

Total phenolic content and antioxidant capacity in *Muscodor* spp. inoculated seedlings of coffee cultivars of Brazil

Bárbara Mourão¹ , Suzan Kelly Vilela Bertolucci¹ , Sarah da Silva Costa Guimarães^{2*} , Adriane Duarte Coelho¹ , João Pedro Miranda Rocha¹ , Júlia Helena Gomes de Carvalho² , Márcio Pozzobon Pedroso³ , Patrícia Gomes Cardoso² 

¹Universidade Federal de Lavras/UFLA, Departamento de Agricultura, Lavras, MG, Brasil

²Universidade Federal de Lavras/UFLA, Departamento de Biologia, Lavras, MG, Brasil

³Universidade Federal de Lavras/UFLA, Departamento de Química, Lavras, MG, Brasil

*Corresponding author: sarahscosta80@gmail.com

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ABSTRACT

Fungi of the genus *Muscodor* have received considerable attention for producing volatile and non-volatile organic compounds with biological activity. The objective of this study was to determine the total phenolic content and antioxidant capacity in two coffee cultivars (Catuaí Vermelho and Topázio) inoculated with the endophytic fungi *Muscodor coffeanum* and *Muscodor* sp. and in fungal extracts, as well as to characterize the phenolic compounds and trigonelline produced by isolates of *Muscodor*. Seeds of *Coffea arabica* (Catuaí Vermelho and Topázio cultivars) were inoculated with twelve fungal isolates and grown in a greenhouse for 24 months. The results showed that the cultivars inoculated with *M. coffeanum* (CML 4019) had the highest production of total phenolic content and antioxidant capacity. The presence of total phenolic content showed a direct correlation with antioxidant activity. Total phenolic content and antioxidant capacity varied among different *Muscodor* isolates. The phenolic profile of *Muscodor* extract determined by HPLC-DAD showed the production of catechin, vanillin, and the caffeic, chlorogenic, ferulic, gallic, o-coumaric, p-coumaric, and syringic acids and the alkaloid trigonelline. These endophyte fungi associated with the coffee fruit can improve the quality of the beverage.

Key words: Antioxidant potential; bioactive compounds; endophytic fungi; *Muscodor coffeanum*; trigonelline.

1 INTRODUCTION

Coffee is one of the most important Brazilian commodities, with *Coffea arabica* and *Coffea canephora* Pierre ex A. Froehner being the most commercially important and most consumed beverages in the world (Dong et al., 2017). The coffee drink is an important dietary source of phenolic compounds, and due to their antioxidant properties, these compounds confer to the regular consumer of the drink the reduction of chronic diseases and metabolic syndromes (Soares et al., 2020; Soares et al., 2022). In addition, the association of some phenols determines the quality of coffee, playing a fundamental role in the formation of the specific aroma and taste of the drink (Variyar et al., 2003; Farah et al., 2006; Farah; Donangelo, 2006).

The endophytic fungus of the genus *Muscodor* was isolated from *Coffea arabica* L. grown in a secondary forest in Brazil (Guimarães et al., 2021). Based on the analysis of the phylogeny of three loci (ITS, *rpb2*, and *tub2*), morphological characterization, and production of volatile organic compounds (VOCs) by *Muscodor* isolates, Guimarães et al. (2021) identified ten isolates of *Muscodor coffeanum* and two of *Muscodor* sp. isolated from stems and leaves of *Coffea arabica*. This species produces VOCs with efficient antifungal, antibacterial, and anthelmintic activities and is able to inhibit phytopathogens (Monteiro et al., 2017; Guimarães et al., 2021; Mota et al., 2021). The ethyl

acetate extracts of *Muscodor* spp. show bioactivity, acting as modulators of the blood coagulation process along with different classes of proteases, playing a role in maintaining human hemostasis and as producers of extracellular enzymes (Bastos et al., 2020; Monteiro et al., 2020; Cardoso et al., 2022). Coffee seeds inoculated with *Muscodor* spp. and grown for 24 months showed higher values for plant height, stem diameter, number of leaves, main-root length, main-root diameter, and total dry biomass than uninoculated plants. In addition, six months after sowing, the greenhouse was infested with *Leucoptera coffeella*, and the plants inoculated with *Muscodor* showed less damage caused by the insect compared to uninoculated plants, showing that the interaction between *Muscodor* and coffee plants increased plant resistance to the leaf miner (Mourão et al., 2025).

