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Scientific Article

In vitro compatibility of fungicides with *Metarhizium rileyi* in soybean disease management

Compatibilidade in vitro de fungicidas e *Metarhizium rileyi* no manejo de doenças em soja

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ABSTRACT – Compatibility between chemical fungicides and biological insecticides is essential for maintaining plant health and preserving entomopathogenic fungi that induce epizootics in arthropod pests in soybean crops. The objective of this study was to evaluate the effects of the fungicides Fox Xpro®, Sphere Max®, Orkestra®, Elatus®, Vessarya®, and the adjuvant Aureo® on the in vitro development of the entomopathogenic fungus *Metarhizium rileyi* (strain UFMS 03). The effects of these products on vegetative growth (cm), conidial production, and germination (%) of *M. rileyi* were assessed. The obtained data were used to calculate the compatibility factor based on the biological index. The fungicide Elatus® and the adjuvant Aureo® were compatible with *M. rileyi*, showing no adverse effects on its vegetative growth. The fungicide Orkestra® was moderately toxic to *M. rileyi*, negatively affecting its vegetative growth and sporulation. The fungicides Fox Xpro®, Sphere Max®, and Vessarya® were toxic to *M. rileyi*, reducing its vegetative growth and sporulation. Although all products reduced *M. rileyi* sporulation, Elatus® and Aureo® were compatible with *M. rileyi* and can be integrated into disease management strategies aimed at conserving this biological control agent.

Keywords: *Glycine max*. Lepidoptera. Biological control. Entomopathogenic fungi. Selectivity.

RESUMO – A compatibilidade entre fungicidas químicos e inseticidas biológicos é essencial para a manutenção da sanidade de plantas e a preservação de fungos entomopatogênicos que induzem epizootias em pragas de artrópodes em cultivos de soja. O objetivo deste estudo foi avaliar os efeitos dos fungicidas Fox Xpro®, Sphere Max®, Orkestra®, Elatus®, Vessarya® e do adjuvante Aureo® sobre o desenvolvimento in vitro do fungo entomopatogênico *Metarhizium rileyi* (cepa UFMS 03). Foram avaliados os efeitos desses produtos no crescimento vegetativo (cm), produção de conídios e germinação (%) de *M. rileyi*. Os dados obtidos foram utilizados para calcular o fator de compatibilidade com base no índice biológico. O fungicida Elatus® e o adjuvante Aureo® demonstraram compatibilidade com *M. rileyi*, sem efeitos adversos no seu crescimento vegetativo. O fungicida Orkestra® foi moderadamente tóxico para *M. rileyi*, afetando negativamente seu crescimento vegetativo e esporulação. Os fungicidas Fox Xpro®, Sphere Max® e Vessarya® foram tóxicos para *M. rileyi*, reduzindo seu crescimento vegetativo e esporulação. Embora todos os produtos tenham reduzido a esporulação de *M. rileyi*, Elatus® e Aureo® apresentaram compatibilidade com *M. rileyi* e podem ser recomendados no manejo de doenças da soja, visando a conservação desse agente de controle biológico.

Palavras-chave: *Glycine max*. Lepidoptera. Controle biológico. Fungos entomopatogênicos. Seletividade.

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INTRODUCTION

Brazilian soybean (*Glycine max*) production has exhibited exponential growth, with a significant increase in exports over the past two decades (CONAB, 2024). The expansion of agricultural areas and the adoption of advanced technologies and inputs have significantly increased agricultural production (PICOLI et al., 2020).

Although technological advancements and inputs have significantly enhanced agricultural production, climate change, shifts in cultivation systems, and the indiscriminate use of pesticides have led to challenges, including disease outbreaks, resurgence of key pests, emergence of secondary pests, and the selection of resistant arthropod populations (SKENDŽIĆ et al., 2021). In Brazil, soybean disease management initially relied on resistant varieties; however, after 1997, outbreaks of fungal diseases caused by *Microsphaera diffusa* Cooke & Peck and *Phakopsora pachyrhizi* Sydow & P. Sydow necessitated the use of chemical fungicides (CHANDER et al., 2019; ZAMBOLIM et al., 2022).

