Effectiveness of fungicide seed treatment in the control of soybean seedling damping-off caused by *Rhizoctonia solani* under greenhouse conditions

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ABSTRACT

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The present study was conducted to evaluate the efficacy of fungicide seed treatment in the control of soybean seedling damping-off caused by *Rhizoctonia solani* under greenhouse conditions. The experiment was carried out in Dourados, Mato Grosso do Sul State, Brazil. Treated and untreated soybean seeds were sown in previously sterilized sand, contained in plastic trays, in equidistant individual wells at 3cm depth. The soybean sowing density was 200 seeds/plastic tray. Inoculation with *R. solani* was done by homogeneous distribution of the fungal inoculum onto the substrate surface (5.0g/tray). The fungus was grown for 35 days on autoclaved black oat seeds ground to powder with a mill (1mm). Seedling emergence, pre and post-emergence damping-off, injured seedlings and disease index (severity) were evaluated according to McKinney formula. Fungicide seed treatments had a significant effect ($P \leq 0.05$) on seedling emergence, pre and post-emergence damping-off, injured seedlings and disease index (severity). The best results were obtained for fludioxonil+metalaxyl-M+thiabendazole and fipronil+pyraclostrobin+thiophanate-methyl, followed by carbendazim+thiram+pencycuron and thiophanate-methyl+fluazinam. The least efficient treatments were carbendazim+thiram, fludioxonil+metalaxyl-M and carboxin+thiram. Pearson correlation analysis indicated that injured seedlings and disease index were positively correlated (R=0.92). 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 soybean seedlings.

Keywords: *Glycine max*, damping-off, emergence, chemical control, seed pathology, soil-borne disease, soil-borne fungus.

RESUMO

Goulart, A.C.P. Eficácia do tratamento de sementes com fungicidas no controle do tombamento de plântulas de soja causado por *Rhizoctonia solani* sob condições casa de vegetação. ***Summa Phytopathologica,*** v.48, n.3, p.121-125, 2022.

Esse estudo foi desenvolvido para avaliar a eficácia do tratamento de sementes de soja com fungicidas no controle do tombamento causado por *Rhizoctonia solani*, em condições de casa de vegetação. O experimento foi conduzido em Dourados, MS. Sementes de soja tratadas e não tratadas com os fungicidas foram semeadas em areia previamente esterilizada, contida em bandejas plásticas, dispostas em orifícios individuais, equidistantes e a 3 cm de profundidade. Foram semeadas 200 sementes/bandeja plástica. A inoculação com *R. solani* foi feita pela distribuição homogênea do inóculo do fungo na superfície do substrato (5,0g/bandeja). O fungo foi cultivado por 35 dias em sementes de aveia preta autoclavadas e trituradas em moinho (1mm). Foram avaliados a emergência de plântulas, tombamento de pré e pós emergência, plântulas lesionadas e o índice de doença (severidade) de acordo com a formula de McKinney. Foi observado efeito significativo ($P \leq 0.05$) do tratamento de sementes com fungicidas em relação a emergência de plântulas, tombamento de pré e pós emergência, plântulas lesionadas e o índice de doença (severidade). Os melhores resultados foram obtidos com fludioxonil+metalaxyl-M+thiabendazole e fipronil+pyraclostrobin+tiofanato metílico, seguido de carbendazim+thiram+pencycuron and tiofanato metílico+fluazinam. Os tratamentos menos eficientes foram carbendazim+thiram, fludioxonil+metalaxyl-M and carboxin+thiram. A análise da correlação de Pearson indicou correlação positiva entre plântulas lesionadas e índice de doença (R=0,92). 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 soja.

Palavras chaves:*Glycine max,* tombamento, emergência, controle químico, patologia de sementes, doença de solo, fungo de solo.

Seedling diseases caused by a complex of soil-borne fungi are one of the major problems for soybean production worldwide (2, 5, 6, 26). According to Goulart (9, 10) and Paula Júnior et al. (19), the most important pathogen considering the soybean seedling disease complex in Brazil is *Rhizoctonia solani* Kühn anastomosis group AG-4 [teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk].

