



Muscodor coffeanum as a strategy for organic tomato (*Solanum lycopersicum*) cultivation for tolerance to *Ralstonia solanacearum*

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Abstract Tomato is one of the most economically important crops in Brazil. However, bacterial wilt, caused by *Ralstonia solanacearum*, is one of the devastating diseases in tomatoes. Endophytic fungi of the genus *Muscodor* are well known for their bioactivity against phytopathogens, and also have the potential to promote plant growth. The objectives of this study were to verify the inhibition of *R. solanacearum* phylotype II sequevar 1 (race 3, biovar 2) growth by

volatile organic compounds (VOCs) produced by seven isolates of *M. coffeanum* and two isolates of *Muscodor* sp in vitro and to evaluate the effects of inoculation of fungal isolates on increasing productivity and tomato fruit quality *in vivo*. The VOCs of *M. coffeanum* (CML 4009) showed total inhibition of the pathogen's growth. This isolate also promoted an increase in root dry biomass of tomato plants and has the potential to increase tomato plant tolerance to *R. solanacearum*, contributing to the observed numerical increases of 24% in total soluble solids and 26.8% in fruit yield compared to the control. This gain in fruit yield, when extrapolated to organic commercial tomato farming, will lead to greater profitability.

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Introduction

The tomato (*Solanum lycopersicum* L.) is an economically important crop in Brazil. With increasing production over the last five years, in 2023, the country produced 4,166,017 tons, with the state of Goiás being the largest producer (IBGE 2023). However, bacterial diseases can affect tomato productivity. Different bacterial diseases have been described in tomato plants, such as bacterial wilt, caused by *Ralstonia solanacearum*, bacterial speck,

caused by *Pseudomonas syringae* pv. *Tomato*, and bacterial black spot, caused by *Xanthomonas vesicatoria*, *X. euvesicatoria* pv. *euvesicatoria*, *X. euvesicatoria* pv. *perforans* and *X. hortorum* pv. *gardneri* (Jones et al. 2004; Morinière et al. 2020; Harrison et al. 2023).

Ralstonia solanacearum ranks among the top 10 bacterial species in terms of economic importance in plant diseases (Yuliar and Toyota 2015). This species was traditionally classified into five races based on host range and six biovars based on biochemical characteristics. However, multilocus sequence analysis (MLSA) has since shown that this pathogen is a species complex. This complex is now referred to as the *Ralstonia solanacearum* species complex (RSSC) and comprises four distinct phylotypes. These phylotypes correspond to three species: *R. solanacearum* (phylotype II), *R. pseudosolanacearum* (phylotypes I and III), and *R. syzygii* (phylotype IV). Tomato and other solanaceous hosts can be affected by strains from all four phylotypes, which highlights the complex's broad host range and genetic diversity (Prior and Fegan 2005). *Ralstonia solanacearum* phylotype II sequevar 1 (race 3 biovar 2) and *R. pseudosolanacearum* phylotype I sequevar 14 causing bacterial wilt on tomato in America (Roman-Reyna et al. 2021; Magar et al. 2025).

Ralstonia solanacearum is one of the most common soil-borne pathogens of many crops, including tomato. This bacterium causes bacterial wilt, compromising the plant's vascular system, leading to yield losses of up to 91%. It has become a growing threat in protected crops during the rainy summer, especially in the North and Northeast regions of Brazil, as humidity aids its spread and infection. (Lopes 2009; Lopes et al. 2015; Yuliar and Toyota 2015; Aslam et al. 2017). Effective control of this bacterium has been a challenge as it remains on the ground for a long time after its initial introduction to the field. Copper has been used to inhibit the growth of bacterial pathogens in tomatoes. However, it has not been recommended against *R. Solanacearum* as this chemist has no systemic action (La Torre et al. 2018). Biological control agents are solutions that have been gaining interest among researchers and rural producers because of their suppression mechanisms, ability to promote plant growth, enhancement of agricultural productivity, and environmental protection (Yuliar and Toyota 2015; Koskey et al. 2021).

