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Original Article

Agronomic performance of soybean seeds treated with chemical and biological phytosanitary products

Desempenho agronômico de sementes de soja tratadas com produtos fitossanitários químicos e biológicos

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Abstract

Soybean is one of the major crops in Brazil, and its quality and yield can be compromised by pests. Seed treatment with chemical products is widely used to prevent damage caused by these organisms; however, excessive use can lead to selection of resistant pathogens and negative environmental impacts. Biological products, in contrast, have proven to be a cost-effective and sustainable alternative for maintaining crop yield potential. This study evaluated the effects of treating seeds with combinations of biological and chemical products on soybean agronomic parameters. The experimental area consisted of four 20-ha randomized blocks, with treatments comprising different combinations of chemical insecticides and fungicides (clothianidin, fipronil, pyraclostrobin, methyl thiophanate, and fluazinam) and biological agents [(*Bacillus subtilis*, *B. firmus*, *B. licheniformis,* and *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*)], plus an untreated control. Plant population, height, and leaf disease incidence were assessed at 7, 14, 21, and 28 days after planting (DAP) by randomly sampling 10 plants per block. Data were subjected to analysis of variance (ANOVA), and treatment means were compared using the Scott-Knott test ($p \leq 0.05$). Drones and digital image analysis were used for data collection and processing. Significant differences among treatments were observed for plant height, but no differences were detected for plant health at 7 and 14 DAP. Treatment 2 (*B. firmus* + clothianidin + pyraclostrobin + methyl thiophanate + fipronil) resulted in higher plant stand, height, and health, grain yield indicating that the combination of chemical and biological products enhances soybean development.

Keywords: *Bacillus*, clothianidin, fipronil, methyl thiophanate, *Purpureocillium lilacinum*, pyraclostrobin.

Resumo

A soja é uma das principais culturas agrícolas do Brasil, e sua qualidade e produtividade podem ser comprometidas por pragas e patógenos. O tratamento de sementes com produtos químicos é amplamente utilizado para prevenir danos causados por esses organismos; contudo, seu uso excessivo pode resultar em seleção de patógenos resistentes e a impactos ambientais negativos. Em contrapartida, produtos biológicos têm se mostrado uma alternativa sustentável e economicamente viável a manutenção do potencial produtivo das culturas. O objetivo deste estudo foi avaliar os efeitos do tratamento de sementes com combinações de produtos biológicos e químicos sobre parâmetros agronômicos da cultura da soja. A área experimental foi composta por quatro blocos randomizados (20 ha cada), com tratamentos consistindo de diferentes combinações de inseticidas e fungicidas químicos (clotianidina, fipronil, piraclostrobina, tiofanato metílico e fluazinam) e agentes biológicos [(*Bacillus subtilis*, *B. firmus, B. licheniformis* e *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*)], além de uma testemunha sem tratamento. População e altura de plantas e incidência de doenças foliares foram avaliadas aos 7, 14, 21 e 28 dias após o plantio (DAP), por amostragem aleatória de 10 plantas por bloco. Os dados foram submetidos à análise de variância (ANOVA), e as médias foram comparadas pelo teste de Scott-Knott ($p \leq 0{,}05$). Drones e ferramentas de análise foram utilizados para a coleta e o processamento de imagens digitais. Diferenças significativas entre tratamentos foram observadas para altura de plantas, enquanto que sanidade de plantas não apresentou diferenças aos 7 e 14 DAP. O tratamento 2 (*B. firmus* + clotianidina + piraclostrobina + tiofanato metílico + fipronil) resultou em maior estande, altura e sanidade das plantas e produtividade de grãos, indicando que a combinação de produtos químicos e biológicos favorece o desenvolvimento da soja.

Palavras-chave: *Bacillus*, clotianidina, fipronil, tiofanato metílico, *Purpureocillium lilacinum*, piraclostrobina.

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1. Introduction

The cultivation of soybean [*Glycine max* (L.) Merr.] is of major importance to Brazilian agriculture due to scientific and technological advances that have made it the country's leading agricultural commodity (EMBRAPA, 2022). In the 2024/2025 growing season, Brazil produced 154.8 million Mg (CONAB, 2025). High grain yield depends on proper crop management and favorable environmental conditions. However, pests and diseases can reduce grain quality and yield, and may cause plant mortality and severe crop losses when not adequately managed (CEPEA, 2019).

Agricultural modernization has enabled the use of digital tools for improved crop management. Remote sensing platforms allow the identification of specific areas requiring intervention, supporting strategic decision-making and reducing excessive use of phytosanitary products (Vianna, 2020). These technologies also improve data collection, resource management, and pest and nematode control, leading to increased efficiency and reduced environmental impacts (Tonnang et al., 2022).

Plant-parasitic nematodes reduce soybean yield by damaging roots, decreasing plant vigor, and favoring infection by pathogens. Their importance in Brazilian production systems has increased, making them one of the main phytosanitary constraints; in severe cases, they render cultivation unfeasible. In addition to direct damage, they contribute to disease complexes by creating infection sites, modifying the rhizosphere, serving as vectors of viruses, bacteria, and fungi, and altering host susceptibility through physiological changes (Buzdar et al., 2025).

Seed treatment with chemical and biological products is one of the main tools for managing nematodes and insects (Paula et al., 2024). Seeds of uncertified origin can reduce germination and vigor, thus limiting yield, increasing costs, and facilitating the dissemination and accumulation of pathogens in cultivated areas (Maculan et al., 2021). Chemical treatment effectively protects seeds and prevents fungal diseases; however, excessive use can promote the selection of resistant pathogen populations and cause environmental impacts, indicating the need for appropriate application rates and responsible use (Sinha et al., 2017; Paulikienė et al., 2025).

