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# Effect of Fungicides on the Decontamination and Survival of Coffea Arabica L. Explants. In Vitro Cultures

*Effect Of Fungicides On Decontamination And Survival Of In Vitro Cultured Coffea Arabica L. Explants*

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**Summary**

Coffee stands out as one of Brazil's main agricultural commodities, being Coffea arabica.
L. is of major economic importance. In vitro micropropagation is an essential tool for the production of uniform and healthy seedlings; however, microbial contamination remains the main obstacle in the establishment of crops. This study aimed to evaluate the effect of different fungicides and concentrations on surface disinfection and survival of *C. arabica* explants cultivated in vitro. Leaf and meristematic explants were subjected to a surface asepsis protocol and inoculated in Woody Plant Medium (WPM) culture medium, under eight treatments containing the fungicides tebuconazole and azoxystrobin, alone or in combination, with four replicates each. The variations tested included fungal and bacterial contamination and survival for 28 days. The data were subjected to Generalized Linear Models (GLM) with binomial distribution and paired comparisons using Fisher's exact test with Holm correction. The results indicated a significant effect ($p < 0.05$) of the treatments for all variables. Although paired comparisons did not show isolated differences after correction for multiple tests, biological superiority was observed in the treatment with azoxystrobin (1 mL·Lÿ¹), which showed a total absence of contaminants and 100% survival. These findings suggest that strobilurins favor the balance between sanitary control and the physiological integrity of the explant.

It is concluded that the strategic use of fungicides in the culture medium is promising for improving the in vitro establishment of coffee plants, and a larger sample size is recommended to validate the ideal dosages and mitigate the effects of oxidative phytotoxicity.
**Keywords:** Plant asepsis, Endophytic contamination, Tissue culture.

**Abstract**

Coffee stands out as one of Brazil's main agricultural commodities, with Coffea arabica L. being the most economically important. In vitro micropropagation is an essential tool for producing uniform and healthy seedlings; however, microbial contamination remains the main obstacle to crop establishment. This study aimed to evaluate the effect of different fungicides and concentrations on surface disinfection and survival of C. arabica explants cultivated in vitro. Leaf and meristematic explants were subjected to a surface asepsis protocol and inoculated in Woody Plant Medium (WPM) culture medium, under eight treatments containing the fungicides tebuconazole and azoxystrobin, alone or in combination, with four replicates each. The variations tested included fungal and bacterial contamination and survival for 28 days. The data were subjected to Generalized Linear Models (GLM) with binomial distribution and paired comparisons using Fisher's exact test with Holm correction. The results indicated a significant effect ($p < 0.05$) of the treatments for all variables.
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**Keywords:** plant asepsis; endophytic contamination; plant tissue culture.

## 1. Introduction

Brazil is the world's largest producer and exporter of coffee, exhibiting high... economic relevance in the global agricultural landscape. In 2024, the country exported approximately 50.5 Millions of 60 kg bags of the product, starting the year 2025 with positive results in the market. internationally (CONAB, 2025). Among the cultivated species, *Coffea arabica* L. stands out for the quality of its beans and its significant share of Brazilian exports (CECAFÉ, 2026).

However, coffee production can be compromised by several biotic factors and abiotic factors, among which fungal diseases stand out. Among them, *Phoma leaf spot,* caused *Phoma* species occur in virtually all producing regions of the country. causing symptoms such as leaf blade deformities, necrosis, branch dieback, and abortion. young fruits, which can significantly reduce crop productivity (CAIXETA et al., 2024).

In this context, plant biotechnology has established itself as an important... A tool for producing pathogen-free seedlings. Among the available techniques, the Micropropagation allows for the rapid and controlled multiplication of plants using explants. grown under aseptic conditions, ensuring greater genetic and phytosanitary uniformity. (VIEIRA; KOBAYASHI, 2002).