Endophytic fungi of the genus *Muscodora* (*Induratia*) have been reported to be efficient biocontrol agents for the synthesis of volatile organic compounds (VOCs). The VOCs are gas-phase mixtures of hydrophobic, carbon-based molecules. These compounds can serve as airborne signals that are sensed by plants, playing a crucial role in triggering signaling cascades that influence secondary metabolism, development, and growth (Razo-Belmán et al. 2023). *Muscodora* spp. produce a mixture of these organic molecules, hydrophobic, of low molecular weight, high vapor pressure, and which can diffuse easily through the atmosphere and soil. Some species are commercially available as biological control agents, such as Ennoble™ Biofumigant, a product derived from the *Muscodora albus* (Marrone Bio Innovations) in the USA (Strobel et al. 2001; Worapong et al. 2001; Mercier and Jimenez 2004; Mercier and Smilanick 2005; Grimme et al. 2007; Guimarães et al. 2021; Cedeño-Sánchez et al. 2023, Manathunga et al. 2024). Despite the potent antimicrobial action of fungi of the genus *Muscodora*, little is known about their mechanism of toxicity. The prevailing hypothesis for the inhibition of microbial growth by *M. albus* was a synergistic effect between multiple VOCs (Strobel et al. 2001). However, the production of N-methyl-N-nitrosoisobutyramide, a dominant factor in the toxicity of different *Muscodora* isolates, inhibited target organisms through its conversion to an alkylating or nitrosylating agent that damages DNA in the long term (Hutchings et al. 2017).

In 2020, *Muscodora* species were transferred to the *Induratia* genus based on two fungal species with teleomorphs strikingly similar to *Induratia apiospora*, *M. thailandicus* ($\equiv$ *I. thailandica*), and *M. ziziphi* ($\equiv$ *I. ziziphi*) (Samarakoon et al. 2020). However, the authors did not include DNA sequences of *Induratia* spp. for comparison. In 2023, Cedeño-Sánchez et al. included an ex-holotype strain of *I. apiospora* in the ATCC collection, detailing its morphology and molecular phylogeny. Phylogenetic analyses of multigene sequences revealed a close relationship of *I. apiospora* to the Barrmaeliaceae, whereas *Muscodora* showed no relationship with *I. apiospora*. Therefore, the genus *Muscodora* was resurrected and belongs to the family Xylariaceae. The *Induratia* species were formally transferred to *Muscodora*.

Muscodora species have been reported in Brazil, including *M. coffeanum*, *Muscodora* sp. *M.*

yucatanensis, *M. vitigenus* isolated from the stems and leaves of *Coffea arabica* (Hongsanan et al. 2015; Monteiro et al. 2017; Guimarães et al. 2021; Mota et al. 2021; Gomes et al. 2023), and *M. brasiliensis*, isolated from the leaves of *Schinus terebinthifolius* (Pena et al. 2019). *M. coffeanum* and *Muscodor* sp. produced the VOCs Pyrimidine 2-chloro-4-ethyl-6-methyl-, Cyclosativene, Tricyclo[4.3.1.1(3,8)]undecane- 3-methoxy-, 4-Amino-3,5-diethylpyridine, Imidazo [5,1-f][1,2,4] triazine-2,7-diamine, Dimethyl-[4-[2-(3-methylisoxazol-5-yl)vinyl]phenyl]amine, 4(1H)-Pyrimidinone, 2,3-dihydro-1-methyl- 6-(4-pyridinyl)-2-thioxo-, 2-Phenyl-6-chloro-benzofuran, 1-Methyl-2-nitro-4-(1,2,2-trimethyl-cyclopentyl)-benzene (Guimarães et al. 2021; Gomes et al. 2023). The VOCs had antifungal activity against *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani*, *F. verticillioides*, *Phoma* spp., *Cercospora coffeicola*, *Pestalotia longisetula*, *Aspergillus ochraceus* (Monteiro et al. 2017), *Penicillium digitatum* (Pena et al. 2019), *Botrytis cinerea* (Guimarães et al. 2021), *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum*, and *Pseudocercospora griseola* (Mota et al. 2021). *Muscodor coffeanum* isolates from coffee plants presented antimicrobial activity against *Aspergillus ochraceus*, *A. sclerotiorum*, *A. elegans*, *A. foetidus*, *A. flavus*, *A. tamari*, *A. tubingensis*, *A. sydowii*, *A. niger*, *A. caespitosus*, *A. versicolor*, and *Penicillium expansum* (Gomes et al. 2023). Some isolates of *M. coffeanum* showed nematocidal activity against *Meloidogyne incognita* and antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and *E. faecium*, and reduced preformed

biofilms of *S. aureus* and *S. epidermidis* (Guimarães et al. 2021). Moreover, *Muscodor* species also produced non-volatile secondary metabolites, including extracellular amylase, cellulase, lipase, pectinase, phytase, protease, endo β -1,4-glucanase, as well as exo β -1,4-glucanase, and molecules that can modulate enzymes involved in human hemostasis (Bastos et al. 2020; Monteiro et al. 2020). The inoculation of *Muscodor* species from *Coffea arabica* in common bean seeds was effective in enhancing grain yield (Hayashibara et al. 2022). This study was pioneering and a promising strategy to improve bean productivity and plant health, showing the possibility of successfully introducing *Muscodor* spp. on a plant different from the original host.