The association of different product classes reduces the number of crop management operations, increasing efficiency, safety, and convenience. Seed treatment commonly combines fungicides, insecticides, and nematicides (Moumni et al., 2023; Loureiro et al., 2026). Despite advances in biotechnology, agricultural sustainability remains a global concern that continues to receive increasing attention. In this context, biological control of pests and diseases has expanded worldwide at rates of 10%–20% per year and in Brazil at approximately 47%, driven by consumer demand for safer products with fewer synthetic residues and lower environmental toxicity risks (Van Lenteren et al., 2018; Andreata et al., 2025).

Bacteria of the genus *Bacillus* are important components of the rhizosphere and effectively control nematodes, pathogens, and insects. These bacteria exhibit multiple modes of action, which confer high efficiency and versatility. They colonize plant roots, produce antagonistic compounds against nematodes and pathogens, form biofilms that hinder nematode penetration, promote plant growth, and induce resistance (Bettiol et al., 2022; Padilha et al., 2025). Fernandes et al. (2021) reported that *Bacillus* spp. and antagonistic fungi can inhibit the mycelial growth of several pathogenic fungi in soybean, thereby reducing pathogen incidence. Therefore, this study evaluated the effects of treating seeds with combinations of chemical and biological phytosanitary products on soybean yield and plant health.

2. Materials and Methods

2.1. Experimental setup and cultural practices

The study was conducted during the 2024/2025 growing season at Bacuri Farm in Costa Rica, Mato Grosso do Sul (MS), Brazil (18°19'26.0"S, 53°5'41.3"W; 810 m altitude), in an area of 880 ha surrounded by commercial soybean fields. The regional climate is classified as Aw (tropical savanna), according to the Köppen classification, with a well-defined dry season, a mean annual temperature of 25 °C and an annual rainfall of 1,600–1,800 mm (Peel et al., 2007). The predominant soil type in the region is Typic Hapludox (Latossolo Vermelho Distrófico típico) (Soil Survey Staff, 2022; Santos et al., 2025)

The soybean cultivar Brasmax Bonus IPRO RR2 Intacta (indeterminate growth habit, maturity group 7.9) was used (Brasmax, 2025). Seeds were treated on the day of planting, 23 October 2024. Sowing was performed with a vacuum precision planter at a row spacing of 0.45 m, with a target population of 14 plants per linear meter.

Basal fertilization consisted of the application of organomineral fertilizers. Potassium was applied 30 days after soybean emergence at a rate of 60 Kg ha^{-1} of K_2O using KCl (60% K_2O). Cultural practices followed recommendations for soybean crops, with deficiencies corrected to avoid interference with the products in the evaluated treatments (Grigolli and Grigolli, 2019). Three fungicide applications were performed: pyraclostrobin plus fluxapyroxad (0.3 mL ha^{-1}) at 45 days after emergence (DAE); fluxapyroxad (0.3 mL ha^{-1}) plus prothioconazole (0.25 mL ha^{-1}) at 60 DAE; and fenpropimorph (0.3 mL ha^{-1}) at 75 DAE. Thereafter, the need for an additional fungicide application was evaluated, but none was required.

2.2. Combination of chemical and biological phytosanitary products in soybean management

The experiment was conducted in a randomized block design with four blocks, each with 20 ha, for a total experimental area of 80 ha. The combinations of chemical and biological products for seed treatments are described in Table 1. Soybean seeds were treated separately for each treatment by adding the products at the recommended rates and then homogenizing the seed lot to ensure uniform distribution. Rates were based on the recommendation for each product per 100 Kg of seeds.

The number of healthy plants (those without disease symptoms), the number of plants showing disease symptoms, and the presence of nematode lesions in roots (history of *Pratylenchus brachyurus* infestation) were

Table 1. Experimental seed treatments with combinations of biological and chemical products in soybean.

Treatment	Class	Active ingredient	Mode of action	Trade name	Applied rate
T1	Fungicide	Pyraclostrobin	Inhibition of complex III (cytochrome bc1, ubiquinol oxidase at the Qo site)	Standak® Top	2 mL kg^{-1}
	Fungicide	Methyl thiophanate	β-tubulin assembly inhibition during mitosis		
	Insecticide	Fipronil	Contact and ingestion activity		
T2	Bionematicide	*Bacillus firmus*	Microbiological	Votivo® Prime	1 mL kg^{-1}
	Insecticide	Clothianidin	Systemic	Poncho®	1 mL kg^{-1}
	Fungicides + insecticide	Pyraclostrobin + methyl thiophanate + fipronil	Multiple modes of action	Standak® Top	2 mL kg^{-1}
T3	Insecticide/ termiticide	Fipronil	Contact and ingestion activity	Nortox® 800	2 mL kg^{-1}
	Fungicide/ nematicide	Methyl thiophanate + fluazinam	Systemic and contact	Certeza® N	2 mL kg^{-1}
	Bionematicide	*B. subtilis* + *B. licheniformis* + *Purpureocillium lilacinum* (= *Paecilomyces lilacinus*)	Microbiological	Profix®	75 g ha^{-1}
T4	Untreated control	–	–	–	–

evaluated. Evaluations were performed in each block at 7, 14, 21 and 28 days after planting (DAP) by sampling 10 randomly selected points per block, with 10 plants collected per point to assess stand (number of plants m^{-2}), plant height (cm), and plant health status.

2.3. Digital image analysis

Drones and digital platforms were used for data collection and analysis based on two systems: Xarvio® Field Manager for area demarcation and assessment of insect damage, drought stress, and fungal diseases; and Climate FieldView™ for monitoring crop yield values across all plots. An iPad was connected to the harvester in each 20-ha area to record the location data and ensure accurate yield estimation. After harvesting the designated area, the data were automatically stored and made available by the platform.

