

Open Minds

Internacional Journal

ISSN 2675-5157

vol. 2, n. 6, 2026

●●● ARTICLE 9

Acceptance date: 10/03/2026

ARBUSCULAR MYCORRHIZAL FUNGI IN THE SUPPRESSION OF PATHOGENIC FUNGI ASSOCIATED WITH SOYBEAN CULTIVATION

Adrielly Rosa da Silva

Agricultural Engineer, Palmeiras de Goiás, GO, Brazil.
<https://orcid.org/0009-0003-2829-1671>

Alliny das Graças Amaral

Professor and Researcher, State University of Goiás – CET, Anápolis, GO, Brazil.
<https://orcid.org/0000-0002-1418-9698>

Roberto Gomes Vital

Professor and Researcher, State University of Goiás – Palmeiras de Goiás University Unit, Palmeiras de Goiás, GO, Brazil. <https://orcid.org/0000-0002-7907-076X>

Táis Ferreira de Almeida

Professor and Researcher, State University of Goiás – Palmeiras de Goiás University Unit, Palmeiras de Goiás, GO, Brazil.
<https://orcid.org/0000-0002-6102-4781>



All content published in this journal is licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0).



Abstract: The objective of this study was to evaluate the potential of the arbuscular mycorrhizal fungus (AMF) isolate ISOE 032 in suppressing phytopathogenic fungi of high relevance to soybean (*Glycine max* L.) cultivation. The study was conducted at the Plant Pathology Laboratory of the State University of Goiás, where the bottom-to-bottom plate method was used to evaluate the effect of volatile compounds emitted by isolate ISOE 032 on the mycelial growth and sporulation of different pathogenic isolates, namely: *Macrophomina* sp., *Rhizoctonia solani*, *Phomopsis* sp., *Sclerotinia* sp., *Colletotrichum dematium* var. *truncata*, *Cercospora kikuchii*, and *C. sojina*. FMA ISOE 032 showed significant performance in controlling phytopathogens, promoting high rates of mycelial inhibition, with *Macrophomina* sp. standing out with 81%, followed by *C. kikuchii* (77.5%) and *C. sojina* (77.25%). In addition, a significant reduction in the sporulation capacity of the pathogens was observed, demonstrating that the compounds released by the isolate not only affect vegetative growth but also interfere with the reproduction of the tested fungi. The results highlight the potential of volatile metabolites produced by FMAs as antifungal agents, with effects comparable to those of synthetic fungicides, but without the environmental impacts associated with the continuous use of chemical pesticides. Thus, FMA ISOE 032 has biotechnological potential for integration into sustainable management strategies.

Keywords: bioinputs; *Glycine max*; *Macrophomina* sp.; sustainable management; secondary metabolites.

INTRODUCTION

Soybean (*Glycine max* L.), belonging to the Fabaceae family, is a plant native to China and has established itself as one of the most important crops in the global agricultural scenario. According to Embrapa (2021), from an economic perspective, soybeans played a central role in the development of Brazilian commercial agriculture, driving mechanization, modernizing transportation systems, expanding agricultural frontiers, professionalizing international trade, enriching the diet, and strengthening agribusiness, especially in the pork and poultry chains.

Among the producing states, Goiás stood out for its performance above the national average. In the first half of 2025, the state shipped 8.3 million tons of soybeans, an increase of 7.8% compared to 2024. By August, Goiás had already set a new record, with 12.4 million tons exported, an increase of 6.6% (Seapa, 2025).

Global studies indicate that pests and diseases are one of the main factors limiting soybean productivity, causing estimated losses of 21.4% of the crop's potential yield. Thus, the losses imposed by fungi translate not only into quantitative grain losses but also into additional costs for management, prevention, and mitigation technologies, affecting the profitability of soybean crops and the production chain that extends from seed to export (Savary *et al.*, 2019).

