

Evaluation of fungicide efficacy and agronomic viability for controlling *Rhizoctonia solani*-induced damping-off in soybean

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Abstract

This study aimed to evaluate the agronomic efficacy of different fungicides applied via seed treatment for the control of *Rhizoctonia solani* AG-4 in soybean (*Glycine max* (L.) Merrill). The experiment was carried out in a greenhouse in Rio Verde, GO, Brazil, during the 2023/2024 growing season, using the Bônus 8579RSF IPRO cultivar previously inoculated with the pathogen. Nine treatments, including controls, were tested at the manufacturers' recommended doses. The evaluated parameters included emergence, vigor, shoot height, root length, pre- and post-emergence damping-off, disease index, and product efficacy. The experimental design was completely randomized with four replications. Results showed that the treatments Fludioxonil + Metalaxyl-M + Thiabendazole and Thiophanate-methyl + Fluazinam exhibited the highest control efficacy, reducing the disease index by 73.79% and 58.39%, respectively. It is concluded that seed treatment is an effective and viable strategy for the preventive management of *Rhizoctonia solani* during the initial development stages of soybean cultivation.

Keywords: soybean, fungicidal activity, *Rhizoctonia*, Thiabendazole, grain yield.

Avaliação da eficácia e da viabilidade agrônômica de fungicidas no controle do tombamento por *Rhizoctonia solani* em soja

Resumo

Este trabalho teve como objetivo avaliar a eficácia agrônômica de diferentes fungicidas aplicados via tratamento de sementes no controle de *Rhizoctonia solani* AG-4 na cultura da soja (*Glycine max* (L.) Merrill). O experimento foi conduzido em casa de vegetação no município de Rio Verde, GO, Brasil, durante a safra 2023/2024, utilizando a cultivar Bônus 8579RSF IPRO previamente inoculada com o patógeno. Foram avaliados nove tratamentos, incluindo controles, aplicados nas doses recomendadas pelos fabricantes. Os parâmetros analisados incluíram emergência, vigor, altura da parte aérea, comprimento da raiz, tombamento pré e pós-emergência, índice de doença e eficácia dos produtos. O delineamento experimental adotado foi inteiramente casualizado, com quatro repetições. Os resultados demonstraram que os tratamentos com Fludioxonil + Metalaxil-M + Tiabendazol e Tiofanato-metilico + Fluazinam apresentaram os melhores desempenhos, promovendo reduções no índice de doença de 73,79% e 58,39%, respectivamente. Conclui-se que o tratamento de sementes constitui uma estratégia eficiente e viável para o manejo preventivo de *Rhizoctonia solani* na fase inicial de desenvolvimento da cultura da soja.

Palavras-chave: soja, ação fungicida, gênero *Rhizoctonia*, Tiabendazol, produção de grãos.

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is currently the most important leguminous crop in Brazil, accounting for a

significant share of the country's grain production and exports (Guo et al., 2022; CONAB, 2024). Its broad adaptability, high yield potential, and major economic relevance have established Brazil as one of the world's leading producers and exporters of soybeans and derived products such as oil, feed, and protein (Zaaboul et al., 2022; Guo et al., 2022). However, the continuous expansion of soybean cultivation into new agricultural frontiers has contributed to increased pressure from pests and diseases, particularly those associated with the soil (Russ et al., 2024).

Among the main soilborne pathogens that affect early crop establishment, *Rhizoctonia solani* AG-4 stands out as the causal agent of seedling damping-off (Akber; Fang, 2024). This fungus exhibits necrotrophic and saprophytic behavior, with high survival capacity in soil and plant residues, infecting seedlings both before and after emergence. These infections often lead to stand failure and compromise early plant development (Singh et al., 2016; Goulart et al., 2001; Goulart, 2022; EMBRAPA, 2023). Disease outbreaks are favored by high soil moisture and mild temperatures, conditions frequently observed during the early sowing period in several soybean-producing regions of Brazil (Russ et al., 2024).

