COFFEE SCIENCE
ISSN: 1984-3909
eISSN: 1984-3909
Coffee Science, e172049, 2022

Doi: https://doi.org/10.25186/.v17i.2049

Management of coffee leaf rust using L-glutamic acid biofertilizer combined with fungicide

Samanta Aparecida Cristiano Cabral[1], Natália Moreira Mafra[2], Beatriz Fagundes de Carvalho[1], Dalilla Carvalho Rezende[1]

[1]Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais/IFSULDEMINAS, *Campus* Machado, Machado, MG, Brasil
[2]Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais/IFSULDEMINAS, *Campus* Carmo de Minas, Carmo de Minas, MG, Brasil
Contact authors: samantaapcristiano@gmail.com; natalia.mafra@ifsuldeminas.edu.br; bfcagro@gmail.com; dalilla.rezende@ifsuldeminas.edu.br
Received in June 21, 2022 and approved in December 12, 2022

ABSTRACT

The use of biofertilizers is a potential tool for the management of crop diseases. Coffee leaf rust, which is commonly controlled by triazole and strobilurin fungicides, is one of the main phytosanitary challenges associated with coffee cultivation. However, the indiscriminate use of such fungicides may be harmful to the environment and human health, in addition to having a negative impact on coffee exports. The aim of this study was to evaluate the effect of foliar application of L-glutamic acid on the incidence and severity of coffee leaf rust in the southern region of Minas Gerais, Brazil. A biofertilizer made of sugarcane molasses fermented by the bacterium *Corynebacterium glutamicum* was used in combination with 25% L-glutamic acid and a fungicide of the triazole group registered for crops. The experimental design adopted was randomized blocks with four replications (eight plants per replicate) and seven treatments: fungicide, control test, 0.8 L ha^{-1} of biofertilizer, combinations of 0.04, 0.06, 0.08, and 0.1 L ha^{-1} of biofertilizer and fungicide. Three treatments were initially applied in a preventive way, before the rainy season, and then at intervals of 60 days. After seven months of treatment, we observed that coffee leaf rust incidence was significantly lower in coffee plants treated with combined products than in treatments of either fungicide or biofertilizer only, with a reduction of 56% and 45%, respectively, being observed. Among the combinations of biofertilizer and fungicide, coffee leaf rust incidence reduced with an increase in the biofertilizer dose, such that the disease incidence in plants treated with a dose of 0.1 L ha^{-1}was 58% lower than that in plants treated with fungicide only. No differences in disease severity were observed among the treatments. In conclusion, the use of a combination of biofertilizer and fungicide is more effective for the management of coffee leaf rust than the use of the isolated products.

Key words: Amino acid; *Hemileia vastatrix*; Incidence; Severity.

1 INTRODUCTION

In Brazil, coffee cultivation has historical importance, including creation of thousands of jobs along the supply chain, and 24% of the coffee produced is exported (Moreira et al., 2019). According to the National Supply Company (CONAB) data, Brazil produces 2.2 million hectares of coffee, with the state of Minas Gerais producing approximately 1.3 million hectares (Companhia Nacional de Abastecimento - CONAB, 2022). Brazilian coffee exports in February 2022 amounted to 3.441 million of 60 kg coffee bags, an equivalent of foreign exchange earnings of 782.6 million dollars (Brazilian Coffee Exporters Council – CECAFE, 2022), which highlights the importance of coffee cultivation to the national economy.

The coffee tree is biennial and its highest production years are more susceptible to diseases due to the quantity of foliar area and nutrients required for crop growth (Pozza; Pozza, 2003). Coffee leaf rust is caused by the biotrophic fungus *Hemileia vastatrix* and it is one of the major leaf diseases of the crop (Alfonsi et al., 2019). The fungus was first found in Sri Lanka (former Ceylon) in 1869 and was first reported in 1970 in Bahia (Bergamim Filho et al., 2018).

Currently, coffee leaf rust is present in all coffee-producing regions, and the majority of coffee cultivars are susceptible to the pathogen. Disease damage is indirect, such as defoliation and death of plagiotropic branches, resulting in the loss of leaf photosynthetic area. A reduction in flowers and poor fruit formation occurs when the leaves fall before floral induction or during fruit formation, which may cause a 50% reduction in coffee productivity (Alfonsi et al., 2019).

Chemical control using triazole and strobilurin fungicides is currently the most commonly used method to manage coffee leaf rust. However, the intense and indiscriminate use of agrochemical products in coffee cultivation has a negative impact on coffee exports and product cost, in addition to their potential harm to the environment and human health (Carvalho; Cunha; Silva, 2012).

Biofertilizers are used in plants as resistance inducers to combat diseases. Such products have been an ally to the producer, as they contain bioactive compounds that can induce resistance and/or exhibit antifungal properties (Medeiros; Lopes, 2006).

L-glutamic acid, which is an amino acid obtained by the fermentation of sugarcane molasses and a precursor of photosynthesis, facilitates the absorption of cations by plants, and improves the efficacy and uptake of nutrients (Dreyer; Coelho; Mondiel, 2000). According to Pozza and Pozza (2003), plants are more resistant to pathogens when well-nourished.

