F. incarnatum* was efficiently restricted by the Carboxina; Tiram and Carbendazim; Tiram, with LD_{50} values below 1 µg/mL. A similar effect was observed by MARTINS (2009) on the inhibition of three isolates of *Fusarium* fungi when testing these same fungicides. The presence of phytopathogenic fungi in *Paspalum* seeds is a source of concern, because some of these fungi may reduce seed viability. Fast-growing and aggressive fungi such as *Fusarium* sp., can cause seed death even before germination (MENTEN et al., 2010). The confirmation of the efficiency of these products for the control of *Fusarium* species suggests greater safety in the management of contaminated *Paspalum* seeds. The efficiency of the aforementioned fungicides to control *Fusarium* is due in particular to Thiram, which belongs to the chemical group dimethyl dithiocarbamate. It is a contact fungicide with a broad spectrum of action. Formulated by various chemical companies, it is recommended for the treatment of seeds of various cultures against various fungi, among them *Fusarium moniliforme*, *F. avenaceum*, *F. solani* and *F. oxysporum* (BRASIL, 2019).

In a study of grasses such as sugarcane, *E. sorghinum* was among the most common fungal species associated with seeds (MARTINS et al., 2009). These authors advocated investigations to

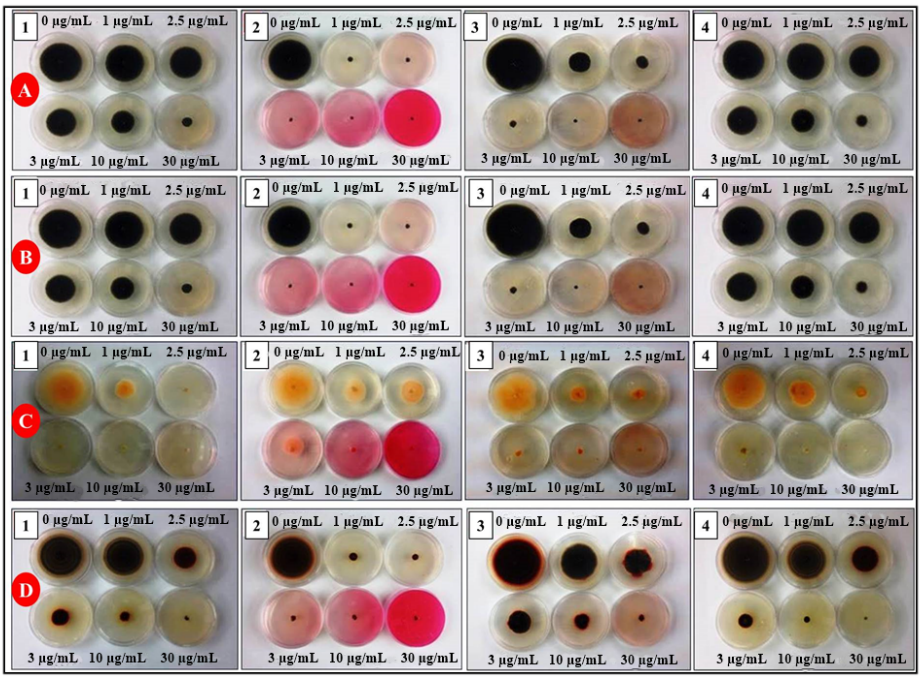

Figure 2 - Mycelial growth of *Bipolaris micropus* (A), *Curvularia geniculata* (B), *Fusarium incarnatum* (C) and *Epicoccum sorghinum* (D) on potato-sucrose-agar and streptomycin culture medium with various concentrations (µg/mL) of Carbendazim; Tiram (1), Fludioxonil; Metalaxil-M (2), Carboxina; Tiram (3) and Tiofanato-metílico (4).

establish chemical treatments aiming to reduce this pathogen in sugarcane and limiting seedling mortality rates. Of the four fungicides tested in this study, only Fludioxonil; Metalaxil-M efficiently controlled *E. sorghinum*, with LD_{50} below 1 µg/mL. In addition to *Epicoccum*, Fludioxonil; Metalaxil-M effectively controlled *Bipolaris* and *Curvularia*. MARTINS et al. (2009) also reported Fludioxonil; Metalaxil-M inhibiting 50% of mycelial growth of isolates of *Bipolaris*, *Curvularia* and *Phoma* at concentrations below 1 µg/mL. Despite the fact that we only investigated the chemical treatment of *Paspalum* seeds, our results provide useful information for controlling these pathogens, with possible application to other forage crops.