The production of phenolic compounds by endophytic fungi isolated from medicinal plants and their antioxidant capacity have been reported. *Aspergillus nidulans* var. *dentatus*, *Chaetomium globosum*, *Fusarium oxysporum*, and *Trichoderma longibrachiatum* isolated from *Passiflora incarnata* as endophytes produced phenolic compounds with high antioxidant activity (Da Silva et al., 2020). *Curvularia* sp. G6-32, an endophyte isolated from the medicinal plant *Sapindus saponaria* L., produced gallic acid (Polli et al., 2020). *Penicillium canescens* and *Fusarium chlamydosporum*, isolated as endophytes from *Polygonum chinense* L., produced vanillic acid and ferulic acid, respectively (Das et al., 2018).

Another substance with antioxidant activity is trigonelline (1-methylpyridinium-3-carboxylate) (Abdel-Farid et al., 2021). It is a phytohormone that helps plants survive and grow by performing a variety of regulatory functions, including cell cycle regulation, nodulation, and oxidative stress (Garg, 2016). Trigonelline accumulates in large amounts in coffee seeds and contributes to the bitter taste of coffee. Therefore, the metabolic formation of trigonelline may be useful in the technology of producing high quality coffee beans (Ashihara et al., 2011). Trigonelline is an alkaloid compound, and alkaloids are the major group of plant defense molecules that act against pathogens and predators due to their toxicity (Yeshe et al., 2022). Some fungi can alter the concentration of metabolites in plants (Machiani et al., 2022), such as *Gigaspora rosea*, which increased the concentration of trigonelline in the roots of *Prosopis laevigata*, a semi-arid legume, by 1.8-fold (Rojas-Andrade et al., 2003).

The production of phenolics and trigonelline by endophytic fungi of the genus *Muscodor* is poorly understood, and we know that *Muscodor*-plant interaction alters some host plant metabolites. Therefore, we determined the total phenolic content and their antioxidant capacity in two coffee cultivars (Catuaí Vermelho and Topázio) inoculated with isolates of *M. coffeanum* and *Muscodor* sp. and in fungal extracts. We also characterized the phenolic compounds and trigonelline in the *Muscodor* extract.

2 MATERIAL AND METHODS

2.1 Endophytic fungi

The endophytic fungi were isolated from fresh and healthy leaves and stems of coffee plants (*Coffea arabica*) growing spontaneously in a secondary forest in Mata do Paraíso (20° 48' 03.4" S, 42° 51' 42.6" W), Zona da Mata region, Viçosa, Minas Gerais, Brazil, and identified by Guimarães et al. (2021) as *Muscodor coffeanum* (CML 4009, CML 4010, CML 4011, CML 4012, CML 4014, CML 4016, CML 4017, CML 4018, CML 4019, CML 4020) and *Muscodor* sp. (CML 4013, CML 4015). The isolates were cryopreserved as a mycelial suspension in 15% glycerol at -80 °C and maintained in the Departamento de Biologia, Universidade Federal de Lavras, Brazil. The fungi were reactivated in Petri dishes containing potato dextrose agar (PDA: broth from 200 g of potatoes, 20 g of dextrose, 20 g of agar, and 1 liter of distilled water) for 30 days at room temperature under a 12 h photoperiod.

2.2 Seed inoculation

Discs of approximately 9 mm in diameter from the fungal colonies were cultivated in 2 L Erlenmeyer flasks with dextrose potato broth (PD: broth from 200 g of potatoes, 20 g of dextrose, and 1 liter of distilled water) for 30 days at room

temperature. Coffee seeds, of the Catuaí Vermelho (IAC 144) and Topázio (MG 1190) cultivars, were disinfested to eliminate the epiphytic organisms by immersion and constant agitation in 70% ethanol for 2 minutes, in sodium hypochlorite (2% active chlorine) for 3 minutes, and again in 70% ethanol for 1 minute. Then, the material was triple-washed in autoclaved distilled water. The dry seeds were transferred to Petri dishes containing PD medium and crushed mycelia of endophytic fungi, being immersed for 6 hours (Mourão et al., 2025).