The use of biofertilizers combined with traditional chemical products (fungicides) could be a potential approach to mitigating the harmful effects of crop diseases in the field and promoting sustainable agriculture. Therefore, the present study aimed to evaluate the efficacy of L-glutamic acid biofertilizer with or without a fungicide for the management of coffee leaf rust in a field in the southern region of Minas Gerais, Brazil.

2 MATERIAL AND METHODS

The experiment was carried out from November 2020 to March 2021 on a coffee farm in the town of Campo do Meio (latitude 21° 08’05″ S, longitude 45° 47’52″ W) in the southern region of Minas Gerais, Brazil. The area was planted with three-year-old coffee trees (Mundo Novo group) that are susceptible to coffee leaf rust. The plantation was considered to have proper nutritional management based on the leaves as well as soil chemical analysis, with plant spacing of 3.0 × 0.8 m and a stand of 4,100 plants per hectare. The experimental design used randomized blocks with seven treatments, four replications, and eight plants per replicate, from which the first and last plants were discarded as borders.

A biofertilizer made of sugarcane molasses fermented by the bacterium *Corynebacterium glutamicum* was used with 25% (300 g L^{-1}) of L-glutamic acid and 4.0% (48 g L^{-1}) of water-soluble N, which was available in the market. In addition, a triazole fungicide was added to the biofertilizer at a concentration of 0.4 L ha^{-1} (flutriafol 500 g L^{-1}), which is recommended by the manufacturer for coffee tree cultivation.

The foliar pulverizations (Table 1) followed the treatments: T1, fungicide flutriafol 0.4 L ha^{-1}; T2, no application of fungicide or biofertilizer; T3, 0.08 L ha^{-1} of biofertilizer; T4, flutriafol fungicide with 0.04 L ha^{-1} of biofertilizer; T5, flutriafol fungicide combined with 0.06 L ha^{-1} of biofertilizer; T6, flutriafol combined with 0.08 L $ha^{-1}$of biofertilizer.

The treatments were pulverized in three stages: the initial treatment was applied in a preventive way, before the rainy season, which is critical for coffee leaf rust development, and subsequent treatments were applied at 60-day intervals.

The treatments were sprayed using a backpack pump equipped with a conical jet spray tip at a pressure of 30–60 psi and a standardized flow rate of 400 L ha^{-1}.

Evaluations were carried out monthly, with the first being conducted before product pulverization and the others between November and April, totaling seven assessments. Disease incidence and severity were evaluated in the lower third of the plants. Two branches on both sides of each plant were marked, totaling four branches per plant, and the same branch was used for the evaluations.

Coffee leaf rust incidence was obtained using the following formula:

$$I\,(\%) = (NFD/NFT)\ {*}100$$

I (%): Incidence (%)
NFD: Number of affected leaves on the branches
NFT: Total number of leaves on the branches

Coffee leaf rust severity index was determined according to a diagrammatic scale proposed by Cunha et al. (2001). The 3rd and 4th pairs of leaves from the same branch where disease incidence was assessed were selected, and percentages were assigned based on the severity level of disease symptoms, which ranged from 0 (no presence of pustules) to 5 (20–25% of the leaves had pustules) according to the progress of the disease.

In addition, the areas under the disease progress curve for incidence (AUDPC) and severity (AACPDS) were calculated using the equation proposed by Shaner and Finney (1977):

$$AUDPC\ or\ AACPDS = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)\left(t_{i+1} - t_i\right)$$

AUDPC: Area under the incidence progress curve
AACPDS: Area under the severity progress curve
Yi: Proportion of disease or foliage n i-th observation
Ti: Time in days in the i-th observation
n: Total number of observation

Analysis of variance assumptions of the normality of the residuals and homogeneity of variances were analyzed using SISVAR (Ferreira, 2019). The variables were subjected to variance analysis ($p \leq 0.05$), their means were compared using the Scott–Knott test (Scott; Knott, 1974), and the treatment variables obtained with the combination of products were subjected to regression analysis.

Table 1: Treatments and their application dates on coffee trees used in the field experiment in the southern region of Minas Gerais, Brazil.

Treatment		Dose (L ha^{-1})	1st application	2nd application	3rd application
Fungicide	T1	0.4	11/07/2020	01/22/2021	03/19/2021
Control Test	T2	x	11/07/2020	01/22/2021	03/19/2021
Biofertilizer	T3	0.08	11/07/2020	01/22/2021	03/19/2021
Biofertilizer + fungicide	T4	0.04 + 0.4	11/07/2020	01/22/2021	03/19/2021
Biofertilizer + fungicide	T5	0.06 + 0.4	11/07/2020	01/22/2021	03/19/2021
Biofertilizer + fungicide	T6	0.08 + 0.4	11/07/2020	01/22/2021	03/19/2021
Biofertilizer + fungicide	T7	0.1 + 0.4	11/07/2020	01/22/2021	03/19/2021

Source: Authors.

3 RESULTS

A combination of the fungicide and L-glutamic acid biofertilizer was more effective in the management of coffee leaf rust than the treatments using isolated products. A comparison of treatments by pairwise testing revealed that the superiority of the combinations over the other treatments was notable, regardless of the dose used (Figure 1).

