Ciência

Control of Microdochium albescens in pre-germinated and dryland rice seeds

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ABSTRACT: The fungus Microdochium albescens can interfere in the germination of seeds and in the death of rice seedlings; however, there is not technical indication for its control by seed treatment. This research evaluated the efficiency of fungicides in the treatment of pre-germinated and dryland rice seeds in the control of M. albescens. Seeds of the cultivars, Epagri 109, SCS116 Satoru, SCS121 CL and SCS122 Miura were treated with the following fungicides (g a.i./100 kg of seeds): fluazinam + thiophanate methyl (9.45 + 63), pyraclostrobin + thiophanate methyl (5 + 45), carboxin + thiram (60 + 60), metalaxyl-M + thiabendazole + fludioxonil (3 + 22.5 + 3.75), carbendazim + thiram (45 + 105), carbendazim (45). Seeds without fungicide treatment constituted the control. The treated seeds were sown in potato-sucrose-agar (PSA) culture medium and incubated at 25 ± 2 °C and photoperiod of 12 hours for 14 days. Treated seeds were also submitted to the germination test. Fluazinam + thiophanate methyl and metalaxyl-M + thiabendazole + fludioxonil showed greater control of M. albescens, not differing statistically between them, in the five cultivars and in the two cultivation systems. The tested fungicides provided seed germination above 90% in the four cultivars.

Key words: Fungicide, Oryza sativa, Seed treatment.

Controle de Microdochium albescens em sementes de arroz pré-germinado e sequeiro

RESUMO: O fungo Microdochium albescens pode interferir na germinação de sementes e na emergência de plântulas de arroz, porém não há indicação técnica para seu controle via tratamento de semente. Este trabalho teve como objetivo avaliar a eficiência de fungicidas em tratamento de sementes de arroz pré-germinado e sequeiro no controle de M. albescens. Sementes das cultivares Epagri 109, SCS116 Satoru, SCS121 CL e SCS122 Miura foram tratadas com os seguintes fungicidas (g i.a./100 kg de sementes): fluazinam + tiofanato metílico (9,45 + 63), piraclostrobina + tiofanato metílico (5 + 45), carboxina + tiram (60 + 60), metalaxil-M + tiabendazol + fludioxonil (3 + 22,5 + 3,75), carbendazim + tiram (45 + 105), carbendazim (45). Sementes sem tratamento com fungicida constituíram a testemunha. As sementes tratadas foram semeadas em meio de cultura de batata-sacarose-ágar (BSA) e incubadas a $25 \pm 2^{\circ}$ C e fotoperíodo de 12 horas por 14 dias. Sementes tratadas tratadas foram submetidas ao teste de germinação. Fluazinam + tiofanato metílico + tiabendazol + fludioxonil 4 e datat-sacarose-ágar (BSA) e incubadas a $25 \pm 2^{\circ}$ C e fotoperíodo de 12 horas por 14 dias. Sementes tratadas também foram submetidas ao teste de germinação. Fluazinam + tiofanato metílico e metalaxil-M + tiabendazol + fludioxonil apresentaram maior controle de M. albescens, não diferindo estatisticamente entre si, nas cinco cultivares e nos dois sistemas de cultivares e nos dois sistemas de cultivares. **Palavras-chaves**: Fungicida, Oryza sativa, Tratamento de ementes.

INTRODUCTION

Leaf scald, caused by the fungus *Microdochium albescens* Thüm (Syn. *Microdochium oryzae* Hashioka & Yokogi; *Gerlachia oryzae* Hashioka & Yokogi), has been reported in all rice producing regions in the world (FARR et al., 2008). Seeds are considered a source of primary inoculum (OU, 1985; WEBSTER & GUNNELL, 1992), and the transmission of the fungus in dryland cultivation occurs through infected seeds causing discoloration in the seedlings (FILIPPE et al., 2005; GUTIÉRREZ,

2008). Leaf scald reduces the number, weight and physiological quality of seeds (MOURA et al., 2014), causing up to 30% damage to yield (THOMAS, 1984). Companies seek to meet the standards of seed commercialization; and therefore, adopted measures for quality control using physiological tests, such as germination and vigor tests, and alternatives for maintaining quality through seed treatment.