To mitigate the damage caused by this pathogen, seed treatment with fungicides has become one of the most effective and economically viable strategies (Bradley, 2008; Greenhouse study, 2024). This technique offers protection to seedlings during the critical early growth stages, with relatively low cost and ease of application (Utiamada et al., 2024). However, the efficacy of fungicides may vary depending on the active ingredient, product formulation, and environmental interactions, highlighting the importance of comparative evaluations to support rational and efficient disease management strategies (McKinney, 1923; Utiamada et al., 2024).

In this context, the present study aimed to evaluate, under greenhouse conditions, the efficacy and agronomic feasibility of different fungicides used in seed treatment for controlling damping-off caused by *Rhizoctonia solani* in soybean cultivation.

2. Materials and Methods

2.1 Experimental site

The experiment was conducted in a greenhouse at Agro Carregal Research and Plant Protection, located in the municipality of Rio Verde, Goiás, during the 2023/2024 growing season. The geographical coordinates of the experimental site are 17°47'0" South latitude and 51°0'6" West longitude, with an altitude of 766.7 meters.

2.2 Cultivation, inoculation, and experimental installation

Soybean seeds of the Bônus 8579RSF IPRO cultivar, batch 2020BO53, from the 2022/2023 crop season, were used. The seeds were artificially infected with the fungus *R. solani*. Seed treatment was carried out on the day the experiment was set up, December 29, 2023, and evaluations continued until February 11, 2024. The treatments applied are listed in (Table 1). Before inoculation, the seeds underwent surface disinfection by being washed in a 0.5% sodium hypochlorite solution and then air-dried in the shade. This procedure aims to eliminate pathogens present on the outer surface of the seeds.

Table 1. Treatments used in the experiment for controlling the pathogen *Rhizoctonia solani* in soybean cultivation.

Treatments	Dose	Time of application
	100 Kg ⁻¹ mL	
1 – Inoculated control	-	ST
2 – Non-inoculated control	-	ST
3 – Carboxin + Thiram	250	ST
4 – Thiram + Ipconazole	250	ST
5 – Iptaconazole	5,6	ST
6 – Chlorothalonil + Thiophanate-methyl	350	ST
7 - Thiophanate-methyl + Fluazinam	200	ST
8 – Thiophanate-methyl + Fluazinam	215	ST
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	ST

Note: ST = (Seed treatment). Source: Authors, 2025.

2.3 Production of *Rhizoctonia solani* inoculum

The inoculum production of *R. solani* was carried out according to the methodology proposed by Goulart (2006). Pure cultures of the pathogen, isolated from lesions on the collar of soybean seedlings, were used as inoculum and maintained on BDA (potato dextrose agar) medium for 48 h, the time required for fungal growth.

After this period, the fungus was transferred to a substrate composed of 2 kg of black oat seeds and 500 mL of water, previously autoclaved in a 2.0 L *Erlenmeyer* flask for 30 min. This autoclaving procedure was repeated for three consecutive days at 127 °C (1.5 atm of pressure). The inoculated substrate was then kept under ambient conditions for 35 days to allow full fungal colonization. On the 35th day, the colonized oat seeds were removed from the *Erlenmeyer* flask and air-dried in the shade for ten days. At the end of this period, the substrate (oat + *R. solani*) was ground using a 1 mm sieve mill to obtain the pathogen inoculum in powdered form.

2.4 Planting, cultural practices, and morphological analyses

Treated and untreated seeds with fungicides were sown in a greenhouse in plastic trays (56 × 35 × 10 cm) filled with washed sand. The seeds were placed in individual, equidistant holes at a depth of 3 cm. Before closing the holes, *Rhizoctonia solani* was inoculated by evenly distributing 8 g of fungal inoculum per tray on the substrate surface, ensuring direct contact with the seeds.

Each tray received 100 seeds. At the end of the experimental period, 14 days after sowing (DAS), the following assessments were performed: pre- and post-emergence damping-off, seedlings with lesions, initial and final seedling emergence, disease index, and selectivity. To confirm the presence of *R. solani* in symptomatic seedlings, the affected plants were collected, washed under running water, surface-disinfected with a 1.5% sodium hypochlorite solution for three minutes, and placed in a moist chamber. After five days of incubation at 22 °C under a 12-hour light/12-hour dark photoperiod, pathogen identification was conducted.