During *in vitro* cultivation , however, contamination by fungi and bacteria is one of the major challenges for establishing and maintaining crops. Therefore, the use of Antimicrobial agents combined with proper aseptic protocols become essential for the obtaining viable explants free of contaminants. In addition, the use of regulators of Plant growth hormones (phytohormones) play a fundamental role in inducing processes. morphogenic processes, such as callus formation, sprouting, and rooting, are essential for the success of micropropagation (ALMEIDA, 2023).

According to Mamata et al. (2022), efficient disease management programs They often combine chemical methods with other control strategies. In the context of plant tissue culture, evaluation of different antifungal compounds and their concentrations This can contribute to the development of more efficient decontamination protocols and establishment of crops.

**Year VI, v.1 2026 | Submission: 03/15/2026 | Accepted: 03/17/2026 | Publication: 03/19/2026**

Given this, the objective of this study was to evaluate the effect of different fungicides and their concentrations in the asepsis, survival and early development of Coffea explants arabica L. cultivated in vitro.

## 2. Materials and Methods

The micropropagation and *in vitro* culture experiment was carried out in the Laboratory of Biotechnology from the University Center of Várzea Grande – UNIVAG, based on the collection of limbus. Leaf and meristem analysis performed on a specimen of *Coffea arabica* L. from a field cultivation. Experimental program at UNIVAG.

Leaf and meristematic explants of *Coffea arabica* L. were used , obtained from of mother plants with a history of poor phytosanitary health and occurrence of contamination Fungal infection associated with the genus *Phoma* spp. Explants were collected from plants grown in field conditions and subsequently transferred to the laboratory for culture establishment . *vitro.*

The explants were subjected to an aseptic process in a laminar flow hood. Following these steps:

1. Immersion in 70% ethyl alcohol for 3 minutes;
2. Immersion in a 2% sodium hypochlorite solution for 3 minutes;
3. Three consecutive washes with distilled and sterilized water;
4. Immersion in a solution containing the products corresponding to each experimental treatment during 1 hour.

After the disinfection procedure, the explants were inoculated into flasks. containing WPM *(Woody Plant Medium)* culture medium , frequently used for species woody.

Two systemic fungicides widely used in pest management were evaluated. Phytosanitary product: Tebuconazole and Azoxystrobin, applied to the composition of the culture medium considering the concentrations of the commercial products. These products were tested individually and in combination. combination, in different concentrations, as shown in Table 1. Each treatment was consisting of four replicates, each replicate consisting of a vial containing three meristematic explants.

**Table** 1. Experimental design of treatments composed of commercial products and their respective dosages, used to control contaminants in *C. arabica* L. explants.

| Treatment | Tebuconazole (mL·Lÿ¹) | Azoxystrobin (mL ·Lÿ¹) |
|---|---|---|
| w | 0 | 0 |

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| | | |
|---|---|---|
| **T1** | 0.5 | 0 |
| **T2** | 1 | 0 |
| **T3** | 1.5 | 0 |
| **T4** | 0 | 0.5 |
| **T5** | 0 | 1 |
| **T6** | 0 | 1.5 |
| **T7** | 0.5 | 0.5 |

The flasks were kept in a growth chamber under the following conditions: Temperature of 25 ± 2 °C; photoperiod of 16 hours; approximate light intensity of 40 µmol mÿ².sÿ¹.

The assessments were carried out weekly for 28 days, recording the following... Variables: fungal contamination, bacterial contamination, explant survival, formation of Calluses or sprouts, tissue oxidation, observable morphological variations. Each variable was Recorded on a binary scale, where: 0 = absence of the characteristic; 1 = presence of the characteristic.

The data obtained were organized in a spreadsheet and subsequently analyzed. statistically. For each treatment, the following were calculated: absolute frequency of occurrence (k), total number of observations (n) and observed proportion (k/n). The proportions were used to Comparison between treatments and interpretation of the biological results of the experiment.