Fungicides are among the primary factors affecting the incidence of entomopathogenic fungi, such as *Metarhizium rileyi* (Farlow) Samson (Ascomycota: *Clavicipitaceae*), which causes white muscardine disease, and Entomophthorales fungi, such as *Pandora* sp. and *Zoophthora* sp., which cause brown muscardine disease, leading to pest outbreaks and resurgence due to the suppression of these natural enemies (LOPES et al., 2023).

Entomopathogenic fungi are important natural enemies with a broad spectrum of action and the capacity to induce epizootic diseases (MANTZOUKAS et al., 2022). *Metarhizium rileyi* exhibits high environmental adaptability, parasitizing over 60 arthropod species, particularly Lepidoptera (SOUZA et al., 2024). Species susceptible to *M. rileyi*, the lepidopteran pest complex of the Noctuidae family, prevalent in soybean crops, include *Anticarsia gemmatalis* Hübner, *Chrysodeixis includens* Walker, and *Rachiplusia nu* Guenée (ANDRADE; RANGEL, 2021). Epizootics caused by *M. rileyi* have been reported in crops in Mato Grosso do Sul, Brazil, reducing populations of *Spodoptera frugiperda* J.E. Smith, *Spodoptera cosmioides* Walker (Lepidoptera: Noctuidae), and *Helicoverpa armigera* Hübner (LOUREIRO et al., 2024).

The use of phytosanitary products compatible with natural enemies, such as entomopathogenic fungi, is a safe and effective strategy for integrated pest and disease management (BAMISILE et al., 2021; LOUREIRO et al., 2024). However, product selection does not always prioritize the selectivity or compatibility with natural enemies (CARVALHO et al., 2019). Pesticides can inhibit the development of entomopathogenic fungi, as incompatible fungicides may interfere with vegetative growth and conidial germination, and potentially induce genetic mutations (ZAMBOLIM et al., 2022). An in vitro study indicated that many fungicides used in soybean production are toxic to *M. rileyi* (KHUN et al., 2021).

Understanding the selectivity and compatibility of fungicides with entomopathogenic fungi enables the optimization of their use in applied or conservative biological control through the strategic timing of fungicide and fungal applications (CHANDER et al., 2019). Studies investigating the compatibility of phytosanitary products with microorganisms enhance the efficacy of integrated pest and disease management by supporting the survival of natural enemies that contribute to biological control in agroecosystems. In this context, the objective of this study was to evaluate the effects of chemical fungicides (Fox Xpro®, Sphere Max®, Orkestra®, Elatus®, Vessarya®) and the adjuvant Aureo® on the in vitro development of the entomopathogenic fungus *Metarhizium rileyi* (strain UFMS 03).

MATERIAL AND METHODS

Fungicides, adjuvant, and *Metarhizium rileyi*

Approved fungicides for disease control in soybean crops (Fox Xpro®, Sphere Max®, Orkestra®, Elatus®, Vessarya®) and the adjuvant Aureo® were applied at recommended rates according to the manufacturers' instructions, using a fixed spray volume of 100 L ha^{-1} (Table 1) (AGROFIT, 2024). The strain UFMS 03 of the entomopathogenic fungus *Metarhizium rileyi*, maintained in the entomopathogenic fungal collection of the Entomology Laboratory, Federal University of Mato Grosso do Sul (UFMS), Chapadão do Sul, MS, Brazil, was used.

Table 1. Description of fungicides and adjuvant approved for disease control in soybean crops and the recommended rates according to the manufacturers' instructions.