Health tests for irrigated rice seeds from Santa Catarina crops, performed at the Phytopathology Laboratory of the Universitdade do Estado de Santa Catarina in the 2015/16, 2016/17 and 2017/18

Received 07.08.20 Approved 06.25.21 Returned by the author 08.31.21 CR-2020-0639.R1 Editor: Leandro Souza da Silva i harvests revealed a 100% prevalence of *M. albescens* and average incidence greater than 50% (SCHEIDT et al., 2020a) of the lots analyzed. According to the Technical Department of Cooperativa Cravil, responsible for the largest production of irrigated rice seeds in the state of Santa Catarina, the occurrence of leaf scald has increased considerably in irrigated rice crops in the pre-germinated cultivation system.

In crops such as soybeans and corn, seed treatment (ST) has been used to control fungi associated with the seed; however, in rice this practice does not occur with the same frequency, especially in the pre-germinated cultivation system, where there is no information and indications (SOSBAI, 2018).

The ST of rice aimed to control fungi by infecting and infesting seeds, soil fungi and also insects in case of the use of insecticide in addition to the fungicide, thus protecting the seedling in the early stages of the crop. Its use is essential to ensure germination, emergence, seedling formation, and consequent plant population, associated with crop yield (MENTEN & MORAES, 2010).

ST is considered one of the low-cost cultural practices in the control of pathogens in upland rice (KIMATI et al., 1997); however, there is no information on the effect of fungicides on ST to control *M. albescens* in the system cultivation of pre-germinated rice (SOSBAI, 2018). It is believed that the product is diluted in water and has reduced efficiency and / or that there is a possible elimination of pathogens in the pre-germination process and subsequent seeding in water depths in the fields. However, the fungus is transmitted from seed to seedling in this system (SCHEIDT et al., 2020a), indicating that the elimination of pathogens from seeds does not occur by immersing them in water.

This research evaluated the efficiency of fungicides in the treatment of pre-germinated and dryland rice seeds in the control of *M. albescens*.

MATERIALS AND METHODS

The studies were conducted *in vitro* at the plant pathology laboratory of the University of the State of Santa Catarina (UDESC), Lages-SC, under a completely randomized design, in a 2 x 7 x 2 trifactorial scheme (cultivation systems x seed treatment x evaluation days of the TS effect).

Seeds from cultivars Epagri 109, SCS116 Satoru, SCS121 CL and SCS122 Miura were used, produced in the Alto Vale do Itajaí in the 2017/18 harvest. Each cultivar constituted an isolated study. For the choice of fungicides, the molecules available on the market for ST in wheat and corn were considered, mainly for Fusarium control. Seven seed treatments were evaluated for each cultivar, six fungicides and one control, where: T1-fluazinam + thiophanate methyl (9.45 + 63 g a.i) (Certeza[®]); T2-pyraclostrobin + thiophanate methyl (5 + 45)g a.i) (Standak Top®); T3- carboxin + thiram (60 + 60 g a.i) (Vitavax Thiram[®]); T4- metalaxyl-M + thiabendazole + fludioxonil (3 + 22.5 + 3.75 g a.i)(Maxim Advanced®); T5- carbendazim + thiram (45 + 105 g a.i) (Derosal Plus®); T6- carbendazim (45 g a.i) (Carbendazim Nortox®) in doses of commercial producer per 100 kg of seed-1 and T7- control, untreated. The seed treatment was carried out in the laboratory, wet (2% water), using 1 kg of seeds per treatment. After the application of the products, the seeds were packed in plastic bags, for homogenization. Then, they were put to dry in the shade and packed in paper bags, until the beginning of the tests.