The disease index was evaluated at the end of the experiment, 15 days after seedling emergence, by assessing symptom severity. Seedlings were carefully removed from the trays with minimal root damage, washed under running water, and taken to the laboratory for evaluation. Each seedling was rated for severity of *Rhizoctonia solani* symptoms using the scale shown in (Table 2).

Table 2. Severity rating scale for *Rhizoctonia solani* symptoms in soybean seedlings.

Note	Description
0 – Absent – Lesion type 0	Healthy seedlings
1 – Mild – Lesion Type 1	Only a small reddish-brown lesion at the collar, with or without constriction
2 – Moderate – Lesion Type 2	Larger lesions at the collar (one or more), with or without constriction, dark coloration
3 – Severe – Lesion Type 3	Extensive lesions at the collar (over 2 cm), with constriction
4 – Very severe – Lesion type 4	Pre-emergence damping-off

Source: Authors, 2025.

The disease index (DI) was calculated according to McKinney (1923). This index is determined based on an infection severity rating scale using the following formula:

$$DI = \sum (f \cdot n) \cdot 100 / F \cdot N \text{ Eq. (1)}$$

Where:

DI = Disease index

f = number of plants in each severity class

n = infection severity score

F = total number of evaluated (inoculated) plants

N = maximum score on the severity scale

For the calculation of DI, the following values were assigned:

Score “0” for asymptomatic seedlings

Score “2.5” for type 1 lesions

Score “5.0” for type 2 lesions

Score “7.5” for type 3 lesions

Score “10.0” for type 4 lesions (pre-emergence damping-off) (Goulart; McKinney, 1923).

To ensure that the results reflected exclusively the effect of *Rhizoctonia solani* on seedling emergence and damping-off, a seed lot free from any fungal contamination was used, selected based on results from multiple seed health tests.

Seed treatment was performed by applying the fungicides to 500 g of seeds placed in 2.0-liter plastic bags, followed by the addition of water to reach a slurry volume equivalent to 500 mL per 100 kg of seeds. The bags were then shaken for a few minutes until complete coverage of the seeds by the products was achieved. The experiments were set up on the same day as the seed treatment.

2.5 Statistical analysis

The experimental design used was a completely randomized design (CRD) with four replicates. Analysis of variance (ANOVA) was performed, and means were compared using the *Scott-Knott* test at a 5% significance level, with the assistance of SAS-M software. Data transformation was applied when variance homogeneity was not observed (Canteri et al., 2001).

3. Results

3.1 Greenhouse conditions

Regarding seedling emergence, the treatments with Fludioxonil + Metalaxyl-M + Thiabendazole and Thiophanate-methyl + Fluazinam showed the best performance, with emergence rates above 85%. In contrast, the inoculated control showed an emergence rate below 50%, highlighting both the aggressiveness of the pathogen and the effectiveness of the fungicidal treatments in protecting the seeds (Table 3).

Table 3. Seedling emergence (%) at 7 and 14 days after sowing (DAS) in response to seed treatments for the control of *Rhizoctonia solani* in soybean.

Treatments	Dose	Emergence	
	mL 100 Kg ⁻¹ seeds	7 DAS	14 DAS
1 – Inoculated control	-	56.25 b	59.25 a
2 – Non-inoculated control	-	81.00 d	90.50 d
3 – Carboxin + Thiram	250	58.00 b	81.50 c
4 – Thiram + Ipconazole	250	72.25 c	78.50 c
5 – Ipconazole	5.6	44.25 a	58.75 a
6 – Chlorothalonil + Thiophanate-methyl	350	69.50 c	79.75 c
7 – Thiophanate-methyl + Fluazinam	200	66.75 c	71.00 b
8 – Thiophanate-methyl + Fluazinam	215	69.00 c	81.25 c
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	76.50 d	96.25 d
CV %		10.02	9.9

Notes: DAS = Days after sowing. Source: Authors, 2025.

