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Research Article

Compatibility of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) with phytosanitary products used in soybean crops

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Abstract. Soybean (*Glycine max* (L.) Merr.) is an agricultural crop of great importance, but it faces challenges such as pests and invasive plants. Conventional control of these threats relies on phytosanitary products that can impact beneficial organisms, such as the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuill., an important agent in the biological control of mites and insect pests. Given the relevance of these interactions, bioassays were performed *in vitro* to evaluate the effect of two fungicides (Cronnos® and Fox Xpro®), two herbicides (Select® and Preciso xK®), and three insecticides (Avatar®, Engeo®, and Tiger®) on the viability, vegetative growth, and conidiogenesis of the fungus *B. bassiana* isolate Unioeste 76, on Petri dish culture medium. The two fungicides, Cronnos® and Fox Xpro®, were toxic to *B. bassiana*, compromising all evaluated parameters. The herbicide Select® showed high toxicity. The herbicide Preciso xK® and the three tested insecticides were compatible. The careful selection of phytosanitary products compatible with entomopathogenic fungi reduces dependence on chemical pesticides and the risks associated with the selection of resistant populations, also favoring the preservation of biodiversity and the sustainability of soybean crops.

Keywords: Biological control, Susceptibility, Integrated Pest Management, IPM.

Introduction

Soybean, *Glycine max* (L.) Merr. (Fabales: Fabaceae) is an economically crop in Brazil. In the 2024/2025 harvest, the grain reached a record production in the country, with 345.2 million tons (CONAB 2025). Soybean has several applications, used in human and animal food, in the composition of biofuels, and other industrial derivatives, making it an important agricultural commodity worldwide (Ávila & Gazzoni 2021).

Soybean cultivation faces several phytosanitary challenges. The brown stink bug (BSB), *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae), is the main and most abundant pest, causing severe damage to pod and consequently reducing grain yield and quality (Panizzi et al. 2022; Saldanha et al. 2024). Chemical control is still predominant due to its rapid effect and high efficiency. However, it can select resistant genotypes and requiring new molecules for efficient management (Sosa-Gómez & Silva 2010). Natural enemies are also reported in soybean cultivation, such as parasitoids and entomopathogenic fungi (Corrêa-Ferreira & Panizzi 1999; Bueno et al. 2024) which can be affected by these insecticides. Furthermore, the large-scale use of chemical insecticides increases the resistance selection in populations of *E. heros*, resulting in management difficulties (Souza Neto et al. 2021; Panizzi et al. 2022).

Weed species compete with soybean plants for resources and cause allelopathic effects (Fleck & Candemil 1995). For their control, herbicides are used, their prolonged use in conventional management can affect soil microbial communities and selection of resistant biotypes (Cerdeira et al. 2011).

In addition, plant pathogens such as the fungus *Phakopsora pachyrhizi* Syd. & P. Syd. (Pucciniales: Phakopsoraceae), the causal agent of Asian rust, and *Fusarium solani* (Mart.) Sacc. (Hypocreales: Nectriaceae), the causal agent of red root rot, also cause significant losses (Freitas et al. 2004; Fontes et al. 2003). The excessive use of fungicides may select resistant fungal populations and cause environmental impacts (Dela Cruz et al. 2024; Naqvi et al. 2025).

In general, phytosanitary products, despite their importance, may compromise the sustainability of soybean production (Santos et al. 2003; Godoy et al. 2016; Stacke et al. 2020). In this context, and due to the risks of these products to health and the environment, more sustainable alternatives have been studied, especially biological control. Entomopathogenic fungi, such as *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), are important biological control agents and are present in dozens of commercial products registered in Brazil to the BSB biocontrol (AGROFIT 2026). In this context, Integrated Pest Management (IPM) aims to reduce the impacts of intensive pesticide use, by combining chemical, biological and cultural control to reduce dependence on pesticides (Carson 1962; Zhou et al. 2024).