Based on the dosage, we observed that coffee leaf rust incidence decreased with an increase in the biofertilizer concentration (Figure 2). Among the doses used in the present study, a dose of 0.10 ha^{-1} considerably inhibited coffee leaf rust incidence, which presented an average incidence of 1.08%, corresponding to a 58.6% lower incidence than the treatment with fungicide only.

No coffee leaf rust signs (pustules) were observed in the first evaluation, which was carried out prior to the first pulverization (Figure 3).

In the third evaluation, we observed that T6 (combination of 0.08 L ha^{-1} of biofertilizer and flutriafol fungicide) and T7 (combination of 0.1 L ha^{-1} of biofertilizer and flutriafol fungicide) treatments resulted in an increase in the incidence rates of the disease, while incidence rates in T2 treatments (control test with no addition of biofertilizer or fungicide) remained stable. The monthly incidence rates of coffee leaf rust in T4 (combination of 0.04 L ha^{-1} of biofertilizer and flutriafol fungicide), T5 (combination of 0.06 L ha^{-1} of biofertilizer and flutriafol fungicide), T3 (biofertilizer only 0.08L ha^{-1}), and T1 (flutriafol fungicide only) treatments decreased.

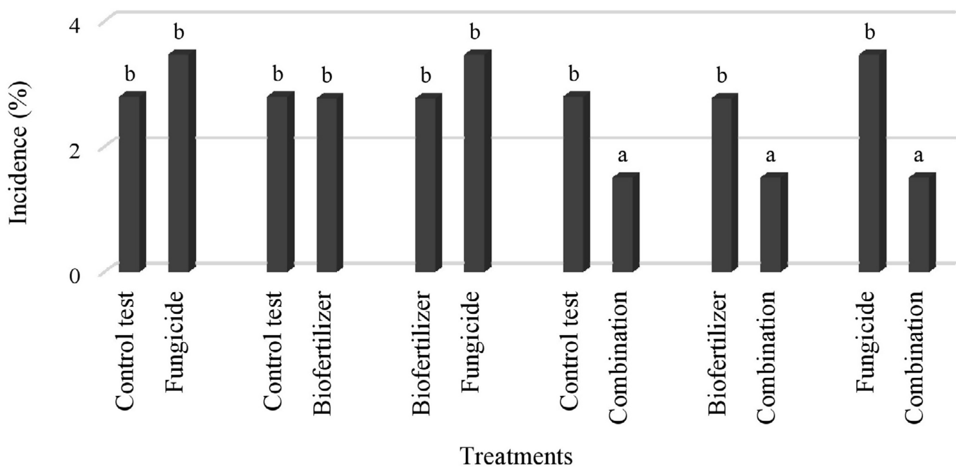

Figure 1: Incidence of coffee leaf rust (*Hemileia vastatrix*) in coffee trees subjected to fungicide (flutriafol at 0.4L ha^{-1}), control test (no fungicide or biofertilizer), L-glutamic acid biofertilizer (0.08L ha^{-1}), combined biofertilizer (sum of the means 0.04, 0.06, 0.08 and 0.10L ha^{-1} of biofertilizer + flutriafol fungicide) treatments. Means followed by different letters are significantly different according to a contrast test at 5% significance.

Source: Authors.

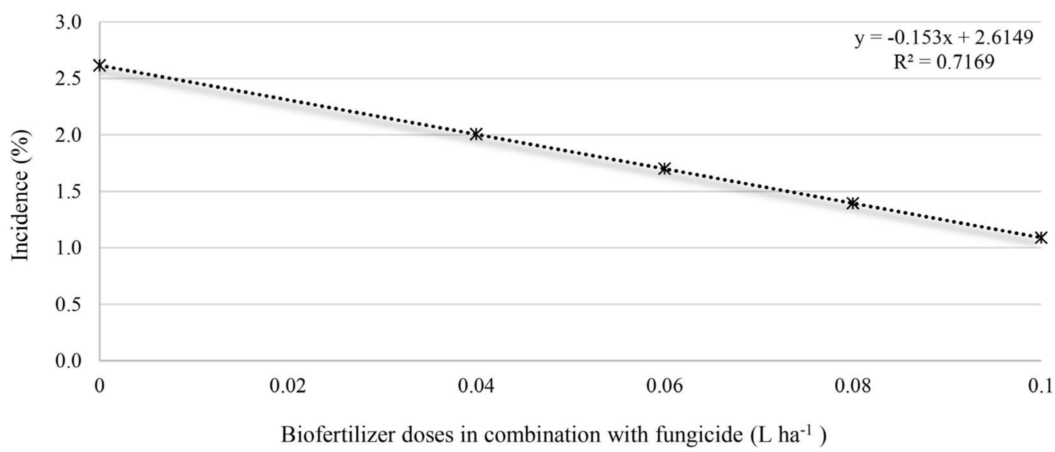

Figure 2: Coffee leaf rust incidence averages (*Hemileia vastatrix*) among coffee trees at doses of 0, 0.04, 0.06, 0.08, and 0.10 L ha^{-1} of L-glutamic acid biofertilizer combined with flutriafol fungicide. The equation represents the analysis of linear regression.