Conventional management of these diseases is largely dependent on the use of synthetic fungicides, which, although effective, have disadvantages such as environmental impact, residual toxicity, and the development of resistance by pathogens (FRAC, 2025). In this context, the use of

biological agents emerges as a sustainable and ecologically viable alternative.

Biological control mediated by arbuscular mycorrhizal fungi (AMF) is an efficient alternative to the use of chemicals in plant disease management. AMF establish a symbiotic relationship with the root system of plants, whose extraradical mycelium branches out in the soil, promoting highly efficient absorption of water and nutrients, especially phosphorus (P) (Souza *et al.*, 2011). In addition to promoting the absorption of water and nutrients, such as phosphorus and zinc, AMF can exert an antagonistic effect on phytopathogenic fungi through mechanisms of competition, antibiosis, parasitism, and induction of systemic resistance (Etesami; Beyrami, 2021). Studies indicate that FMAs are capable of reducing the colonization and growth of pathogens in different crops, including soybeans, through the emission of volatile compounds and secondary metabolites that interfere with the development of phytopathogenic fungi (Jung *et al.*, 2012).

This combination of effects promotes plants that are more resistant to disease, less dependent on chemical inputs, and with superior productive performance. The adoption and encouragement of mycorrhizal associations represents a promising strategy within the integrated management of soybeans and other crops, contributing to the efficiency of agricultural systems.

Given this context, the present study aimed to determine the ability to suppress mycelial growth and sporulation of phytopathogenic fungi associated with soybeans through volatile compounds produced by an AMF isolate under *in vitro* conditions.

DEVELOPMENT

The studies were conducted in the phytopathology laboratory of the State University of Goiás, Palmeiras de Goiás University Unit, in 2025.

FMA isolate

The FMA isolate belongs to the working collection of Professor Taís Ferreira de Almeida, ISOE 032/18 (*Waitea* spp.), obtained in 2018 from native orchids in the municipality of Araçu, Goiás, multiplied in BDA (potato dextrose agar) medium and mineral oil.

Pathogenic isolates

Isolates of *Macrophomina* sp., *Rhizoctonia solani*, *Phomopsis* sp., *Sclerotinia* sp., *Colletotrichum dematium* var. *truncata*, *Cercospora kikuchii*, and *C. sojina* were used, belonging to the Mycology Collection of the Department of Plant Pathology at UNESP – Jaboticabal/SP, preserved in BDA medium and oil.

Mycelial development assay

The evaluation of mycelial growth suppression by volatile compounds was performed using the double plate assay method, as described by Dennis and Webster (1971) and later adapted by Fiddaman and Rossall (1993) and Ryu *et al.* (2003), using BDA medium. Twenty-four hours after preparing the plates with the medium, a 5 mm diameter disc of ISOE 032 was placed centrally in each plate. The same process was performed for the pathogenic isolates.

After preparing the plates, both were positioned bottom-to-bottom (BTB) on a laminar flow bench so that the volati-

les emitted by the FMAISOE 032 culture could diffuse into the airspace without direct contact between the pathogenic microorganisms (PM). The edges were sealed with plastic film to prevent the dispersion of volatile compounds. Each FMA-PM set was incubated under controlled conditions (25 ± 2 °C, 12-hour photoperiod) for 10 days, and the diameter of the fungal colonies was measured with a digital caliper 7 and 10 days after the test was set up. Mycelial growth inhibition (%) was calculated according to the formula:

$$\text{Inibição (\%)} = \frac{(C - T)}{C} \times 100$$

Where: C is the average colony diameter in the control (without exposure to volatiles) and T is the average diameter in the presence of volatile compounds.

Sporulation test

After determining mycelial growth, sporulation of the phytopathogenic agents

was determined. Sporulation is quantified based on the count of spores produced per colony area.

The spore suspension was obtained by adding 5 to 10 mL of sterile distilled water to the surface of the colony. Then, with the aid of a stiff bristle brush, the mycelium and spores were gently scraped off. The suspension obtained was filtered through sterile gauze to remove mycelium fragments.

