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ARTICLE

Chemical seed treatments for the control of cotton seedling damping-off caused by *Rhizoctonia solani* under greenhouse conditions

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ABSTRACT: The aim of this study was to determine the effectiveness of cotton seed treatment with fungicides in the control of seedling damping-off caused by *Rhizoctonia solani*, under greenhouse conditions. Treated and untreated delinted seeds of the cultivar DeltaOpal were sowed in sand contained in plastic trays, placed in individual and equidistant wells, 3 cm deep. Into each plastic tray were put 3.0 g of the pathogen inoculum. The inoculation of *R. solani* AG-4 was done by the homogeneous distribution of the fungus inoculum onto the substrate. The fungus was grown for 35 days on autoclaved oat seeds and then ground to powder using a mill (1 mm). Fungicide seed treatments had a significant effect ($P \leq 0.05$) on initial and final seedling emergence, pre and post-emergence damping-off, injured seedlings and disease index according to McKinney formula (lesion severity). The best results were obtained by fludioxonil + metalaxyl-M + azoxystrobin, fipronil + pyraclostrobin + methyl-thiophanate, carbendazim + thiram + pencycuron + baytan and carbendazim + thiram + pencycuron + baytan + procymidone. Pearson correlation analysis indicated that injured seedlings and disease index were positively correlated ($R = 0.95$). This means that the greater the number of injured seedlings, the greater the disease index and the less efficient the fungicide. The fungicides used in the present study did not have any phytotoxic effect on cotton seedlings.

Index terms: *Gossypium hirsutum* L., fungicides, inoculation, soil-borne disease, soil-borne fungus.

RESUMO: O objetivo deste trabalho foi avaliar a eficácia do tratamento de sementes de algodoeiro com fungicidas no controle do tombamento de plântulas causado por *Rhizoctonia solani*, sob condições de casa de vegetação. Sementes de algodão deslintadas da cultivar DeltaOpal, tratadas e não tratadas, foram semeadas em areia contida em bandejas plásticas, dispostas em orifícios individuais, equidistantes e a 3 cm de profundidade. A inoculação com *R. solani* foi feita pela distribuição homogênea do inóculo do fungo na superfície do substrato. O fungo foi cultivado por 35 dias em sementes de aveia preta autoclavadas e trituradas em moinho (1mm). Foi observado efeito significativo ($P \leq 0.05$) do tratamento de sementes com fungicidas em relação a emergência inicial e final de plântulas, tombamento de pré e pós emergência, plântulas lesionadas e o índice de doença (severidade) de acordo com a fórmula de McKinney. Os melhores resultados foram obtidos com fludioxonil + metalaxyl-M + azoxystrobin, fipronil + pyraclostrobin + methyl-thiophanate, carbendazim + thiram + pencycuron + baytan e carbendazim + thiram + pencycuron + baytan + procymidone. A análise da correlação de Pearson indicou correlação positiva entre plântulas lesionadas e índice de doença ($R = 0,95$). Isso significa que quanto maior o número de plântulas lesionadas maior será o índice de doença e menos eficiente o fungicida. Os fungicidas usados nesse estudo não apresentaram qualquer tipo de efeito fitotóxico nas plântulas de algodão.

Termos para indexação: *Gossypium hirsutum* L., fungicidas, inoculação, doença de solo, fungo de solo.

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INTRODUCTION

Seedling diseases of cotton, caused by a complex of soil-borne fungi, are a very serious problem all over the world where cotton is grown (Paplomatas and Elena, 1998; Zaki et al., 1998; Zeun et al., 2013). This seedling disease complex can vary greatly, in terms of disease severity and fungi involved, from field to field, and from year to year, depending upon several cultural and environmental conditions.

According to Paula-Júnior et al. (2001) and Goulart (2021a), in Brazil, the most important soil-borne pathogen involved in this complex of seedling diseases is *Rhizoctonia solani* Kühn, anastomosis group AG-4 [teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk]. This fungus is the major cause of seedling damping-off affecting cotton crops and is widely distributed particularly in the cerrado regions of the states of Mato Grosso, Mato Grosso do Sul, Minas Gerais, Goiás and Bahia (Goulart, 2008a; 2008b, 2016). This disease has frequently caused damage in the early stages of crop establishment by reducing plant population, as stated by Rizvi and Yang (1996), Goulart (2018) and Dorrance et al. (2003).