Commercial name	Active ingredients	Chemical group	Formulation	Rate L ha^{-1}	Class
Fox Xpro®	Bixafen; Prothioconazole; Trifloxystrobin	Carboxamide, Triazolinthione and Strobilurin	SC	0.5	F
Sphere Max®	Trifloxystrobin; Cyproconazole	Strobilurin and Triazole	SC	0.2	F
Orkestra®	Pyraclostrobin; Fluxapyroxad	Strobilurin e Carboxamide	SC	0.35	F
Elatus®	Azoxystrobin; Benzovindiflupyr	Strobilurin and Pyrazole Carboxamide	WG	0.2	F
Vessarya®	Picoxystrobin; Benzovindiflupyr	Strobilurin and Pyrazole Carboxamide	EC	0.6	F
Aureo®	—	Soy oil methyl ester	EC	0.25% v v^{-1}	A

F = fungicide; A = adjuvant; Formulations: SC = Concentrated Suspension, WG = Dispersible Granule, EC = Emulsifiable Concentrate. Source: AGROFIT (2024).

Compatibility bioassays

The effects of the five fungicides and the adjuvant on vegetative growth (cm), conidial production (sporulation), and germination of the isolated fungus *M. rileyi* (strain UFMS 03) were evaluated through in vitro experiments to assess compatibility.

Vegetative growth and conidial production of *M. rileyi* were evaluated by incorporating the products into 200 mL of non-solidified potato dextrose agar (PDA) culture medium, followed by agitation using a Vortex tube shaker (Biomixer®) at 2,800 rpm. The resulting mixtures were poured into Petri dishes with a diameter of 9 cm (3 dishes per treatment), labeled according to the treatments. After medium solidification, *M. rileyi* (strain UFMS 03) was inoculated using a platinum needle at three equidistant points (9 points per treatment) on each plate. The plates were sealed with PVC film, incubated for 7 days in a biochemical oxygen demand (BOD) chamber at 25 ± 1 °C, with relative humidity of 70 ± 10%, and photoperiod of 12 hours to facilitate colony growth, following the methodology of Oliveira et al. (2018) and Loureiro et al. (2024).

Vegetative growth was assessed by measuring colony diameters along two orthogonal axes, with the mean diameter (cm) calculated for each colony. Conidial production was determined by excising colonies from the plates using a sterile surgical scalpel and transferring them to test tubes containing 10 mL of distilled water with 0.01% Tween 80®. The conidia were disaggregated, and the suspensions were agitated using a Vortex shaker, followed by dilution for conidial counting in a Neubauer chamber (Bright-Line; Boeco®) under an optical microscope (100×), according to the methodology of Loureiro et al. (2024).

Germination was assessed in a 100 mL suspension containing 1.0×10^8 conidia mL^{-1} of *M. rileyi* (strain UFMS 03), which was incubated for one hour in a mixture with the tested fungicides and adjuvant at the recommended rates (Table 1). Subsequently, 1.0 mL of the suspension was transferred using a graduated pipette onto Petri dishes and spread over the PDA culture medium with a Drigalsky handle. After inoculation, the plates were labeled, sealed with PVC film, and incubated for 20 hours in an air-conditioned BOD chamber at 25 ± 1 °C, with relative humidity of 70 ± 10%, and photoperiod of 12 hours (LOUREIRO et al., 2024).

After incubation, the plates were divided into four quadrants, and 100 conidia were counted in each quadrant to determine the germination percentage. The results of the germination analysis were classified according to the standards of the Biological Control Laboratory of the Instituto Biológico de Campinas as: high germination (80–100%), medium–high germination (60–79%), average germination (50–59%), medium–low germination (30–49%), and low germination (0–29%) (ZAPPELINI; ALMEIDA; GASSEN, 2005). Three replicates of the bioassay were conducted to evaluate the compatibility of the fungicides with the fungus *M. rileyi* (strain UFMS 03).

The data were utilized to calculate the compatibility factor based on the biological index proposed by Rossi-Zalaf et al. (2008). This index categorizes products according to their toxicity by assessing the impacts of the evaluated parameters (vegetative growth, conidial production, and germination). The biological index values were compared with established thresholds to determine the toxicity of the studied products, classified as toxic (0 to 41), moderately toxic (42 to 66), or compatible (> 66).