For counting, a 100 μL aliquot was removed and deposited in a Neubauer chamber for counting with the aid of an optical microscope (40x). Six replicates were made per pathogen, with each plate considered one replicate and the value converted to 1 cm^2 (Leslie; Summerell, 2006).

Experimental design

The results were expressed as a mean in a completely randomized design and analyzed statistically by ANOVA and Tukey's test ($p < 0.05$) for comparison between treatments.

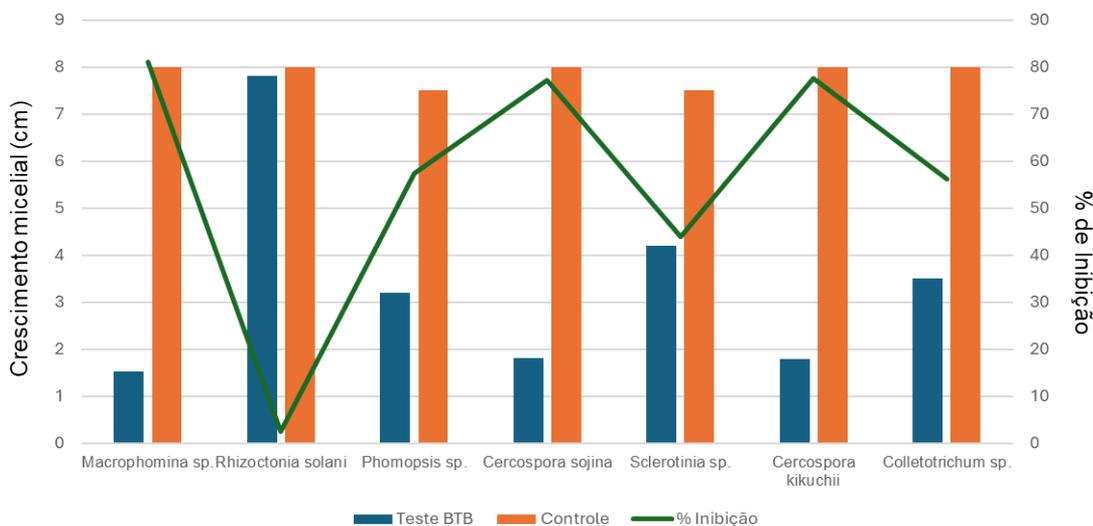


Figure 1. Inhibition of mycelial growth of phytopathogenic fungi by volatile compounds emitted by isolate FMA ISOE 032.

Source: Author.

Results and discussion

Figure 1 shows the mean values obtained for mycelial growth (in centimeters) and in the BTB test, as well as the percentage of inhibition calculated for each pathogen. Figure 2 shows the activity of the isolate on the development of some fungal colonies evaluated.

It was observed that the mycorrhizal isolate had a significant inhibitory effect on most of the phytopathogens evaluated, especially *Macrophomina* sp. (81%), *Cercospora sojina* (77.25%), and *C. kikuchii* (77.5%). These values fall within the range considered to be highly effective for biocontrol, according to parameters commonly used for evaluating synthetic fungicides, in which inhibitions greater than 70% indicate satisfactory control of the pathogen (Garcia *et al.*, 2018).

An intermediate situation was observed for *Sclerotinia* sp., with 44% inhibition, characterizing a moderate level of efficiency, comparable to chemical formulations of average performance under *in vitro* conditions (Leite *et al.*, 2014).

On the other hand, the mycorrhizal isolate showed low inhibition against *Rhizoctonia solani* (2.5%), suggesting that this pathogen is less sensitive to the volatile metabolites emitted by arbuscular mycorrhizal fungi (AMF), which has also been reported in studies involving biocontrol agents against more aggressive and structurally resistant pathogens (Melo; Azevedo, 2017).

