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RESEARCH ARTICLE

Compatibility of biological agents with chemical seed treatments in soybean assessed by seed colonization and minimum inhibitory concentration assays

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Abstract

Chemical seed treatment is widely used to manage diseases and pests during the early stages of crop development. However, the integration of this practice with biological agents may be limited by incompatibility between chemical products and beneficial microorganisms. The objective of this study was to evaluate the compatibility of chemical seed treatments with the biological agents *Trichoderma harzianum*, *Paecilomyces lilacinus*, *Bacillus subtilis*, *Azospirillum brasilense*, and *Bradyrhizobium japonicum* using seeds of soybean cultivar NS 6700. Seeds were subjected to different chemical treatments and subsequently inoculated with the microorganisms. Compatibility was assessed through the percentage of microbial growth on treated seeds incubated on culture medium and by determining the minimum inhibitory concentration (MIC) using the broth microdilution method. The results demonstrated that compatibility was highly dependent on the chemical formulation evaluated. The treatment containing thiophanate-methyl + fluazinam showed high compatibility with the fungal antagonists, allowing 88% growth of both *T. harzianum* and *P. lilacinus*, whereas the formulation composed of pyraclostrobin + thiophanate-methyl + fipronil supported 83% growth of *T. harzianum* and 100% growth of *B. subtilis*. In contrast, treatments containing carbendazim + thiram and thiophanate-methyl + fluazinam + fipronil completely inhibited the growth of all biological agents evaluated. MIC assays complemented the results observed in seed colonization, revealing marked differences in the sensitivity of biological agents to the chemical treatments. It can be concluded that compatibility between biological products and chemical seed treatments depends on the formulation used, and prior compatibility assessments are essential to ensure the successful integration of these technologies in agricultural production systems.

Keywords: biological control; agricultural microbiology; microbial inoculants; phytosanitary formulations.

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1. Introduction

Soybean (*Glycine max* L. Merrill) is one of the most important agricultural commodities worldwide, playing a strategic role in global food, feed, and biofuel production. However, the crop is frequently affected by seed- and soil-borne pathogens that compromise seed quality, plant establishment, and grain yield. Under favorable environmental conditions, these pathogens can rapidly infect and spread throughout the crop, resulting in significant economic losses (Ribeiro et al., 2017).

Chemical seed treatment has become one of the main strategies for disease management in soybean production due to its relatively low cost, ease of application, and high efficiency in protecting seeds and seedlings during the early stages of crop development (Xia et al., 2006). In Brazil, the adoption of seed treatment has increased substantially over the last decades. Initially used in only a small proportion of soybean-growing areas, chemical seed treatment currently approaches full adoption in commercial production systems, including the combined use of fungicides and insecticides (Baudet & Villela, 2012; Richetti & Goulart, 2019).

Despite its effectiveness, the intensive and repeated use of fungicides has contributed to the selection of

resistant pathogen populations. The emergence of resistant strains has been associated with the continuous use of compounds with similar modes of action, reducing disease control efficiency and increasing the need for integrated management strategies (Lucas et al., 2015).

In recent years, the use of biological products in soybean production systems has expanded considerably. Biological agents such as *Trichoderma* spp., *Bacillus* spp., *Azospirillum brasilense*, and *Bradyrhizobium japonicum* have been widely studied because of their ability to promote plant growth, improve nutrient acquisition, induce plant resistance, and suppress phytopathogens. Recent studies have demonstrated that microbial seed inoculation and biopriming can improve soybean performance under both biotic and abiotic stress conditions (Silva et al., 2024).

The integration of biological agents with conventional chemical seed treatment represents a promising strategy for soybean production. However, fungicides and insecticides applied to seeds may negatively affect the survival, growth, colonization capacity, and physiological activity of beneficial microorganisms. These effects may vary according to the active ingredient, dose, exposure period, microbial species, and chemical formulation used.
Although previous studies have investigated interactions between chemical seed treatments and microbial inoculants, much of the available information has focused on plant performance and nodulation responses. Studies directly evaluating the effects of complete commercial seed-treatment formulations on the viability and growth of different groups of beneficial microorganisms remain scarce, particularly in soybean. Commercial formulations may contain combinations of fungicides, insecticides, micronutrients, polymers, and other formulation components, making their overall effects on microbial viability difficult to predict solely from the response to individual active ingredients. Therefore, direct compatibility assessments of complete commercial formulations are needed to support the safe and effective integration of chemical and biological seed treatments.

Therefore, the present study aimed to evaluate the compatibility of commercial chemical products commonly used in soybean seed treatment with the biological agents *Trichoderma harzianum*, *Paecilomyces lilacinus*, *Bacillus subtilis*, *Azospirillum brasilense*, and *Bradyrhizobium japonicum* using complementary seed-colonization and minimum inhibitory concentration assays.

2. Methodology

2.1 Experimental site

The experiments were conducted in the Plant Health and Seed Analysis Laboratories of the Federal University of Technology – Paraná (UTFPR), Paraná State, Brazil.