In the seedling vigor assessment, no significant variation was observed at 7 and 14 days after emergence, with all treatments presenting a fixed value of 5%. The coefficient of variation (5%) indicates good data uniformity, and the absence of statistical differences suggests that the treatments did not affect the initial seedling vigor. This may be attributed to the cultivar's tolerance or the low severity of *Rhizoctonia solani* infection under the experimental conditions (Table 4).

According to Table 5, a significant difference was observed in seedling height at 14 days after emergence as a function of the applied treatments. Treatments 7 (Thiophanate-methyl + Fluazinam – 200 mL) and 9 (Fludioxonil + Metalaxyl-M + Thiabendazole – 100 mL) resulted in greater seedling growth, with an average height of 16 cm, and were statistically superior to the other treatments.

These results suggest that certain treatments not only control *R. solani* effectively but also contribute positively to early seedling development, possibly by reducing biotic stress and protecting the root system.

Table 4. Seedling vigor (%) at 7 and 14 days after emergence (DAE) in response to *Rhizoctonia solani* control in soybean. Agro Carregal Research and Plant Protection Experimental Station – Rio Verde, GO.

Treatments	Dose mL 100 Kg ⁻¹ seeds	Vigor	
		7 DAE	14 DAE
1 – Inoculated control	-	5.00 n.s	5.00 n.s
2 – Non-inoculated control	-	5	5
3 – Carboxin + Thiram	250	5	5
4 – Thiram + Ipconazole	250	5	5
5 – Ipconazole	5.6	5	5
6 – Chlorothalonil + Thiophanate-methyl	350	5	5
7 – Thiophanate-methyl + Fluazinam	200	5	5
8 – Thiophanate-methyl + Fluazinam	215	5	5
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	5	5
CV %		5.16	5.16

Note: Means followed by the same letter do not differ significantly according to the Scott-Knott test at 5% probability. DAE = Days after emergence. Source: Authors, 2025.

Table 5. Seedling height at 14 days after emergence (DAE) as a function of soybean seed treatments.

Treatments	Dose mL 100 Kg ⁻¹ seeds	Height (cm)
		14 DAE
1 – Inoculated control	-	10.50 a
2 – Non-inoculated control	-	11.00 a
3 – Carboxin + Thiram	250	11.50 a
4 – Thiram + Ipconazole	250	12.00 a
5 – Ipconazole	5.6	12.75 a
6 – Chlorothalonil + Thiophanate-methyl	350	13.50 a
7 – Thiophanate-methyl + Fluazinam	200	16.50 b
8 – Thiophanate-methyl + Fluazinam	215	14.25 b
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	16.50 b
CV %		11.83

Note: NS – Not significant according to the Scott-Knott test at 5% probability. DAE = Days after emergence. Source: Authors, 2025.

In the seedling growth evaluation, the most effective treatments also promoted greater root and shoot lengths, reflecting the overall health of the plants and reduced interference from the pathogen in the root system (Table 6).

Table 6. Root length at 14 days after emergence (DAE) as a function of soybean seed treatments.

Treatments	Dose	Root length
	mL 100 Kg ⁻¹ seeds	14 DAE
1 – Inoculated control	-	13.50 a
2 – Non-inoculated control	-	16.75 a
3 – Carboxin + Thiram	250	15.00 a
4 – Thiram + Ipconazole	250	14.00 a
5 – Ipconazole	5.6	29.00 b
6 – Chlorothalonil + Thiophanate-methyl	350	15.25 a
7 – Thiophanate-methyl + Fluazinam	200	13.00 a
8 – Thiophanate-methyl + Fluazinam	215	14.50 a
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	16.00 a
CV %		11.88

Note: NS – Not significant according to the *Scott-Knott* test at 5% probability. DAE = Days after emergence. Source: Authors, 2025.

Regarding pre- and post-emergence damping-off, the same treatments stood out, with disease index reductions of up to 73% and 58%, respectively, when compared to the inoculated control (Table 7).

Table 7. Pre- and post-emergence damping-off (%) of soybean seedlings as a function of seed treatments for *Rhizoctonia solani* control.