2.3 Coffee growing

After the inoculation procedure, the seeds were planted in 12 L pots, with a substrate composed of 70% sieved soil and 30% tanned bovine manure; for each cubic meter, 5 kg of simple superphosphate, 1 kg of potassium chloride, and 1.5 kg of limestone were added. Soil analysis showed that the substrate mixture used had the following chemical attributes: pH = 4.8; P, K and Na (mg dm⁻³) = 1272.81; 207.07 and 61.00; Ca, Mg, Al, H + Al, SB, t e T (cmolc dm⁻³) = 4.89; 1.28; 0.10; 4.20; 9.43; 9.43 and 13.63; m and V (%) = 1.05 and 69.21; organic matter = 6.72 dag kg⁻¹; P-Rem = 30.20 mg L⁻¹; Zn, Fe, Mn, Cu, B and S (mg dm⁻³) = 5.40; 35.60; 15.30; 0.85; 0.18 and 455.00. Substrate texture: clayey, with 64.0 dag kg⁻¹ of clay, 16.0 dag kg⁻¹ of silt, and 20.0 dag kg⁻¹ of sand. At the time of planting, PD medium with crushed mycelia was also added to the holes next to the seeds with parchment. The pots were kept in a greenhouse for 2 years, at room temperature, with automatic irrigation and a 12 h photoperiod. The experiment was conducted in a completely randomized design with three replicates for each treatment in a factorial scheme. The factors consisted of two coffee cultivars, 12 *Muscodor* isolates, and two additional controls [(2 × 12) + 2].

2.4 Preparation of plant and fungal extracts

Plant extracts were obtained from 50 mg of frozen liquid nitrogen and pulverized aliquots of Catuaí Vermelho and Topázio coffee leaves. Plant materials were sonicated for 30 min in 2 mL of 70% hydroethanolic solution. The extracts were purified by centrifuging at 10,000 rpm for 10 min. The supernatants were collected and diluted (from 1 to 32 times).

Fungal extracts were prepared from five discs of approximately 5 mm in diameter from the fungal colonies that were transferred to a 2 L Erlenmeyer with 1 L of potato-dextrose medium, which were left static for 30 days at room temperature. Cultures in liquid medium were vacuum filtered and extracted twice by liquid-liquid partition with ethyl acetate (1:2). The extracts were concentrated and dried in a rotary evaporator at 40 °C.

2.5 Quantification of total phenolic content and antioxidant capacities

The total phenolic content and antioxidant capacities were determined using an Infinite 200 spectrophotometer (A-

5082; Tecan, Grödig, Salzburg). The fungal (10 mg/mL) and plant (25 mg/mL) hydroethanolic extracts were sequentially diluted up to a proportion of 1/128 in 96-well microplates. All treatments were evaluated in triplicate.

2.5.1 Total Phenolic Content (TPC)

The total phenolic content was performed according to the Folin-Ciocalteu method, with modifications, for fungal extracts and plant extracts individually (Singleton; Rossi, 1965). An aliquot of fungal extracts (50 µL) or plant extracts (20 µL) was transferred to 96-well microplates. Both were mixed with 120 µL of Folin-Ciocalteu ethanolic solution (10%) and 120 µL of sodium carbonate (7%). Then, at room temperature, the samples were incubated for 2 hours away from light, and the absorbances were read at 760 nm. The calibration curve of gallic acid (Sigma-Aldrich®, Brazil, 98% purity) comprised a concentration range from 0.0078 to 0.25 mg/mL ($y = 6.6918 + 0.2452x$, $R^2 = 0.9947$) that was employed to determine total phenolic content. The results were expressed in milligrams of gallic acid equivalent per gram of extract (mgEAG/g).

2.5.2 Free Radical Elimination Activity (DPPH)

The antioxidant capacity of fungal and plant extracts, the DPPH (2,2-diphenyl-2-picryl-hydrazyl) radical scavenging, was performed using the methodology of Brand-Williams, Cuvelier and Berset (1995). Sequential dilutions in ethanol were performed for each sample, from the extract in the original concentration to a proportion of 1/128. In 96-well microplates, 250 µL aliquots of DPPH ethanolic solution (60 µg/mL) were added to 50 µL of extracts. Subsequently, the samples were incubated for 60 minutes in the dark and at room temperature. Ethanol was used as a negative control. The absorbance of the samples was measured in a spectrophotometer at 517 nm. The percentage of inhibition of DPPH free radicals (I%) was calculated according to the equation $I(\%) = [(A_{\text{standard}} - A_{\text{sample}}) / A_{\text{standard}}] \times 100$, where A_{standard} corresponds to the absorbance of the DPPH ethanolic solution and A_{sample} corresponds to the absorbance of the DPPH solution + extract. Free radical scavenging capacity was expressed by IC_{50} (µg/mL), corresponding to the extract concentration capable of inhibiting 50% of DPPH radicals.