Goulart (11) pointed out that the damages caused by this pathogen, in the early stage of seedling development, are related to seedling population reduction (stand losses) due to damping-off. It is worth mentioning that a high incidence of soybean seedling disease causes poor stands, which can result in added costs of replanting and reduced yields because of late planting (5, 21).

Agriculture has experienced great technological advances with the incorporation of new technologies, and the most recent practices related to the industry of seeds and fungicides deserve special mention (12). According to Gaspar et. al. (6), soybean seed treatment adoption has increased dramatically over the past decade in addition to the number of pesticide components within commercially available seed treatments.

The use of soybean seed treatment with fungicides in Brazil has been growing every year. According to Richetti & Goulart (20), in the 1991/1992 crop season, only 5% of the soybean area was sown with treated seeds. Until the 2016/2017 crop season, 98.2% of the soybean seeds were treated with fungicides whether in the industry (Industrial Seed Treatment - TSI - 25.6%) or on farms (72.6%). As regards costs, those same researchers showed that such a practice represented only 2.2% of the production cost for 1ha soybean crop. Currently in Brazil, the fungicide mixtures registered and recommended by the Ministry of Agriculture, Livestock and Supply (MAPA) for soybean seed treatment are carbendazim+thiram, carboxin+thiram, fludioxonil+mefenoxam, fipronil+pyraclostrobin+thiophanate-methyl, fludioxonil+mefenoxan+thiabendazole, thiophanate-methyl +fluazinam and chlorothalonil+thiophanate-methyl (12). It is worth mentioning that all these fungicides are registered for soybean seed treatment in the control of *R. solani*.

There are a number of approaches to soybean seedling disease management. Among them, fungicide seed treatment is widely used in grain crops. Few references have been found related to the control of soybean seedling damping-off caused by *R. solani* using seed dressing (2, 6, 26), however, fungicide seed treatment can be considered one of the most appropriate and effective tools for integrated management programs (2, 9, 10, 12a). Thus, further research is needed to fill this gap.

The present study was conducted to evaluate the efficacy of fungicide seed dressing in the control of soybean seedling damping-off caused by *R. solani* under greenhouse conditions.

MATERIAL AND METHODS

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

The pathogen inoculum was composed of black oat seeds colonized by *R. solani* AG-4, isolated from infected soybean seedlings. *Rhizoctonia solani* anastomosis group AG-4 was chosen since this group is responsible for causing damping-off on soybean seedlings, as shown in previous 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 2.0kg black oat seeds and ½ liter water. After 35-day incubation in a BOD chamber at 22°C and alternating 12-h period of darkness and fluorescent white light, the oat seeds colonized by *R. solani* were allowed to dry on trays for 10 days and subsequently ground to powder with a mill (1 mm).

Seed treatment was conducted in polyethylene bags. The products were added to disposable syringes and the bags were vigorously shaken for homogeneous distribution of the spray solution over the seeds. Treated and untreated soybean seeds were sown in sterilized sand contained in plastic trays (56x35x10 cm), in equidistant wells, at 3cm depth. The soybean sowing density was 200 seeds/plastic tray. A pre-established amount of pathogen inoculum was mixed in 1.0 kg sand for the tests in plastic trays. Before closing the wells, *R. solani* was inoculated by homogeneous distribution onto the substrate. Inoculum densities were adjusted to 5.0 g/plastic tray, based on pathogenicity. Such inoculum quantities were adjusted to provide a maximum of 50% soybean seedling damping-off, according to the methodology proposed by Weinhold (28) and Wang & Davis (27). Seedling emergence, pre and post-emergence damping-off and injured seedlings were evaluated based on symptom development and seedling survival, using the "growing on test". Plastic trays were maintained in a greenhouse for 21 days and watered daily.

The obtained results were also used to calculate the disease index (severity) for the remaining seedlings. The disease index was obtained for roots and hypocotyls of each sampled seedling, which received a score based on the lesion severity caused by *R. solani*, according to the rating scale for assessing the severity of *R. solani* lesions on cotton, soybean and common bean seedlings proposed by Goulart (11). The disease index (ID) was calculated according to McKinney (16), using the following equation:

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

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

To confirm the pathogen identity, injured seedlings were collected and taken to the laboratory for analysis. Hypocotyl fragments from these seedlings were incubated in a humid chamber. Five days after incubation, the fragments were evaluated and the presence of *R. solani* was confirmed.