Considering the importance of the *R. solanacearum*/tomato pathosystem and the biocontrol potential of fungi of the genus *Muscodor*, we hypothesize that isolates of *M. coffeanum* and *Muscodor* sp. can be used as plant biostimulants to support organic tomato farming, to increase the tolerance of tomato plants to *R. solanacearum*, and improve tomato yield and quality.

Materials and methods

Fungal and bacterial cultures

Seven *M. coffeanum* and two *Muscodor* sp. (Table 1), isolated from healthy leaves and stems of *Coffea arabica* and deposited in the Coleção Micológica de Lavras, Universidade Federal de Lavras (UFLA), Brazil (<http://www.dfp.ufla.br/cml>), were used in this

Table 1 *Muscodor* isolates used in this study and GenBank accessions

Species	Isolates code	Coffee tissue	GenBank accession number*		
			ITS	<i>RPB2</i>	<i>TUB2</i>
<i>M. coffeanum</i>	CML 4009	Leaf	MN658674	MN689579	MN689587
<i>M. coffeanum</i>	CML 4010	Leaf	MN658675	MN689580	MN689588
<i>M. coffeanum</i>	CML 4011	Leaf	MN658676	MN689581	MN689589
<i>M. coffeanum</i>	CML 4012	Stem	MN658677	MN689582	MN689590
<i>Muscodor</i> sp.	CML 4013	Stem	MN658681	MN689583	MN689591
<i>M. coffeanum</i>	CML 4014	Stem	MN658683	MN746440	MN746443
<i>Muscodor</i> sp.	CML 4015	Stem	MN658682	MN689584	MN689592
<i>M. coffeanum</i>	CML 4016	leaf	MN658684	MN746441	MN746444
<i>M. coffeanum</i>	CML 4017	Leaf	MN658685	MN746442	MN746445

*ITS: internal transcribed spacer; *RPB2*: Second largest subunit of RNA polymerase II; *TUB2*: β -tubulin. Sequences generated by Guimarães et al. (2021)

study (Guimarães et al. 2021). *R. solanacearum* phylo-type II sequevar 1 (race 3, biovar 2) provided by the Vegetal Bacteriology Laboratory of UFLA was isolated from an infected tomato plant cultivated in the municipality of Carandaí, MG, Brazil. The *Muscodor* isolates were cultivated on potato dextrose agar (PDA—Merck 110,130) at 25 ± 2 °C for 15 days, and *R. solanacearum* was activated on 523 medium (Kado & Heskett 1970) at 28 ± 2 °C for 72 h.

VOCs from *Muscodor* spp. in growth in the *R. solanacearum*

The production of volatile organic compounds (VOCs) by *Muscodor* isolates with activity against *R. solanacearum* was evaluated using bipartite Petri dishes (9 cm). The fungi were grown for 7 days in PDA medium (Merck 110,130) at 25 ± 2 °C, and the bacterium was cultured on 523 broth medium in a Shaker incubator (Moxcare MCOS-UPAB) for 24 h at 120 rpm at 28 ± 2 °C. A plug of fungus was transferred to one side of the plate containing PDA, and 100 µL of a bacterial suspension ($OD_{600\text{ nm}} = 0.1 \approx 10^8$ CFU mL⁻¹) was pipetted to the other side of the plate containing 523 solid medium, followed by incubation for 72 h. The experiment was carried out in a randomized design, with ten treatments and four replicates. The control was a bacterial suspension in a 523 solid medium. The assessment of antagonism compared the bacterial growth in the presence and absence of the fungus (control). No colonies of bacteria indicated total inhibition, and a number of colonies lower than in the control group indicated partial inhibition. To confirm the bactericidal effect, the

fungus was removed, and only the pathogen was left for another 72 h of incubation. The experiment was performed twice to confirm the results.