2.2 Chemical seed treatments and biological agents

Soybean seeds of the cultivar NS 6700 were used in all experiments. For each treatment, 40 seeds were placed in previously sterilized polyethylene bags and treated with the chemical products described in Table 1. Sixty minutes after chemical treatment application, the seeds were inoculated with the biological agents *Trichoderma harzianum* (isolate IBLF 006), *Paecilomyces lilacinus* (isolate UEL Pae 10), *Bacillus subtilis* (strain QST 713), *Azospirillum brasilense* (strains AbV5 and AbV6), and *Bradyrhizobium japonicum* (strains SEMIA 5079 and SEMIA 5080), according to the commercial rates recommended for soybean seed treatment. The biological products, microorganism concentrations, strains or isolates, and application rates are presented in Table 2 (Figure 1).

2.3 Compatibility between chemical seed treatments and biological agents on soybean seeds

Treated seeds were distributed into four replicates of ten seeds each. Under aseptic conditions in a laminar-flow hood, the seeds were transferred to Petri dishes containing approximately 20 mL of potato dextrose agar (PDA), ensuring adequate spacing to avoid contact among seeds.
The experiment was arranged in a completely randomized design consisting of seven chemical seed treatments and an untreated control, totaling eight treatments with four replicates per treatment. Each Petri dish containing ten seeds constituted one experimental unit. An independent experiment was conducted for each biological agent to be evaluated. After three days of incubation, the percentage of seeds exhibiting growth of the respective biological agent was assessed. Evaluations were performed using a stereomicroscope at 80× magnification. Results were expressed as the percentage of microbial growth on treated seeds.

2.4 Statistical analysis

Data were initially subjected to the Lilliefors normality test and Bartlett's for homogeneity. When necessary, data were transformed using the Box–Cox procedure implemented in Action Stat® software. After verifying the assumptions of normality and homogeneity of variance, data were analyzed by analysis of variance (ANOVA), and treatment means

were compared using Tukey's test at the 5% significance level. Statistical analyses were performed using Genes® software.

2.5 Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of each chemical seed treatment was determined using the broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI M38-A). The effects of the chemical treatments were evaluated against *T. harzianum*, *P. lilacinus*, *B. subtilis*, *A. brasilense*, and *B. japonicum*, with a separate experiment conducted for each microorganism.

All procedures were performed in a laminar-flow hood previously disinfected with 10% sodium hypochlorite solution, 70% ethanol (v/v), and ultraviolet light exposure for 30 min. Chemical products were prepared at the commercial rates recommended by the manufacturers (Table 1).

Biological suspensions and inoculum concentrations were prepared according to the specifications presented in Table 2.

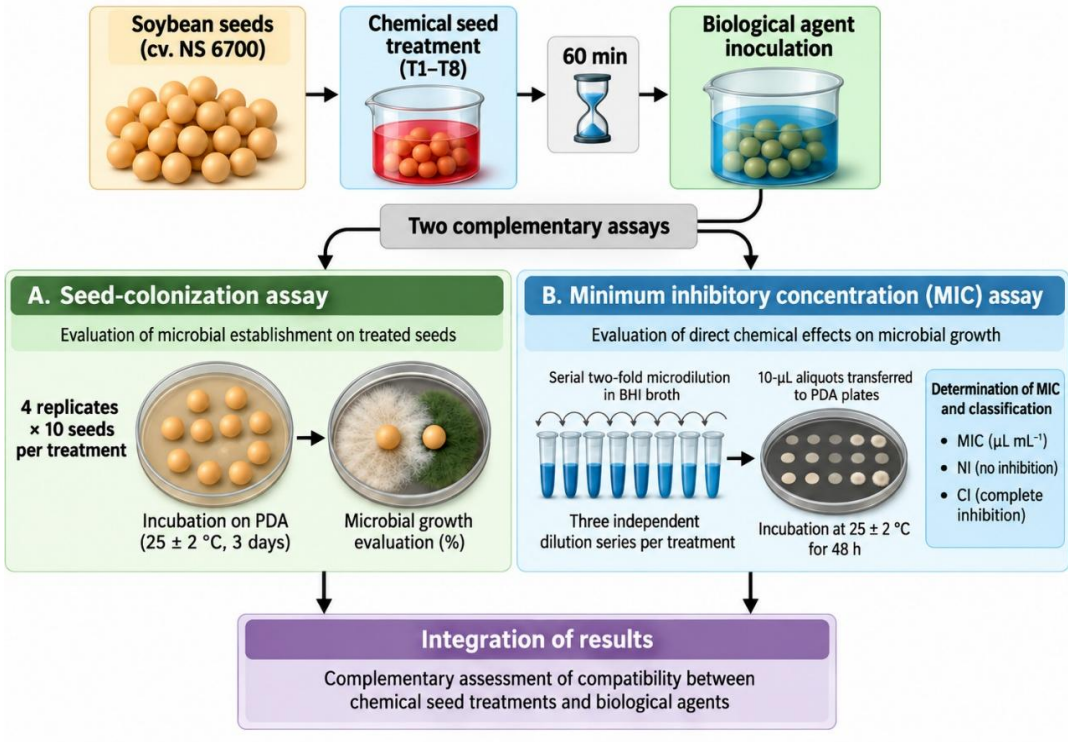


Figure 1. Experimental workflow used to evaluate the compatibility between chemical seed treatments and biological agents. Soybean seeds (cv. NS 6700) were subjected to chemical seed treatment and, after 60 min, inoculated with the biological agents. Two complementary approaches were subsequently performed: (A) a seed-colonization assay to evaluate microbial establishment on chemically treated seeds and (B) a minimum inhibitory concentration (MIC) assay based on serial microdilution followed by spot-test confirmation. The results obtained from both assays were integrated to assess the compatibility between chemical seed treatments and biological agents.