However, the integration of entomopathogenic fungi with chemical products requires previous compatibility evaluation, as these products can negatively affect fungal growth and reduce fungal virulence against the target pest (Alves et al. 1998). *In vitro* compatibility studies are important because they expose the microorganism directly to the products, helping predict field selectivity (Mamprim et al. 2014; Oliveira et al. 2016).

Although the compatibility between entomopathogenic fungi and phytosanitary products has been investigated (Alves et al. 1998; Celar & Kos 2016; Khun et al. 2020; Phatak et al. 2025), the list of products recommended for use in soybean crops is periodically revised. This process results in the exclusion of certain products and the inclusion of new active ingredients; the interaction of these new ingredients with the fungi—whether harmful or not—remains unknown (Mascarin & Jaronski 2016; Khun et al. 2020; Zeina & Laing 2025).

Furthermore, while many *B. bassiana*-based biological insecticides are registered for the control of the green-belly stink bug (BSB), the Unioeste 76 isolate is not yet included in any of them. This isolate was recently selected due to its high virulence against adult BSB and extremely high conidial yield (Silva-Santana et al. 2022). Additionally, field experiments in soybean crops have demonstrated its efficacy in controlling BSB populations (Oliveira et al. 2026). Despite this, information regarding the compatibility of this fungal strain with phytosanitary products currently used in soybean crops is still lacking; such information is necessary for guiding decisions within integrated pest management programs for soybean.

Therefore, this study evaluates the compatibility of chemical pesticides currently in use in soybean crops with *B. bassiana* strain Unioeste 76, through their effects on conidial germination, vegetative growth, conidiogenesis and biological index.

Material and Methods

Entomopathogenic fungus and phytosanitary products. The isolate Unioeste 76 of *B. bassiana* from the Entomopathogenic Fungi Collection of the Agricultural Biotechnology Laboratory (CFEUnioeste) was previously selected for its high virulence against *E. heros* and high conidia production capacity (Silva-Santana et al. 2022). The fungus was multiplied in culture medium for conidia production (ME) (1.58 g $NaNO_3$; 0.36 g KH_2PO_4; 0.60 g $MgSO_4.7H_2O$; 1.05 g $NaHPO_4.7H_2O$; 1.00 g KCl; 5.0 g yeast extract; 10.0 g glucose; 20.0 g agar; 1000 mL distilled water) in 9 cm diameter Petri dishes (7 to 10 days; 26 ± 2 °C; 14 h photophase). Conidia were collected and stored in a freezer at -8 °C for use in bioassays. Two fungicides (Cronnos® and Fox Xpro®), 3 insecticides (Avatar®, Tiger®, and Engeo®), and 2 herbicides (Select® and Preciso xK®) were tested, at the concentrations and spray volume recommended by their respective manufacturers (Tab. 1).

Conidia viability. Conidia suspension (250 µL; 1 × 10^8 conidia/mL) were inoculated with an automatic pipette onto the surface of PDA medium (potato 200 g, dextrose 20 g, agar 15 g, and 1000 mL distilled water), in Rodac plates. After drying in a laminar flow hood, 250 µL of the test product solutions were sprayed on the culture media with an airbrush coupled to an air compressor (constant pressure of 0.6 Kgf/cm^2). The plates were incubated (26 ± 2 °C, 12h photoperiod, for 16 to 20 hours), and then the count of viable and non-viable conidia was performed under an optical microscope at 100× magnification, with a conidium considered viable if it presented a germ tube with a length twice its diameter (Oliveira et al. 2015).

Vegetative growth. Petri dishes (9 cm diameter) were prepared with PDA medium. The fungus was inoculated with a platinum loop at three equidistant points. Subsequently, the plates were incubated for 48 hours (26 ± 2 °C; 14 h photophase). After this period, 250 µL of the product spray mixtures were sprayed onto the surface of the plates, as described in the previous section. The plates were incubated for another 5 days under the same conditions. After the total incubation time, two colonies with the greatest similarity were evaluated in each Petri dish (12 colonies per treatment). Using calipers, colony diameters were measured in orthogonal directions, obtaining the average diameter in centimeters.