Possible phytotoxic effects on soybean seedlings due to seed treatment with fungicides were evaluated, including: slow germination and seedling emergence, low percentage of seedling emergence, hypocotyl thickening and shortening, reduced seedling size and epicotyl etiolation (such thickening often occurs together with fissures in the hypocotyl), atrophy of the root system, winding root problems, "snail" type, "umbrella handle" curvature of the main root, presence of burned-margined cotyledons, seedlings with yellowed and burned-margined cotyledons, and early fall of cotyledons.

The fungicide mixtures used as soybean seed treatments in the current study were commercially available seed treatments registered for *R. solani* control and were applied at the recommended rates (Table 1).

Complete randomized block design was used, including four replicates and nine treatments. Experimental plot consisted of one plastic tray containing 200 seeds. The percentage data were transformed in arc sen $\sqrt{x}/100$. Scott Knott test (P=0.05) was chosen. All analyses were conducted in ASSISTAT (23). For comparison, two control treatments were adopted: one inoculated treatment and one non-inoculated treatment.

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

Treatments	Dose (a.i. / 100 kg seeds)
Fludioxonil+metalaxyl-M+thiabendazole	2.5+2.0+75.0
Fipronil+pyraclostrobin+thiophanate-methyl	50+5+45
Carbendazim+thiram	70+30
Carbendazim+thiram+pencycuron	70+30+50
Thiophanate-methyl+fluazinam	70+10.6
Fludioxonil+metalaxyl-M	2.5+1.0
Carboxin+thiram	75+75
Inoculated control	-
Non-inoculated control	-

RESULTS AND DISCUSSION

The results obtained in the current experiment are presented in Tables 2 and 3.

Fungicide seed treatments had a significant effect ($P \leq 0.05$) on seedling emergence, pre and post-emergence damping-off, injured seedlings and disease index (severity), compared to untreated controls, indicating that they all provided some level of protection (Table 2). The best results were found for fludioxonil+metalaxyl-M+thiabendazole and fipronil+pyraclostrobin+thiophanate-methyl, followed by carbendazim+thiram+pencycuron and thiophanate-methyl+fluazinam. The least efficient treatments were carbendazim+thiram, fludioxonil+metalaxyl-M and carboxin+thiram.

Pencycuron is a phenylurea fungicide which has a contact effect when used as seed dressing. It has specific activity against the plant pathogen *R. solani*, for which it was developed. This explains the improved control efficiency of the mixture carbendazim+thiram in the present study when pencycuron was added, corroborating with results obtained by Campion et al (3), Sumner (24) and Thind et al. (25), who also reported the efficacy of pencycuron against *R. solani*. On the other hand, the best control obtained in the present experiment by fludioxonil+metalaxyl-M+thiabendazole, compared to fludioxonil+metalaxyl-M, suggests synergistic interactions of thiabendazole with these fungicides. Similar results indicating synergistic interactions between thiabendazole and other fungicides were found by Gisi et al. (8), Gisi (7) and Schirra et al. (22). 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. According to Gisi (7), among other reasons, fungicides are often combined in mixtures to promote additive and synergistic interactions between them, which increase the overall activity and can reduce the concentrations of compounds without activity loss.

The benefits of fungicide seed treatments on soybean stand establishment and *R. solani* control have been previously documented. Bradley (2) and Urrea et al. (26) had similar results and reported that soybean seed treatment with broad-spectrum fungicide combinations led to higher plant stands and efficient control of soybean seedling damping-off caused by *R. solani*, compared to untreated seeds. Wang & Davis (27) found that seed treatment to control *R. solani* damping-off improved the stand in all their greenhouse trials, compared to untreated seeds. Goulart (13) and Rothrock et al. (21) reported similar results in experiments conducted on cotton.

It is worth mentioning that, although some level of protection was achieved, high percentages of injured seedlings were also observed (Table 3), leading to significant levels of symptom severity (disease index). Pearson correlation analysis indicated that injured seedlings and disease index were positively correlated (R=0.92). This means that the greater the number of injured seedlings, especially in level 3 (severe intensity lesion) of the scale proposed by Goulart (11), the greater the disease

Table 2: Seedling emergence and seedling damping-off (pre and post-emergence), according to fungicide seed dressing.