Source: Authors.

In the fourth evaluation, a reduction in coffee leaf rust incidence rates was only observed in coffee branches in T7 treatment (a combination of 0.1 L ha^{-1} of biofertilizer and flutriafol fungicide). However, the treatments with isolated products exhibited relatively high incidence rates, which are attributed to the rains in December based on the data acquired from the meteorological station at Campo Verde Farm (Campo Verde, 2021) (Figure 4). The rains facilitated disease establishment. In the fifth evaluation, the incidence rates of the disease in T7 treatment (combination of 0.1 L ha^{-1} of biofertilizer and flutriafol fungicide) were stable, while the other treatments showed a decline in the incidence rates.

In general, the evaluation of coffee leaf rust incidence rates illustrated in Figure 3 revealed relatively low rates in T5 (combination of 0.06 L ha^{-1} of biofertilizer and flutriafol fungicide), T6 (combination of 0.08 L ha^{-1} of biofertilizer and flutriafol fungicide), and T7 (combination of 0.1 L ha^{-1} of biofertilizer and flutriafol fungicide) treatments. Conversely, relatively high coffee leaf rust incidence rates were observed in T1 (flutriafol fungicide only) and T3 (biofertilizer 0.08 L ha^{-1} only) treatments.

Based on the AUDPC analysis (Figure 5), we observed a relatively low expression of the disease in treatments where combinations of fungicide and biofertilizer were used (T4, T5, T6, and T7).

As shown in Figure 6, we observed, from February onward, low disease rates severity and stability in the treatments with a combination of biofertilizer and fungicide.

In addition, the area under the disease progress curve (AACPDS) was calculated (Figure 7) to determine disease severity; no significant difference was observed among the treatments.

4 DISCUSSION

The application of L-glutamic acid biofertilizer combined with the fungicide was more effective in the management of coffee leaf rust than other isolated products. Similar results were obtained by Morales, Santos, and Tomazeli (2012) who investigated diseases in aerial parts of wheat in which the complementary nutritional foliar effect was potentiated by the fungicide, enhancing plant resistance and, consequently, reducing phytopathogenic agents when compared to the use of isolated products.

Despite the absence of coffee leaf rust pustules on the under leaf surfaces in the first evaluation carried out before the application of the treatments, spores and reproductive structures may have been present in the areas because the disease is endemic (Bergamin Filho et al., 2018). The finding was consistent with the observation made in the present study, as some chlorotic spots appeared on coffee tree leaves in all treatments in later evaluations, with the spots being more pronounced in the control test and mild in the treatment with a combination of the fungicide and L-glutamic acid.

The ideal conditions for the development of coffee leaf rust are temperatures in the range of 20–24°C combined with high relative humidity, which is caused by frequent rain, shading, and high crop density (Bergamin Filho et al., 2018). December received the highest amount of rain during the experimental period, which explains the subsequent increase in the incidence of coffee leaf rust. The evaluation carried out at the beginning of January reflected the progression of the disease in the previous month that had high precipitation and leaf wetness.