2.5.3 Oxygen Radical Absorption Capacity (ORAC)

The ORAC was determined by the method of Ou et al. (2001). In a 96-well black microplate, 25 µL of fungal or plant extracts and 150 µL of fluorescein (70 mM) prepared in phosphate buffer (75 mM; pH 7.4) were added. The microplate was pre-incubated for 10 minutes at 37 °C in the spectrophotometer. Subsequently, 30 µL of the radical AAPH [2,2'-Azobis (2-amidinopropane) dihydrochloride] (12 mM) was added. The microplate fluorescence was recorded every minute for 150

minutes. The Trolox standard was used as a positive control. As a negative control, 30 µL of potassium phosphate buffer (75 mM; pH 7.4) was used instead of AAPH. ORAC values were calculated using a regression equation for the Trolox concentration and the liquid area under the curve (AUC) ($AUC_{\text{sample}} - AUC_{\text{control}}$) using PRISM® software (version 9.5.1) and expressed in micromoles of Trolox equivalent per gram of extract (µmolET/g).

2.5.4 Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined by the molybdate reduction protocol according to Prieto et al. (1999). In 2 mL microtubes, 50 µL of fungal or plant extracts and 1 mL of TAC reagent solution (monobasic sodium phosphate (28 mM); ammonium molybdate (4 mM); sulfuric acid (0.6 M), in equal proportions). The microtubes were incubated for 90 minutes in a water bath at 95°C. After reaching room temperature, a 200 µL aliquot of the samples was pipette into 96-well microplates and its absorbance was measured in a spectrophotometer at 695 nm. The calibration curve was created using ascorbic acid standard (Sigma-Aldrich®, Brazil, 99.7% purity) at concentrations from 0.002 to 0.5 mg/mL, generating the equation: $y = 6.80x + 0.0716$, $R^2 = 0.9946$. The results were expressed in milligrams of ascorbic acid equivalents per gram of extract (mgEAA/g).

2.6 HPLC-DAD analysis of phenolic compounds and trigonelline in fungal extracts

HPLC-DAD analysis of fungal extracts was performed on a Shimadzu liquid chromatography (Shimadzu Corp., Japan), equipped with four high-pressure pumps (model LC-20AT), with a diode array detector (model SPD-M20A), degasser (model DGU-20A5), CBM-20A interface, CTO-20AC oven, and automatic sampler (model SIL-20A). Separations were performed using a Shimadzu Shim-pack ODS GVP-C18 column (4.6 x 250 mm, 5 mm) connected to a pre-column (Shimadzu-pack ODS GVP-C18, 4.6 x 10 mm, 5 µm) and 20 µL of each sample (automatic injection). The mobile phase used was (A) water/acetic acid (98:2; v/v) and (B) methanol/water/acetic acid (70:28:2; v/v/v). The oven temperature was 35 °C. The gradient was linear at a flow rate of 1.0 mL/min from 0% to 20% solvent B for 5 minutes, from 20% to 40% solvent B for 20 min, 40% to 45% solvent B for 18 min, 45% to 80% solvent B for 7 minutes, and 80% to 0% solvent B for 5 min, followed by washing with solvent A and column equilibration for 10 min. Diode array detection was performed at 280 nm. The analyzed phenolic compounds and trigonelline were identified by comparing their retention times with their respective standards. The reference substances (catechin, resveratrol, trigonelline, vanillin, and also the caffeic, chlorogenic, ferulic, gallic, m-coumaric, o-coumaric, p-coumaric, rosmarinic, syringic, and trans-cinnamic acids) were weighed separately, directly into microcentrifuge bottles,

followed by solubilization in 1,000 µL of HPLC grade MeOH, at a concentration of 3.0 mg/mL.

2.7 Statistical analysis

The experimental data were first analyzed for homogeneity of variance and normality. Subsequently, the data from the analyses of DPPH, ORAC, TAC and TPC were subjected to analysis of variance (ANOVA), and the treatments were compared using the Scott-Knott test ($p < 0.05$).