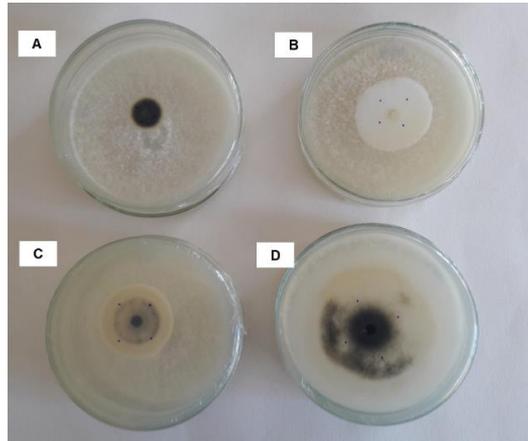


Figure 2. Development of fungal colonies subjected to the BTB test to evaluate mycelial growth inhibition. A – *Cercospora sojina*; B – *Phomopsis* sp.; C – *Colletotrichum* sp.; D – *Cercospora kikuchii*.

Source: Author.

These results indicate that the volatile compounds produced by AMF have effective antifungal action against fungi associated with important soybean diseases, such as gray stem rot and purple spot. These results corroborate the findings of Zhang *et al.* (2021), who reported the production of volatile organic compounds with fungistatic action in crops colonized by FMAs, reducing pathogen growth by up to 80%.

The inhibition observed is comparable to that obtained in studies with broad-spectrum chemical fungicides, such as thiophanate-methyl and carbendazim, which have average inhibition rates of 80 to 90% on the same fungal genera in *in vitro* tests (Garcia *et al.*, 2018; Leite *et al.*, 2014). However, unlike synthetic compounds, the action of FMAs occurs without residual toxicity and without negative impact on beneficial soil

microbiota, representing a more ecologically safe alternative (Silva *et al.*, 2020).

In the case of *Macrophomina* sp., responsible for soybean charcoal rot, the 81% inhibition observed with isolate ISOE 032 is close to the values obtained with the use of strobilurin-based chemical fungicides (80-85%) (Ferreira *et al.*, 2020). However, while chemical control requires successive applications and presents a risk of fungal resistance, the effect of FMAs tends to be more lasting, as it results from the symbiotic and continuous interaction between the plant and the beneficial fungus, strengthening the root system and inducing systemic resistance (Hung *et al.*, 2013).

For the pathogens *Cercospora sojina* and *C. kikuchii*, which cause leaf spot and purple seed spot, respectively, the inhibition percentages observed (77.25% and 77.5%) are also promising. Studies with chemical control report an average efficacy of 70 to 90% with triazole and strobilurin fungicides, depending on the concentration and environmental conditions (Dalla Lana *et al.*, 2018; Godoy *et al.*, 2016). Thus, the antifungal activity of the volatile compounds emitted by FMA ISOE 032 reveals potential comparable to conventional chemical control, with the advantage of not generating residues and contributing to the biological balance of the soil.

On the other hand, the low inhibition on *R. solani* (2.5%) indicates physiological resistance of the pathogen to volatile compounds produced by FMA. This species is known for its high colonization capacity and wide range of adaptation, which reduces the effectiveness of biological agents and even some contact fungicides, which vary in effectiveness between 30 and 60%, as reported by Caires *et al.* (2019). For *Sclerotinia*

sp., the moderate effect (44%) suggests that inhibition depends on the concentration and composition of the secondary metabolites emitted, reinforcing the need for further studies to optimize the use of FMA in combination with other control strategies (Bettiol; Morandi, 2009).

In addition to inhibiting mycelial growth, a reduction in sporulation was observed in pathogens exposed to volatile compounds from FMAs, indicating interference in the reproductive phase and dissemination of these fungi. Similar results were described by Gao *et al.* (2022), who observed a reduction of more than 60% in the production of *Fusarium oxysporum* spores in the presence of FMAs. This action is possibly due to the production of volatile alcohols and fatty acids with antifungal properties (Jung *et al.*, 2012).