Conidia production in colonies. The previously selected colonies were cut from the culture medium using a stainless-steel spatula and stored at -8 °C in glass tubes sealed with plastic film for later quantification. For quantification, the colonies were first disintegrated inside the tubes using a metal spatula. Then, 10 mL of sterile distilled water + Tween 80 (0.01%) were added to each tube. After vortexing for 30 seconds, the conidia concentration in the suspension was quantified in a Neubauer chamber under an optical microscope (400×).

In all bioassays, for each product tested and the control, 6 plates were prepared, each considered a replicate.

Statistical analysis. Data were tested for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) ($p < 0.05$). When assumptions were met, the data were analyzed using ANOVA, followed by Tukey's test ($p < 0.05$). When variance homogeneity was not observed, the Games-Howell post-hoc test ($p < 0.05$) was applied. Analyses were performed using R software version 4.2.2 (R Core Team 2025).

The type of interaction between different phytosanitary products and the fungus *B. bassiana* Unioeste 76, was evaluated based on the biological index (BI), also referred to as the Compatibility Factor, as proposed by Rossi-Zalaf et al. (2008), using the following formula:

$$BI = \frac{47[CV] + 43[ESP] + 10[GER]}{100}$$

Where: BI = biological index; CV (colony diameter) = percentage of vegetative colony growth after 7 days, compared to the control; ESP (conidia production) = percentage of colony sporulation after 7 days, compared to the control; GER (viability) = percentage of conidia germination after 20 hours. The CV, ESP, and GER values were first corrected in relation to their respective controls.

The calculated BI values were compared with the established limits: 0 to 30 = highly toxic; 31 to 45 = toxic; 46 to 60 = moderately toxic; > 60 = compatible, with the objective of indicating the selectivity and compatibility of the products with the fungus, in order to support possible usage recommendations in integrated pest management.

Results and Discussion

The phytosanitary products showed different effects in the viability and growth of the fungus. Conidial germination was completely inhibited by the fungicides Cronnos® and Fox Xpro® (Tab. 2), indicating high toxicity. Similar results were reported by Soares et al. (2017) who observed reduced viability of the fungus *B. bassiana* with mancozeb-based products. Mancozeb acts as a nonspecific enzyme inhibitor, affecting metabolic processes involved in fungal growth (Loureiro et al. 2002). Furthermore, the Triazole group may affect spore germination

Table 1. Products used with their respective classes, active ingredients, mode of action, formulation, dose, and spray volume indicated by the manufacturer (adapted from product leaflets).

Product	Active Ingredient	Chemical Group	Formulation	Dose (L/ha)	Spray Volume (L/ha)
Cronnos® (Fungicide)	picoxystrobin, tebuconazole and mancozeb	strobilurin, triazole and alkylenobis	oil dispersion (OD)	2500-3000	20–40
Fox Xpro® (Fungicide)	bixafen, prothioconazole and trifloxystrobin	carboxamide, triazolinthione and strobilurin	suspension concentrate (SC)	500	70-150
Avatar® (Insecticide)	indoxacarb	oxadiazine	emulsifiable concentrate (EC)	300-400	150-200
Engeo® (Insecticide)	thiamethoxam and lambda-cyhalothrin	neonicotinoid and pyrethroid	mixture of CS and SC (ZC)	150-200	200
Tiger® (Insecticide)	pyriproxyfen and xylene	pyridyloxypropyl ether and aromatic hydrocarbon	emulsifiable concentrate (EC)	250	200-300
Select® (Herbicide)	clethodim and alkylbenzene	cyclohexanedione and light naphtha aromatic hydrocarbon	emulsifiable concentrate (EC)	0,40-0,45	100-250
Preciso xK® (Herbicide)	glyphosate	substituted glycine	soluble concentrate (SL)	2,0-2,50	2-6

and mycelial growth, reducing fungal development (Carzaniga et al. 1991; Phatak et al. 2025).