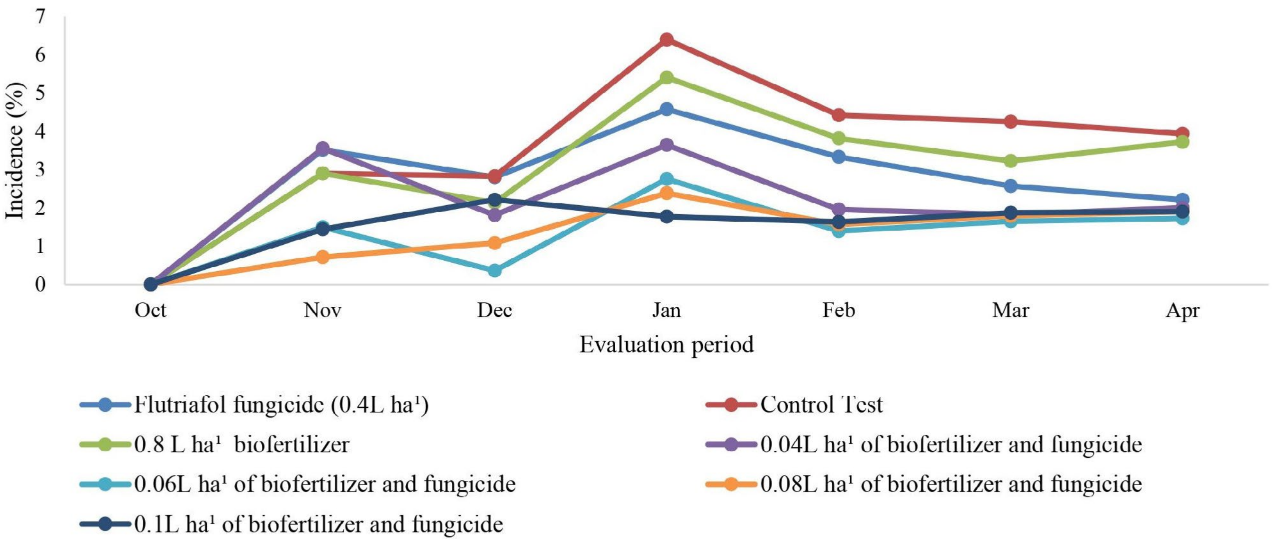

Figure 3: Average monthly incidence rates of coffee leaf rust (*Hemileia vastratix*) from October 2020 to April 2021 in coffee trees subjected to flutriafol fungicide (T1), control test with no addition of fungicide or biofertilizer (T2), 0.08L ha^{-1} of L-glutamic acid biofertilizer (T3), combined doses of 0.04L ha^{-1} of biofertilizer and fungicide (T4), 0.06L ha^{-1} of biofertilizer and fungicide (T5), 0.08L ha^{-1} of biofertilizer and fungicide (T6), and 0.1L ha^{-1} of biofertilizer and fungicide (T7) treatments.

Source: Authors.

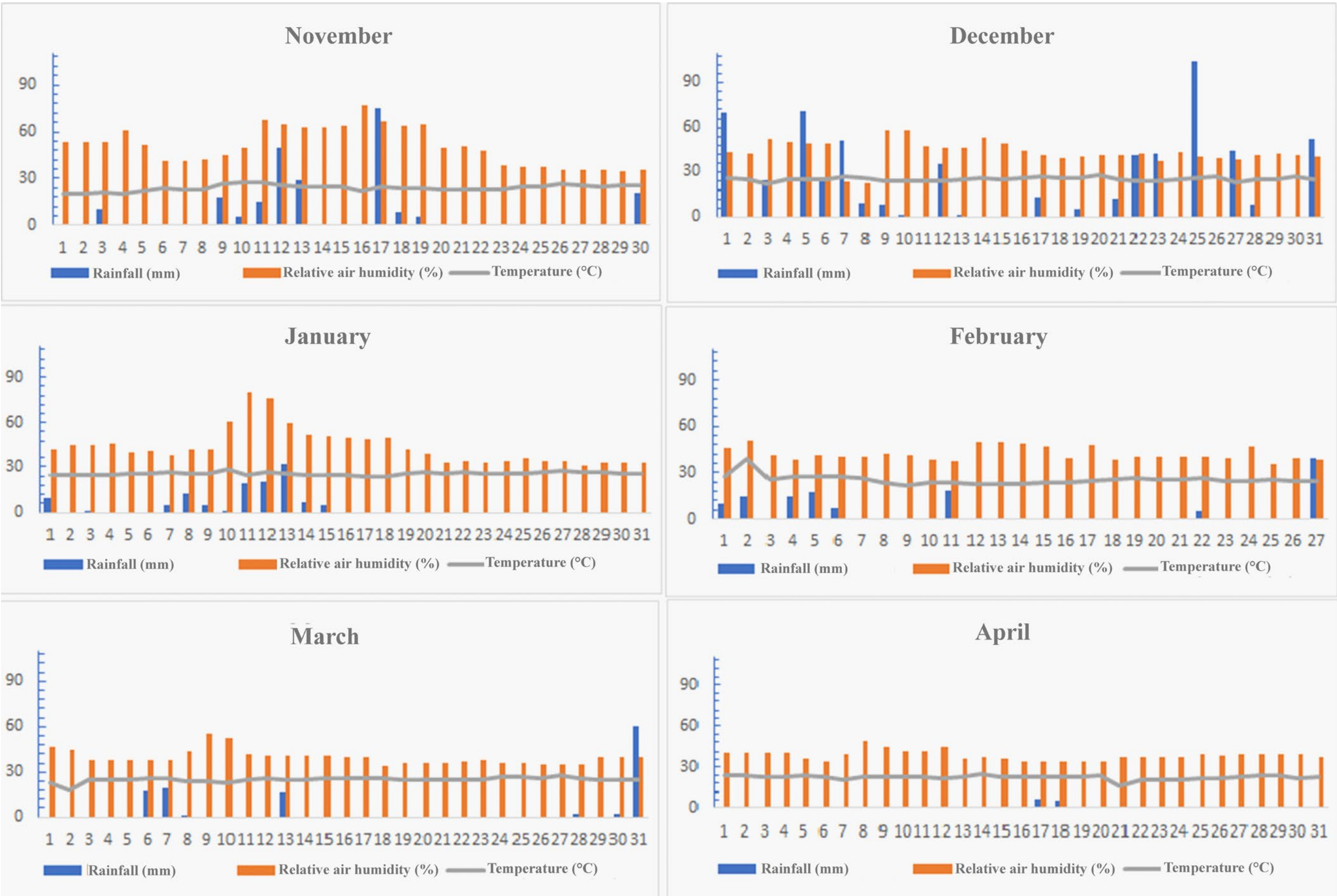

Figure 4: Average temperature (ºC), relative air humidity (%), and rainfall (mm) from November 2020 to April 2021 in Campo do Meio region (South of Minas Gerais, Brazil).

Source: Data collected personally from the Meteorological Station at Campo Verde Farm – Jodil Group in Campo do Meio, MG, Brazil, located approximately 12 km from the experimental area.