After being treated, 400 seeds of each treatment were separated to simulate each cultivation system. In the pre-germinated system, the seeds were previously immersed in water (1:10 ratio, seed: water) for 30 hours, with subsequent removal and maintenance of them in a plastic tray in the shadow for the same period to induce the germination process (seeds at the "needle point"), phenological stage for sowing S3 (COUNCE, 2000; SOSBAI, 2018). For the dryland system the seeds were treated and not submitted to the pre-germination process.

The treated seeds were subjected to the health test by sowing them in Petri dishes containing PSA culture medium, plus antibiotics (streptomycin sulfate, 0.05%). The dishes were incubated for seven and fourteen days in a growth chamber at a temperature of $25 \pm 2^{\circ}$ C and a photoperiod of 12 hours. The seeds under which it was possible to identify colonies and/or structures of the fungus *M. albescens* observed under a binocular loupe and/or slide under an optical microscope were considered to be infected, the final incidence of the pathogen in treatments could be determined. However, to evaluate the efficiency of fungicides in treatments, the incidences were verified at 7 and 14 days after incubation in comparison to the control (untreated).

Germination was evaluated according to the Rules for Seed Analysis (BRASIL, 2009). Four replications of 100 seeds sown on Germitest[®] paper, moistened with distilled water at three times the weight of the paper, were used for each treatment, maintained at 25°C in germination chambers. The normal seedlings were evalueted after seven and fourteen days. The variables evaluated were subjected to analysis of variance and when found interactions or simple effects significant to the means were compared by the Tukey test, with a 5% probability of error. The assumptions of normality of residues and homogeneity of variance were verified through diagnostic analysis. When necessary, transformations to arc sen $\sqrt{(x / 100)}$ were performed to meet the assumptions. Statistical analyzes were performed using the statistical programs R software, version 3.4.1 (R CORE TEAM, 2017).

RESULTS AND DISCUSSIONS

All cultivars showed germination higher than 80% in both treatments, which is the minimum required for the commercialization of rice seeds in Brazil (BRASIL, 2013). No ST caused reduction in germination compared to the control, demonstrating that there was no phytotoxicity in the doses used (Table 1). Phytotoxicity was also not observed by LOBO (2008) when using TS with carboxin + thiram (60 + 60g a.i), tricyclazole (225 g a.i), azoxystrobin (100g a.i) and pyroquilon (400 g a.i). Fluazinam + thiophanate methyl, carboxin + thiram, metalaxyl-M + thiabendazole + fludioxonil, carbendazim + thiram and carbendazim maintained the best germination percentages for the cultivar SCS121 CL; however, piraclostrobin + thiophanate methyl and carboxin + thiram did not show any difference (Table 1).

For cultivar SCS122 Miura, carboxin + thiram, metalaxyl-M + thiabendazole + fludioxonil and carbendazim + thiram present bed the highest and pyraclostrobin + thiophanate methyl the lowest germination percentage, not differing from the control (Table 1). According to PRABHU & VIEIRA (1989) rice seeds, treated with carboxin + thiram showed maintenance of germination and better seedling health. The same was observed in cotton seeds (FARIA et al., 2003), and sorghum (NETTO et al., 1997) using the same assets. LENZ (2008), using seed treatment with carboxin + thiram observed that it did not interfere with the germination, emergence and speed of emergence index of rice seedlings. For cultivars SCS122 Miura and Epagri 109, all treatments had the