3 RESULTS

3.1 Total phenolic content and antioxidant capacity of coffee cultivars inoculated with *Muscodora* spp. and uninoculated plants

The total phenolic content, free radical elimination activity, oxygen radical absorption capacity, and total antioxidant capacity of coffee plants of the Catuaí Vermelho and Topázio cultivars inoculated with *Muscodora* spp. after 2 years at a concentration of 25 mg/mL are shown in Table 1. Coffee plants from the Catuaí Vermelho cultivar inoculated with *M. coffeanum* (CML 4019 and CML 4014) showed the highest TPC, 1.71 mgEAG/g and 1.31 mgEAG/g, respectively. Plants inoculated with the other isolates showed values below 0.85 mgEAG/g. For the Topázio cultivar, plant extracts from plants inoculated with *M. coffeanum* (CML 4019, 4018, and 4014) showed the highest TPC: 0.91 mgEAG/g, 0.62 mgEAG/g, and 0.61 mgEAG/g, respectively.

The results of the DPPH free radical scavenging activity showed that coffee plants from the Catuaí Vermelho cultivar inoculated with *M. coffeanum* (CML 4019) presented an IC_{50} of 136.51 µg/mL, which was significantly lower than plants inoculated with the other isolates of *Muscodora*, showing a greater scavenging capacity of the radical DPPH. The DPPH radical scavenging activity was not significant for the coffee plants of the Topázio cultivar.

The oxygen radical scavenging capacity showed that coffee plants of the Catuaí Vermelho cultivar inoculated with *M. coffeanum* (CML 4020 and CML 4019) had the highest antioxidant capacities (29.87 µmol ET/g and 27.31 µmol ET/g, respectively). Coffee plants of the Topázio cultivar inoculated with *M. coffeanum* (CML 4011, 4016 and 4018) showed the highest oxygen radical scavenging capacities (30.63 µmol ET/g, 13.36 µmol ET/g and 13.43 µmol ET/g, respectively).

The total antioxidant capacity showed that coffee plants from the control cultivar Catuaí Vermelho presented the highest TAC value (2.97 mgEAA/g), while plants inoculated with the *M. coffeanum* isolates CML 4017 and CML 4019 presented 2.76 mgEAA/g and 2.16 mgEAA/g, respectively. Coffee plants of the Topázio cultivar inoculated with *M. coffeanum* (CML 4014) and *Muscodora* sp. (CML 4013) produced 0.66 mgEAA/g and 0.64

mgEAA/g, respectively, representing the highest TAC values compared to plants inoculated with the other isolates of *Muscodora*.

3.2 Total phenolic content and antioxidant capacity of *Muscodora* spp.

Among the twelve isolated fungi, *M. coffeanum* (CML 4019, CML 4018) and *Muscodora* sp. (CML 4013) produced the highest total phenolic content of 53.6 mgEAG/g, 52.3 mgEAG/g and 52.1 mgEAG/g, respectively (Table 2). The other fungal isolates showed values between 41.0 and 48.8 mgEAG/g.

Muscodora sp. (CML 4013) and *M. coffeanum* (CML 4019) presented the best capacities of oxygen radical absorption and DPPH free radical scavenging activity. The DPPH free radical scavenging activity of *M. coffeanum* (CML 4019) presented an IC_{50} of 276.7 µg/mL, which was significantly lower than the other extracts, highlighting a capacity for scavenging the DPPH radical. The oxygen radical scavenging capacity analysis of *M. coffeanum* (CML 4019 and CML 4018) and *Muscodora* sp. (CML 4013) showed the highest ORAC of 154.3 µmolET/g, 150.3 µmolET/g and 149.9 µmolET/g, respectively. The highest total antioxidant capacity was obtained from *M. coffeanum* (CML 4012) and *Muscodora* sp. (CML 4013) with 46.6 mgEAA/G and 44.5 mgEAA/G, respectively (Table 2).

3.3 Compounds characterization

From the comparison of retention times of 14 standards, the results of the preliminary screening by HPLC-DAD analysis indicated the presence of trigonelline and nine phenolic compounds in the analyzed fungal extracts: catechin, vanillin, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, o-coumaric acid, p-coumaric acid, and syringic acid. The ferulic acid and trigonelline were produced by 10 of the 12 fungi analyzed, caffeic acid by seven, and the remaining phenolic compounds produced by five or fewer fungi (Table 3).