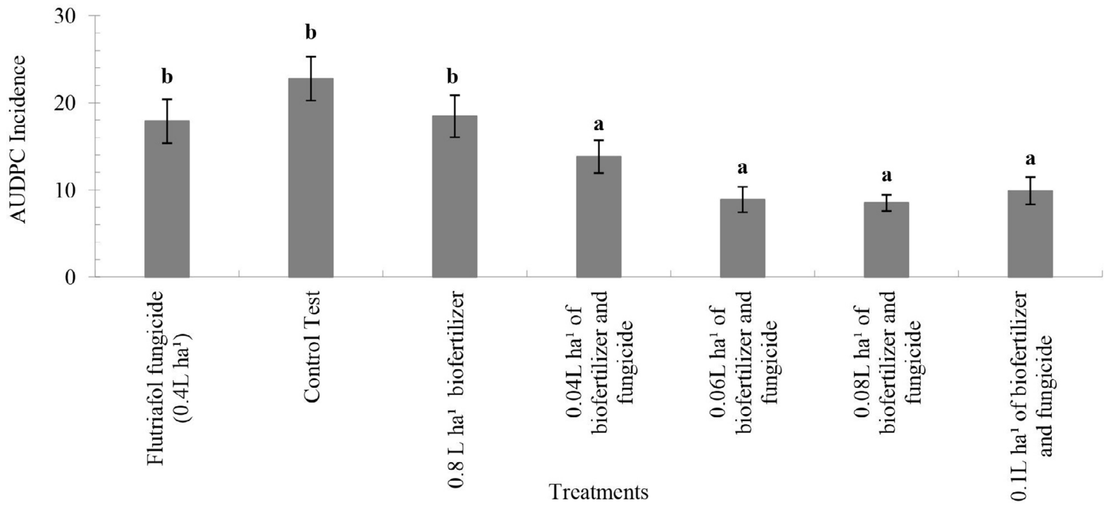

Figure 5: Mean values of the areas under the disease progress curve (AUDPC) for coffee leaf rust (*Hemileia vastatrix*) incidence from October 2020 to April 2021 in coffee trees subjected to flutriafol fungicide (T1), control test with no addition of fungicide or biofertilizer (T2), 0.08L ha^{-1} of L-glutamic acid biofertilizer (T3), combined doses of 0.04L ha^{-1} of biofertilizer and fungicide (T4), 0.06L ha^{-1} of biofertilizer and fungicide (T5), 0.08L ha^{-1} of biofertilizer and fungicide (T6), and 0.1L ha^{-1} of biofertilizer and fungicide (T7) treatments. Means followed by different letters are significantly different according to a Scott- Knott test at 5% significance.

Source: Authors.

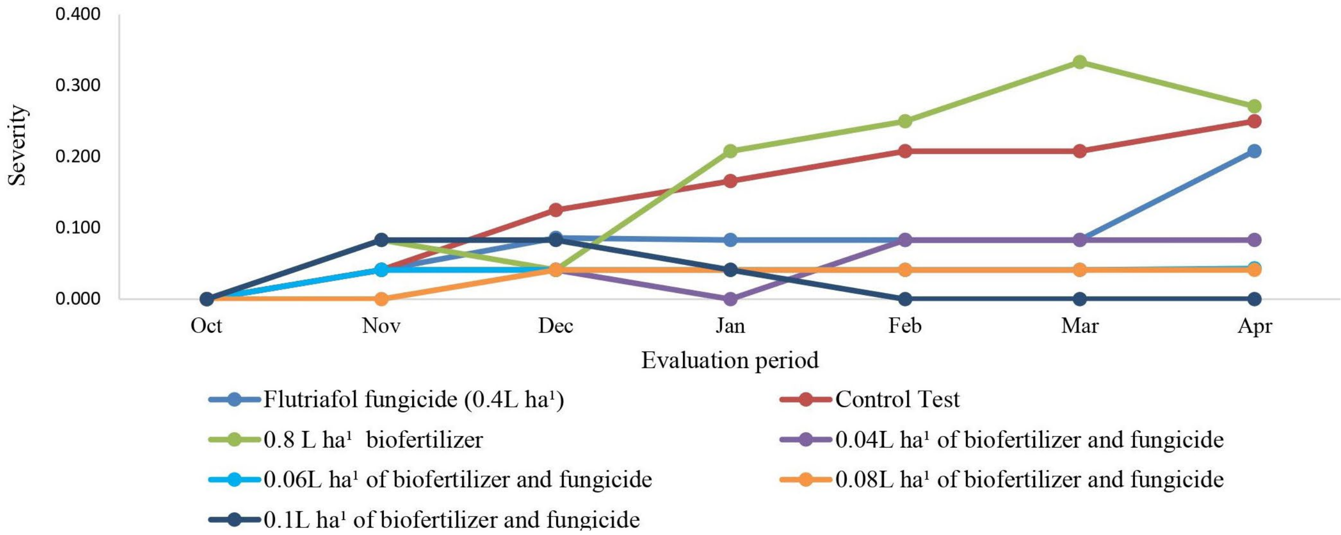

Figure 6: Average monthly progression of coffee leaf rust *(Hemileia vastatrix)* severity among coffee trees subjected to flutriafol fungicide (T1), control test with no addition of fungicide or biofertilizer (T2), 0.08L ha^{-1} of L-glutamic acid biofertilizer (T3), combined doses of 0.04L ha^{-1} of biofertilizer and fungicide (T4), 0.06L ha^{-1} of biofertilizer and fungicide (T5), 0.08L ha^{-1} of biofertilizer and fungicide (T6), and 0.1L ha^{-1} of biofertilizer and fungicide (T7) treatments. The scores correspond to the severity of the disease on the third leaves from the lower branches, starting from 0 (no presence of pustules) to 5 (20–25% of the leaves have pustules) between October 2020 and April 2021 in the southern region of Minas Gerais, Brazil.