Statistical analysis

A completely randomized experimental design was used, comprising seven treatments (five fungicides, one adjuvant, and a control). The vegetative growth and conidial production assays were conducted using three plates per treatment, each containing nine colonies, of which six were randomly assigned, resulting in six replications per treatment. Data on vegetative growth and conidial production were analyzed using Tukey's test ($p = 0.05$). Conidial germination was assessed with four replications, consisting of four Petri dishes divided into four quadrants each. Germination data were analyzed using Tukey's test ($p = 0.05$). Statistical analyses were performed using Sisvar software.

RESULTS AND DISCUSSION

Among the phytosanitary products tested, only the fungicide Elatus® and the adjuvant Aureo® did not inhibit the vegetative growth of *M. rileyi* (strain UFMS 03), showing no significant difference from the control treatment ($p > 0.05$; Table 2). The colony diameters of *M. rileyi* (strain UFMS 03) in the presence of the fungicides Fox Xpro®, Sphere Max®, Orkestra®, and Vessarya® showed significant differences compared to the control treatment and the products Elatus® and Aureo® ($p < 0.05$; Table 2).

No vegetative growth of *M. rileyi* (strain UFMS 03) was observed in the presence of the fungicide Vessarya®, with a significant difference from all other treatments ($p < 0.05$; Table 2). All tested phytosanitary products reduced conidial production compared to the control ($p < 0.05$) (Table 2). Based on the germination classification adopted in this methodology, all treatments exhibited low germination of *M. rileyi* (strain UFMS 03) conidia; however, the fungicides Fox Xpro® and Vessarya® significantly reduced germination compared to the control ($p < 0.05$; Table 2).

Table 2. Mean ± standard error (SE) for vegetative growth, conidial production, and germination of *M. rileyi* in the presence of different fungicides and adjuvant, maintained at 25 ± 1 °C, with relative humidity of 70 ± 10% and photoperiod of 12 hours.

Treatment	Diameter (cm ± SE)	Conidia ($\times 10^7 \pm$ SE)	Germination
Control	2.32 ± 0.12 a	4.98 ± 1.33 a	8.87 ± 0.14 a (low)
Fox Xpro®	0.75 ± 0.03 b	0.13 ± 0.06 b	1.12 ± 0.02 b (low)
Sphere Max®	0.99 ± 0.03 b	0.67 ± 0.09 b	3.56 ± 0.04 ab (low)
Orkestra®	1.21 ± 0.05 b	1.87 ± 0.07 b	2.37 ± 0.03 ab (low)
Elatus®	2.25 ± 0.06 a	2.35 ± 0.12 b	3.81± 0.04 ab (low)
Vessarya®	0.00 ± 0.00 c	0.09 ± 0.05 b	0.43± 0.02 b (low)
Aureo®	2.40 ± 0.35 a	1.77 ± 0.47 b	3.37 ± 0.06 ab (low)
CV%	25.46	79.11	79.57

Means followed by the same letter within columns are not significantly different from each other according to Tukey's test at a 5% probability level. *Germination was classified according to Zappelini, Almeida and Gassen (2005) as high (80–100%), medium–high (60–79%), medium (50–59%), medium–low (30–49%), and low (0–29%).

Based on the compatibility factor (biological index), the fungicides Fox Xpro®, Sphere Max®, and Vessarya® were toxic to the fungus *M. rileyi* (strain UFMS 03) in in vitro tests (Table 3). The product Orkestra® was moderately toxic, whereas the fungicide Elatus® and the adjuvant Aureo® were compatible with *M. rileyi* (strain UFMS 03) (Table 3).

Table 3. Classification of fungicides and adjuvants based on their toxicity (biological index), according to the methodology of Rossi-Zalaf et al. (2008).