4 DISCUSSION

The results showed that *Muscodora* spp. significantly increased the total phenolic content and antioxidant capacity in coffee cultivars (Catuaí Vermelho and Topázio). Coffee plants inoculated with *M. coffeanum* (CML 4019) exhibited the highest total phenolic content, a greater scavenging capacity against the DPPH radical, and the highest antioxidant capacities. These results suggest that this fungus may contribute to improved antioxidant responses in coffee plants. This fungus not only induced changes in the plants but also increased the total phenolic content and showed enhanced antioxidant activity. The correlation between antioxidant capacity and phenolic content has been observed in other studies with endophytic fungi (Cakir et al., 2003; Proestos et al., 2006; Huang et al., 2007; Liu et al., 2007; Kandasamy; Kandasamy, 2014; Tavares et al., 2018; Yehia et al., 2025).

Table 1: Total phenolic content and antioxidant capacity of *C. arabica* Catuaí and Topázio cultivars inoculated with *Muscodora* spp. and non-inoculated.

Treatment	TPC (mgEAG/g)		ORAC ($\mu\text{mol ET/g}$)		TAC (mgEAA/g)		DPPH IC ₅₀ ($\mu\text{g/g}$)
	Catuaí	Topázio	Catuaí	Topázio	Catuaí	Topázio	Catuaí
Control	0.76d	0.45e	21.64c	7.88e	2.97a	0.32f	702.96d
<i>M. coffeanum</i>							
CML 4009	0.69e	0.55c	19.11d	10.01d	0.91h	0.45d	234.71a
CML 4010	0.61f	0.44e	21.64c	8.84e	1.33f	0.56b	269.41b
CML 4011	0.47h	0.48d	15.41e	30.63a	0.59i	0.57b	319.46b
CML 4012	0.53g	0.55c	18.46d	11.21c	1.31f	0.43c	262.13b
CML 4014	1.31b	0.61b	21.91c	11.19c	1.69e	0.66a	186.06a
CML 4016	0.42i	0.57c	18.06d	13.36b	0.67i	0.45d	272.81b
CML 4017	0.58f	0.46e	19.06d	9.61d	2.76b	0.59b	206.26a
CML 4018	0.71e	0.62b	21.36c	13.43b	1.11g	0.58b	275.51b
CML 4019	1.71a	0.91a	27.31b	10.17d	2.16c	0.53c	136.51a
CML 4020	0.84c	0.33f	29.87a	11.37c	1.84d	0.42e	297.06b
<i>Muscodora</i> sp.							
CML 4013	0.36j	0.56c	18.43d	10.26d	0.41j	0.64a	401.16c
CML 4015	0.82c	0.33f	21.14c	8.75e	1.58e	0.46d	145.63a
CV (%)	2.08	2.99	7.07	4.99	5.47	3.31	17.55

Means followed by the same letters in the column do not differ according to the Scott-Knott test ($p < 0.05$).

TPC: Total phenolic content expressed in mg of gallic acid equivalents/g of extract (mg GA/g). DPPH: Free radical elimination activity expressed in IC₅₀ ($\mu\text{g/mL}$), corresponding to the extract concentration capable of inhibiting 50% of DPPH radicals. ORAC: Oxygen Radical Absorption Capacity expressed in micromole of Trolox equivalents/g of extract ($\mu\text{mol ET/g}$). TAC: Total antioxidant capacity expressed in mg of ascorbic acid equivalents/g of extract (mg AA/g).

Table 2: Total phenolic content and antioxidant capacity of *Muscodora* spp.

Fungal extracts	TPC (mgEAG/g)	DPPH (IC ₅₀ , $\mu\text{g/mL}$)	ORAC ($\mu\text{mol ET/g}$)	TAC (mgEAA/g)
<i>M. coffeanum</i>				
CML 4009	46.1c	685.0f	132.5c	11.2e
CML 4010	46.7c	1177.0i	134.5c	7.9f
CML 4011	45.7c	433.3c	131.4c	11.4e
CML 4012	45.8c	416.0c	131.9c	46.6a
CML 4014	48.8b	1088.7h	140.5b	26.4b
CML 4016	42.4f	572.3e	122.1f	7.1f
CML 4017	44.4d	599.7e	127.8d	7.9f
CML 4018	52.3a	472.3d	150.3a	23.3c
CML 4019	53.6a	276.7a	154.3a	26.6b
CML 4020	41.0h	1020.0g	119.1h	18.2d
<i>Muscodora</i> sp.				
CML 4013	52.1a	334.3b	149.9a	44.5a
CML 4015	43.5e	657.0f	125.0e	22.1c
CV (%)	1.42	2.63	1.41	2.71