Source: Authors.

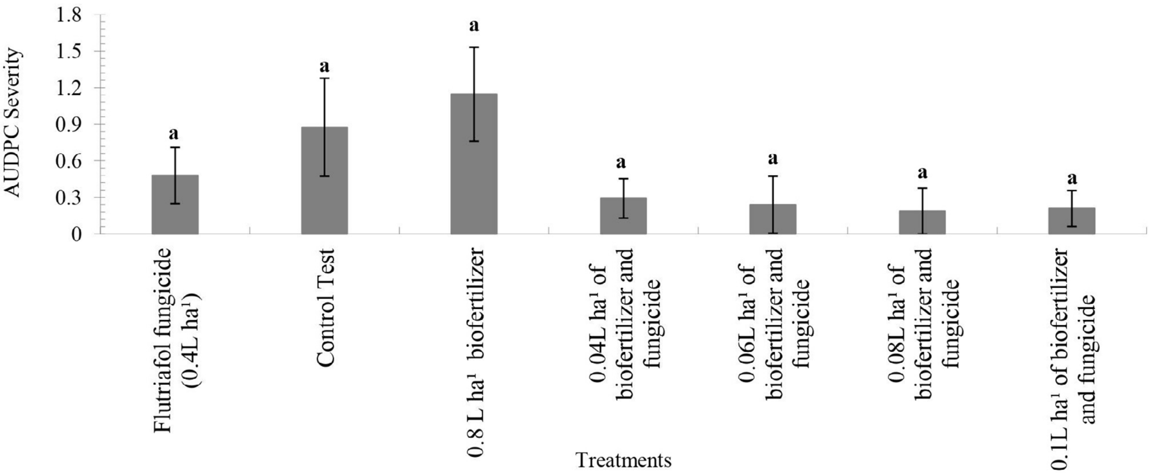

Figure 7: Area under the disease progress curve for coffee leaf rust (*Hemileia vastatrix*) severity (AUDPC) from October 2020 to April 2021 on the abaxial leaf surfaces of coffee trees subjected to flutriafol fungicide (T1), control test with no addition of fungicide or biofertilizer (T2), 0.08L ha^{-1} of L-glutamic acid biofertilizer (T3), combined doses of 0.04L ha^{-1} of biofertilizer and fungicide (T4), 0.06L ha^{-1} of biofertilizer and fungicide (T5), 0.08L ha^{-1} of biofertilizer and fungicide (T6), and 0.1L ha^{-1} of biofertilizer and fungicide (T7) treatments. Means followed by different letters are significantly different according to a Scott- Knott test at 5% significance.

Source: Authors.

Combinations of biofertilizer and fungicide resulted in relatively low rates of coffee leaf rust incidence and AUDPC values. In addition, Carvalho, Cunha and Silva (2012) reported that the use of alternative products significantly reduced AUDPC and rates of the incidence and severity of coffee leaf rust over two consecutive years. The authors used viçosa syrup as an alternative control, which promotes nutritional balance in addition to increasing plant resistance, enhances foliar limb thickness, prevents fungal penetration of leaves, inhibits spore germination, and is effective in the control of diseases and foliage preservation. In a study carried out by Androcioli et al. (2012), AUDPC of coffee leaf rust decreased with the use of alternative products such as silicate clay, propolis extract biofertilizer, viçosa syrup, and kaolin.

The effect of foliar fertilizers is associated with the nutritional aspect of the plant, which indirectly becomes more resistant to diseases and modifies the physical-chemical environment that is reflected by the limitation of pathogen survival (Costa; Zambolim; Rodrigues, 2007). Nutritional imbalance can cause physiological disorders in plants and facilitate pathogen attack, making the infection process easier and establishing parasitic relationships in plant tissues. In such a context, it is important to emphasize the need for adequate nutritional management so that plant physiological processes can occur in a way that creates unfavorable conditions that limit pathogen infection and reduce disease progression (Belan et al., 2006).

Low rates of severity and disease stability were observed in the present study from February to April. Ruiz-Cárdenas (2015) mentioned that the development of coffee leaf rust is associated with the occurrence of favorable conditions for the pathogen, such as the occurrence of rain or high humidity. In the absence of one of the components, the infection is compromised and the disease cycle is not completed. Therefore, the pathogen has difficulties completing its life cycle, in turn, preventing its reproduction and dissemination, and consequently, the establishment of the disease, resulting in low levels of severity during the experimental period, which could be attributed to unfavorable environmental conditions for the pathogen.