The results referring to the concentration of spores found in 1 cm show that the volatile compounds emitted by the mycorrhizal isolate FMA ISOE 032 had a significant inhibitory effect on the sporulation of the phytopathogenic fungi evaluated. A significant reduction in the number of spores produced was observed in comparison to the control, indicating the antifungal action of the isolate. The highest percentage of inhibition was observed for *Cercospora kikuchii* and *C. sojina*, with reductions of approximately 65.6% and 59.8%, respectively, compared to the control. For *Colletotrichum*, the reduction was 52.5%, also demonstrating a considerable inhibitory effect.

These results suggest that isolate FMA ISOE 032 releases volatile metabolites with biocontrol potential over the fungi studied. Volatile compounds, such as alcohols, ketones, and terpenes, produced by arbus-

cular mycorrhizal fungi and rhizobacteria, are recognized for their ability to interfere with spore germination and mycelial growth of phytopathogens (Hung *et al.*, 2013; Wonglom *et al.*, 2018). The reduction in sporulation is a relevant factor, as it limits the spread and survival of pathogens in the environment, contributing to the natural suppression of diseases (Zhang *et al.*, 2021).

The significant inhibition of *Cercospora* species is particularly relevant, as it is associated with diseases responsible for losses of up to 15% in productivity when not properly controlled (Godoy *et al.*, 2020). Thus, the use of microorganisms that produce bioactive compounds, such as FMA ISOE 032, may represent a sustainable alternative to the intensive use of chemical fungicides.

The results of this study reinforce the potential of the ISOE 032 isolate as a broad-spectrum biocontrol agent, with performance equivalent or close to that of chemical fungicides, but with relevant environmental and agronomic advantages. The adoption of FMAs in integrated management programs can reduce the number of fungicide applications and lower production costs. However, the transition to the effective use of these biological agents requires investment in applied research, since factors such as environmental variability, compatibility with cultivars, and management practices can interfere with symbiotic efficiency (Brazil, 2024).

The use of FMAs in disease management represents a sustainable alternative, capable of reducing the use of chemical pesticides, preserving soil microbiota, and contributing to the sustainability of production systems. In addition, the adoption of these microorganisms can promote greater ecological balance, strengthening plants through

symbiosis and stimulating natural resistance mechanisms.

Thus, this study reinforces that arbuscular mycorrhizal fungi represent a viable and sustainable alternative to traditional chemical control, with the potential to integrate soil disease management strategies, reducing dependence on synthetic products and promoting greater ecological balance in agricultural systems.

FINAL CONSIDERATIONS

The results obtained in this study demonstrate that the arbuscular mycorrhizal fungus (AMF) isolate ISOE 032 has potential as a biocontrol agent for phytopathogenic fungi associated with soybean cultivation.

The action of the volatile compounds produced by the isolate promoted significant inhibition of mycelial growth and a significant reduction in the sporulation of pathogens such as *Macrophomina sp.*, *Cercospora kikuchii*, and *C. sojina*, demonstrating its efficiency comparable to that of synthetic fungicides.

Acknowledgments

The authors report that this study was funded by the State University of Goiás – Platform for Research and Innovation in Bio-inputs (UEG/PPIBio).