Treatments	Dose	Pre-emergence	Pros-emergence
	mL 100 Kg ⁻¹ seeds	7 DAS	14 DAS
1 – Inoculated control	-	40.75 d	0
2 – Non-inoculated control	-	0.00 a	0
3 – Carboxin + Thiram	250	18.50 b	0
4 – Thiram + Ipconazole	250	21.50 b	0
5 – Ipconazole	5.6	41.25 d	0
6 – Chlorothalonil + Thiophanate-methyl	350	20.25 b	0
7 – Thiophanate-methyl + Fluazinam	200	29.00 c	0
8 – Thiophanate-methyl + Fluazinam	215	18.75 b	0
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	3.75 a	0
CV %		36	

Note: *Means followed by the same letter in each column do not differ significantly according to the *Scott-Knott* test at 5% probability. Data transformed using the square root of (x + 0.1). DAS = Days after sowing. Source: Authors, 2025.

The evaluation of injured seedlings showed significant differences in the percentage of affected seedlings at 14 days after sowing (DAS), in response to seed treatments. The inoculated control exhibited the highest percentage of lesions (84.50%), confirming the aggressiveness of *Rhizoctonia solani*. In contrast, the non-inoculated control recorded 0%, demonstrating the effectiveness of pathogen exclusion. Among the treatments, the best performances were observed with Fludioxonil + Metalaxyl-M + Thiabendazole (37%) and Thiophanate-methyl + Fluazinam (39%), which differed statistically from the others, indicating greater effectiveness in disease control (Table 8).

Table 8. Percentage of injured soybean seedlings at 14 days after sowing (DAS) as a function of seed treatments for *Rhizoctonia solani* control.

Treatments	Dose	Injured seedlings
	mL 100 Kg ⁻¹ seeds	14 DAS
1 – Inoculated control	-	84.50 f
2 – Non-inoculated control	-	0.00 a
3 – Carboxin + Thiram	250	62.25 e
4 – Thiram + Ipconazole	250	42.25 c
5 – Ipconazole	5.6	53.25 d
6 – Chlorothalonil + Thiophanate-methyl	350	50.50 d
7 – Thiophanate-methyl + Fluazinam	200	47.25 d
8 – Thiophanate-methyl + Fluazinam	215	39.50 c
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	37.25 c
CV %		16.81

Note: Means followed by the same letter do not differ significantly according to the Scott-Knott test at 5% probability. DAS = Days after sowing. Source: Authors, 2023/2024.

The calculation of control efficacy based on the disease index showed that the treatment with Fludioxonil + Metalaxyl-M + Thiabendazole was the most effective, followed by Thiophanate-methyl + Fluazinam (Table 9).

Table 9. Disease index (DI, %) calculated using McKinney's formula at 14 days after sowing (DAS), and control efficacy (%) as a function of soybean seed treatments for *Rhizoctonia solani* control.

Treatments	Dose	Disease index	Efficacy (%)
	mL 100 Kg ⁻¹ seeds	14 DAS	
1 – Inoculated control	-	59.63 e	-
2 – Non-inoculated control	-	0.00 a	100
3 – Carboxin + Thiram	250	36.75 c	38.37
4 – Thiram + Ipconazole	250	28.94 c	51.47
5 – Ipconazole	5.6	45.25 d	24.12
6 – Chlorothalonil + Thiophanate-methyl	350	33.25 c	44.24
7 – Thiophanate-methyl + Fluazinam	200	35.13 c	41.09
8 – Thiophanate-methyl + Fluazinam	215	24.81 b	58.39
9 – Fludioxonil + Metalaxyl-M + Thiabendazole	100	15.63 b	73.79
CV %		21.03	

Note: Means followed by the same letter in each column do not differ significantly according to the Scott-Knott test at 5% probability. DAS = Days after sowing. Sources: Authors, 2025.

4. Discussion

Seed treatments with fungicides showed a significant effect ($p \leq 0.05$) on the phytosanitary parameters evaluated in this study, particularly regarding seedling emergence, damping-off, and disease index. These results confirm the effectiveness of seed treatment as a preventive tool for controlling *R. solani* AG-4 in soybean cultivation, corroborating previous studies conducted under controlled conditions (Goulart, 2000; Bradley, 2008; Utimada et al., 2024).