Evaluations using digital platforms were based on biomass maps generated from satellite imagery, with colors ranging from green to orange, yellow, and red; greener areas indicated more developed plants with greater leaf area, whereas red areas indicated plants with smaller leaf area. The different colors corresponded to biomass percentages ranging from 0 to 100% and indicated the presence of symptoms on plants, which could be caused by nematodes, insects, or diseases. Additionally, biomass monitoring maps were generated to evaluate daily changes in plant development and progression toward harvest as tracked by the platform. All evaluations were based on normalized difference vegetation index (NDVI) images to enable comparisons among treatments. The steps followed to generate the evaluation maps were: 1) the location of the field was verified; 2) the area was registered on the digital platform; and 3) the field boundaries were delineated, after which evaluations were performed within 48 hours, followed by access to the images and georeferenced points.

Harvest was conducted on 13 March 2025**,** with each 20-ha treatment harvested separately using a John Deere® S430 combine harvester to estimate grain yield expressed in 60-kg bags ha^{-1}. After harvest, soybeans were unloaded onto a truck and weighed on a platform scale, and the harvested area was then measured using GPS. Grain yield was calculated after correction to 13% moisture. Data were subjected to analysis of variance (ANOVA), and treatment means were compared using the Scott-Knott test at $p \leq 0.05$.

3. Results and Discussion

Biomass maps showed that soybean plants exhibited an intense green color, indicating better development, at 7 and 14 days after planting (DAP). However, at 21 and 28 DAP, the percentage of plants with green color decreased (Figure 1).

All seed treatments resulted in higher plant stand (number of plants m^{-2}) than the untreated control (T4), with T2 (*Bacillus firmus* + clothianidin + pyraclostrobin + methyl thiophanate + fipronil) providing the best results across all evaluation periods, followed by T1 (pyraclostrobin + methyl thiophanate + fipronil) and T3 (fipronil + methyl thiophanate + fluazinam + *B. subtilis* + *B. licheniformis* + *Purpureocillium lilacinum*), which did not differ from each other (Table 2). Seed treatment with either chemical or biological products is directly related to seed health and, consequently, to plant development, as it provides protection against pathogens, pests, and diseases, thereby ensuring germination and proper plant establishment (Sousa et al., 2021; André et al., 2025). Plant stand establishment is a critical process in soybean production

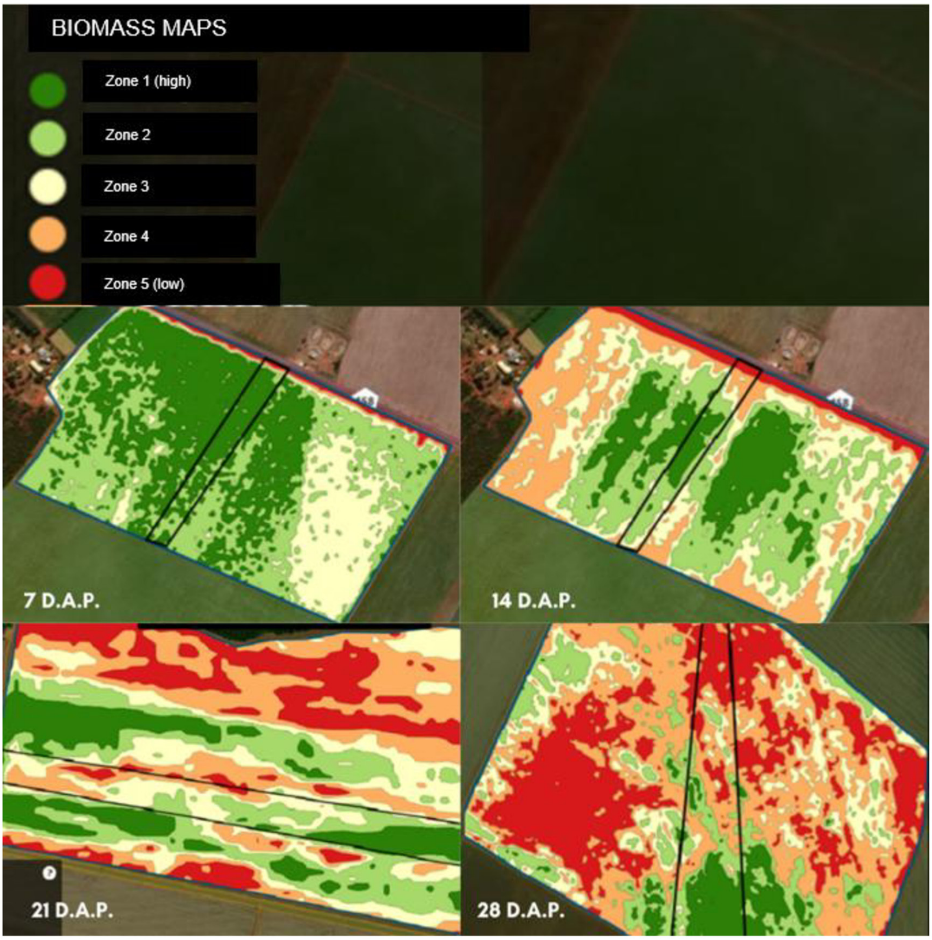

Figure 1. Biomass maps of soybean crops at different days after planting (DAP) from seeds treated with combinations of chemical and biological products.

Table 2. Mean values (± standard error) of plant stand (number of plants m^{-2}) of soybean grown from seeds treated with combinations of chemical and biological products, evaluated at 7, 14, 21, and 28 days after planting (DAP).