Similar results were reported by Wong & Wilcox (2001), for the control *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni ex Sacc. (Peronosporales: Peronosporaceae), the causal agent of grapevine downy mildew. Despite taxonomic differences, similar antifungal effects were observed, especially in the case of mancozeb, which acts by contact and has a multisite activity, preventing the germination of sporangia germination similarly to the effect observed in this study on *B. bassiana* conidia.

The action of the fungicides Cronnos® and Fox Xpro® was reflected in the vegetative growth of the fungus, causing a significant reduction in colony area. Colonies treated with Cronnos®, the colonies reached 0.90 cm², while those treated with Fox Xpro® reached only 0.55 cm² (Tab. 2), Fox Xpro® showed a greater inhibitory effect on mycelial growth. Previous studies with triazole-based products added to the culture medium also indicated total inhibition of vegetative growth of *B. bassiana* (Loureiro et al. 2002; Phatak et al. 2025), because triazoles are present in the fungicides evaluated in the present study.

Cronnos® also reduced conidia production from colonies. In this treatment, conidia production reached 2.94 × 10^4 conidia/colony, whereas colonies treated with Fox Xpro® the production was 10 times higher (26.75 × 10^4 conidia/colony) (Tab. 2). Inhibition of conidia production in *B. bassiana* in treatments that also reduced vegetative growth, was reported by Loureiro et al. (2002), like the effects observed with Cronnos®. However, the inhibition of vegetative growth does not necessarily indicate reduced conidia production or viability, as observed with Fox Xpro® (Oliveira et al. 2018).

Studies with phytopathogenic fungi (Carzaniga et al. 1991; Wong & Wilcox 2001) reported similar effects in the present study with an entomopathogenic fungus. In general, fungicides containing mancozeb, triazoles, and strobilurins affect fungal growth and reproductive structure formation, by affecting cellular respiration, ergosterol biosynthesis, cell division, and protein synthesis, depending on their chemical group and mechanism of action (FRAC 2025).

Furthermore, Soares et al. (2017) observed lower population density of *B. bassiana* was significantly lower when a strobilurin-based product was added to the soil one hour before and one hour after fungal inoculation. In contrast, the application of the same product 48 hours after inoculation did not negatively affect the fungus. This behavior is associated with the preventive activity of strobilurins, which is more effective before fungal establishment, inhibiting the initial colonization of *B. bassiana*.

Similarly, mancozeb is more effective when applied before fungal establishment. These results highlight the inhibitory effects of triazoles and strobilurins chemical groups on *B. bassiana* development when applied close to inoculation. However, when applied 48 hours after inoculation, as used in this study, the toxic effect was attenuated, allowing conidial germination, although other stages of fungal development were still affected.

The fungicides Cronnos® and Fox Xpro®, tested in this study, were classified as toxic to *B. bassiana* Unioeste 76 with a BI value of 16.21% and 10.05%, respectively (Tab. 2), like results reported by Loureiro et al. (2002) and Soares et al. (2017).

The insecticides Avatar®, Engeo®, and Tiger® did not affect conidial viability, but differed in conidia production, vegetative growth, and BI (Tab. 3). However, the products Avatar® and Engeo® significantly reduced colony growth (3.05 cm² and 3.07 cm², respectively). However, the insecticide Tiger® showed an intermediate effect and did not differ statistically from control or the other treatments, suggesting a lower effect on this parameter (Tab. 3).