For each variable evaluated, 95% confidence intervals were estimated. proportions, using Wilson's method, considered more suitable for experiments with small sample size. This approach allows for more robust estimates of the associated uncertainty. to the observed proportions.

A model was used to evaluate the effect of the treatments on the variables analyzed. Generalized Linear Matrix (GLM) with binomial distribution and logit link function.

The model used was:

***Y ~ Treatment***

where:

Y = response variable (survival or contamination);

Treatment = categorical factor with eight levels (C, T1–T7).

The significance of the treatment effect was assessed using the ratio test. likelihood (Likelihood Ratio Test – LRT).

To assess specific differences between treatments, comparisons were made. paired using Fisher's exact test, appropriate for binary data with a small number of observations. Regarding the control of type I error associated with multiple comparisons, the following was applied Holm's adjustment to the p-values.

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Due to the reduced number of repetitions per treatment (n = 4), a statistical power estimation through simulation, with the objective of evaluating the probability of The experiment detected differences between treatments. The estimate was made using simulations. based on the proportions observed in the experiment, considering global independence tests. between treatment and variable response.

In addition to inferential statistical tests, an exploratory analysis was performed on A relationship was constructed between: explant survival and incidence of contamination. A graphical representation of the scatter plot between survival and total contamination, allowing to visualize biological patterns between treatments and identify potential *trade-off* relationships between them. survival and contamination.

Statistical analyses and graphical visualizations were performed using *Python,* with with the help of the following libraries: Pandas (data organization), Statsmodels (statistical models), SciPy (statistical tests) and Matplotlib (graphical visualization).

## 3. Results and Discussion

Temporal analysis demonstrated that standard surface disinfection (alcohol and hypochlorite) was Effective only in the initial phase, since there were no signs of contamination in the first week. However, the emergence of fungi and bacteria from the second and third weeks onwards, especially The results of treatments T1, T3, and T4 suggest that the microorganisms were located in endophytic niches. or in a latent state. In woody species such as *C. arabica,* the persistence of contaminants Internal infections are a recurring challenge, such as those caused by *Phoma* spp., as vascular tissue can harbor pathogens. that escape contact disinfectants (VIEIRA; KOBAYASHI, 2002).

The Generalized Linear Model (GLM) revealed that the treatments exerted influence. significant impact on fungal contamination (p = 0.039), bacterial contamination (p = 0.0016) and survival (p = 0.009). Although Fisher's exact test, after Holm's correction, did not isolate differences Paired due to the sample size (n = 4), the observed biological trends suggest differences. between treatments.

Thus, the distribution of the proportions of fungal contamination among the treatments can be as observed in Figure 1, and the distribution of bacterial contamination among the treatments follows. shown in Figure **2.**

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**Figure 1.** Proportion of fungal contamination observed in *Coffea arabica* L. explants grown *in vitro* under different fungicide treatments. The bars represent the observed proportions and 95% confidence intervals.