Table 1
Chemical products used in soybean seed treatment according to the manufacturer's recommended dose

Treatment	Active ingredients	Dose (mL a.i./100 kg seed)	Spray volume (mL)
1	Thiophanate-methyl + Fluazinam	215	500
2	Metalaxyl-M + Thiabendazole + Fludioxonil	125	500
3	Carbendazim + Thiram	200	400
4	Pyraclostrobin + Thiophanate-methyl + Fipronil	200	500
5	Micronutrient + (Citric acid + Potassium hydroxide + Imidacloprid + Thiodicarb) + Carbendazim + Thiram + Polymer	100+300+200+100	400
6	Micronutrient + (Citric acid + Potassium hydroxide + Thiamethoxam) + Metalaxyl-M + Thiabendazole + Fludioxonil + Polymer	100+200+300+100	500
7	(Thiophanate-methyl + Fluazinam) + Fipronil + Polymer	200+100+200	500
8	Untreated control	0	0

Table 2
Commercial biological products, concentration of active microorganisms, and applied doses used in soybean seed treatment. CFU = colony-forming units

Product	Biological agent	Concentration of active ingredient	Applied dose
Ecotrich®	*Trichoderma harzianum*	1×10^{10} CFU g^{-1}	1 g kg^{-1} seed
Nemat®	*Paecilomyces lilacinus*	7.5×10^{9} CFU g^{-1}	1 g kg^{-1} seed
Serenade®	*Bacillus subtilis*	1×10^{9} CFU g^{-1}	4 mL kg^{-1} seed
Azzofix®	*Azospirillum brasilense*	2×10^{8} CFU mL^{-1}	4 mL kg^{-1} seed
Atmo®	*Bradyrhizobium japonicum*	5×10^{9} CFU mL^{-1}	4 mL kg^{-1} seed

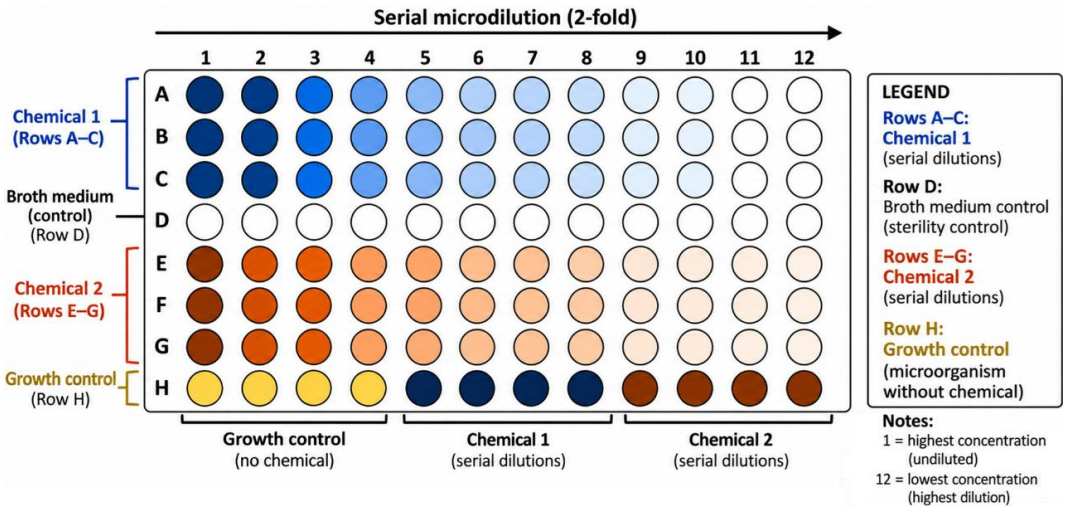


Figure 2. Serial microdilution setup used to determine the minimum inhibitory concentration (MIC).

2.6 Serial microdilution assay and spot test confirmation

Serial microdilution assays were performed in sterile 96-well microplates containing 100 µL of Brain Heart Infusion (BHI) broth per well. The experiment was arranged in a completely randomized design, and each chemical treatment was evaluated in three independent replicates for each biological agent. Each replicate consisted of an independent serial dilution series performed along one row of the microplate.

Initially, 100 µL of each chemical treatment was added to the first well of each replicate. After homogenization, serial two-fold dilutions were prepared by transferring 100 µL from one well to the next along the corresponding row, maintaining a constant final volume in all wells. Subsequently, 10 µL of the corresponding biological agent suspension was added to each well. Growth controls consisted of wells containing only BHI medium and the respective microorganism (Figure 2).