Among the products evaluated, the treatment with Fludioxonil + Metalaxyl-M + Thiabendazole demonstrated the best performance, achieving 73% efficacy in reducing the disease index, followed by the Thiophanate-methyl + Fluazinam formulation (58%). These results suggest that the addition of Thiabendazole to the mixture with Fludioxonil and Metalaxyl-M provided a synergistic control effect, as also reported by Gisi et al. (1996), Rasgado (1998), and Schirra et al. (2004), who highlighted Thiabendazole's ability to enhance the action of systemic fungicides in triple mixtures.

In addition to direct pathogen control, these treatments also improved seedling development, resulting in greater shoot height and root length, indicating reduced biotic stress during early growth stages. Similar findings were reported by Goulart (2000) and Rothrock et al. (2005), who attributed these benefits to the protection conferred by seed treatments against initial *R. solani* infections.

Symptom severity in seedlings, measured by the disease index, showed a direct correlation with the number of infected seedlings, especially at higher severity levels (levels 3 and 4), confirming the disease index as a reliable parameter for efficacy assessment. Pearson correlation analysis revealed a strong relationship between infected seedlings and disease index ($R^2 = 0.92$), indicating that fungicides are more effective in preventing lesions also results in lower disease index values, as demonstrated by Devay et al. (1982) and Kurm et al. (2023).

In this study, a higher incidence of damping-off was observed during the pre-emergence phase compared to post-emergence, a typical pattern for infections caused by *R. solani* (Urrea et al., 2003). This underscores the importance of early fungicidal action in the rhizosphere environment and justifies the need for products with both contact and systemic effects that act rapidly and persistently.

Less effective treatments, such as Iproconazole alone and Carboxin + Thiram, although showing some reduction

in disease index, were statistically outperformed by mixtures combining multiple modes of action. This aligns with Gisi (1996), who emphasized that the efficacy of fungicide mixtures is related to the complementarity of mechanisms of action and their ability to provide broad and prolonged protection against soilborne pathogens.

It is important to highlight that *R. solani* is considered a monocyclic pathogen, meaning it does not produce secondary inoculum during the crop cycle. Therefore, the later the disease manifestation occurs, the less damage it causes (Goulart et al., 2006). Thus, seed treatment fungicides need to ensure systemic activity and persistence for at least 15 days, which remains a limitation of currently available products, as discussed by Utimada et al. (2024).

The experimental model used in this study, conducted in a greenhouse with artificially inoculated substrate, allowed for the standardization of inoculum density and control of environmental factors, providing consistent and reproducible results. According to Devay et al. (1982) and Mertz et al. (2019), this approach is a reliable alternative for estimating fungicide efficacy against soilborne pathogens, given the variability encountered under field conditions. Therefore, the results obtained here are consistent with those reported in the national and international scientific literature and reinforce the importance of fungicide seed treatment as an essential agronomic measure for controlling *R. solani* in soybean cultivation.

5. Conclusions

Based on the results obtained, it is concluded that seed treatment with fungicides is an effective and agronomically feasible strategy for managing damping-off caused by *Rhizoctonia solani* AG-4 in soybean cultivation, especially during the early crop establishment stage. Among the products tested, the combinations of Fludioxonil + Metalaxyl-M + Thiabendazole and Thiophanate-methyl + Fluazinam stood out by promoting higher seedling emergence rates, greater vigor, initial growth, and a significant reduction in disease incidence, reaching control efficacy levels of up to 73%.

The protection provided by these fungicides resulted in more uniform and healthier seedlings, creating favorable conditions for early plant development — a critical phase for achieving high productivity throughout the crop cycle. Therefore, the findings of this study reinforce the importance of seed treatment as a preventive tool in the integrated management of soil-borne diseases in soybean cultivation, contributing technically to more assertive decision-making regarding the selection of the most effective and promising fungicidal products under field conditions.

6. Authors' Contributions

Bruno Pereira de Eça Vivas: data collection, experimental execution, preliminary data analysis. Matheus Vinícius Abadia Ventura: data analysis, manuscript writing, critical review. Antonio Carlos Pereira de Menezes Filho: overall supervision, advisor, final manuscript revision.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. References

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