Inoculation of *Muscodor* spp. in tomato seeds and of *R. solanacearum* in tomato plants

Muscodor isolates were reactivated in Petri dishes containing potato dextrose agar (PDA). Fungal colony discs, approximately 5 mm in diameter, were transferred to PDA medium (-1.2 MPa), and ten superficially disinfested tomato seeds (hybrid N-901) were arranged equidistantly from each other and 1 cm from the fungal mycelial disk. The dish plates were incubated at 25 ± 2 °C for 10 days in the dark. Inoculated tomato seeds (Fig. 1) were sown in expanded polystyrene trays consisting of 5×10 cells with a cell size of 3.5×3.5 cm² filled with Tropstrato™, two seeds per cell, and grown in a greenhouse for 30 days. Then, the plants were transplanted to 4 L pots. After 15 days (plants 45 days old), they were infected via the soil through root injury, followed by irrigation with 30 mL of *R. solanacearum* bacterial suspension ($OD_{600\text{ nm}} = 0.1 \approx 10^8$ UFC mL⁻¹). Non-inoculated seeds were used in negative control. The plants were cultivated in a semi-hydroponic system and fertigated with a nutritive solution daily and trained on a single stem in a greenhouse with controlled humidity ($\approx 65\%$) and temperature (27 ± 2 °C). Fertigation (100 mL per pot) alternated with manual localized irrigation until the pot reached capacity, maintaining an electrical conductivity below 3 dS cm⁻¹. The



Fig. 1 Tomato seed inoculation. **A** The mycelium of *M. coffeanum* covering the tomato seed after inoculation. **B** Tomato seeds superficially colonized by *M. coffeanum*. **C** Control, tomato seed non-inoculated

experiment was carried out in a randomized design, with ten treatments in four replicates each.

Effect of *Muscodor* spp. on the reduction of bacterial wilt severity, on production of fruits and dry mass of the aerial part and root of tomato plants after *R. solanacearum* infection”

Disease severity was evaluated at 15 days after inoculation of the pathogen using the disease scale: 1, healthy plant; 2, plant with 1/3 of leaves wilted; 3, plant with 2/3 of leaves wilted; 4, totally wilted plant; and 5, dead plant (Empig et al. 1962). The °Brix value was assessed in naturally ripened fruits by collecting the pulp of each fruit for digital refractometer readings. After 90 days of cultivation, the dry mass of the aerial part and root (dried in an oven at 65°C for four days) and the production of fresh fruits were measured (g).

Statistical analysis

For all assays, the experimental design was completely randomized. The experimental data on disease severity, total soluble solids content, and aerial and root dry mass of tomato plants were first evaluated for normality and homogeneity of variance, and then subjected to analysis of variance (ANOVA). Treatment means were compared using the Scott–Knott test at a 5% significance level, performed with SISVAR software, version 5.6. (Ferreira 2011).

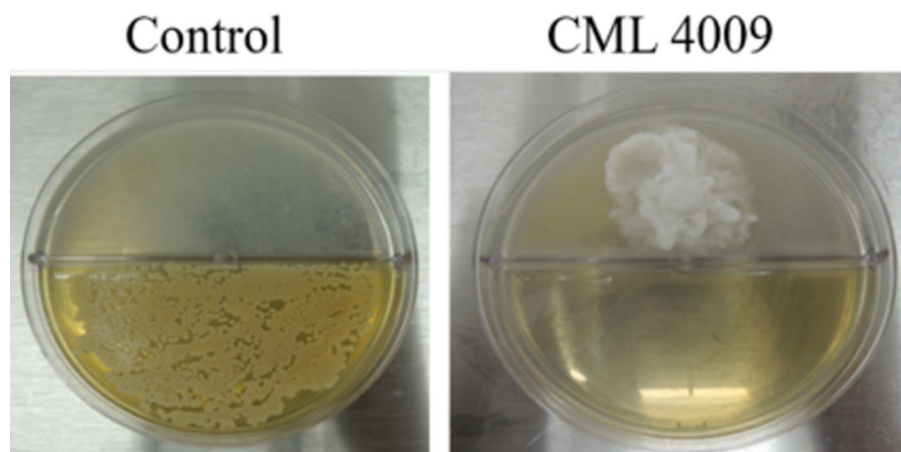
Results

Muscodor coffeanum (CML 4009) completely inhibited the growth of *R. solanacearum* (Fig. 2). Partial inhibition was observed for *M. coffeanum* (CML 4011 and CML 4014) and *Muscodor* sp. (CML 4013). The remaining isolates exhibited bacterial growth similar to that of the control, suggesting no inhibitory effect. The bactericidal effect was confirmed for *M. coffeanum* (CML 4009) after 72 h, no colony of the pathogen grew in the absence of the fungus.