Microplates were incubated in a BOD growth chamber at 25 ± 2 °C under a 12-h photoperiod for 48 h. Following incubation, MIC determination was confirmed using an adapted spot-test procedure (Figure 3). Aliquots of 10 µL from each well were transferred onto Petri dishes containing approximately 30 mL of PDA medium. BHI broth was used in the microdilution assay as a nutrient-rich medium to support microbial growth during exposure to the chemical treatments, whereas PDA was used in the subsequent spot-test as a solid medium for qualitative visualization of microbial growth recovery. Thus, the PDA step was used as a qualitative confirmation of microbial viability following chemical exposure rather than as an optimized medium for bacterial cultivation.

The plates were incubated under the same conditions for an additional 48 h. Microbial growth was then assessed visually. The MIC was defined as the lowest concentration of the chemical treatment capable of completely inhibiting microbial growth. The concentration corresponding to the last dilution showing complete inhibition immediately before growth reestablishment was considered the minimum inhibitory concentration.

3. Results and discussion

3.1 Compatibility of biological agents on chemically treated seeds

Analysis of variance revealed a significant effect of chemical seed treatments on the growth of all biological agents evaluated (Table 3), indicating that compatibility between chemical products and beneficial microorganisms is strongly dependent on the formulation used (Figures 4 and 5).

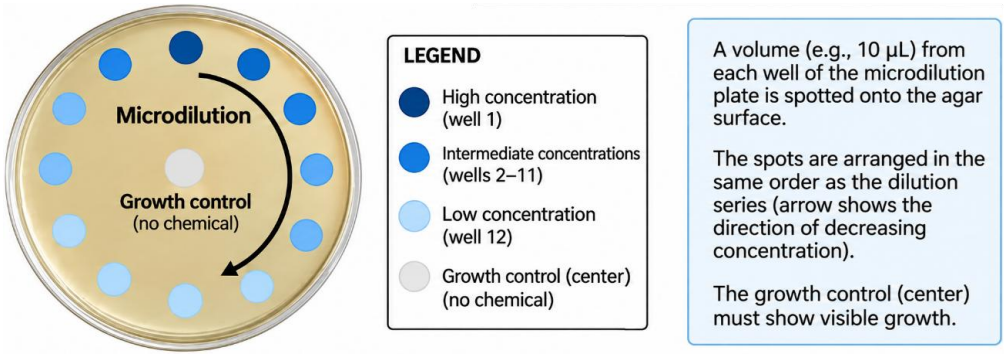


Figure 3. Spot test used to confirm the results of the serial microdilution. The experiment was arranged in a completely randomized design with eight chemical treatments and three replicates for each biological agent.

Table 3
Growth percentage of *Trichoderma harzianum*, *Paecilomyces lilacinus*, *Bacillus subtilis*, *Azospirillum brasiliense*, and *Bradyrhizobium japonicum* on seeds subjected to chemical treatments separately

Treatment	*T. harzianum* (%)	*P. lilacinus* (%)	*B. subtilis* (%)	*A. brasilense* (%)	*B. japonicum* (%)
1	88 a	88 a	10 d	0 d	0 d
2	58 b	45 b	30 c	40 b	0 d
3	0 c	0 c	0 d	0 d	0 d
4	83 a	0 c	100 a	30 b	40 b
5	0 c	0 c	45 b	0 d	0 d
6	0 c	0 c	0 d	33 b	25 c
7	0 c	0 c	0 d	0 d	0 d
8	90 a	90 a	100 a	100 a	93 a

Different lowercase letters indicate significant differences by Tukey test (5% probability).

Overall, a wide variation in microbial growth was observed, ranging from complete inhibition to growth levels similar to those observed in the untreated control. This variability demonstrates that the response of biological agents cannot be generalized based solely on the presence of a specific active ingredient, highlighting the importance of evaluating commercial formulations under practical application conditions.

Among the chemical treatments, T1 (Thiophanate-methyl + Fluazinam) showed the highest compatibility with the fungal biological control agents, allowing 88% growth of both *Trichoderma harzianum* and *Paecilomyces lilacinus*. These results indicate that, despite the fungicidal nature of the formulation, substantial survival of both fungal antagonists was maintained after seed treatment.

In contrast, T4 (Pyraclostrobin + Thiophanate-methyl + Fipronil) exhibited a selective compatibility profile. While *T. harzianum* maintained high growth (83%) and *Bacillus subtilis* reached 100% growth, *P. lilacinus* was completely inhibited. This finding demonstrates that microorganisms belonging to the same functional group may respond differently to the same chemical formulation, emphasizing the importance of species-specific compatibility assessments.

A particularly contrasting response was observed for T4 (pyraclostrobin + thiophanate-methyl + fipronil), which allowed 83% growth of *T. harzianum* but completely inhibited *P. lilacinus*. This differential response indicates that compatibility with chemical seed treatments cannot be generalized even among fungal biological control agents. Similar species-specific responses have been reported by Loureiro et al. (2020), who evaluated *T. harzianum and P. lilacinum* exposed to chemical products used in soybean seed treatment. Although the evaluated chemical products allowed fungal growth, their effects differed between the two biological agents; for example, Avicta® was classified as compatible with *T. harzianum* but moderately toxic to *P. lilacinum*. These findings support the hypothesis that fungal biological control agents may exhibit distinct sensitivities to the same chemical exposure.