Treatment	7 DAP	14 DAP	21 DAP	28 DAP
T1	10.9 ± 0.45 b	12.2 ± 0.71 b	11.8 ± 0.34 b	12.0 ± 0.25 b
T2	13.4 ± 0.21 a	13.1 ± 0.55 a	13.1 ± 0.67 a	13.2 ± 0.85 a
T3	12.3 ± 0.29 b	12.5 ± 0.65 b	12.3 ± 0.41 b	12.5 ± 0.77 b
T4 (untreated control)	10.2 ± 0.44 c	10.0 ± 0.78 c	10.0 ± 0.58 c	10.5 ± 0.36 c
CV (%)	3.19	2.56	2.67	3.62

T1 = pyraclostrobin + methyl thiophanate + fipronil. T2 = *Bacillus firmus* + clothianidin + pyraclostrobin + methyl thiophanate + fipronil; and T3 = fipronil + methyl thiophanate + fluazinam + *B. subtilis* + *B. licheniformis* + *Purpureocillium lilacinum*. CV = coefficient of variation; Means followed by the same letter within a column do not differ significantly according to the Scott-Knott test ($p \leq 0.05$).

that involves seed germination and subsequent plant development. The use of chemical and biological seed treatments is and effective strategy to enhance this process.

Seed treatment with biological agents (beneficial microorganisms such as bacteria and fungi) or chemical products involves the application of fungicides, nematicides, and insecticides that protect seeds and young plants against pathogens and insects. According to Vurro et al. (2019), chemical seed treatment effectively prevents the incidence of fungal diseases that can negatively affect plant health.

Sousa et al. (2021) reported that the combination of chemical and biological treatments can produce synergistic effects and that the interaction between beneficial microorganisms and chemical products can result in healthier, more pathogen-resistant plants, and higher crop yields.

Plant height was not significantly affected by the treatments at 7 DAP. Significant differences among treatments were observed from 14 DAP onward, with T2 resulting in the highest value (Table 3).

Plant height is an important characteristic that reflects crop growth and development. Differences in plant height can be attributed to several factors, including seed treatment, which significantly influences plant height (Sousa et al., 2021; Garziz et al., 2025). Recent research has shown that specific chemical seed treatments produce significantly greater seedling height than untreated seeds (Sousa et al., 2021; Martin et al., 2026), indicating improved early seedling growth.

However, seed treatment can also negatively affect seedling height, as reported by Leite et al. (2024), who observed that excessive use of chemical treatment can inhibit seedling growth, resulting in shorter plants than untreated seeds. Therefore, the application of seed treatments should be balanced. According to Ludwig et al. (2011), seed treatment enhances the physiological quality of seeds and seedling development, positively influencing agronomic parameters, hormonal balance through bioregulators, recovery after water stress, resistance to pests, diseases and nematodes, nutrient uptake, and overall physiological development.

All treatments were superior to the untreated control in terms of plant health at 7 and 14 DAP, with no significant differences among them (Table 4). However, T2 and T3 (combinations of chemical and biological products) resulted in better plant health at 21 and 28 DAP. Plant health was assessed visually, with plants without symptoms indicative of diseases or nutritional deficiencies considered healthy.

Plant height and health are influenced by environmental factors such as temperature, soil moisture, and nutrient availability, as well as by seed treatment. The interaction between seed treatment and environmental factors can be complex and requires further studies to elucidate its effects (Atabaki et al., 2023).

Plant height and health can vary according to the treatment applied due to differences in chemical compounds, seed preparation techniques, interactions with environmental factors, and the modes of action of the products. Beneficial microorganisms contribute to plant health by protecting plants against diseases and improving nutrient uptake (Singh et al., 2020). These microorganisms can colonize roots, enhance root growth, and increase disease resistance, thus resulting in more robust plants (Rai et al., 2020).

Treatment T2 resulted in greater plant height and stand compared with the other treatments. This result can be attributed to the combination of biological agents (*Bacilus firmus*) with chemical fungicides (pyraclostrobin and methyl thiophanate) and insecticides (clothianidin and fipronil). *B. firmus* reduces the hatching of juvenile plant-parasitic nematodes through the production of toxins that primarily affect eggshell formation, causing embryonic death (Matsuo et al., 2015; Ghahremani et al., 2020; Dias-

Table 3. Mean values (± standard error) of plant height of soybean grown from seeds treated with combinations of chemical and biological products, measured at 7, 14, 21 and 28 days after planting (DAP).

Treatment	7 DAP	14 DAP	21 DAP	28 DAP
T1	9.1 ± 0.27 a	12.6 ± 0.48 b	23.0 ± 0.97 b	38.9 ± 0.65 b
T2	9.3 ± 0.33 a	13.5 ± 0.37 a	25.6 ± 0.42 a	41.0 ± 0.15 a
T3	9.0 ± 0.21 a	12.8 ± 0.41 b	23.6 ± 0.91 b	38.0 ± 0.60 b
T4 (untreated control)	8.6 ± 0.57 a	11.7 ± 0.55 c	22.8 ± 0.29 b	38.4 ± 0.69 b
CV (%)	3.96	2.70	2.58	1.53

T1 = pyraclostrobin + methyl thiophanate + fipronil. T2 = *Bacillus firmus* + clothianidin + pyraclostrobin + methyl thiophanate + fipronil; and T3 = fipronil + methyl thiophanate + fluazinam + *B. subtilis* + *B. licheniformis* + *Purpureocillium lilacinum*. CV = coefficient of variation; Means followed by the same letter within a column do not differ significantly according to the Scott-Knott test ($p \leq 0.05$).

Table 4. Mean (± standard error) number of healthy plants (without disease symptoms) at 7, 14, 21, and 28 days after planting (DAP) of soybean from seeds treated with combinations of chemical and biological products.