Plants inoculated with *M. coffeanum* (CML 4009) exhibited partial wilting, affecting approximately two-thirds of the leaves, yet remained alive and relatively healthy (Fig. 3A). In contrast, plants treated with *M. coffeanum* isolates CML 4011 and CML 4012 were nearly completely wilted. The other isolates, as well as the control plant (pathogen only), were completely wilted and dead, indicating that there was no protective effect against *R. solanacearum* (Table 2).

The weight and total soluble solids (°Brix) of tomato fruits were evaluated after the complete maturation stage. Although the results did not show a statistical difference, an increase in the weight and brix of fruits from plants associated with endophytic fungi was observed when compared to the fruits of control plants (Table 2). *Muscodor coffeanum* (CML 4009) promoted a 26.8% increase in fruit yield, followed by *M. coffeanum* (CML 4011) (17.3%) and *M. coffeanum* (CML 4012) (17.1%). *Muscodor coffeanum* (CML 4009) promoted a 24% increase in total soluble solids (°Brix), relative to fruits produced by control

Fig. 2 Effect of the VOCs of *M. coffeanum* (CML 4009) on *R. solanacearum* growth



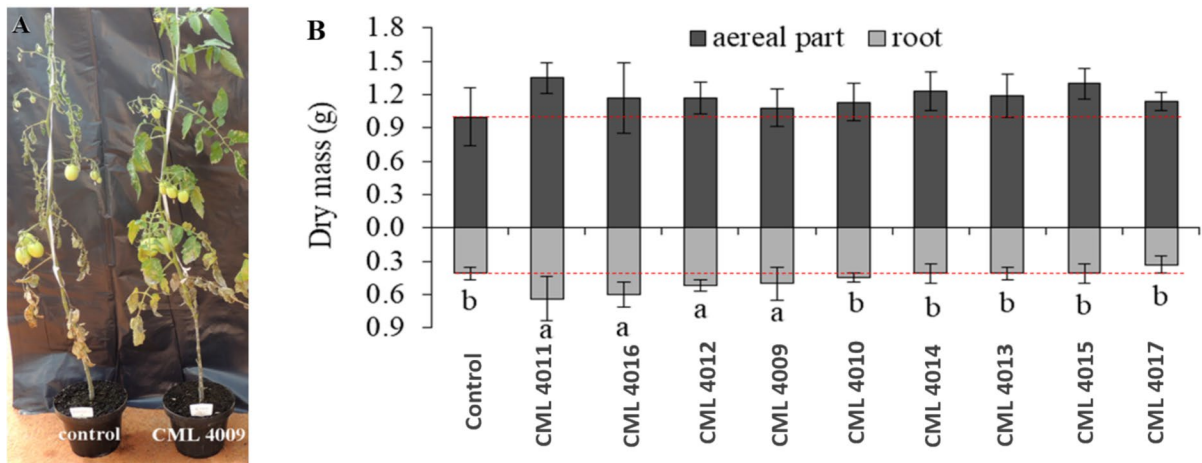


Fig. 3 *Muscodor*-tomato plant interaction and control treatments. **A** Visual symptoms in tomato plants 15 days after inoculation with *R. solanacearum*. **B** Plant growth parameters in tomato plants treated with *Muscodor* isolates compared to

the control under *R. solanacearum* infection. Values indicate means, error bars the standard deviation and small letters indicate significant difference at 5% of probability (ANOVA, Scott-Knott test)

Table 2 Effect of *Muscodor* spp. on bacterial wilt severity, weight, and total soluble solids (°Brix) of tomato fruits