Treatment	SCS121 CL			SCS122 Miura				
	Normal	Abnormal	Dead	Normal	Abnormal	Dead		
T1	90 ab	4 ab	6 ab	91 b	4 c	5 b		
T2	88 bc	6 ab	6 ab	85 c	10 a	5 b		
Т3	90 ab	3 bc	7 a	94 ab	3 c	3 b		
T4	92 a	1 c	7 a	95 a	0 d	5 b		
T5	92 a	3 bc	5 ab	92 ab	4 c	4 b		
T6	91 ab	5 ab	4 b	91 b	5 bc	4 b		
Τ7	86 c	7 a	7 a	83 c	8 ab	9 a		
Average*	90 A	4 B	6 B	90A	5B	5B		
	EPAGRI 109				SCS116 Satoru			
T1	92 bc	6 a	2 b	92 bc	4 ab	4 ab		
T2	91 c	5 ab	4 b	88 cd	7 a	5 ab		
Т3	94 ab	3 bc	3 b	94 b	3 b	3 b		
T4	96 a	1 c	3 b	97 a	1 c	2 b		
T5	92 bc	5 ab	3 b	92 bc	4 ab	4 ab		
T6	93 bc	3 bc	4 b	93 b	4 ab	3 ab		
Τ7	86 d	6 a	8 a	88 d	6 a	6 a		
Average*	92 A	4 B	4 B	92 A	4 B	4 B		
CV (%)		16.4			16.4			

Table 1 - Percentage of germination	of rice seeds of cultiv	ars SCS121 CL	, SCS122 CL,	Epagri 109	and SCS116 Satoru	, submitted to
different treatments.						

T1) fluazinam + thiophanate methyl; T2) pyraclostrobin + thiophanate methyl; T3) carboxin + thiram; T4) metalaxyl-M + thiabendazole + fludioxonil; T5) carbendazim + thiram; T6) carbendazim; T7) control. * Averages followed by the same letter in the columns do not differ by Tukey's test (P < 0.05).

lowest percentage of seedlings death compared to the control. The highest percentage of abnormal seedlings was reported in the cultivar SCS122 Miura with the treatment pyraclostrobin + thiophanate methyl, where it did not differ from the control.

In the cultivars Epagri 109 and SCS116 Satoru the ST with metalaxyl-M + thiabendazole + fludioxonil stood out with the highest percentage of germination; however, in the cultivar Epagri 109, fluazinam + thiophanate methyl, pyraclostrobin + thiophanate methyl, carbendazim + thiram and carbendazim did not have statistical differences. To cultivate SCS116 Satoru, pyraclostrobin + thiophanate methyl obtained the lowest germination values, not differing from the control (Table 1).

Regardless of the cultivar, the fungicide carbendazim had the same incidence of the fungus and control at 7 and 14 days. This can be explained because at seven days all the seeds of the Petri dish had some fungus present in them, mainly fungi of the family Dematiaceae, in which carbendazim has no fungitoxic action on them. The incidence of *M. albescens* present in the seeds used in the experiment was enough to discriminate the efficiency of the fungicides (Table 2). All treatments tested obtained percentages of control of the fungus associated with the seeds; however, there were significant differences in control according to the active ingredient (Table 3).

Regardless of the cultivar, the active ingredients fluazinam + thiophanate methyl and metalaxyl-M + thiabendazole + fludioxonil did not present statistical differences for the variables days and cultivation systems, obtaining the lowest percentages of incidence (Table 2) and greater control of the fungus (Table 3), proving the greater efficiency and persistence of these products in relation to the others. However, treatments with pyraclostrobin+thiophanate methyl and carbendazim showed a higher percentage of fungi incidence in the seeds, characterizing less control, for both systems. According to GARCIA JUNIOR (2008), the fungicides thiabendazole and thiophanate methyl were superior to triflumizole and triadimenol in the treatment of wheat seeds to