The relatively high growth of *T. harzianum* observed under T4 may also be related to the intrinsic ability of some Trichoderma strains to tolerate fungicidal compounds. Kim et al. (2023) demonstrated that *T. atroviride* ATR697 and *T. longibrachiatum* LON701 maintained mycelial growth on PDA supplemented with pyraclostrobin, one of the fungicidal active ingredients present in T4, although the extent of growth differed between isolates. Likewise, Izidoro-Morimitsu et al. (2026) reported a significant fungicide × Trichoderma strain interaction, demonstrating that sensitivity to fungicides varies considerably among strains. In their study, some Trichoderma strains maintained

high mycelial growth under chemical exposure, whereas the commercial *T. harzianum* strain was severely inhibited by some fungicides. Together, these findings reinforce that fungicide compatibility is strongly dependent on the fungal species and strain and should therefore be determined experimentally rather than inferred solely from the chemical active ingredient.

Differences in fungicide tolerance among *Trichoderma* strains may also have a physiological and genetic basis. Membrane transport systems represent one potential mechanism contributing to fungal tolerance to antimicrobial compounds. Liu et al. (2012) identified the major facilitator superfamily transporter gene Thmfs1 in *T. harzianum* and demonstrated that its overexpression increased fungicide tolerance, whereas disruption of the gene resulted in the opposite response. Although the present study did not investigate molecular mechanisms and therefore cannot attribute the tolerance of *T. harzianum* IBLF 006 to this specific pathway, such evidence demonstrates that intrinsic mechanisms of fungicide tolerance occur in *T. harzianum*. Thus, the contrasting responses of *T. harzianum* and *P. lilacinus* to T4 may reflect species- and strain-specific physiological and genetic differences in susceptibility to one or more components of the commercial formulation. Further studies evaluating the individual active ingredients and molecular responses of these strains would be required to identify the mechanisms responsible for this differential compatibility.

The highest levels of incompatibility were observed for T3 (Carbendazim + Thiram) and T7 [(Thiophanate-methyl + Fluazinam) + Fipronil + Polymer], which completely inhibited the growth of all biological agents evaluated. The broad-spectrum toxicity observed in these treatments suggests a strong inhibitory effect on both fungal and bacterial microorganisms, indicating limited potential for simultaneous use with biological products applied via seed treatment.

The T2 (Metalaxyl-M + Thiabendazole + Fludioxonil) exhibited intermediate compatibility, allowing partial growth of *T. harzianum* (58%), *P. lilacinus* (45%), *B. subtilis* (30%), and *Azospirillum brasilense* (40%), although no growth of *Bradyrhizobium japonicum* was detected. These results suggest that formulation exerts selective pressure on different microbial groups, affecting species differently according to their physiological characteristics.