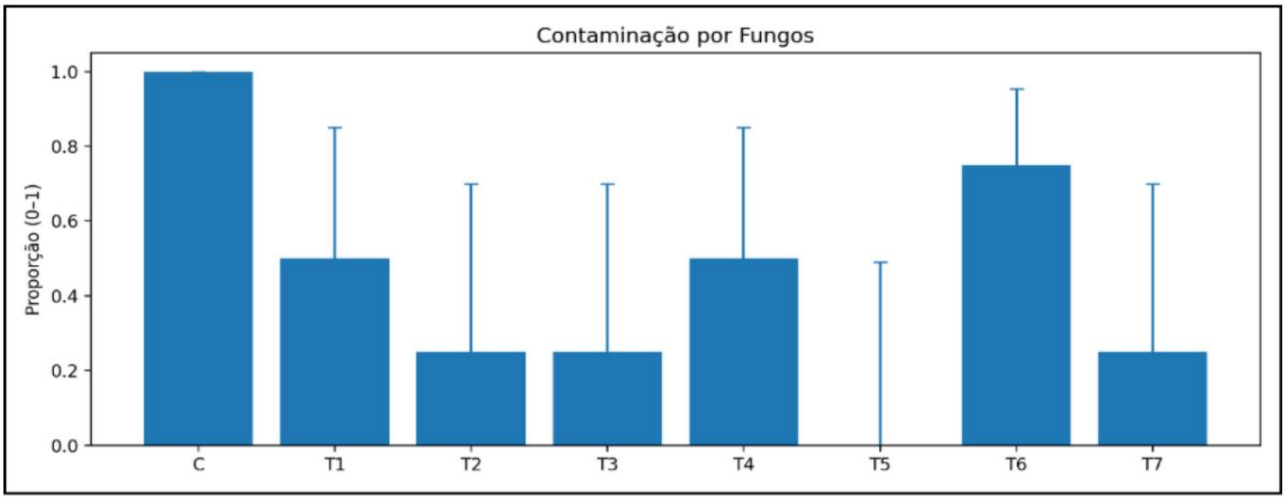
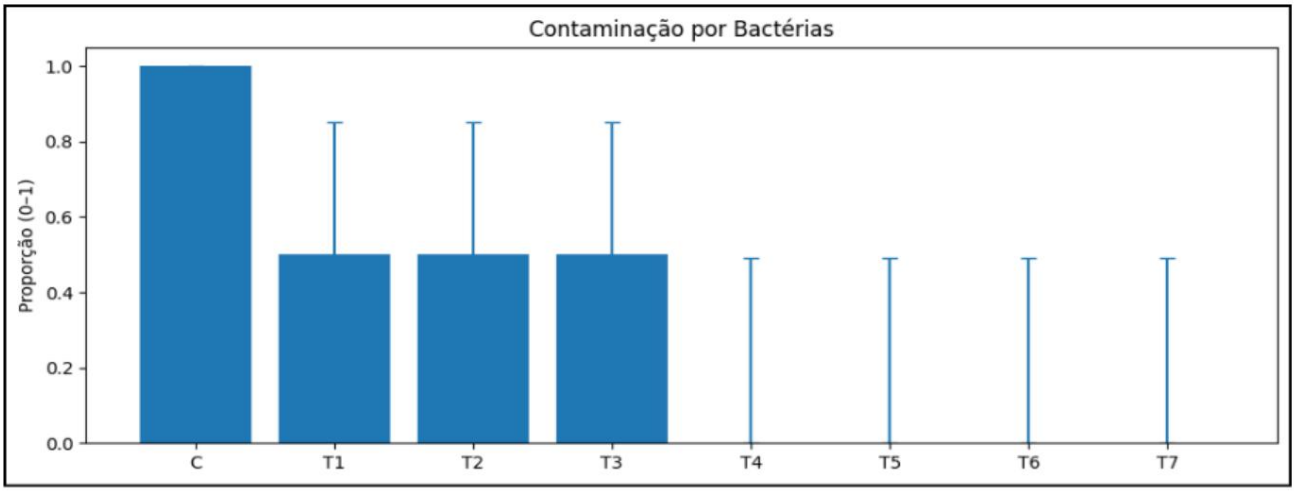



**Figure 2.** Proportion of bacterial contamination found in explants of Coffea arabica L. grown in vitro under different fungicide treatments.




Treatment T5 (Azoxystrobin 1 mL Lÿ¹) stood out for the total absence of contaminants. (Figures 1 and 2) and a 100% survival rate (Figure 3). The effectiveness of azoxystrobin is due to its role as an inhibitor of electron transport in the bc1 complex of fungal mitochondria (SANTOS et al., 2023). In addition to sanitary control, the vigor observed in the explants can be explained by The phenomenon of the "green effect" of strobilurins. According to Klaic et al. (2021), these compounds delay Leaf senescence reduces ethylene synthesis and modulates the activity of enzymes such as superoxide dismutase (SOD) and catalase (CAT) mitigate damage to cell membranes during the critical phase of *in vitro adaptation.*