Economic losses caused by root pathogens have been reported in the global agriculture. According to McLean and Gazaway (2000), in the USA, in 1995, the reduction of cotton yield due to early-season diseases has been estimated in 180,000 tons, representing loss of 2.8% per year. When only the fungus *R. solani* is considered, data obtained by the Cotton Disease Council in the last 50 years, showed that significant losses in the cotton crop yield due to incidence of damping-off caused by this pathogen ranged from 2% to 3% (Sales-Junior et al., 2005). This survey has not been done yet in Brazil.

Control of cotton seedling damping-off caused by *R. solani* can be achieved with the establishment of integrated management programs, which have fungicide seed treatment as the major tool (McLean and Gazaway, 2000; Howell, 2007; Goulart, 2021b). According to Garber et al. (1980), Davis et al. (1997) and Goulart (2021b), this control method is the most efficient and economical option to minimize the negative effects of this disease, protecting the seeds and the emerging seedling in order to ensure a good stand.

Cotton seeds treatment with fungicides has become an essential practice and play an important role in damping-off management. Due to its benefits, it has been used on a large scale by growers in Brazil. In relation to the cost, this practice represents only 0.22% of the production cost of 1ha of cotton crop (IFAG, 2022).

Currently in Brazil, according to Agrofit/MAPA, 20 commercial fungicide formulations are labeled and recommended for cotton seeds treatment. With the launch of new products in the market to control cotton seedling damping-off caused by *R. solani*, tests of these new active ingredients have become necessary and should be continuous, as well as the re-evaluation of fungicides already recommended.

The aim of this study was to determine the effectiveness of cotton seed treatment with fungicides in the control of seedling damping-off caused by *Rhizoctonia solani*, under greenhouse conditions.

MATERIAL AND METHODS

This experiment was carried out under greenhouse conditions in Dourados, Mato Grosso do Sul State, Brazil.

Production of the R. solani inoculum: the pathogen inoculum was composed of black oat seeds colonized by *R. solani* AG-4, isolated from infected cotton seedlings. *R. solani* AG-4 anastomosis group was used in this study because this group is responsible for causing damping-off disease on cotton seedlings which has been shown in the previous research studies. The fungus was cultivated in potato-dextrose-agar medium (PDA) for 10 days at 25 °C. After this period, 5mm diameter mycelial-agar disks were transferred from the margin of growing colonies to Erlenmeyer flasks containing autoclaved substrate composed of 2 kg of black oat seeds and ½ liter of water. After 35 days of incubation in BOD chamber under 22 °C with alternating 12 h period of darkness and fluorescent white light, the oat seeds colonized by *R. solani* were dried on trays for 10 days and then ground to powder using a mill (1 mm).

Seed delinting process: cotton seeds (cultivar DeltaOpal) used in this work were chemically delinted with sulfuric acid (in the proportion of 7 kg of seeds per liter of acid) and neutralized with 2% calcium carbonate.

Chemical seeds treatment: the quantity of 500 g of seed was used for each one of the different treatments. Seed treatment was made in polyethylene bags. The products were added to the seeds with the aid of graduated syringes and the bags were vigorously shaken for homogeneous distribution of the spray solution over the seeds.

Growing on test: treated and untreated cotton acid-delinted seeds were sowed in autoclaved sand contained in plastic trays (56 x 35 x 10 cm), by placing in equidistant 3-cm-deep wells. The cotton sowing density was 200 seeds.plastic tray^{-1}. A pre-established amount of the pathogen inoculum was mixed in 1.0 kg of sand for the tests. Before closing the wells, the inoculation with *R. solani* was done by the homogeneous distribution onto the substrate. Inoculum densities were adjusted to 3.0 g.plastic tray^{-1}, based on its pathogenicity. These inoculum quantities were adjusted to provide a maximum of 50% of cotton seedling damping-off, according to the methodology proposed by Weinhold (1977) and Wang and Davis (1997). Seedling emergence, pre- and post-emergence damping-off and injured seedlings were evaluated, based on the development of symptoms as well as the seedling survival. Damping-off was daily evaluated from the seventh day after sowing. Plastic trays were maintained in greenhouse for 21 days and watered daily.