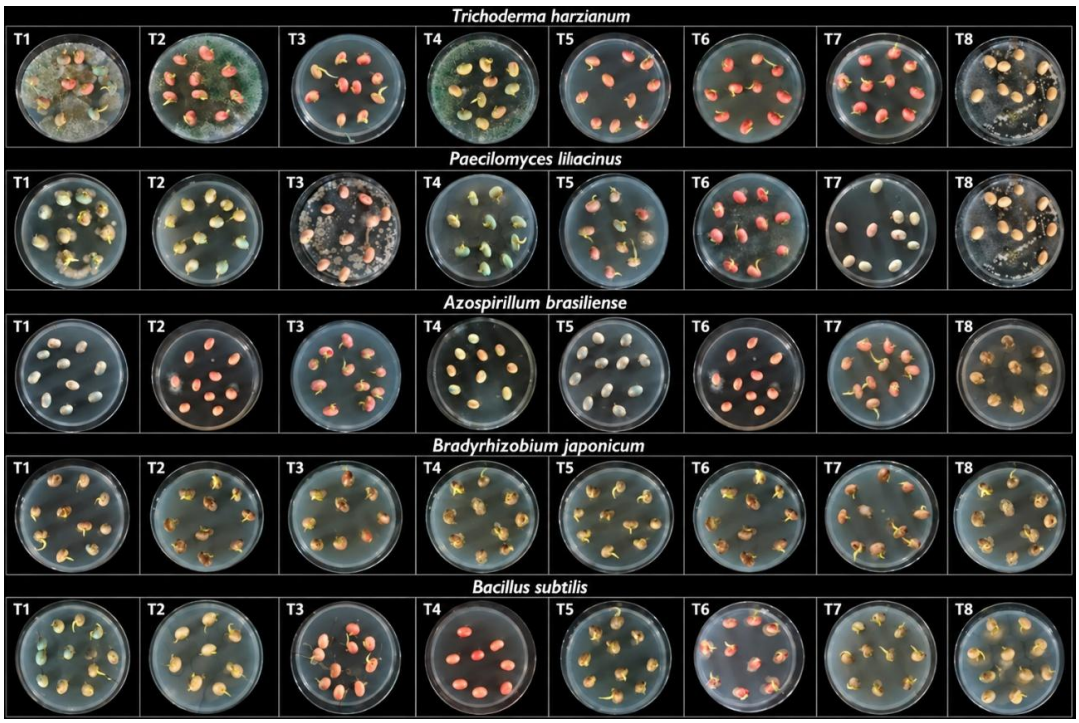


Figure 4. Growth of biological agents on soybean seeds subjected to different chemical seed treatments. Soybean seeds were treated with chemical products (T1–T7) or left untreated (T8) and subsequently inoculated with the biological agents *Trichoderma harzianum*, *Paecilomyces lilacinus*, *Bacillus subtilis*, *Azospirillum brasilense*, and *Bradyrhizobium japonicum*. Seeds were incubated on potato dextrose agar (PDA) medium for three days at 25 ± 2 °C, and microbial growth was assessed under a stereomicroscope (80× magnification). Images represent the visual response of each biological agent to the chemical seed treatments, demonstrating differences in microbial colonization and growth among treatments.

The images presented in Figures 3 and 4 support the quantitative results obtained in Table 3, clearly illustrating the differences in microbial colonization after exposure to the chemical treatments.

A marked difference was observed between fungal and bacterial biological agents. In general, fungal antagonists exhibited greater tolerance to chemical treatments than the bacterial inoculants. While *T. harzianum* maintained substantial growth in T1, T2, and T4, bacterial species showed a more restricted pattern of survival.

For *A. brasilense*, detectable growth was observed only in T2 (40%), T4 (30%), T6 (33%), and the untreated control (100%). A similar trend was observed for *B. japonicum*, which survived only in T4 (40%), T6 (25%), and the untreated control (93%). These results suggest greater sensitivity of nitrogen-fixing and plant growth-promoting bacteria to the active ingredients deposited on the seed surface.

Previous studies have shown that fungicides and insecticides used in seed treatment may negatively affect inoculant survival due to direct contact with active ingredients, changes in pH, and damage to cellular structures (Alcântara Neto et al., 2014). Likewise, Mercante et al. (2007) reported significant reductions in the survival of *Bradyrhizobium japonicum* strains following exposure to fungicides commonly used in soybean seed treatment.

The contrasting responses observed among microorganisms reinforce the importance of evaluating compatibility individually for each biological agent and chemical formulation. The results demonstrate that certain products may allow the integration of biological and chemical technologies, whereas others may completely suppress beneficial microorganisms, compromising the effectiveness of biological seed treatments.

This observation reinforces the importance of evaluating complete commercial formulations rather than individual active ingredients alone. Similar findings were reported by Sánchez-Montesinos et al. (2021), who demonstrated that compatibility between fungicides and *Trichoderma* isolates varied according to the chemical molecule evaluated, indicating that some fungicides can be integrated with biological control agents without significantly affecting their antagonistic activity.

3.2 Minimum inhibitory concentration (MIC) of chemical treatments

The results obtained through the serial microdilution assay complemented the observations from the seed colonization experiment by providing a quantitative assessment of the inhibitory effects of each treatment on microbial growth (Table 4).

The strongest inhibitory effects were observed for T7 [(thiophanate-methyl + fluazinam) + fipronil + polymer], which caused complete inhibition (CI) of *T. harzianum*, *P. lilacinus*, *B. subtilis*, and *A. brasilense*. These results corroborate the complete absence of growth observed in the seed assay and indicate a strong inhibitory effect of this formulation on multiple microbial groups.

Table 4
Minimum inhibitory concentration of chemical seed treatment formulations against biological agents used in seed treatment

Treat-ment	Active ingredients	*T. harzianum* MIC (Dilution)	*P. lilacinus* MIC (Dilution)	*B. subtilis* MIC (Dilution)	*A. brasilense* MIC (Dilution)	*B. japonicum* MIC (Dilution)
1	215	3.36 (7/12)	53.75 (3/12)	3.36 (7/12)	CI	NI
2	125	125 (1/12)	31.25 (3/12)	NI	7.84 (5/12)	NI
3	200	CI	50 (3/12)	100 (2/12)	100 (2/12)	12.5 (5/12)
4	200	NI	50 (3/12)	200 (1/12)	NI	NI
5	100+300+ 200+100	0 (10/12)	CI	NI	3.12+9.37+ 6.25+3.12 (6/12)	CI
6	100+200+ 300+100	NI	0.02+0.04+ 0.05+0.02 (6/12)	NI	1.56+3.12+ 4.69+1.56 (6/12)	NI
7	200+100+ 200	CI	CI	CI	CI	1.56+0.78 +1.56 (8/12)

MIC = minimum inhibitory concentration, defined as the lowest concentration of the chemical treatment capable of completely inhibiting microbial growth. Dilution refers to the dilution level in the serial microdilution assay at which the MIC was determined. NI = no inhibition observed at any tested concentration. CI = complete inhibition at all tested concentrations.