	SCS121 CL				Epagri 109					
Treat	Dr	yland	Pre-germinated		Dryland		Pre-germinated			
	7	14	7	14	7	14	7	14		
T1	5.5 dAI	6.0 dAI	5.0 dAI	6.5 eAI	4.5 cAI	5.5 eAI	5.0 dAI	5.5 eAI		
T2	25.5 cBI	32.0 bAI	55.0 bBV	65.0 aAV	20.0 bBI	26.0 cAI	40.0 bAV	45.5 bAV		
Т3	6.0 dBI	22.0 cAI	20.0 cBV	38.0 cAV	7.5 cBI	16.5 dAI	15.0 cBV	31.5 cAV		
T4	6.5 dBI	11.0 dAI	7.5 dAI	10.0 eAI	8.0 cAI	8.5 deAI	9.0 cdAI	12.0 deAI		
T5	5.5 dBI	21.5 cAI	17.5 cAV	22.0 dAI	8.0 cAI	12.5 deAI	11.5 cdAI	15.5 dAI		
T6	36.0 bAI	36.0 bAI	59.5 bAV	59.5 bAV	27.0 bAI	27.0 bAI	51.5 bAV	51.5 bAV		
T7	52.0 aAI	52.0 aAI	75.0 aAV	75.0 aAV	45.0 aAI	45.0 aAI	60.0 aAV	60.0 aAV		
Average	19.6 BI	27.4 AI	34.2 BV	42.4 AV	17.3 BI	21.4 AI	26.8 BV	31.6 AV		
C.V. (%)	10.73					16.87				
	SCS116 Satoru				SCS122 Miura					
T1	4.5 cAI	6.0 eAI	5.5 dAI	6.5 dAI	5.0 dAI	5.5 dAI	5.0 eAI	6.0 eAI		
T2	28.5 bBI	35.0 bcAI	38.0 cBV	43.0 bAI	22.0 cBI	37.0 bAI	50.0 bBV	57.5 bAV		
Т3	5.5 cBI	18.0 cAI	9.5 dBI	22.0 cAV	6.0 dBI	23.0 cAI	28.5 cBV	37.0 cAI		
T4	7.5 cAI	9.0 deAI	8.5 dAI	10.5 dAI	7.0 dAI	8.5 dAI	5.0 eAI	7.0 eAI		
T5	9.5 cBI	15.0 dAI	13.0 dBI	19.0 cAI	7.0 dBI	20.0 cAI	16.5 dBV	24.0 dAI		
T6	28.0 bAI	28.0 cAI	58.0 bAV	58.0 aAV	32.0 bAI	32.0 bAI	50.0 bAV	50.0 bcAV		
T7	47.0 aAI	47.0 aAI	66.0 aAV	66.0 aAV	62.0 aAI	62.0 aAI	78.0 aAV	78.0 aAV		
Average	18.6 BI	24.8 AI	27.6 BV	33.3 AV	19.7 BI	29.6 AI	32.9 BV	37.6 AV		
C.V (%)	14.03				16.25					

Table 2 - Incidence of *Microdochium albescens* (%) with different treatment of pre-germinated and dryland rice seeds of cultivars SCS121 CL, Epagri 109, SCS116 Satoru and SCS122 Miura.

T1) fluazinam + thiophanate methyl; T2) pyraclostrobin + thiophanate methyl; T3) carboxin + thiram; T4) metalaxyl-M + thiabendazole + fludioxonil; T5) carbendazim + thiram; T6) carbendazim; T7) control. * Means followed by the same letter, lower case in the column, upper case in the line and Roman numbers between systems, do not differ by the Tukey test (P < 0.05).