REFERENCES

- BALAN, A. C. D.; SCOLIN, L. B.; ZANLUCHI, D. G. C.; KRZYZANOWSKI, F. C.; FRANÇA-NETO, J. B.; HENNING, F. A. **Efficiency of soybean seed treatment with fungicides in controlling *Fusarium pallidorozeum* and *Cercospora* spp.** Embrapa, [S.l.], (public series). Available at: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1179176/eficiencia-do-tratamento-de-sementes-de-soja-com-fungicidas-no-controle-de-fusarium-pallidorozeum-e-cercospora-spp>. Accessed on: Nov. 13, 2025.
- BETTIOL, W.; MORANDI, M. A. B. (Ed.). **Biocontrol of plant diseases: use and perspectives.** Jaguariúna: Embrapa Meio Ambiente, 2009. 341 p. ISBN 978-85-85771-48-5. Available at: <https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/579954/1/livrobiocontrole.pdf>. Accessed on: Nov. 13, 2025.
- BRAZIL. Ministry of Agriculture and Livestock. **National Bioinputs Policy: technical report 2024.** Brasília: MAPA, 2024.
- CAIRES, A. R. L. *et al.* Efficacy of fungicides in controlling *Rhizoctonia solani* in soybeans. **Summa Phytopathologica**, v. 45, n. 3, p. 234-241, 2019.
- CAMERON, D. D. *et al.* Mycorrhiza-induced resistance: more than the sum of its parts. **Trends Plant Science**, 2013. Available at: <https://pubmed.ncbi.nlm.nih.gov/23871659/>. Accessed on: Oct. 26, 2025.
- DALLA LANA, F. *et al.* Efficacy of fungicides in controlling late-cycle diseases in soybeans. **Pesquisa Agropecuária Brasileira**, v. 53, n. 10, p. 1127-1137, 2018.
- DENNIS, C.; WEBSTER, J. Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile and volatile antibiotics. **Transactions of the British Mycological Society**, v.57, n.1, 25-39. 1971.
- DUC, N. H.; VO, H.; VAN DOAN, T.; HAMOW, K.; LE, T.; POSTA, K. Volatile organic compounds shape belowground plant-fungi interactions. **Frontiers in Plant Science**, 2022. Available at: <https://pubmed.ncbi.nlm.nih.gov/36561453/>. Accessed on: Nov. 13, 2025.
- EMBRAPA. **Embrapa soybeans in the context of soybean development in Brazil: history and contributions.** Brasília: Embrapa, 2015. Available at: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1043614/1/LivroEmbrapaSojadesenvolvimentoBROL.pdf>. Accessed on: Nov. 10, 2025.
- EMBRAPA. **Socioeconomic importance of soybeans.** Embrapa Portal. 2021. Available at: <https://www.embrapa.br/agencia-de-informacao-tecnologica/cultivos/soja/pre-producao/socioeconomia/importancia-socioeconomica-da-soja>. Accessed on: Nov. 6, 2025.
- ETESAMI, H.; BEYRAMI, M. Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to plant phosphorus acquisition: a review. **Frontiers in Plant Science / Plants** (rev.). 2021. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8280758/>. Accessed on: Sept. 19, 2025.
- FERREIRA, J. M. *et al.* Chemical control of *Macrophomina phaseolina* and biological alternatives in soybeans. **Revista de Ciências Agrárias**, v. 63, n. 2, p. 45-54, 2020.
- FIDDAMAN, P. J.; ROSSALL, S. The production of antifungal volatiles by *Bacillus subtilis*. **Journal of Applied Bacteriology**, v. 74, n. 2, 119-126. 1993.
- FRAC. Fungicide Resistance Action Committee. **Fungicide Resistance Management.** Available at: https://www.frac.info/fungicide-resistance-management/?utm_source=chatgpt.com#open-tour. Accessed on: Aug. 18, 2025.

GAO, Y. *et al.* Antifungal activity of the volatile organic compounds produced by *Ceratocystis fimbriata* strains WSJK-1 and Mby. **Frontiers in Microbiology**, v. 13, art. 1034939, 2022. DOI: 10.3389/fmicb.2022.1034939.

Available at: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.1034939/full>. Accessed on: Nov. 15, 2025.

GARCIA, R. A. *et al.* Efficiency of fungicides in controlling leaf diseases in soybean crops. **Summa Phytopathologica**, Botucatu, v. 44, n. 3, p. 263-271, 2018.

GODOY, C. V.; ALMEIDA, J. R. Production losses caused by soybean diseases. **Summa Phytopathologica**, v. 46, n. 2, p. 125-136, 2020.

GODOY, C. V.; PEREIRA, M. G.; SANTOS, L. J. Soybean leaf spot diseases: management and yield impacts. **Tropical Plant Pathology**, v. 41, n. 4, p. 345-358, 2016.

HUNG, R.; LEE, S.; BENNETT, J. W. Fungal volatile organic compounds and their role in ecosystems. **Applied Microbiology and Biotechnology**, v. 97, p. 1-12, 2013.