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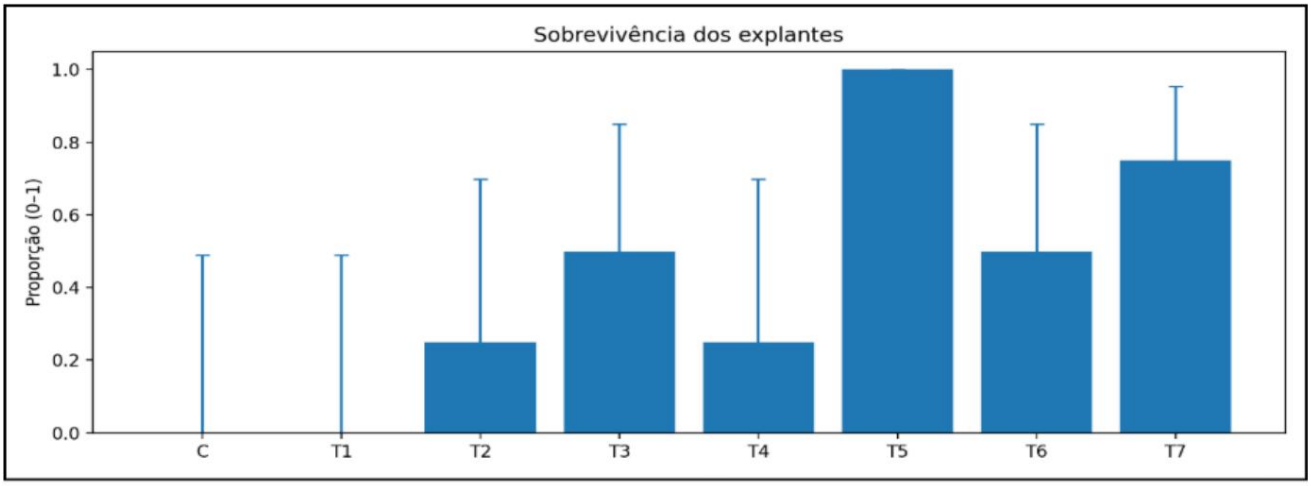
**Figure 3.** Survival rate of *Coffea arabica* L. explants grown *in vitro* under different fungicide treatments.




In contrast, treatments containing Tebuconazole (T1, T2 and T3) and combinations (T7)
This resulted in high oxidation rates and reduced survival. Tebuconazole acts by inhibiting these processes.
of the enzyme 14ÿ-desmethylase, essential in the synthesis of ergosterol in fungi. However, in tissues
In plants, triazoles can exhibit phytotoxic side effects by interfering with the metabolic pathway.
of gibberellins (NARTVARANANT et al., 2024). This hormonal interference frequently leads
due to the accumulation of reactive oxygen species (ROS), triggering lipid peroxidation.
The observed oxidation, manifested by the darkening of the tissues, results from the polymerization of
toxic quinones formed by the action of polyphenol oxidase on phenolic compounds released under
stress (ALMEIDA, 2023; RODRIGUES et al., 2022).

One relevant point was the reduction of bacterial contamination in fungicide treatments (T5).
(T7). Although azoxystrobin and tebuconazole do not possess direct bactericidal properties, studies
Studies indicate that suppressing the fungal microbiota alters the chemical microenvironment of the explant, which may
to exert competitive exclusion (MAMATA et al., 2022). Additionally, authors such as Chen et
al. (2023) suggest that certain systemic fungicides may activate defense signaling pathways, such as
the salicylic acid pathway, increasing the basal resistance of the tissue against opportunistic pathogens.

In addition to inferential statistical analysis, an exploratory trend analysis was performed.
biological (relationships between contamination and explant survival), in which the proportions
The observations revealed a pattern of biological *trade-offs* (Figure 4).