Treatment	7 DAP	14 DAP	21 DAP	28 DAP
T1	8.7 ± 0.43 a	8.4 ± 0.61 a	7.7 ± 0.50 b	7.8 ± 0.99 b
T2	9.2 ± 0.97 a	8.7 ± 0.42 a	8.9 ± 0.73 a	9.0 ± 0.56 a
T3	8.9 ± 0.51 a	8.3 ± 0.65 a	8.0 ± 0.71 a	8.4 ± 0.42 a
T4 (untreated control)	7.6 ± 0.65 b	7.4 ± 0.37 b	6.5 ± 0.49 b	6.9 ± 0.27 b
CV (%)	5.52	7.38	8.38	8.35

T1 = pyraclostrobin + methyl thiophanate + fipronil. T2 = *Bacillus firmus* + clothianidin + pyraclostrobin + methyl thiophanate + fipronil; and T3 = fipronil + methyl thiophanate + fluazinam + *B. subtilis* + *B. licheniformis* + *Purpureocillium lilacinum*. CV = coefficient of variation. Means followed by the same letter within a column do not differ significantly according to the Scott-Knott test ($p \leq 0.05$).

Arieira et al., 2022). This bacterial species also alters root exudates, hindering the ability of parasitic nematodes to locate roots and restricting the mobility of juvenile stages (Machado, 2022). In addition, *B. firmus* induces systemic resistance in plants, providing a protective effect and greater tolerance to abiotic and biotic stresses (Mathew and Opperman, 2020).

The insecticide clothianidin (Poncho®) has both systemic and contact action; it penetrates plant tissues after seed treatment and is translocated through the vascular system. Neonicotinoids act as acetylcholine receptor agonists, causing continuous nerve impulses that lead to hyperexcitation of the nervous system, paralysis, and death of the target organism (Martins et al., 2008). The water-soluble nature of neonicotinoids facilitates their absorption by seeds and translocation during plant development (Elbert et al., 2008), leading to mortality of insects that feed on treated plants.

The commercial product Standak® Top, used in T1 e T2, combines the fungicides pyraclostrobin (protective action) and methyl thiophanate (systemic action) with the insecticide fipronil (contact and ingestion activity). This combination provides multiple and complementary effects during the initial stages of crop development, including suppression of plant-parasitic nematodes, improved root development, uniform stand establishment, and increased tolerance to water stress (BASF, 2025). According to Madalosso (2020), seed treatments with this ready-made mixture of pyraclostrobin, methyl thiophanate, and fipronil (Standak® Top) reduces the initial root inoculum of necrotrophic fungi such as *Macrophomina* spp., *Fusarium* spp., and *Phomopsis* spp. in the management of root diseases, resulting in crop yield increases of up to 2.7 bags ha^{-1}.

Some neonicotinoids can exert a phytotonic effect, promoting faster plant development and better expression of vigor. Experimental treatments with neonicotinoids in soybean have shown increased vigor, yield, leaf area, and root system development, as well as more uniform stand establishment and enhanced initial crop development (Castro et al., 2007).

All seed treatments tested resulted in higher grain yield relative to the untreated control, with T2 and T3 showing the highest values (66.8 and 65.7 bags ha^{-1}, respectively), with T2 exhibiting an increase of 5.6 bags ha^{-1} compared with the untreated control (Table 5 and Figure 2).

Table 5. Mean values (± standard error) of grain yield and yield increase (relative to the untreated control) of soybean from seeds treated with combinations of biological and chemical products.

Treatment	Grain yield (60-kg bags ha^{-1})	Yield increase (60-kg bags ha^{-1})
T1	63.6 ± 1.23 b	2.4
T2	66.8 ± 0.93 a	5.6
T3	65.7 ± 0.44 a	4.5
T4 (untreated control)	61.2 ± 1.78 c	-------
CV (%)	8.74	-------

T1 = pyraclostrobin + methyl thiophanate + fipronil. T2 = *Bacillus firmus* + clothianidin + pyraclostrobin + methyl thiophanate + fipronil; and T3 = fipronil + methyl thiophanate + fluazinam + *B. subtilis* + *B. licheniformis* + *Purpureocillium lilacinum*. CV = coefficient of variation. Means followed by the same letter within a column do not differ significantly according to the Scott-Knott test (p ≤ 0.05).

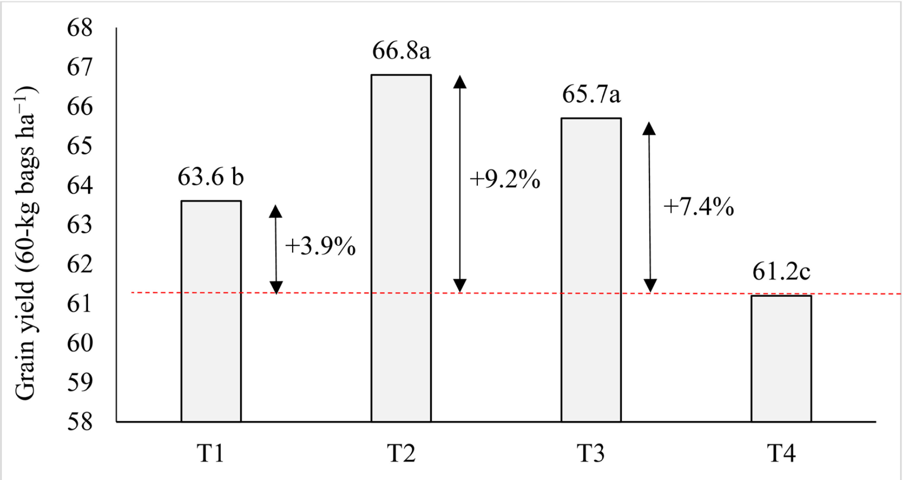

Figure 2. Grain yield (60-kg bags ha^{-1}) of soybean plants grown from seeds treated with different combinations of chemical and biological products. T1 = pyraclostrobin + methyl thiophanate + fipronil. T2 = *Bacillus firmus* + clothianidin + pyraclostrobin + methyl thiophanate + fipronil; T3 = fipronil + methyl thiophanate + fluazinam + *B. subtilis* + *B. licheniformis* + *Purpureocillium lilacinum*; T4 = untreated control.