Disease index: The results obtained were also used to calculate the disease index (severity) in the remaining seedlings. These disease indexes were taken for the roots and hypocotyls of each seedling sampled. For each one, a score was given according to lesion severity of *R. solani*, using the rating scale for assessment of severity of *R. solani* lesions on cotton, soybean and common beans seedlings, proposed by Goulart (2018), as follows: score 0, healthy seedlings (no visible symptom); score 1, light intensity lesion (only one lesion in the hypocotyl, necrotic, reddish-brown colour, which may be circular or elongated, small (less than or equal to 0.5 cm), superficial and without constriction); score 2, moderate intensity lesion (elongated and necrotic lesion, reddish-brown colour, in the root or in the hypocotyls, measuring between 0.5 cm and 2.0 cm, superficial and without constriction); score 3, severe intensity lesion (elongated, necrotic and extensive lesion (greater than 2.0 cm), reddish-brown colour, covering the hypocotyl and the root system, with constriction). The disease index (DI) was calculated according to McKinney (1923), using this equation, as follows:

$$ID = \sum \frac{f.n.}{F.N}.100$$

ID (%) = disease index

f = score assigned to each seedling

n = number of seedlings with this score

F = total number of seedlings evaluated

N = greater score

Confirmation of the pathogen's identity: for this purpose, the injured cotton seedlings were collected and taken to the laboratory for analysis. Fragments of hypocotyl from these seedlings were incubated in humid chamber. Five days after incubation, the fragments were evaluated and the presence of the *R. solani* was confirmed.

Phytotoxic symptoms: possible phytotoxic effects on cotton seedlings due to seed treatment with fungicides were evaluated such as: slow seedling emergence, low percentage of seedling emergence, hypocotyl thickening and shortening with reduced seedling size and atrophy of the root system as well as presence of dark green and coriaceous cotyledonary leaves.

Statistical analysis: the complete randomized block design was used, with four replications and nine treatments. The experimental plot consisted of one plastic tray containing 200 seeds. The data of percentage were transformed in arc sen √x/100. Duncan (P = 0.05) was the test used. All analyses were conducted in ASSISTAT, according to Silva and Azevedo (2009). For purposes of comparison, two control treatments were used, one inoculated and other non-inoculated.

To ensure that the results show only and exclusively the effect of the pathogen *R. solani* on emergence and damping-off, seeds free of any species of fungus that could interfere in the evaluations (choice based on results from several seed health tests) were used.

The fungicide mixture used in the cotton seed treatments in this research were commercially available for seed treatments, registered and recommended by the *Ministério da Agricultura Pecuária e Abastecimento* (MAPA), for the control of *R. solani* and applied at the recommended rates (Table 1).

RESULTS AND DISCUSSION

Fungicide seed treatments had a significant effect ($P \leq 0.05$) on initial and final seedling emergence, pre and post-emergence damping-off, injured seedlings and disease index (lesion severity), in relation to nontreated controls, indicating that they all provided some level of protection (Tables 2, 3 and 4).

Overall, the best results were obtained by fludioxonil + metalaxyl-M + azoxystrobin, fipronil + pyraclostrobin + methyl-thiophanate, carbendazim + thiram + pencycuron + baytan and carbendazim + thiram + pencycuron + baytan + procymidone. These fungicide combinations significantly increased the percentage of emergence (Table 2) and the control of *R. solani* by reducing the damping-off (Table 3), injured seedlings and disease index in cotton seedlings (Table 4). On the other hand, the less efficient treatments were methyl-thiophanate + fluazinam, carboxin+thiram and procymidone. This finding confirms the importance of treating the seeds with fungicides for the integrated management of diseases, significantly contributing to the control of *R. solani* damping-off in cotton seedlings, which corroborates the results obtained by Garber et al. (1980), Davis et al. (1997), Paplomatas and Elena (1998), Chitarra et al. (2009), and Goulart (2008a, 2008b, 2016, 2021a). It is worth pointing out that, in general, the best results were obtained with the premixes fungicide formulations compared to the solely fungicides. The combined use of fungicides with different modes of action has been an effective strategy to control a large number of pathogens (seed-borne and/or soil-borne). Their broad-spectrum mode of action also avoids, to a large extent, the emergence of resistant populations among pathogens. In this way, the use of fungicide mixtures guarantees an ideal plant stand, in the most varied situations. It is important to emphasize that the efficacy of fungicides as well as the benefits of seed treatments for the control of cotton damping-off is influenced by factors such as soil inoculum density and also by interactions with other fungi (Garber et al., 1980; Davis et al., 1997; Goulart, 2006), susceptibility of cotton cultivars (Wang and Davis, 1997; Goulart, 2016) and previous crops to cotton (Paula-Junior et al., 2001; Goulart, 2021b). However, it should be considered that, so far, there is no evidence that the use of fungicides in seed treatment with specific action against *R. solani* can be dispensed in areas with a history of occurrence of this pathogen.

Table 1. Treatments (fungicide active ingredients and untreated control) and doses used in the greenhouse trials.