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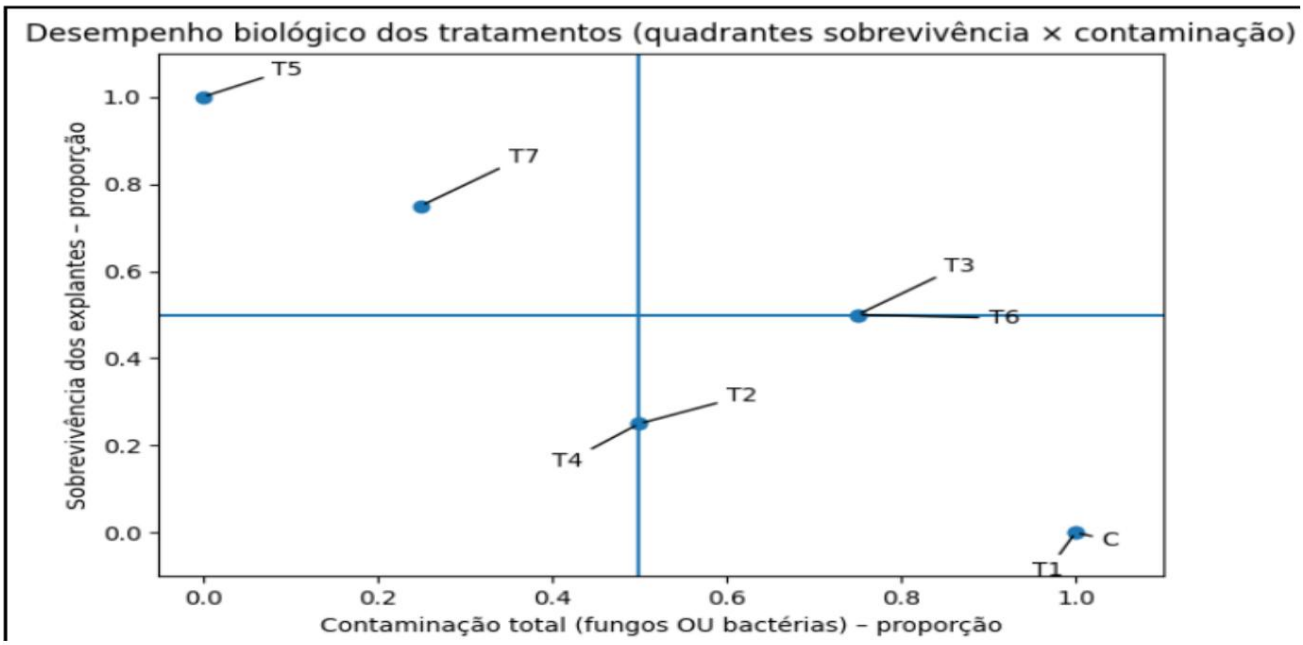
**Figure 4.** Relationship between total contamination and survival of Coffea arabica L. explants.
grown in vitro under different fungicide treatments.




The *trade-off* analysis (Figure 4) confirmed that survival is intrinsically linked to
linked to the balance between the efficiency of pest control and the physiological integrity of the tissue. While
The control (C) succumbed to microbial competition; combined treatments may have
exceeding the chemical tolerance threshold of the coffee plant. Therefore, the use of Azoxystrobin in
Intermediate concentrations are a superior strategy because they balance the pressure.
fungistatic with the maintenance of the plant's antioxidant status (FERREIRA; SOUZA, 2024).

In the meantime, it is recommended to increase the number of experimental repetitions.
The number of experimental repetitions allows for more detailed statistical analyses and greater robustness.
in the comparison between fungicide concentrations, even though no evidence was detected
Differences in pairwise comparisons after correction for multiple tests; the results indicate
A consistent trend of improved performance for certain treatments, suggesting potential.
for optimizing *in vitro* culture protocols .