Microorganisms such as *Bacillus* spp. and *P. lilacinum*, which are present in several commercial products used in plant protection, provide additional benefits to plants, including phosphate solubilization, phytohormone production, nitrogen fixation, and plant growth promotion. These benefits improve seed germination, crop development, and yield (Xiang et al., 2018).

The effects of chemical and biological treatments can vary depending on environmental conditions. Factors such as soil type, climate, and management practices can influence the results; therefore, seed treatment selection should be tailored to specific growing conditions to optimize plant performance (Sousa et al., 2021). Both chemical and biological treatments protect plants against pathogens and insects, promote growth, and improve plant health. Seed treatments combining chemical and biological products can produce synergistic effects. However, the choice of treatments should be adapted to specific environmental conditions to achieve optimal plant stand establishment. The selection between chemical and biological treatments, or their combination, depends on crop requirements, balancing effectiveness against potential environmental impacts, which is essential for productive and sustainable agriculture systems.

4. Conclusions

The seed treatment combining *Bacillus firmus* (Votivo® Prime), clothianidin (Poncho®), pyraclostrobin, methyl thiophanate, and fipronil (Standak® Top) resulted in higher plant stand, height, and health, grain yield indicating that integrating biological agents with chemical insecticides and fungicides enhances soybean development.

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Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

References

ANDRÉ, T.P.P., BARBOSA, M.G., SILVEIRA, F.A., SARMENTO, H.G.S., GODOY, M.S. and PASTORI, P.L., 2025. Efficiency of Bt cotton cultivars without control of different stages of *Spodoptera frugiperda* (Lepidoptera: noctuidae). *Brazilian Journal of Biology*, vol. 85, pp. e291979. https://doi.org/10.1590/1519-6984.291979. PMid:41221930.

ANDREATA, M.F.L., MIAN, S., ANDRADE, G., FREITAS, A.B., VENTURA, M.U., ALMEIDA, J.E.M., IVAN, E.A.F., MOSELA, M., SIMIONATO, A.S., ROBAINA, R.R. and GONÇALVES, L.S.A., 2025. The current increase and future perspectives of the microbial pesticides market in agriculture: the Brazilian example. *Frontiers in Microbiology*, vol. 16, pp. 1574269. https://doi.org/10.3389/fmicb.2025.1574269. PMid:40862135.

ATABAKI, Z.M., GHEREKHLOO, J., GHADERI-FAR, F., ANSARI, O., HASSANPOUR-BOURKHEILI, S. and PRADO, R., 2023. The effect of environmental factors on seed germination and geranium emergence from cut leaves. *Advances in Weed Science*, vol. 41, pp. 1-8. https://doi.org/10.51694/AdvWeedSci/2023;41:00005.

BASF, 2025 [viewed 1 October 2025]. *Standak® Top - Bula e Informações da solução para Tratamento de Sementes* [online]. Available from: https://agriculture.basf.com/br/pt/protecao-de-cultivos-e-sementes/produtos/standak-top.

BETTIOL, W., MEDEIROS, F.H.V., BARROS, J. and MENDES, R., 2022. Advances in screening approaches for the development of microbial bioprotectants to control plant diseases. In: J. KÖHL and W. RAVENSBERG, eds. *Microbial bioprotectors for the management of plant diseases*. Cambridge: Burleigh Dodds Science Publishing, pp. 108.

BRASMAX, 2025 [viewed 7 September 2025]. *Cultivar: Região Cerrado*[online]. Available from: http://www.brasmaxgenetica.com.br/cultivar-regiao-cerrado.

BUZDAR, M.I., AWAN, M.J.A., RAZA, G., NAQVI, R.Z., MANSOOR, S. and AMIN, I., 2025. Potential of conventional and new breeding technologies to manage soybean diseases. *Phytopathology Research*, vol. 7, no. 49, pp. 1-23. https://doi.org/10.1186/s42483-025-00338-0.

CASTRO, P.R.C., PITELLI, A.M.C.M., PERES, L.E.P. and ARAMAKI, P.H., 2007. Analysis of the plant growth regulatory activity of thiamethoxam by means of biotests. *Publicatio UEPG*, vol. 13, no. 3, pp. 25-29.

CENTER FOR ADVANCED STUDIES IN APPLIED ECONOMICS – CEPEA, 2019 [viewed 7 September 2025]. *Efeito do não tratamento de pragas e doenças sobre preços ao consumidor de produtos da cadeia produtiva de soja* [online]. Available from: https://www.cepea.esalq.usp.br/upload/kceditor/files/Cepea_EstudoPragaseDoencas_Parte%203.pdf.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2025 [viewed 29 November 2025]. *CONAB Web: Monitoring of the Brazilian harvest: Grains, Harvest 2024/25, 10th survey* [online]. Available from: https://www.gov.br/conab/pt-br/atuacao/informacoes-agropecuarias/safras/safra-de-graos/boletim-da-safra-de-graos/10o-levantamento-safra-2024-25/10o-levantamento-safra-2024-25.

DIAS-ARIEIRA, C.R., SANTANA-GOMES, S.M., MIAMOTO, A. and MACHADO, A.C.Z., 2022. Biological management of nematodes Bioinputs in soybean crop. In: M.C. MEYER, A.F. BUENO, S. M., MAZARO and J.C. SILVA, eds. *Bioinsumos na cultura da soja*. Brasília: EMBRAPA Soja, pp. 345-358.