Phytosanitary Products	Biological Index	Classification
Fox Xpro®	18.8	Toxic
Sphere Max®	29.9	Toxic
Orkestra®	43.6	Moderately toxic
Elatus®	70.1	Compatible
Vessarya®	1.3	Toxic
Aureo®	67.6	Compatible

The results from this study demonstrate that the fungicide Elatus® and the adjuvant Aureo® do not interfere with the growth of *Metarhizium rileyi* colonies (strain UFMS 03), exhibiting compatibility with the isolate, as indicated by the compatibility factor (biological index). In contrast, the fungicides Fox Xpro®, Sphere Max®, Orkestra®, and Vessarya® are likely to contain active ingredients that inhibit *M. rileyi* development, reducing colony diameters or, in the case of Vessarya®, completely suppressing vegetative growth.

Pesticide selectivity, defined as the differential impact of a pesticide on various organisms within an ecosystem, is a crucial factor influencing the evolutionary development of pesticide resistance, potentially favoring the survival and proliferation of resistant target pests while differentially affecting non-target species, including entomopathogenic fungi (HAWKINS et al., 2019). The mode of action of the active ingredient is a primary factor contributing to the toxicity of phytosanitary products, with systemic fungicides exhibiting greater specificity for phytopathogens (ZAMBOLIM et al., 2022). Compatibility studies of systemic products with active ingredients, such as cyproconazole, have demonstrated high toxicity to entomopathogenic fungi, including *Beauveria bassiana* (Balsamo) Vuillemin (LOUREIRO et al., 2023).

Systemic fungicides target multiple sites within the pathogen, interfering with several vital processes in phytopathogenic fungi (KHUN et al., 2021). All tested fungicides exhibit systemic modes of action, suggesting that their physiological targets may contribute to the toxic effects observed Fox Xpro®, Sphere Max®, Vessarya®, and Orkestra® on *M. rileyi* (strain UFMS 03).

The active ingredient is a crucial factor in pesticide selectivity. All tested fungicides included at least one active ingredient from the strobilurin chemical group. Incompatibility effects of fungicides from the strobilurin group and phenylpyrrole commonly used and approved for fruit species have been reported on the development of five isolates of *Metarhizium anisopliae* var. *anisopliae* (Metch.) Sorokin (DAMIN et al., 2011). Toxic effects and incompatibility of fungicides from the triazole group have been reported for the fungi *B. bassiana*, *M. anisopliae,* and *Lecanicillium lecanii* (Zimm.) Zare & W. Gams (BAMISILE et al., 2021). Studies on fungicide compatibility with entomopathogenic fungi have revealed adverse effects for products containing strobilurin plus triazole, which inhibit the vegetative growth of *Metarhizium* spp. (DAMIN et al., 2011).

The findings from this study regarding the fungicide Sphere Max® corroborate previous observations of adverse effects of Sphere®, which contains trifloxystrobin and cyproconazole, on the development of *M. anisopliae* var. *anisopliae* strains: CG-28 (strain AL) and CG-30 (strain E6) (ONOFRE et al., 2011). Although the fungicide Vessarya® exhibited toxicity to *M. rileyi* (strain UFMS 03), it comprises active ingredients from the strobilurin and pyrazole carboxamide chemical groups, specifically picoxystrobin (100 g L^{-1}) and benzovindiflupyr (50 g L^{-1}).

In comparison to the other tested products, Vessarya® shares chemical groups with Elatus®, containing azoxystrobin (300 g kg^{-1}) (strobilurin) and benzovindiflupyr (150 g kg^{-1}) (pyrazole carboxamide) as active ingredients and, despite this similarity, Elatus® demonstrated compatibility with *M. rileyi* (strain UFMS 03). The presence of the same active ingredient, benzovindiflupyr, in the fungicides Vessarya® and Elatus®, and the incompatibility observed with Vessarya® may be attributed to the formulation of these fungicides, as phytosanitary products include not only active ingredients but also inert ingredients—substances that enhance efficacy and may be toxic to microorganisms (LOPES et al., 2020).