JUNG, S. C. *et al.* **Mycorrhiza-induced resistance and priming of plant defenses.** (Review / PDF). 2012. Available at: <https://grupos.eez.csic.es/mycostress/wp-content/uploads/2021/04/Mycorrhiza-induced-resistance-and-priming-of-plant-defenses-Jung-et-al-2012.pdf>. Accessed on: Nov. 16, 2025.

LEITE, M. S. *et al.* Efficacy of *Trichoderma* spp. isolates in the in vitro control of *Sclerotinia sclerotiorum*. **Summa Phytopathologica**, Botucatu, v. 40, n. 2, p. 150-156, 2014.

LESLIE, J. F.; SUMMERELL, B. A. **The Fusarium Laboratory Manual.** Ames, Iowa: Blackwell Professional, 2006. Available at: <file:///C:/Users/taisf/Downloads/THEFUSARIUMLABORATORYMANUAL.pdf>. Accessed on: Sept. 20, 2025.

MARTINS, R. S. *et al.* Evaluation of fungicides in the control of *Cercospora sojina* and *Cercospora kikuchii*. **Fitopatologia Brasileira**, v. 43, n. 4, p. 385-392, 2018.

MELO, I. S.; AZEVEDO, J. L. Biological control. 4th ed. rev. and expanded. Brasília, DF: Embrapa, 2017.

MINA, D.; FINKEMEIER, I.; WENZEL, M.; WESEMAEL, W. Volatile organic compounds in microbial interactions: a review. **Frontiers in Microbiology**, v. 11, p. 1-12, 2020.

POZO, M. J.; AZCÓN-AGUILAR, C. Unraveling mycorrhiza-induced resistance. **Trends in Plant Science**, v. 12, n. 8, p. 426-427, 2007. Available at: <https://pubmed.ncbi.nlm.nih.gov/17658291/>. Accessed on: Nov. 13, 2025.

RYU, C. M. *et al.* Bacterial volatiles promote growth in *Arabidopsis*. **Proceedings of the National Academy of Sciences**, v.100, n. 8, 4927-4932, 2003.

SAVARY, S. *et al.* The global burden of pathogens and pests on major food crops. **Nature Ecology & Evolution**, vol. 3, 430-439, 2019.

SEAPA. Goiás State Secretariat of Agriculture, Livestock, and Supply. Soybeans – **Agro in Data** – August/2025. Goiás, 2025. Available at: <https://goias.gov.br/agricultura/soja-agro-em-dados-agosto-2025/>. Accessed on: Nov. 12, 2025.

SILVA, R. F.; MATOS, J. S.; PEREIRA, L. Environmental impact of fungicides on soil: review. **Revista de Ciências Agrárias**, v. 43, n. 1, p. 55-72, 2020.

SMITH, S. E.; READ, D. J. **Mycorrhizal Symbiosis.** 3rd ed. London: Academic Press, 2008. Available at: <https://www.elsevier.com/books/mycorrhizal-symbiosis/smith/978-0-12-370526-6>. Accessed on: Aug. 16, 2025.

SOUZA, F. A. *et al.* **Arbuscular mycorrhizae:** prospects for increasing phosphorus (P) acquisition efficiency in Poaceae – grasses. Embrapa Milho e Sorgo, Sete Lagoas. 2011. 30p. Available at: <https://www.infoteca.cnptia.embrapa.br/bitstream/doc/921204/1/doc134.pdf>. Accessed on: Sept. 20, 2025.

WONGLOM, P.; SIRIPATTARAPORNSAK, S.; SRISAWAT, W. *et al.* Sensitivity of *Rhizoctonia solani* isolates to chemical fungicides and biological agents. **Plant Pathology Journal**, v. 34, n. 3, p. 279-289, 2018.

ZHANG, J. *et al.* Volatile organic compounds produced by biocontrol agents and their mechanisms in plant disease suppression. **Biological Control**, v. 160, p. 104682, 2021.