Means followed by the same letters in the column do not differ according to the Scott-Knott test ($p < 0.05$). TPC: Total phenolic content expressed in mg of gallic acid equivalents/g of extract (mg GA/g). DPPH: Free radical elimination activity expressed in IC₅₀ ($\mu\text{g/mL}$), corresponding to the extract concentration capable of inhibiting 50% of DPPH radicals. ORAC: Oxygen Radical Absorption Capacity expressed in micromole of Trolox equivalents/g of extract ($\mu\text{mol ET/g}$). TAC: Total antioxidant capacity expressed in mg of ascorbic acid equivalents/g of extract (mg AA/g).

Table 3: Phenolic compounds and trigonelline produced by *Muscodor* spp.

Fungal extracts	Caffeic Acid	Chlorogenic Acid	Ferulic Acid	Gallic Acid	<i>o</i> -Coumaric Acid	<i>p</i> -Coumaric Acid	Syringic Acid	Catechin	Vanillin	Trigonelline	Total
<i>M. coffeanum</i>											
CML 4009	+		+			+				+	4
CML 4010		+	+				+	+	+	+	6
CML 4011	+		+		+	+				+	5
CML 4012	+		+							+	3
CML 4014	+	+	+	+				+		+	6
CML 4016			+							+	2
CML 4017	+										1
CML 4018			+	+							2
CML 4019				+						+	2
CML 4020		+	+	+						+	4
<i>Muscodor</i> sp.											
CML 4013	+		+			+				+	4
CML 4015	+		+	+		+				+	5

+ Indicates the presence of the compound in fungal extracts as determined by HPLC-DAD analysis.

Microorganisms play a crucial role in enhancing the sensory qualities of coffee by releasing metabolites and degrading phytochemicals (Sousa et al., 2023). They can also increase soluble phenolic content and improve antioxidant capacity (Zhao et al., 2024). For example, *Aspergillus niger*, *Monascus anka*, and *Eurotium cristatum* were shown to increase phenolic content in grape pomace seeds after 8 and 12 days of solid-state fermentation (Zhao et al., 2024). *Eurotium cristatum* and *M. anka* showed the highest ability to release soluble phenolics, with TPC increasing 9.21 and 5.72 times, respectively. Specifically, *M. anka* enhanced the soluble phenolic profile by increasing compounds such as chlorogenic acid, syringic acid, ferulic acid, epicatechin gallate, and resveratrol, resulting in improved antioxidant activity. These results suggest that fungal fermentation can enhance the valorization of winemaking by-products by enriching the soluble phenolic profiles and bioactivities of grape pomace seeds (Zhao et al., 2024). The use of isolated cultures for induced fermentation results in better sensory outcomes. For example, yeast fermentation of coffee beans resulted in increased antioxidant activity and total phenolic content, while also affecting the aroma and flavor profile of coffee (Kwak et al., 2018). Furthermore, *Saccharomyces cerevisiae* has been shown to improve the chemical and sensory qualities of coffee beverages (Sousa et al., 2023). The presence of microorganisms indigenous to coffee may also contribute to spontaneous fermentation during the fermentation process of the coffee fruit, ultimately affecting the final quality of the coffee beverage (Braga et al., 2023; Sousa et al., 2023).

Species as *Enterobacter* sp., *Lactobacillus* sp., *Pantoea* sp., *Cladosporium* sp., and *Candida* sp. were predominant during spontaneous anaerobic coffee fermentation. However, the microbial community composition was influenced by the location of fermentation, and washing the coffee beans before fermentation resulted in a rapid decrease in pH, favoring the growth of *Lactobacillus* sp. and *Candida* sp., which shortened the fermentation time and improved the sensory quality of the coffee. (Braga et al., 2023). *Muscodor coffeanum* (CML 4019), a native coffee fungus, increased the total phenolic content of Catuaí coffee plants by 2.25 times compared to the control. This fungus also produces gallic acid, a potent antioxidant known for its free radical scavenging properties and potential therapeutic benefits in oxidative damage-related diseases (Gao et al., 2019). Therefore, *M. coffeanum* (CML 4019) has promising properties that could improve the quality of coffee beverages and contribute to health benefits.