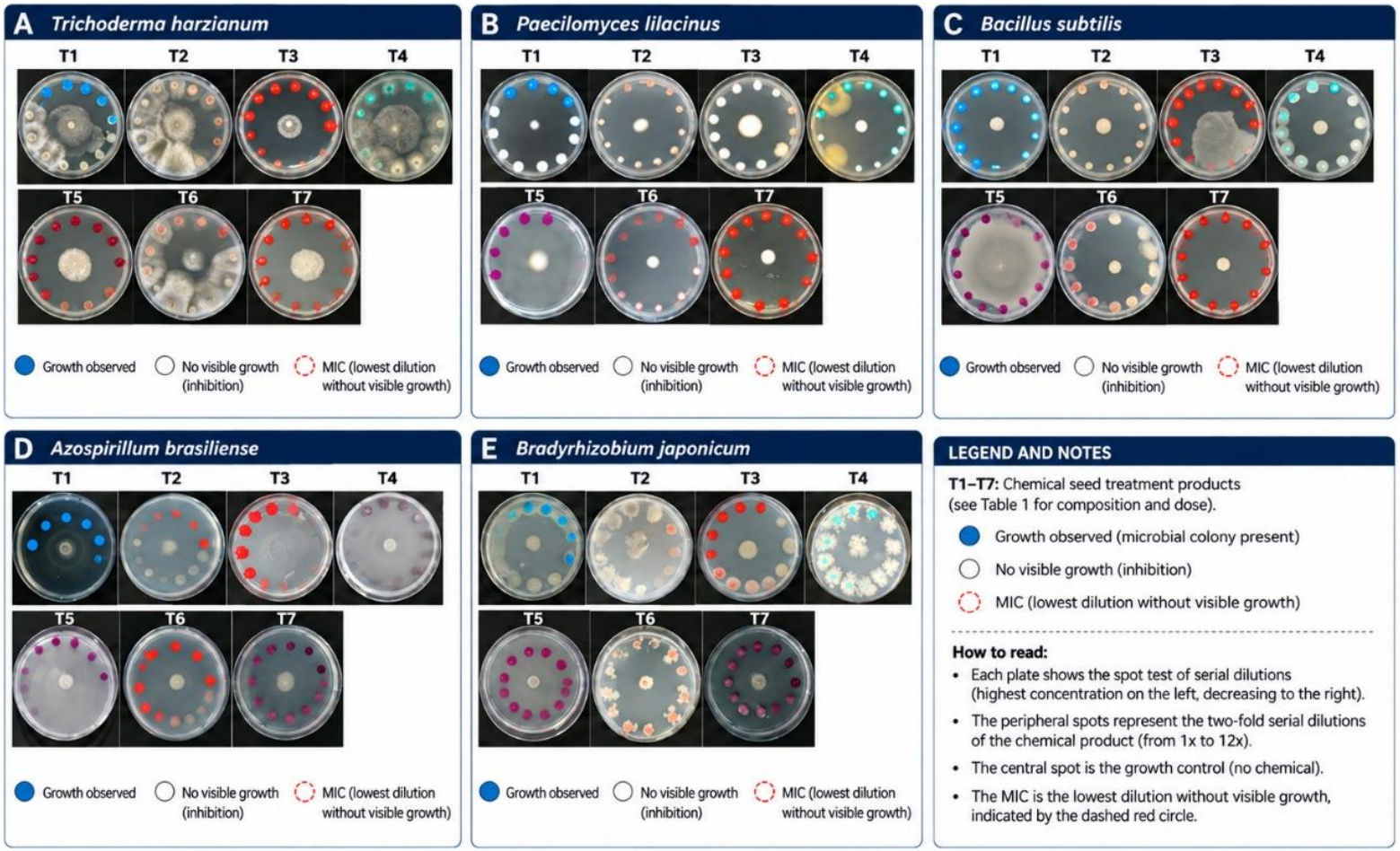


Figure 5. Representative spot-test assay used to confirm the minimum inhibitory concentration (MIC) of chemical seed treatment formulations against beneficial microorganisms. Following serial two-fold microdilution in Brain Heart Infusion (BHI) broth, 10-µL aliquots from each dilution were transferred onto potato dextrose agar (PDA) plates and incubated at 25 ± 2 °C for 48 h. The presence or absence of microbial growth was used to confirm the minimum inhibitory concentration (MIC) for *Trichoderma harzianum*, *Paecilomyces lilacinus*, *Bacillus subtilis*, *Azospirillum brasilense*, and *Bradyrhizobium japonicum*. Representative images show the transition between complete growth inhibition and growth recovery across the dilution series of the evaluated chemical seed treatment formulations.

Similarly, T3 (carbendazim + thiram) caused complete inhibition of *T. harzianum* and inhibited the other microorganisms at different concentrations in the MIC assay, supporting the severe suppression observed in the seed-colonization experiment. The strong antimicrobial activity of this formulation may therefore explain the complete absence of microbial growth recorded on treated seeds.

Conversely, T4 (pyraclostrobin + thiophanate-methyl + fipronil) showed a more microorganism-dependent compatibility profile. No inhibition was observed for *T. harzianum, A. brasilense, or B. japonicum* in the MIC assay, whereas *B. subtilis* showed inhibition at 200 µL mL^{-1}. These findings partially agree with the seed-colonization assay, particularly for *T. harzianum,* which maintained 83% growth. However, differences between the two methodologies were also evident, since P. lilacinus showed no growth on treated seeds, whereas *A. brasilense* and *B. japonicum* showed only partial establishment. These results reinforce that compatibility depends not only on the chemical formulation and biological agent but also on the experimental conditions under which their interaction is evaluated.