Despite these variations, none of the tested insecticides significantly affected the mycelial growth of the fungus. Polanczyk et al. (2025) observed that vegetative growth is less sensitive to pesticides than conidia production, as the latter parameter depends directly on the metabolic integrity of the mycelium. Similarly, Phatak et al. (2025) also found that mycelial inhibition by most of the tested insecticides was relatively low, mainly with neonicotinoid insecticide.

Conidia production was not affected by the insecticides (Tab. 3). Previous studies indicate that the components of the formulation may act as stressors that stimulate conidiogenesis (Sosa-Gómez & Silva 2010). Similarly, Khun et al. (2020) demonstrated that products containing oxadiazines containing indoxacarb show low toxicity to entomopathogenic fungi being compatible with *B. bassiana*, like the results observed in this study.

Soares et al. (2017) reported high conidial production in the presence of thiamethoxam. Likewise, Gassen et al. (2008) also observed that this same active ingredient did not affect the development of *B. bassiana,* as observed in this study. Products containing neonicotinoids and pyrethroids, such as Engeo® are often compatible with biological agents when applied at sublethal concentrations and generally do not affect fungal growth, under favorable conditions for fungal development (Loureiro et al. 2002). In this context, small reductions in conidia production may be acceptable, as long as they do not compromise the fungus's biological cycle, supporting the compatibility of these products in the field (Rossi-Zalaf et al. 2008). Furthermore, it has been suggested that microorganisms, through physiological mechanisms, metabolize toxic compounds into secondary nutrients favoring vegetative growth and conidiogenesis (Loureiro et al. 2002).

Oliveira et al. (2018) observed that pyriproxyfen showed an inhibitory effect on the sporulation of *B. bassiana*. In contrast, in the present study, the insecticide Tiger® did not affect conidial production. These differences may be explained by differences in formulation, inert compounds and adjuvants of each brand may affect the fungus,

Table 2. Effect of the tested fungicides, conidia, vegetative growth, conidiogenesis, and compatibility (BI) with the isolate of *Beauveria bassiana* Unioeste 76 under laboratory conditions (26 °C, 12h photoperiod).

Treatment	Viability (%)	Vegetative growth (cm²)	Conidia/colony (10^4)	BI (%)
Cronnos®	0.00 b	0.90 c	2.94 c	16.21
Fox Xpro®	0.00 b	0.55 b	26.75 b	10.05
Control	72.71 a	2.62 a	5.794 a	-

Means followed by the same letter in the column do not differ from each other (Kruskal-Wallis test and *post-hoc* of Conover with Holm's adjustment, $p < 0.05$). BI = Biological index, according to Rossi-Zalaf et al. (2008).

Table 3. Effects of different insecticides on conidial viability, vegetative growth, conidiogenesis, and compatibility (BI) with the isolate of *Beauveria bassiana* Unioeste 76 under laboratory conditions (26 °C and 12h photoperiod).

Treatment	Viability (%)[1]	Vegetative growth (cm²)[2]	Conidia/colony (10^4)	BI (%)
Avatar®	73.38 b	3.04 b	47.15 a	93.44
Engeo®	72.35 b	3.07 b	33.14 a	80.07
Tiger®	81.05 a	3.64 ab	33.33 a	85.07
Control	75.28 b	4.25 a	46.94 a	-

[1]Means followed by the same lowercase letter in the column do not differ from each other (Games-Howell *post-hoc* test, $p > 0.05$); [2]Means followed by the same lowercase letter in the column do not differ from each other (Tukey-HSD test, $p > 0.05$). BI = Biological index, according to Rossi-Zalaf et al. (2008).

regardless of the active ingredient.

According to Rossi-Zalaf et al. (2008) BI values above 70% indicate compatibility, and the products tested in the present study meet this criterion, particularly Avatar®, which showed the highest BI (93.44%) presented in Tab. 3.

The herbicides showed different effects on the development of *B. bassiana* Unioeste 76. Viability was significantly reduced by both herbicides, compared to the control, causing 98 to 100% inhibition, for Preciso xK® and Select®, respectively (Tab. 4).