We also measured the efficacy of fungicides in control of *C. geniculata* and *B. micropus* in *Paspalum* seeds (Table 1). Two of the four fungicides tested, Carboxina; Tiram and Fludioxonil; Metalaxil-M, equally efficiently controlled these species by presenting LD_{50} values below 1 µg/mL. It is noteworthy that Carboxina; Tiram includes thiram combined with carboxin from the carboxanilide chemical group, with higher fungitoxicity for fungi of the class Basidiomycetes. Fludioxonil; Metalaxil-M contains fludioxonil, from the chemical group phenylpyrroles, in a mixture as fungicide metalaxyl from the acylalaninate chemical group, specific for Oomycetes. The probable efficacy of this control is attributable to the contact and broad-spectrum fungicides present in each of the formulations, because none of the aforementioned fungal species belongs to Basidiomycetes or Oomycetes, but rather to Ascomycetes. These pathogens are transmitted from seed to shoots and cause severe damage to leaves as patches expand and coalesce, culminating in necrotic tissues and loss of mass for grazing or hay, as well as overall reduction of plant vigor. The efficiency of chemical control over *B. micropus* is especially desirable, as its transmission capacity and high incidence in forage seeds could in part explain the increase of leaf spots, seriously compromising pasture sustainability (MALLMANN et al., 2013).

Notwithstanding the very low LD_{50} values for various fungicides tested, figure 1 also illustrates the high growth inhibition rate for some fungi at lower concentrations, particularly 1.0, 2.5 and 5.0 µg/mL. Thus, for *Fusarium incarnatum*, Carboxina;

Tiram, Tiofanato-metílico and Carbendazim; Tiram stood out, just as Fludioxonil; Metalaxil-M stood out for *Epicoccum sorghinum*. Similarly, Carboxina; Tiram and Fludioxonil; Metalaxil-M stood out for *Curvularia geniculata* and *Bipolaris micropus*. Importantly, fungicide concentrations for seed treatment were quite high. For example, when using 300 mL of Carboxina; Tiram per 100 kg of seeds and considering that this product has 50% active ingredients, the concentration used is equivalent to 1500 µg/mL. This result suggested that performance of the fungicide is expected to be even more effective in controlling seed-associated fungi than in inhibiting mycelial growth in culture medium.

Currently, there is growing concern regarding the use of isolated systemic fungicides because they are more likely to select insensitive isolates. For this reason, many of the new products on the market include two or more active ingredients, usually a systemic accompanied by a contact fungicide that generally is broad-spectrum and presents low risk for selecting insensitive isolates. Of the four products tested in this paper, three were mixtures of one systemic and one contact, and Tiofanato-metílico possesses only a single active ingredient. Tiofanato-metílico belongs to the group of benzimidazoles, which are systemic fungicides. Development of pathogen resistance to fungicides in this group has been reported by several groups (RODRIGUES et al., 2007; AVOZANI et al., 2014), suggesting that precautions should be taken when continuing to use products with this feature. In the present study, however, this product was not as efficient as the other fungicides in controlling the tested fungi, with no LD_{50} values lower than 1 µg/mL.

Carboxina; Tiram and Fludioxonil; Metalaxil-M gave LD_{50}s lower than 1 µg/mL for three of the four tested fungi, and were considered the best performing fungicides in this experiment. For this reason, they were chosen, alone or in mixture, to determine their efficiencies in controlling the same fungi previously described via seed treatment. The effectiveness of Carboxina; Tiram and Fludioxonil; Metalaxil-M in reducing and/or eradicating fungi, whether pathogenic or not, has been demonstrated by several researchers in several species (LASCA et al., 2004; PINTO, 2004; MIGLIORINI, 2012). According to GOULART (1998), the combined use of broad-spectrum fungicides has been one of the most effective strategies for controlling numerous seed-associated pathogens.

The improvement provided by the treatment of *Paspalum* seeds with the tested fungicides indirectly highlights the substantial damage caused by the presence of fungi associated with the seeds, suggesting that their health indirectly interferes with their quality standards. The seeds used in this study, which suffered high incidences of associated fungi, offered favorable conditions for the expression of beneficial fungicidal effects. These conditions are representative of the reality in which southern Brazilian farmers find themselves in the pasture seed market.

Of all the parameters evaluated in this research, the seed germination percentage showed the most significant increase. This is one of the most limiting factors in term of seed quality for the majority of currently marketed forage. Results obtained in the present study were similar to those reported by other researchers. Machado (2000) reported positive effects of Carboxina; Tiram in terms of germination percentage, emergence speed and higher seedling health. This fungicide also significantly reduced dead seeds and/or pre-emergence damping-off in peanut seeds (BITTENCOURT et al., 2007) and increased the germination percentage of rice seeds (SCHUCH et al., 2006). MIGLIORINI (2012) reported that Carboxina; Tiram and Fludioxonil; Metalaxil-M gave rise to increased percentage of canola seed germination.

The significant reduction in seed death and, in particular, the reduction in abnormal seedlings is also considerable improvements, particularly for the establishment of healthy plants for grazing. Seeds predisposed to the action of pathogens, when treated, reduce the survival capacity of phytopathogens and promote greater seed longevity, germination, vigor and plant performance. As an example, FIPKE et al. (2019) showed greater emergence of wheat plants in the soil when their seeds were treated with fungicides.

Results presented in this paper underscore the importance of adopting measures to reduce or eliminate pathogens in seeds. Seed treatment, once performed with suitable products, significantly improves seed germination and other physiological parameters, inhibiting the adverse effects attributed to fungi.

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DECLARATION OF CONFLICT OF INTEREST

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.

AUTHOR 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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