Treatments	Dose (mL. of a.i. 100 kg^{-1} of seeds)
Fludioxonil + metalaxil-M + azoxyxtrobin	5 + 15 + 30
Fipronil + pyraclostrobin + methyl-thiophanate	50 + 5 + 45
Methyl-thiophanate + fluazinam	150 + 150
Carboxin + thiram	187.5 + 187.5
Procymidone	150
Carbendazim + thiram + pencycuron + baytan	70 + 30 + 100 + 100
Carbendazim + thiram + pencycuron + baytan + procymidone	70 + 30 + 100 + 100 +150
Non-Inoculated control	-
Inoculated control	-

Table 2. Initial and final seedling emergence according to fungicide seed dressing.

Treatments	Dose (mL. of a.i. 100 kg^{-1} of seeds)	Initial seedling emergence (%)	Final seedling emergence (%)
Fludioxonil+metalaxil-M + azoxyxtrobin	5 + 15 + 30	81.0 a	77.0 a
Fipronil+pyraclostrobin + methyl-thiophanate	50 + 5 + 45	79.0 a	72.5 b
Methyl-thiophanate + fluazinam	150 + 150	63.5 d	40.0 f
Carboxin + thiram	187.5 + 187.5	67.0 c	44.0 e
Procymidone	150	50.0 e	26.5 g
Carbendazim + thiram + pencycuron + baytan	70 + 30 + 100 + 100	73.0 b	67.0 c
Carbendazim + thiram + pencycuron + baytan + procymidone	70 + 30 + 100 + 100 + 150	75.5 b	68.5 c
Non-Inoculated control	-	63.5 d	63.5 d
Inoculated control	-	47.5 e	22.5 h
Average		66.67	53.50
CV (%)		8.82	10.56

Mean values followed by the same lowercase letter in the column do not differ statistically by the Duncan´s test (P = 0.05). CV: Coefficient of variation.

Table 3. Seedling damping-off (pre and pos emergence) according to fungicide seed dressing.

Treatments	Dose (mL. of a.i. 100 kg^{-1} of seeds)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)
Fludioxonil +metalaxil-M + azoxyxtrobin	5 + 15 + 30	3.5 e	8.8 e
Fipronil + pyraclostrobin + methyl-thiophanate	50 + 5 + 45	5.5 e	8.6 e
Methyl-thiophanate + fluazinam	150 + 150	12.7 c	29.1 b
Carboxin + thiram	187.5 + 187.5	9.0 d	17.1 d
Procymidone	150	18.5 b	23.0 c
Carbendazim + thiram + pencycuron + baytan	70 + 30 + 100 + 100	4.0 e	8.2 e
Carbendazim + thiram + pencycuron + baytan + procymidone	70 + 30 + 100 + 100 + 150	5.0 e	8.5 e
Non-inoculated control	-	0.0 f	0.0 f
Inoculated control	-	26.8 a	40.5 a
Average		9.44	15.97
CV (%)		15.64	12.94

Mean values followed by the same lowercase letter in the column do not differ statistically by the Duncan´s test (P = 0.05). CV: Coefficient of variation.

According to Gisi et al. (1985) and Gisi (1996), synergy is a frequent phenomenon in fungicide mixtures. Its magnitude depends on the ratio of the components in the mixture and their modes and mechanisms of action. In such a context, the best results obtained in this experiment, by carbendazim + thiram + pencycuron + baytan + procymidone compared with carbendazim + thiram + pencycuron + baytan, in relation to injured seedlings and disease index (lesion severity), suggest synergistic interactions of procymidone with these fungicides, which corroborates the results obtained by these previously mentioned authors. However, it is worth mentioning that this possible synergistic effect was not observed for seedling emergence and seedling damping-off.

The benefits of fungicide seed treatments on cotton stand establishment due to the *R. solani* control have been previously documented. Our findings are in general agreement with previous studies developed by Howel et al. (1997), Chitarra et al. (2009), Wang and Davis (1997), Davis et al. (1997), Menten and Paradela (1996) and Goulart (2008a, 2008b, 2016, 2021a), which reported that cotton seed treatment with broad-spectrum fungicides combinations resulted in higher plant stands and efficient control of soybean seedling damping-off caused by *R. solani* than nontreated seeds. Bradley (2008) and Urrea et al. (2013) reported similar results in experiments conducted on soybean. The fungus *R. solani* can cause pre and post-emergence damping-off, which was observed under the conditions of the present trial. The evaluation of the final seedling emergence percentage reflects the efficiency of the fungicides in protecting seedlings against *R. solani* infection, as well as the capacity to maintain the stand in order to avoid post-emergence damping-off caused by this pathogen. The drastic effect of the pathogen can clearly be observed when comparing the results obtained in the control treatments with and without inoculation.