T2 (metalaxyl-M + thiabendazole + fludioxonil) also demonstrated microorganism-dependent responses. No inhibition was detected for *B. subtilis* or *B. japonicum* in the MIC assay, whereas the fungal agents and *A. brasilense* were inhibited at specific concentrations. In the seed-colonization assay, however, B. japonicum showed no growth, despite its NI response in the MIC assay. This difference further illustrates that microbial sensitivity under direct exposure in culture medium does not necessarily predict establishment on the treated seed surface.

An additional aspect highlighted by the MIC assay was the occurrence of treatments classified as NI (no inhibition). NI indicates that microbial growth was maintained even at the highest concentration tested, whereas CI (complete inhibition) indicates that microbial growth was completely inhibited at all concentrations evaluated. These classifications therefore represent opposite responses to direct chemical exposure under the conditions of the microdilution assay. NI responses were particularly frequent for B. subtilis, which showed no inhibition in T2, T5, and T6, indicating relatively high tolerance to these formulations under the conditions of the MIC assay.

The combined use of seed-colonization and MIC assays is particularly relevant because different methodologies may generate distinct responses regarding biological compatibility. In a meta-analysis comprising 122 studies and more than 2,000 experimental observations, Folorunso et al. (2024) demonstrated that pesticide concentration, exposure time, temperature, and experimental methodology significantly influence compatibility assessments involving biological control agents. According to the authors, fungicides are among the pesticide groups most likely to interfere with beneficial microorganisms, corroborating the formulation- and microorganism-dependent responses observed in the present study.

The integration of the seed-colonization and MIC assays therefore provides complementary information on the compatibility between chemical treatments and biological agents. The apparent discrepancies between the two assays should not necessarily be interpreted as contradictory results, since they represent distinct exposure conditions. In the seed-colonization assay, microbial establishment occurs on the seed surface, where the chemical treatment is subjected to deposition, distribution, adsorption, and interactions with the seed coat and formulation components. In contrast, the MIC assay exposes the microorganism directly to serial concentrations of the chemical treatment in a nutrient medium, providing more direct and homogeneous contact between the microorganism and the chemical components. Consequently, a treatment classified as NI in the MIC assay may still reduce microbial establishment on treated seeds, while inhibition observed under direct in vitro exposure may not necessarily correspond to the same magnitude of inhibition on the seed surface. Thus, the two assays should be interpreted as complementary rather than interchangeable: the MIC assay characterizes microbial sensitivity under direct chemical exposure, whereas the seed-colonization assay represents a condition more closely related to the practical interaction between chemical and biological products following seed treatment.

From a practical perspective, the MIC results also indicate that reducing product concentration may not always be sufficient to ensure biological compatibility. In several cases, inhibitory effects persisted even after successive dilutions, indicating that some formulations maintain antimicrobial activity against non-target microorganisms at concentrations below the commercial rate.

Taken together, the results of both methodologies demonstrate that compatibility between chemical seed treatments and biological agents is highly dependent on the formulation, microorganism, and exposure conditions. While treatments such as T1 and T4 exhibited compatibility with specific microbial groups, T3 and T7 showed broader inhibitory effects and may compromise the establishment or

performance of biological inoculants and biological control agents when applied simultaneously to soybean seeds. Therefore, compatibility should be evaluated for each chemical–biological combination, and field studies are required to determine whether the responses observed under controlled conditions are maintained under commercial soybean production conditions.

4. Conclusions

Compatibility between chemical seed treatments and biological agents was found to depend on the chemical formulation, the microbial species, and the strain evaluated. Among the tested treatments, T3 (carbendazim + thiram) and T7 [(thiophanate-methyl + fluazinam) + fipronil + polymer] showed the broadest inhibitory effects and should therefore be avoided for simultaneous application with the biological agents evaluated in this study. In contrast, T1 (thiophanate-methyl + fluazinam) showed greater compatibility with *T. harzianum* and *P. lilacinus*, while T4 (pyraclostrobin + thiophanate-methyl + fipronil) showed greater compatibility with selected biological agents, particularly *T. harzianum*. These results demonstrate that the selection of chemical and biological products for combined seed treatment should consider the specific microorganism and strain used, since compatibility observed for one strain cannot necessarily be extrapolated to other strains of the same species. The complementary use of seed-colonization and minimum inhibitory concentration (MIC) assays can support the selection of potentially compatible combinations. However, field validation, including the evaluation of different microbial strains, is required before these combinations can be recommended for commercial soybean production.

CRediT authorship contribution

M. da-Silva: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Validation. **J.-C. Possenti:** Conceptualization, Supervision, Project administration. **M.-C. Schuster-Russiano:** Data curation, Writing – review & editing, Investigation. **C. G. S. Russiano:** Investigation, Writing – review & editing. **S. Bau-Morgan:** Investigation, Writing – review & editing. **C.-N. Salvadori:** Investigation, Writing – review & editing. **Y.-K. Kniss:** Visualization, Validation. **T. de-Campos-Siega:** Visualization, Validation. **S.-M. Mazaro:** Conceptualization, Supervision, Project administration.

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