5 CONCLUSION

The combination of L-glutamic acid biofertilizer with fungicide is effective in the management of coffee leaf rust. A reduction in the disease incidence was positively influenced by an increase in biofertilizer dose. The application of the biofertilizer and fungicide alone or in combination did not reduce the severity of coffee leaf rust under the experimental conditions of the present study.

6 ACKNOWLEDGMENTS

The authors express their gratitude to the Federal Institute of Education, Science, and Technology of South of Minas Gerais (IFSULDEMINAS) for providing financial support to publish this study.

7 AUTHORS' CONTRIBUTION

SACC performed the experiment, collected data and wrote the manuscript, NMM followed the experiment and review the final version of the work, BFC performed the statistical analyses, DCR designed and supervised the experiment. All authors contributed to the interpretation and discussion of the results.

8 REFERENCES

ALFONSI, W. M. et al. Período de incubação da ferrugem do cafeeiro. **Summa Phytopathologica**, 45(2):134-140, 2019.

ANDROCIOLI, H. G. et al. Produtos alternativos no controle da *Hemileia Vastatrix (Berkeley & Broome) e Cercospora Coffeicola (Berkeley & Cooke)* em cafeeiros. **Coffee Science**, 7(2):187-197, 2012.

BELAN, L. L. et al. Manejo nutricional aplicado ao manejo de doenças de plantas: Enfoque na cafeicultura. *In*: FERREIRA, A. et al. **Tópicos especiais em produção vegetal VI.** Alegre: CAUFES, p.377-402, 2016.

BERGAMIN FILHO, A. et al. Epidemiologia de doenças de plantas. *In*: AMORIM. L; REZENDE. J. A. M.; BERGAMIM FILHO. A. **Manual de fitopatologia**: Princípios e conceitos, v. 1, 5 ed. São Paulo: Ceres, p.71-84. 2018,

CAMPO VERDE. **Data base of the Meteorological Station at Campo Verde Farm – Jodil Group**. Campo do Meio, MG, Brazil, 2021.

CARVALHO, V. L.; CUNHA, R. L.; SILVA, N. R. N. Alternativas de controle de doenças do cafeeiro. **Coffee Science**, 7(1):42-49, 2012.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. **Acompanhamento da safra brasileira de café**, v. 9, safra 2022, n. 1, primeiro levantamento janeiro 2022. Brasília: CONAB, 2022, 61p. Available in: <https://www.conab.gov.br/info-agro/safras/cafe>. Access in: March, 25, 2022.

CONSELHO DOS EXPORTADORES DE CAFÉ - CECAFE. **Relatório mensal fevereiro 2022**: Exportações Brasileira. 2022. Available in: <https://www.cecafe.com.br/publicacoes/relatorio-de-exportacoes/>. Access in: March, 25, 2022.

COSTA, M. J. N.; ZAMBOLIM, L.; RODRIGUES, F. A. Avaliação de produtos alternativos no controle da ferrugem do cafeeiro. **Fitopatologia Brasileira**, 32:150-155, 2007.

CUNHA, R. L. et al. Desenvolvimento e validação de uma escala diagramática para avaliar a severidade da ferrugem (*Hemileia vastatr*ix) do cafeeiro. *In*: **Simpósio de pesquisa dos cafés do Brasil**, Vitória, Embrapa Café, p.1001-1008, 2001.

DREYER, A.; COELLO, N.; MONDIEL, E. Utilización de la metodologia de superfície de respuesta de la optimización de um medio de cultivo para la producción de L-lisin por Corynebacterium glutamicum. **Agronomía Tropical**, 50(2):167-188, 2000.

FERREIRA, D. F. Sisvar: A computer analysis system to fixed effects split plot type designs. **Revista Brasileira de Biometria**, 37(4):529-535, 2019.

MEDEIROS M. B.; LOPES J. S. Biofertilizantes líquidos e sustentabilidade agrícola. **Bahia Agrícola**, 7(3):24-25, 2006.

MORALES, R. G. F.; SANTOS, I.; TOMAZELI, V. N. Influência da nutrição mineral foliar sobre doenças da parte aérea da cultura do trigo. **Revista Ceres**, 59(1):71-76, 2012.

MOREIRA, P. C. et al. Produtividade e economia de fatores de produção na cafeicultura brasileira. **Revista de Política Agrícola**, 2(6):6-21, 2019.

POZZA, E. A.; POZZA, A. A. Manejo de doenças de plantas com macro e micronutrientes. **Revista Brasileira de Fitopatologia**, 28(1):52-54, 2003.

RUIZ-CÁRDENAS, R. **A Cafeicultura e sua Relação com o Clima**. 1 ed. Lavras: Artes Gráficas Formato LTDA, 2015. 23p.

SCOTT, A.; KNOTT, M. Cluster-analysis method for grouping means in analysis of variance. **Biometrics**, 30(3):507-512, 1974.

SHANER, G.; FINNEY, R. R. The effect of nitrogen fertilization on the expression of slow-mildewing in knox wheat. **Phytopathology**, 67(8):1052-1056, 1977.