It is worth mentioning that, despite some level of protection has been achieved, high percentages of injured seedlings were also observed (Table 4), leading to significant levels of symptom severity (disease index). Pearson correlation analysis indicated that injured seedlings and disease index were positively correlated (R = 0.95). It means that the greater the number of injured seedlings, especially in level 3 (severe intensity lesion) of the rating scale proposed by Goulart (2018), the greater the disease index and the less efficient the fungicide. The use of diagrammatic scale or rating scales, as a tool to help in the quantification of the severity of a particular disease, is more common for foliar diseases, as stated by Godoy et al. (2006) for soybean rust, Aquino et al. (2008) for areolate mildew on cotton, Lenz et al. (2009) for Isariopsis leaf spot on grapevines and Malagi et al. (2011) for white spot on corn. In relation to soilborne fungi, and specifically regarding to the pathosystem cotton x *R. solani* damping-off, the use of this scale in our experiment made it possible to calculate the disease index (severity), in the remaining seedlings, according to McKinney formula, an evaluation not previously used in cotton seed treatment experiments with fungicides.

Diseases caused by *R. solani* are monocyclic, without secondary inoculum production. That is to say that there is no plant-to-plant transmission and the total inoculum comes from the soil. In this context, the later the disease

Table 4. Injured seedlings and disease index according to fungicide seed dressing.

Tratamentos	Dose (mL. of a.i. 100 kg^{-1} of seeds)	Injured seedlings (%)	Disease index (lesion severity) (%)
Fludioxonil + metalaxil-M + azoxyxtrobin	5 + 15 + 30	10.8 f	5.8 e
Fipronil + pyraclostrobin + methyl-thiophanate	50 + 5 + 45	13.0 e	7.9 d
Methyl-thiophanate + fluazinam	150 + 150	28.5 b	28.7 b
Carboxin + thiram	187.5 + 187.5	22.1 c	24.6 c
Procymidone	150	30.7 b	28.6 b
Carbendazim + thiram + pencycuron + baytan	70 + 30 + 100 + 100	16.3 d	8.8 d
Carbendazim + thiram + pencycuron + baytan + procymidone	70 + 30 + 100 + 100 + 150	14.0 e	6.1 e
Non-inoculated control	-	0.0 g	0.0 f
Inoculated control	-	50.1 a	38.8 a
Average		20.61	16.59
CV (%)		19.43	16.62

Mean values followed by the same lowercase letter in the column do not differ statistically by the Duncan´s test (P = 0.05). CV: Coefficient of variation.

appear, the smaller the damage caused by it. Thus, to achieve such a control level, a desirable fungicide for cotton seed treatment should have higher systemicity in terms of protection (long-lasting effect – more than 15 days). Considering that the currently available fungicides for *R. solani* control do not present this feature, new efficient products are needed to fill this gap.

According to Rothrock et al. (2012), comparisons of seed treatments in the field are often inconsistent, in part due to the low inoculum density of soil-borne pathogens. In this context, Paplomatas and Elena (1998) and Kouyeas and Davatzi-Helena (1980) pointed out that evaluating the effectiveness of cotton seed protectant fungicides in the field is a long and laborious process that does not always guarantee reproducible results, since the pathogen structure in the soil is practically unknown and varies with time. Another approach to obtain the efficiency of seed-treatment fungicides is to evaluate their efficacy under controlled conditions against specific pathogenic fungi. This method is a useful indicator for predicting the effectiveness of these products under field conditions. Several authors have developed research of this nature under greenhouse conditions (Weinhold, 1977; Menten and Paradela, 1996; Wang and Davis, 1997; Goulart, 2006; 2008a; 2016), using the same methodology of substrate inoculation with *R. solani.* Thus, the present results, in addition to those previously mentioned, demonstrate the feasibility of this inoculation method to evaluate the efficiency of cotton seed treatment with fungicides in the control of this pathogen under greenhouse conditions.

The obtained results demonstrated the phytocompatibility of all fungicides used with cotton plants and no symptoms were observed on the seedlings that would reveal the presence of phytotoxic effects from the use of these products.

CONCLUSIONS

Cotton seed treatment with fungicides was efficient in the control of seedling damping-off caused by *R. solani*. The fungicides used in the present study did not have any phytotoxic effect on cotton seedlings. The inoculation method used proved to be feasible for studies on seed treatment with fungicides under controlled conditions.

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