ELBERT, A., HAAS, M., SPRINGER, B., THIELERT, W. and NAUEN, R., 2008. Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science*, vol. 64, no. 11, pp. 1099-1105. https://doi.org/10.1002/ps.1616. PMid:18561166.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA, 2022 [viewed 7 September 2025]. *Science and technology have made Brazil one of the largest food producers in the world* [online]. Available from: https://www.embrapa.br/busca-de-noticias/-/noticia/75085849/ciencia-e-tecnologia-tornaram-o-brasil-um-dos-maiores-produtores-mundiais-de-alimentos.

FERNANDES, M.F.R., RIBEIRO, T.G., ROUWS, J.R., BARROS, L.H.S. and ZILLI, J.E., 2021. Biotechnological potential of bacteria from genera *Bacillus paraburkholderia* and *Pseudomonas* to control seed fungal pathogens. *Brazilian Journal of Microbiology : Publication of the Brazilian Society for Microbiology*, vol. 52, no. 2, pp. 705-714. https://doi.org/10.1007/s42770-021-00448-9. PMid:33594600.

GARZIZ, A.A., HELAL, M., YOUNIS, A.M., EL-BADRY, M.A. and YOSRI, M., 2025. Stimulation of the production of proteases from *Penicillium verrucosum* isolated from Egyptian soil. *Brazilian Journal of Biology*, vol. 85, pp. e296244. https://doi.org/10.1590/1519-6984.296244. PMid:40900503.

GHAHREMANI, Z., ESCUDERO, N., BELTRÁN-ANADÓN, D., SAUS, E., CUNQUERO, M., ANDILLA, J., LOZA-ALVAREZ, P., GABALDÓN, T. and SORRIBAS, F.J., 2020. *Bacillus firmus* strain I-1582, a nematode antagonist by itself and through the plant. *Frontiers in Plant Science*, vol. 11, no. 1, pp. 796. https://doi.org/10.3389/fpls.2020.00796. PMid:32765537.

GRIGOLLI, J.F.J. and GRIGOLLI, M.M.K., 2019 [viewed 7 September 2025]. *Manjeo de doenças na cultura da soja* [online]. Available from: https://www.fundacaoms.org.br/wp-content/uploads/2021/02/Tecnologia-e-Producao-Soja-Safra-20182019.pdf

LEITE, C.A.M., FRANÇA-NETO, J.D.B., KRZYZANOWSKI, F.C., GOMES-JUNIOR, F.G., LOPES, I.D.O.N. and HENNING, F.A., 2024. Validation of the Vigor-S image analysis system for the characterization of phytotoxicities in soybean seedlings. *Journal of Seed Science*, vol. 46, pp. 1-8. https://doi.org/10.1590/2317-1545v46279386.

LOUREIRO, E.S., OLIVEIRA, G.S., RODRIGUES, A.S., PESSOA, L.G.A., AMARAL, T.S., PESSOA, M.B., GREGORI, G.S., SOTO-MESA, N.E. and DIAS, P.M., 2026. Potential of adjuvants and an entomopathogenic fungus for the management of *Helicoverpa armigera. Brazilian Journal of Biology*, vol. 86, pp. e303128. http://doi.org/10.1590/1519-6984.303128. PMid:41849476.

LUDWIG, M.P., LUCCA-FILHO, O.A., BAUDET, L., DUTRA, L.M.C., AVELAR, S.A.G. and CRIZEL, R.L., 2011. Quality of soybean seeds stored after being covered with amino acids, polymers, fungicides, and insecticides. *Revista Brasileira de Sementes*, vol. 33, no. 3, pp. 395-406. https://doi.org/10.1590/S0101-31222011000300002.

MACHADO, A.C.Z., 2022. Bionematicides in Brazil: an emerging and challenging market. *Annual Review of Plant Pathology*, vol. 28, pp. 35-49. https://doi.org/10.31976/0104-038321v280002.

MACULAN, J.F., ENGEL, E., BORTOLOTTO, R.P., PASINI, M.P.B., ZAMBERLAN, J.F., HORZ, D.C., CAMERA, J.N. and FIORIN, J.E., 2021. Componentes de rendimento de genótipos de soja em relação a forma de obtenção da semente. *Holos*, vol. 7, no. 37, pp. 1-17. https://doi.org/10.15628/holos.2021.9998.

MADALOSSO, M., 2020 [viewed 10 October 2025]. *Standak® Top - Proven Benefit* [online]. Available from: https://revistacultivar.com.br/artigos/standak-top-beneficio-comprovado.

MARTIN, T.N., VEY, R.T., STECCA, J.D.L., BRUM, L.N., CIPRIANI, L.P. and BRASIL NETO, E.S., 2026. Applications of *Trichoderma asperelloides* in soybean production: a perspective based on bibliometric and experimental data. *Brazilian Journal of Biology*, vol. 86, pp. e301828. https://doi.org/10.1590/1519-6984.301828. PMid:41880388.

MARTINS, G.M., TOSCANO, L.C., TOMQUELSKI, G.V. and MARUYAMA, W.I., 2008. Eficiência de inseticidas no controle de *Dalbulus maidis* (Hemiptera: Cicadellidae) na cultura do milho. *Revista Caatinga*, vol. 21, no. 4, pp. 196-200.

MATHEW, R. and OPPERMAN, C., 2020. Current insights on migratory endoparasitism: deciphering the biology, parasitism mechanisms, and management strategies of the main migratory endoparasitic nematodes. *Plants*, vol. 9, no. 6, pp. 671. https://doi.org/10.3390/plants9060671. PMid:32466416.