Treatments	Disease severity ^a	Fruit mass (g)	Gain (%)	°Brix ^b
<i>M. coffeanum</i>				
CML 4009	3.0±2.0	97.4±49.1	26.8	5.8±1.2
CML 4010	5.0±0.0	55.6±18.9	-27.6	5.0±1.9
CML 4011	4.0±2.0	90.1±42.3	17.3	4.9±0.4
CML 4012	4.0±2.0	89.9±44.9	17.1	5.3±1.1
CML 4014	5.0±0.0	87.6±16.1	14.0	4.2±0.7
CML 4016	4.8±0.5	74.3±18.2	3.2	4.8±1.1
CML 4017	4.8±0.5	77.9±41.2	1.4	6.2±2.0
<i>Muscodor</i> sp.				
CML 4013	4.8±0.5	59.3±45.2	-22.9	5.6±1.9
CML 4015	5.0±0.5	89.5±67.9	16.5	5.7±1.2
Control	5.0±0.0	76.8±47.0	0	4.7±0.7
CV (%)	23	52	n.p	26

^aDisease severity was evaluated after 15 days of pathogen infection

^bTotal solid soluble fruits in the complete maturation stage

Values indicate means and ± standard deviation. The averages presented no significant difference (ANOVA, Scott-Knott test at 5%). CV Coefficient of variation, n.p. not performed

plants. The results suggest a potential beneficial effect of the endophytic fungus on plant physiological performance under biotic stress.

For the plant growth parameters, significant differences were observed in root dry biomass compared to the control treatment. *Muscodor coffeanum* (CML 4011) promoted 56.1% gain in root

dry biomass, followed by isolates CML 4016, CML 4012, and CML 4009 (Fig. 3B). Regarding the results of the analysis of the dry biomass of the aerial, although of some fungal isolates have promoted a discreet increase, no significant difference was observed between the tomato plants associated with endophytic fungi and the control plants (Fig. 3B).

Discussion

This is the first report that demonstrates the dual role of *Muscodor* spp. as a potential biological control agent against *R. solanacearum* phylotype II, sequence 1 (race 3, biovar 2) and as a biostimulant that improves some physiological parameters of tomato plants. The association between *M. coffeanum* (CML 4009) and tomato plants resulted in both in vitro suppression and increased plant tolerance to *R. solanacearum*, leading to improved root dry biomass of tomato plants.

Muscodor coffeanum (CML 4009) completely inhibited the growth of *R. solanacearum*, in vitro. In the study of Guimarães et al. (2021), this isolate produced VOCs, alcohols as 1-Propanol, 2-methyl-; 1-Butanol, 3-methyl-; 1-Butanol, 2-methyl-; Phenylethyl Alcohol, and Ethyl alcohol, aldehydes such as Hexanal, terpenes (β -Phellandrene, α -Bergamotene, and Sesquiterpene), and esters (Methyl 2-methylpropanoate, Methyl 2-methylbutanoate, Butyric acid, thio-, S-methyl ester. Compounds as butanoic, propanoic acids, hexanal, and many other alcohols have already been reported to exhibit antifungal and antibacterial activities (Strobel et al. 2008; Mari et al. 2016). One or some of these compounds must have been responsible for the antimicrobial action of *M. coffeanum* (CML 4009). Hutchings et al. (2017) described that the *N*-methyl-*N*-nitrosoisobutyramide is the dominant factor of multiple *Muscodor* isolates toxicity and acts primarily through DNA methylation. However, this VOC is undetected in standard gas chromatography–mass spectrometry assays due to its thermal instability. This volatile may also be produced by *M. coffeanum* (CML 4009) and be responsible for the inhibition of *R. solanacearum*.

The application of *M. coffeanum* (CML 4009) increased fruit yield by 26.8% and total soluble solids content by 24%. Although these differences were not statistically significant, the numerical data showed higher values compared to the control, suggesting a biologically relevant trend, particularly considering that the experiment was conducted under controlled and reproducible conditions. These results indicate positive effects of this isolate enhancing tomato fruit quality and yield, which could translate into economic benefits and increased profitability in commercial-scale tomato production. Additionally, the *M. coffeanum* isolates (CML

4011, CML 4016, CML 4012, and CML 4009) showed a significant increase in root dry mass compared to the control, suggesting a beneficial effect of these isolates on the root system under biotic stress. These findings suggest that *M. coffeanum* may support tomato plant development. The capacity of *Muscodor* spp. to modulate plant defense responses, influence hormonal signaling pathways, and enhance physiological efficiency of host plants has been reported in other studies (Guimarães et al. 2021; Hayashibara et al. 2022; Mourão et al. 2025a,b).