## Final Considerations

The results of this study confirm that the inclusion of systemic fungicides in
The *in vitro* establishment process of *Coffea arabica* L. exerts a significant influence on the control
of microbial load and explant viability. The use of a Generalized Linear Model
It allowed us to identify that, although classic surface asepsis is insufficient to mitigate
In the event of latent pathogens, strategic chemical intervention alters the survival profile of crops.

**Year VI, v.1 2026 | Submission: 03/15/2026 | Accepted: 03/17/2026 | Publication: 03/19/2026**

Treatment containing azoxystrobin at a concentration of 1 mL·Lÿ¹ (T5) proved to be the most promising protocol, showing a total absence of observed contamination and a maximum rate survival. This performance suggests that this compound acts selectively, controlling contaminants without inducing the critical levels of tissue oxidation observed in treatments with tebuconazole or in combinations of active ingredients.

Although the reduced number of repetitions limited the detection of differences Paired statistics after correction for multiple tests, biological trends, and the overall effect of Models indicate that azoxystrobin has the potential to optimize the micropropagation of coffee plants. with a compromised phytosanitary history. For future work, expansion of the experimental design and investigation of the residual effects of these fungicides on subsequent organogenic development of the explants.

**References**

ALMEIDA, JG. **Use of hypochlorite in the decontamination of explants and the effect of phytohormones on the in vitro propagation of vanilla *(Vanilla bahiana* Hoehne).** 2023. Undergraduate Thesis (Agronomy) – Pontifical Catholic University of Goiás, Goiânia, 2023.

CAIXETA, E. *et al.* **Integrated pest and disease management of Arabica coffee.** Brasília, DF: Embrapa, 2024. (Technical Circular).

CHEN, L. *et al.* Priming of plant defense responses by systemic fungicides: a molecular approach. **Journal of Agricultural and Food Chemistry,** vol. 71, no. 5, p. 1102-1115, 2023.

National Supply Company (CONAB). **Ministry of Agrarian Development and Family Agriculture.** Brasília: CONAB, 2025.

Brazilian Coffee Exporters Council (CECAFÉ). **Monthly export report – January 2026.** São Paulo: Cecafé, 2026. Available at: https://www.cecafe.com.br/publicacoes/relatorio-de-exportacoes/. Accessed on: February 23, 2026.

FERREIRA, MA; SOUZA, RT Physiology of stress in woody plants cultivated *in vitro.* **Brazilian Journal of Applied Botany,** v. 15, n. 1, p. 45-60, 2024.

KLAIC, M. *et al.* Physiological effects of strobilurins on plant growth and yield: a review. **Agricultural Science,** vol. 12, no. 4, p. 245-258, 2021.

MAMATA, SG *et al.* Assessment of efficacy of potential fungicides and biocontrol agents for efficient management of *Phoma* sp., causing leaf spot of pigeonpea. **Biological Forum – An International Journal,** India, p. 1321-1327, 2022.

NARTVARANANT, P. *et al.* Effects of triazole compounds on plant growth and development in

**Year VI, v.1 2026 | Submission: 03/15/2026 | Accepted: 03/17/2026 | Publication: 03/19/2026**

woody species. **Journal of Plant Growth Regulation,** vol. 43, p. 112-125, 2024.

RODRIGUES, PS *et al.* Phenology and oxidative metabolism in *Coffea arabica* under abiotic stress. **Pesquisa Agropecuária Brasileira,** v. 57, e02845, 2022.

SANTOS, RL *et al.* Mechanisms of action of systemic fungicides in the control of endophytic pathogens. **Brazilian Journal of Phytopathology,** v. 48, n. 2, 2023.

VIEIRA, LGE; KOBAYASHI, AK. Micropropagation of coffee plants. In: **SYMPOSIUM ON COFFEE RESEARCH IN BRAZIL,** Poços de Caldas, MG. **Lectures.** Brasília: Embrapa Café, 2002. p. 147-167.