Treatments	Dose (a.i. / 100 kg seeds)	Seedling emergence (%)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)
Fludioxonil+metalaxyl-M+thiabendazole	2.5+2.0+75.0	89.0 a	1.1 e	1.5 d
Fipronil+pyraclostrobin+thiophanate-methyl	50+5+45	89.5 a	0.6 e	1.2 d
Carbendazim+thiram	70+30	82.0 c	8.9 b	3.2 b
Carbendazim+thiram+pencycuron	70+30+50	87.5 b	2.8 d	1.9 c
Thiophanate-methyl+fluazinam	70+10.6	88.0 b	2.2 d	2.0 c
Fludioxonil+metalaxyl-M	2.5+1.0	83.0 c	7.8 c	3.0 b
Carboxin+thiram	75+75	83.5 c	7.6 c	2.8 b
Inoculated control	-	66.5 d	26.1 a	7.6 a
Non-inoculated control	-	90.0 a	0.0 f	0.0 e
Average		84.33	6.34	2.57
C.V. (%)		8.82	10.55	10.34

Mean values followed by the same lowercase letter in the column do not differ statistically according to Scott Knott test (P=0.05)

Table 3: Injured seedlings and disease index according to fungicide seed dressing.

Treatments	Dose (a.i. / 100 kg seeds)	Injured seedlings (%)	Disease index (lesion severity) (%)
Fludioxonil+metalaxyl-M+thiabendazole	2.5+2.0+75.0	28.5 e	6.0 e
Fipronil+pyraclostrobin+thiophanate-methyl	50+5+45	27.3 e	4.8 f
Carbendazim+thiram	70+30	35.5 b	10.4 b
Carbendazim+thiram+pencycuron	70+30+50	30.8 d	7.9 c
Thiophanate-methyl+fluazinam	70+10.6	31.5 d	7.2 d
Fludioxonil+metalaxyl-M	2.5+1.0	33.8 c	10.1 b
Carboxin+thiram	75+75	33.3 c	9.8 b
Inoculated control	-	53.7 a	23.4 a
Non-inoculated control	-	0.0 f	0.0 g
Average		**30.48**	**8.84**
C.V. (%)		**9.23**	**8.52**

Mean values followed by the same lowercase letter in the column do not differ statistically according to Scott Knott test (P=0.05)

index and the less efficient the fungicide.

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 appear, the smaller the damage caused by it. Thus, to achieve such a control level, a desirable fungicide for soybean 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.

Soybean seedling damping-off usually occurs in pre-emergence and in post-emergence, and both were observed in the current experiment. The damping-off levels in pre-emergence were higher than those in post-emergence , which corroborates the results obtained by Urrea et al. (26). The number of emerged seedlings reflects the efficiency of fungicides in the control of *R. solani*, as well as the stand maintenance capability of avoiding post-emergence damping-off. The drastic effect of the pathogen can be observed by comparing the results obtained for the untreated control (inoculated and non-inoculated).

According to Rothrock et al. (21), comparisons of seed treatments in the field are often inconsistent, in part due to the low inoculum density of soil-borne pathogens. This approach can be unreliable in practice if the employed field locations are insufficient or the conditions are not sufficiently favorable for seedling diseases (15). Another approach to obtain the efficiency of seed-treatment fungicides is to evaluate their efficacy under controlled conditions against specific pathogenic fungi (18). In this context, Devay et al. (4) pointed out that experiments carried out under greenhouse conditions, to determine the performance of fungicides applied as seed dressing, were a useful indicator for predicting the effectiveness of these products under field conditions.

Several authors have developed research of this nature under greenhouse conditions (1, 13, 14, 17, 18, 27, 28), using the same methodology of substrate inoculation with *R. solani*. The present results, in addition to those previously mentioned, demonstrate the feasibility of this inoculation method to evaluate the efficiency of soybean seed treatment with fungicides in the control of this pathogen under greenhouse conditions. Such research provides highly reliable results, for working under controlled conditions with a pre-established inoculum density, allowing accurate assessment of the benefits of soybean seed treatment with fungicides.

Soybean 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 soybean seedlings.

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