SCS121 CL					Epagri 109					
Treat	Dryland		Pre-germinated		Dryland		Pre-germinated			
	7	14	7	14	7	14	7	14		
T1	89.4	88.5	93.3	91.3	90.0	87.8	91.0	90.8		
T2	50.9	38.0	26.7	13.3	55.5	42.2	33.3	24.2		
Т3	88.5	57.0	73.3	50.7	83.3	63.3	75.0	47.5		
T4	87.5	78.8	90.0	86.7	82.2	81.1	85.0	80.0		
Т5	89.4	58.6	76.6	70.7	82.2	72.2	80.0	74.2		
Т6	30.8	30.8	20.7	20.7	40.0	40.0	14.2	14.2		
Average (%)	72.8	58.6	63.4	55.6	72.2	64.4	63.1	55.2		
	SCS116 Satoru					SCS122 Miura				
T1	90.4	87.2	91.7	90.2	91.0	91.1	93.0	92.3		
T2	39.4	25.5	42.4	34.8	64.5	40.3	35.8	26.3		
Т3	88.3	61.0	85.6	66.0	90.3	62.9	63.5	52.6		
T4	84.0	80.8	87.1	84.1	88.7	86.3	93.6	91.0		
Т5	79.7	68.1	80.3	71.2	88.7	67.7	78.8	69.2		
T6	40.4	40.4	12.12	12.12	48.4	48.4	35.9	35.9		
Average (%)	70.4	60.5	66.5	59.7	78.6	66.1	66.8	61.2		

Table 3 - Percentage of control of *Microdochium albescens* with different treatment of pre-germinated and dryland rice seeds of cultivars SCS121 CL, Epagri 109, SCS116 Satoru and SCS122 Miura.

T1) fluazinam + thiophanate methyl; T2) pyraclostrobin + thiophanate methyl; T3) carboxin + thiram; T4) metalaxyl-M + thiabendazole + fludioxonil; T5) carbendazim + thiram; T6) carbendazim; T7) control.

control *Fusarium graminearum Schwabe*. According to AGOSTINETTO et al. (2018) fungicide mixtures with the presence of carbendazim and / or carboxin + thiram had greater control of *F. graminearum* in barley seeds. Likewise, HENNING et al. (2009), using black oat seed treatment, determined that the carbendazim + thiram fungicides removed the fungi of the *Fusarium* genus from the seeds.

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The active ingredients carboxin + thiram and carbendazim + thiram were more efficient in the control up to seven days, in the dryland cultivation system compared to the pre-germinated system, but even in dryland, they were not persistent until the 14 days (Table 2 and 3). LOBO (2008), when evaluating the phytosanitary quality of rice seeds under the chemical treatment with carboxin + thiram (60 + 60 g a.i) and tricyclazole (225 g a.i), observed a 100% control for *M. albescens* when it affected 22.5 % of seeds (control). The average incidence of *M. albescens* in this study is high (greater than or equal to 45%), which reduced the possibility of controls occurring by 100%.

The average incidence of *M. albescens* in the control treatment was higher in the pre-germinated cultivation system, regardless of the cultivar. This can be explained by placing the seeds to pre-germinate,

the embryo, initially protected and possibly infected, is externalized. In this way, there is an increase in the probability of detection in relation to dryland seeds, since the fungus inside the seed is exteriorized close to the root (Tables 2).

There is no information for the chemical treatment of irrigated rice seeds in the pre-germinated cultivation system. Thus, the information obtained in this study is of great importance for the management of M. albescens in this system. It is recommended to evaluate active ingredients in isolation as well as different doses in order to give greater support to this research.

CONCLUSIONS

Seed treatments with fluazinam + thiophanate methyl, carboxin + thiram, metalaxyl-M + thiabendazole + fludioxonil, carbendazim + thiram show control over 70% of the fungus *M. albescens* up to seven days after sowing pre-germinated rice seeds and dry in PSA+A culture medium.

The active ingredients fluazinam + thiophanate methyl and metalaxyl-M + thiabendazole + fludioxonil show greater efficiency and persistence at 7 and 14 days after sowing pre-germinated and dryland rice seeds in PSA+A culture medium compared to pyraclostrobin + thiophanate methyl, carboxin + thiram, carbendazim + thiram and carbendazim with control over 80%.

Seed treatment is a crucial tool in integrated disease management for both cropping systems.

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DECLARATION OF CONFLICT OF INTEREST

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

AUTHORS' CONTRIBUTIONS

BTS, RTC and EZF conceived and designed experiments. BTS, FCM and MMDP performed the experiments and carried out the lab analysis. BTS, JB prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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