All tested fungicides adversely affected the sporulation and germination of *M. rileyi* (strain UFMS 03), thereby impairing the host colonization process and reducing inoculum potential (SILVA; NEVES; SANTORO, 2005). This suggests the high sensitivity of this isolate to chemical fungicides and the influence of their diverse active ingredients on conidiogenesis. Research indicates that the fungitoxic activity of phytosanitary products varies depending on the fungal species, isolate, entomopathogenic fungus structure, concentration, and chemical nature of the product. The results obtained showed that the effects of phytosanitary products on vegetative growth and conidial production differed based on the chemical nature of the product and the entomopathogenic species. The presence of emulsifiers and other additives contributes to the incompatibility of fungicides with entomopathogens, a factor considered significant in the development of new commercial product formulations (ZAMBOLIM et al., 2022).

The significant increase in agricultural inputs and the use of non-selective products contribute to environmental imbalance by rendering fungal propagules unviable, thereby affecting the quality of microbiological agents such as entomopathogenic fungi (SOUZA et al., 2024). According to the germination classification proposed by Zappelini, Almeida and Gassen (2005), all treatments exhibited low germination. The observation that the control treatment also exhibited low germination does not invalidate this study, as the in vitro test assesses critical parameters and, despite not reflecting field conditions, provides results indicating possible interactions, compatibility, or fungitoxic action (SILVA; NEVES; SANTORO, 2005, MONTECALVO; NAVASERO, 2024). Moreover, the compatibility observed in an in vitro test suggests possible selectivity in the field (LOPES et al., 2020). However, further investigations into the germination of the UFMS 03 strain in the presence of these products are essential, as this is a primary parameter for the evaluation of entomopathogenic fungi (SILVA; NEVES; SANTORO, 2005; OLIVEIRA et al., 2018).

Although this study evaluated only one adjuvant, Aureo®, investigating the compatibility of microorganisms with adjuvants and spreaders is crucial for the development of bioinsecticide formulations (LOPES et al., 2020). Since *M. rileyi* formulations are not commercially produced on a large scale, this study addressed key aspects relevant for advancing the development of new bioproduct formulations, offering insights into potential management recommendations considering the incompatibility of certain fungicides. Understanding the selectivity and compatibility of fungicides with entomopathogenic fungi facilitates optimized use of this microorganism in applied and conservation biological control, which can be achieved through the strategic timing of fungicide and fungal applications (SOUZA et al., 2024).

The toxicity results observed for the other fungicides highlight the need for cautious use of these products in disease management, employing spatially or temporally separated applications when applying entomopathogenic fungi or during entomopathogenic epizootics in the area. Selecting phytosanitary products that are selective toward microorganisms, such as the fungicide Elatus® and the adjuvant Aureo®, is essential for the conservation of *M. rileyi*. This is consistent with efforts toward efficient integrated pest and disease management combined with sustainability.

The findings of this study are highly valuable for integrated pest and disease management, considering the environmental impacts identified during the technological evolution of agriculture and the naturally occurring epizootics involving *M. rileyi*. The need for further studies to assess these phytosanitary products, along with other active ingredients and classes, under field conditions is evident to better elucidate the potential effects on *M. rileyi* (strain UFMS 03).

CONCLUSION

The fungicide Elatus® and the adjuvant Aureo® demonstrated compatibility with the entomopathogenic fungus *Metarhizium rileyi* (strain UFMS 03) in in vitro tests. The fungicide Orkestra® exhibited moderate toxicity, while the fungicides Fox Xpro®, Sphere Max®, and Vessarya® were toxic to *M. rileyi* (strain UFMS 03). The fungicide Vessarya® completely inhibited the vegetative growth of *M. rileyi* (strain UFMS 03).

All phytosanitary products impacted the sporulation of *M. rileyi* (strain UFMS 03); however, due to the compatibility of the Elatus® and Aureo®, these products can be recommended for disease management in soybean crops, prioritizing the conservation of *M. rileyi*.

The observed differences in compatibility among products with shared active ingredients emphasize the influence of formulation components on the selectivity of fungicides toward entomopathogenic fungi.

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