The active ingredient Clethodim (oxime cyclohexanedione group) present in the herbicide Select®, interferes with fungal lipid synthesis, being an ACCase inhibitor, impairing mycelial development (Celar & Kos 2016), which may also explain the significant reduction in vegetative growth (0.43 cm²). Hyphal destabilization consequently interfered with conidia production in colonies treated with Select® (58.61 × 10^4 conidia/colony). On the other hand, the product Preciso xK®, based on glyphosate, although it negatively affected fungal growth, showed lower effects than Select® on vegetative growth (1.27 cm²). Previous studies reported that glyphosate-based products at sublethal concentrations minimally affected this parameter (Ratcliff et al. 2006).

Conidial production was not significantly impacted (143.15 × 10^4 conidia/colony). The BI reflected the effects of herbicides on the fungus. The product Select®, with a BI of 41.05%, indicates compatibility, reinforcing incompatibility. In contrast, the product Preciso xK®, with a BI of 84.47%, was considered compatible, indicating compatibility for integrated use (Rossi-Zalaf et al. 2008). These results are promising, particularly because this product is widely used in soybean production.

To improve pest management, it is essential to understand in vitro interactions between entomopathogenic fungi and pesticides, since pesticides may affect fungal efficacy, interfering with growth, sporulation, and virulence (Mascarin & Jaronski 2016). Conventional pesticides frequently have a negative impact on predators, parasitoids, and entomopathogens (Copping & Menn 2000), highlighting the importance of compatible agrochemicals to improve pest control efficiency and minimize negative effects on biodiversity.

The development of new products and differences in their action, among active ingredients or formulations, depending on fungal species and strain, highlight the need for continued studies (Alves et al. 1998; Stacke et al. 2020). Therefore, the success of epizootics depends on insect population density, conidia viability, and infection efficiency (Meyling & Eilenberg 2007). In addition, entomopathogenic fungi leave fewer residues, help preserve biodiversity, and are economically viable (Sousa et al. 2023).

Conclusion

The fungicides Cronnos® and Fox Xpro® showed high toxicity to the fungus *B. bassiana* Unioeste 76, with deleterious effects preventing their combined use with the biological control and highlight the importance of application intervals for insect pests and phytopathogen control. In contrast, insecticides, their compatibility supports their use in pest management, and the stimulation of conidiogenesis further supports their integrated use. Results with herbicides suggest caution for integrated use; some active ingredients may be compatible, supporting more sustainable management, but others require applications at different times, such as Select®.

Furthermore, *in vitro* incompatibility does not always occur under field conditions, as under field conditions, the toxicity of pesticides to the biological agent can vary. Therefore, even with complete inhibition of germination in the laboratory, the fungal efficacy may still occur under field conditions, especially when application intervals are respected.

Table 4. Effects of different herbicides on conidial viability, vegetative growth, conidiogenesis, and compatibility (BI) with the isolate of *Beauveria bassiana* Unioeste 76 under laboratory conditions (26 °C and 12h photoperiod).

Treatment	Viability (%)[1]	Vegetative growth (cm²)[2]	Conidia/colony (10^4)	BI (%)
Select®	0 c	0.43 c	58.61 b	41.05
Precise xK®	1.87 b	1.27 b	143.15 a	84.47
Control	87.27 a	1.85 a	136.64 a	-

[1]Means followed by the same lowercase letter in the column do not differ among themselves (Kruskal-Wallis test; [2]Means followed by the same lowercase letter in the column do not differ among themselves (Tukey-HSD test, $p < 0.05$). BI = Biological index, according to Rossi-Zalaf et al. (2008).

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Authors' Contribution

JVCC: Conceptualization, Investigation, Writing - original draft; LFAA: Supervision, Methodology, Writing-review & editing.; AJBM: Formal analysis, Methodology, Validation; UMR: Methodology, Validation.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest or competing interests.

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