Endophytic fungi can stimulate plant growth by increasing the availability of nutrients, the action of extracellular enzymes, or soil pH regulation, and by producing or inducing hormones such as indole-3-acetic acid (AIA), gibberellins (GAs), and cytokinins, and can alter the concentration of metabolites in plants (Shoresh et al. 2010; Kanchiswamy et al. 2015; Suwannarach et al. 2015). A study developed by our group, using the same fungal isolates, showed a greater production of GAs in *Coffea arabica* inoculated with *Muscodor* spp. (Mourão et al. 2025b). In tomato plants, *Muscodor* isolates can alter the synthesis of some phytohormones, inducing plant defence mechanisms for greater tolerance to bacterial wilt. The phytohormones also act as signalling components and play important roles in regulating defence responses against various biotrophic and necrotrophic pathogens (Harllen and Bettiol 2009; Khan et al. 2015). GA has proven efficiency in stimulating the vegetative phase until flowering, determining sexual expression, improving seed development, and increasing the total yield of tomato crops and fruiting, in addition to faster fruit growth (Pramanik et al. 2017; 2018), as well as induce greater tolerance of tomato plants to bacterial wilt (Rodrigues et al. 2018).

Muscodor spp. produced different phenolic compounds with direct correlation with antioxidant activity (Mourão et al. 2025b). The tomato quality can be improved when there is an increase in the levels of phenolic compounds (Lima et al. 2023). The inoculation of *Muscodor* spp. in tomato plants may contribute to the efficiency of the antioxidant system of tomato plants, inducing positive responses to the defence mechanism of tomato plants, increasing tolerance to *R. solanacearum*, and improving tomato quality.

Muscodor isolates produced the extracellular enzymes cellulase, pectinase, protease, phytase, lipase, amylase endo β -1,4-glucanase, and exo β -1,4-glucanase (Monteiro et al. 2020). These enzymes can cause degradation of the host tissue, leading to the emission of danger signals called damage-associated molecular patterns (DAMPs), which include cell wall or extracellular protein fragments, peptides, nucleotides, and amino acids. Biological control agents may induce resistance or prime enhanced resistance against pathogen infections in plant tissues without directly interacting with the pathogen (Conrath et al. 2015). The specific molecular signatures of biological control agents, microbe-associated molecular patterns (MAMPs) and DAMPs, are recognized by the corresponding extracellular pattern-recognition receptors (PRRs), which relay these signals to multiple intracellular signalling modules (Köhl et al. 2019). Thus, MAMPs and DAMPs activate immune signalling pathways, such as pattern-triggered immunity (PTI), which plays an important role in reducing pathogen invasion and maintaining homeostasis of endophytic leaf microbiota of plants (Li et al. 2016; Hou et al. 2019; Köhl et al. 2019). Molecular patterns like chitin and enzymes from endophytic fungi can act as elicitors, inducing plant resistance and stimulating other endophytes (both fungi and bacteria) to produce bioactive secondary metabolites, potentially influencing host protection (Zabalgogezcoa 2008; Gao et al. 2010; Kusari et al. 2012).

Microbial control has been gaining increasing global attention as an alternative disease control strategy aimed at promoting sustainable food production with minimal ecological impact. Biological control of plant disease-causing bacteria has been based on the use of bacteria as biocontrol agents. *Bacillus amyloliquefaciens* SQR-9 produced VOCs that significantly inhibited the growth of *R. solanacearum*. The VOCs also inhibited the motility traits, production of antioxidant enzymes and exopolysaccharides, biofilm formation, and tomato root colonization by *R. solanacearum* (Raza et al. 2016). Biological preparations based on *Bacillus subtilis* have been recommended for controlling bacterial diseases such as black spots caused by *Xanthomonas vesicatoria* and bacterial cancer induced by *Clavibacter michiganensis* subsp. *michiganensis* (Kolomiets et al. 2017). *Muscodor coffenaum* (CML

4009) can also be a strategy in the control of *R. solanacearum* since it stands out a robust and versatile bio-control agent.

Organic agriculture has become important for the production of healthy and sustainable food, and the association of *M. coffeannum* with tomato plants can bring benefits, such as greater resistance of plants to the pathogen *R. solanacearum*, and greater quality and productivity of fruits in organic tomato production.

Conclusion

Muscodor has shown a promising strategy in the control of *R. solanacearum*. However, further research is needed to clarify the functional role of volatile metabolites and compounds produced by fungi against this pathogen. These endophytes also show promise as a biostimulant for organic tomato cultivation, and the understanding of ecological relationships in this association is essential for optimizing the performance under field conditions.

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Declarations

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