Muscodor coffeanum (CML 4019, CML 4018) and *Muscodor* sp. (CML 4013) produced three times more phenolic content (53.6 mgEAG/g, 52.3 mgEAG/g and 52.1 mgEAG/g, respectively) than *Alternaria alternata* isolated from *C. arabica* (Fernandes et al., 2009). *M. coffeanum* (CML 4019) showed a higher capacity to scavenge the DPPH radical, and *Muscodor* sp. (CML 4013) showed a much higher total antioxidant capacity than the other isolates. These fungi also showed the highest oxygen radical scavenging capacities. This demonstrates a direct relationship between the higher amount of total phenolic content and the antioxidant capacity of these fungal extracts. Antioxidant activity has been reported in

Muscodor species such as *M. indica*, *M. strobilii*, *M. tigerii*, *M. ghoomensis*, *M. camphora*, *M. darjeelingensis* and *M. kashayum* (Kapoor; Saxena, 2016).

The oxygen radical scavenging capacity of endophytic fungi is probably related to their life history strategy to overcome the metabolically aggressive environment of the host plant (Hamilton et al., 2012; Kapoor; Saxena, 2016). The fungal extract in ethyl acetate of *Phanerochaete* sp. showed an ORAC of 19 $\mu\text{mol ET/g}$ (Mena et al., 2021). *Phanerochaete* sp. did not maintain an endophytic-plant relationship and presented a very low oxygen radical inhibitory capacity compared to the results obtained with *Muscodor* isolates. According to Kapoor and Saxena (2016), the oxygen radical inhibitory capacity may be related to the antioxidant activity of the fungus, which is associated with overcoming oxidative stress based on plant defense mechanisms.

HPLC-DAD analysis revealed the presence of nine different phenolic compounds and the alkaloid trigonelline in the *Muscodor* spp. extract. The phenolic compounds contribute to the quality of coffee by enhancing its aroma and flavor (Farah; Donangelo, 2006; Kitzberger et al., 2014; Ribeiro et al., 2016). Trigonelline is a natural alkaloid found in plants as green coffee beans and fenugreek (Nguyen et al., 2024). It has antioxidant properties, contributes to coffee quality, and is associated with overall antioxidant capacity (Ribeiro et al., 2018; Abdel-Farid et al., 2021). In addition, trigonelline offers therapeutic benefits, including neuroprotective and hypoglycemic effects (Nguyen et al., 2024). In addition, phenolic compounds play a role in plant defense against environmental stresses, pathogens, and herbivores (Khanday et al., 2024), while trigonelline shows antimicrobial activity, affecting colony structure, motility, biofilm architecture, and virulence factor production of *Pseudomonas aeruginosa* (Kar et al., 2024). These secondary metabolites, both phenolic and alkaloidal, are likely to serve as plant protection agents against pathogens and pests. *Muscodor coffeanum* (CML 4019) has shown significant efficacy in host plant defense, particularly in the control of diseases caused by fungi, bacteria, and nematodes, as well as in the control of *L. coffeella* (Guimarães et al., 2021; Mota et al., 2021; Mourão et al., 2025). It also exhibits potent antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and *E. faecium*, with trigonelline production likely contributing to this effect (Guimarães et al., 2021). This isolate may therefore be a promising candidate for biological control.

Muscodor coffeanum (CML 4014) produced chlorogenic, caffeic, and ferulic acids. Chlorogenic acid plays a critical role in helping plants adapt to stress, suggesting that it may have specific physiological functions in coffee plants, such as regulating germination and cell growth by modulating indoleacetic acid levels (Farah; Donangelo, 2006). In coffee, chlorogenic acid has been shown to reduce oxidative stress

in diseased cells and protect against DNA damage in breast cancer cells due to its antioxidant activity (Alves et al., 2009; Nuhu, 2014; Durán et al., 2017; Laukaleja; Kruma, 2018). Caffeic and ferulic acids also exhibit photoprotective and antioxidant effects, with studies indicating their potential to prevent dermatological problems and inhibit the growth of colon and rectal cancer (Magnani et al., 2014).

5 CONCLUSIONS

This study demonstrates that inoculating Catuaí Vermelho and Topázio coffee cultivars with endophytic fungi, particularly *M. coffeanum* (CML 4019), increases their antioxidant capacity compared to uninoculated plants. The observed increases in total phenolic content, DPPH radical scavenging activity, and oxygen radical absorbance capacity highlight the role of *Muscodor* in stimulating antioxidant responses and suggest its potential to improve the quality of coffee beverages.

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7 AUTHORS' CONTRIBUTION

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