MATSUO, E., SEDIYAMA, T., DIAS, W.P. and GLASENAPP, J.S., 2015. Resistência a nematóides. In: T. SEDIYAMA, ed. *Melhoramento Genético da soja*. Londrina: Mecenas, pp. 211-238.

MOUMNI, M., BRODAL, G. and ROMANAZZI, G., 2023. Recent innovative seed treatment methods in the management of seedborne pathogens. *Food Security*, vol. 15, no. 5, pp. 1365-1382. https://doi.org/10.1007/s12571-023-01384-2.

PADILHA, K.S.M.A., PEGORARO, R.F., BARROSO, A.M.F., SOARES, V.A.S.F., ALVES, P.G.L., FERREIRA, E.A. and SOUZA, R.P.D., 2025. Damage caused by caterpillars in chickpea crops in tropical regions. *Brazilian Journal of Biology*, vol. 85, pp. e290139. https://doi.org/10.1590/1519-6984.290139. PMid:40435073.

PAULA, L.L., CAMPOS, V.P., TERRA, W.C., BRUM, D., JACOBS, D.C., BUI, H.X. and DESAEGER, J.A., 2024. The combination of *Bacillus amyloliquefaciens* and *Purpureocillium lilacinum* in the control of *Meloidogyne enterolobii. Biological Control*, vol. 189, no. 105439, pp. 1-9. https://doi.org/10.1016/j.biocontrol.2023.105438.

PAULIKIENĖ, S., BENESEVIČIUS, D., BENESEVIČIENĖ, K. and ŪKSAS, T., 2025. Seed treatment: importance, application, impact, and opportunities for increasing sustainability. *Agronomy (Basel)*, vol. 15, no. 7, pp. 1-28. https://doi.org/10.3390/agronomy15071689.

PEEL, M.C., FINLAYSON, B.L. and MCMAHON, T.A., 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*, vol. 11, no. 5, pp. 1633-1644. https://doi.org/10.5194/hess-11-1633-2007.

RAI, P.K., SINGH, M., ANAND, K., SAURABHJ, S., KAUR, T. and KOUR, D., 2020. Role and potential applications of plant growth promotion in rhizobacteria for sustainable agriculture. In: A. A. RASTEGARI, A. N. YADAV and N. YADAV, eds. *Trends in microbial biotechnology for sustainable agriculture and biomedical systems: diversity and functional perspectives.* Amsterdam: Elsevier, pp. 49-60.

SANTOS, H.G., JACOMINE, P.K.T., ANJOS, L.H.C., OLIVEIRA, V.A., LUMBRERAS, J.F., COELHO, M.R., ALMEIDA, J.A., ARAÚJO FILHO, J.C., LIMA, H.N., MARQUES, F.A., OLIVEIRA, J.B. and CUNHA, T.J.F., 2025. *Sistema Brasileiro de Classificação de Solos*. 6. ed. Brasília, DF: Embrapa, 393 p.

SINGH, M.K., SINGH, N.K. and SINGH, S.P., 2020. Impact of herbicide use on soil microorganisms. In: P. Singh, S. K. Singh and S.M. Prasad, eds. *Plant responses to soil pollution*. Singapore: Springer, pp. 179-194.. https://doi.org/10.1007/978-981-15-4964-9_11.

SINHA, E., MICHALAK, A.M. and BALAJI, V., 2017. Eutrophication will increase during the 21st century as a result of precipitation changes. *Science*, vol. 357, no. 6349, pp. 405-408. https://doi.org/10.1126/science.aan2409. PMid:28751610.

SOIL SURVEY STAFF, 2022. *Keys to Soil Taxonomy*. 13th ed. Washington, DC: Department of Agriculture, Natural Resources Conservation Service, 430 p.

SOUSA, W.N., BRITO, N.F., FELSEMBURGH, C.A., VIEIRA, T.A. and LUSTOSA, D.C., 2021. Evaluation of *Trichoderma* spp. isolates in cocoa seed treatment and seedling production. *Plants*, vol. 10, no. 9, pp. 1-10. https://doi.org/10.3390/plants10091964. PMid:34579496.

TONNANG, H.E., SALIFU, D., MUDERERI, B.T., TANUI, J., ESPIRA, A., DUBOIS, T. and ABDEL-RAHMAN, E.M., 2022. Advances in data-collection tools and analytics for crop pest and disease management. *Current Opinion in Insect Science*, vol. 54, pp. 100964. https://doi.org/10.1016/j.cois.2022.100964. PMid:36055644.

VAN-LENTEREN, J.C., BOLCKMANS, K., KÖHL, J., RAVENSBERG, W.J. and URBANEJA, A., 2018. Biological control using invertebrates and microorganisms: many new opportunities. *BioControl*, vol. 63, no. 1, pp. 39-59. https://doi.org/10.1007/s10526-017-9801-4.

VIANNA, M.S., 2020. *Evaluation of vegetation indices from aerial images in soybean breeding*. Piracicaba: Universidade de São Paulo, 70 p. Master's Dissertation in Genetics and Plant Breeding.

VURRO, M., MIGUEL-ROJAS, C. and PÉREZ-DE-LUQUE, A., 2019. Safe nanotechnologies to increase the effectiveness of environmentally friendly natural agrochemicals. *Pest Management Science*, vol. 75, no. 9, pp. 2403-2412. https://doi.org/10.1002/ps.5348. PMid:30672106.

XIANG, N.I., LAWRENCE, K.S. and DONALD, P.A., 2018. Biological control potential of plant growth-promoting rhizobacteria suppression of *Meloidogyne incognita* on cotton and *Heterodera glycines* on soybean: a review. *Journal of Phytopathology*, vol. 166, no. 7-8, pp. 449-458. https://doi.org/10.1111/jph.12712.