ÍTALO AUGUSTO FÉRRER MELO SANTOS

***Muscodor* spp.:** PROMOTES TOMATO GROWTH, PROTECTS AGAINST BACTERIAL WILT AND PRODUCES BIOACTIVE VOLATILE COMPOUNDS

LAVRAS – MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola para a obtenção do título de Doutor.

Prof(a). Dr(a). Patrícia Gomes Cardoso

Orientador(a)

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APROVADA em 14 de dezembro de 2018.

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À minha família.

Dedico

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“A alegria que se tem em pensar e aprender faz-nos pensar e aprender ainda mais.”
(Aristóteles)

RESUMO

Uma ampla diversidade de fungos endofíticos produzem metabólicos com atividade antimicrobiana. Além disso, interagem com plantas superiores, podendo desempenhar funções indispensáveis na natureza, tais como promoção do crescimento e proteção contra doenças. Destes, fungos do gênero *Muscodor* têm sido descritos por produzirem uma mistura de compostos orgânicos voláteis (COVs) letal contra vários microrganismos, além de melhorar o desenvolvimento de algumas plantas. No entanto, a aplicação de fungos deste gênero na agricultura tem como desafio a não esporulação. Portanto, visando explorar o potencial de nove isolados de três espécies de *Muscodor* (*M. vitigenu*s, *M. coffeanum* e *M. yucatanensis*) avaliamos a atividade *in vitro* dos seus COVs contra as fitobactérias *Ralstonia solanacearum* raça 3, *Xanthomas vesicatoria* e *Pseudomonas syringae* pv. *tomato*, com posterior caracterização dos voláteis por cromatografia gasosa acoplada à espectro de massas (GC-MS). Efeito alelopático dos voláteis sobre radículas de tomate também foi avaliado. Foram testados dois métodos de inoculação em plantas de tomate, em muda, em dois experimentos independentes, via suspensão de micélio, e em semente, através de adaptações na técnica de osmocondicionamento, no qual foi avaliada promoção do crescimento, além da resistência de plantas, inoculadas via semente, à murcha bacteriana causada por *R. solanacearum* raça 3, respectivamente. Vários isolados de diferentes espécies produziram voláteis com atividade antibacteriana e alelopática, destaque para os VOCs do fungo *M. coffeanum* COAD1900, com efeito letal sobre todas as fitobactérias, exceto sobre *P. syringae* pv. *tomato* que cresceu parcialmente, além de alelopatia desde o segundo dia de crescimento. Ao oitavo dia de crescimento, 18 COVs foram caracterizados, dentre eles: ácidos; ésteres; álcoois; terpenos; aldeído e amida. A inoculação via suspensão de micélio de cinco dos nove fungos, promoveu incrementos no rendimento de frutos frescos de tomate de mesa, nos dois experimentos realizados, com os maiores ganhos promovidos pelos fungos *M. coffeanum* COAD1842 (20%), *M. vitigenus* CML4015 (19%) e *M. vitigenus* CML4014 (15%). Ganhos de biomassa seca de raiz e parte aérea foram observados apenas no segundo experimento, realizado no inverno. O osmocondicionamento mostrou ser uma técnica de inoculação eficaz para fungos *Muscodor*, promovendo colonização efetiva das sementes sem danos ao crescimento do fungo. Porém, alguns isolados afetaram negativamente a germinação, altura e a razão parte aérea/raiz de mudas. No entanto, *M. coffeanum* COAD1842 e *M. yucatanensis* CML4016 aumentaram significativamente a biomassa seca de raiz, além das mudas apresentarem menor relação parte aérea/raiz que o controle. Plantas de tomate industrial inoculadas com *M. coffeanum* COAD1900 apresentaram resistência moderada à murcha bacteriana, além de incremento no rendimento de frutos frescos e teor de sólidos solúveis totais. A observação da colonização da radícula sugere a capacidade de colonização de fungos *Muscodor* em raízes de tomate. Adicionalmente, os fungos *M. coffeanum* COAD1842 e *M. yucatanensis* CML4016 apresentaram compatibilidade com o tratamento de semente (metalaxil+deltametrina). Nossos resultados reforçam o potencial de utilização de fungos *Muscodor* na cultura do tomate, com desenvolvimento de novas estratégias de aplicação em muda e semente visando o desenvolvimento vegetal e controle da murcha bacteriana.

Palavras-chave: COVs. Atividade antibacteriana. Osmocondicionamento. *Solanum lycopersicum*. Controle biológico. Xylariaceae.

ABSTRACT

Several endophytic fungi are producers of metabolites with antimicrobial activity. In addition, they interact with higher plants and can perform several indispensable functions in nature, such as growth promotion and protection against diseases. Of these, fungi of the genus *Muscodor* have been reported as producers of a mixture of lethal volatile organic compounds (VOCs) against a wide amount of microorganisms, in addition to improving the development of some plants. However, the application of fungi of this genus in agriculture has the challenge of non-sporulation. Therefore, aimed to explore the potential of nine isolates of three species of *Muscodor* (*M. vitigenus*, *M. coffeanum* and *M. yucatanensis*) we evaluated the *in vitro* activity of their VOCs against the phytobacteria *Ralstonia solanacearum* race 3, *Xanthomas vesicatoria* and *Pseudomonas syringae* pv. *tomato*, with subsequent characterization of the volatiles produced by gas chromatography-mass spectrometry (GC-MS). Allelopathy of volatiles on tomato radicles was also evaluated. Two methods of inoculation were tested in tomato, in seedling, in two independent experiments, with mycelium suspension, and seed, through adaptations in the water restriction technique, in which growth promotion was evaluated, as well as plant resistance, inoculated in seed, to bacterial wilt caused by *R. solanacearum* race 3, respectively. Several isolates of different species produced volatiles with antibacterial and allelopathic activity, especially the VOCs of the fungus *M. coffeanum* COAD1900, with a lethal effect against all phytobacteria, except *P. syringae* that grew partially, and phytoinhibition from the second day of growth. After eight days of growth, 18 VOCs were characterized, among them: acids; esters; alcohols; terpenes; aldehyde and amide. The inoculation with mycelial suspension of five fungi increased the yield of fresh fruits of tomato in the two experiments, with the highest gains promoted by the fungi *M. coffeanum* COAD1842 (20%), *M. vitigenus* CML4015 (19%) and *M. vitigenus* CML4014 (15%). Dry biomass gains of root and shoot were observed only in the second experiment, carried out in winter. Osmoconditioning proved to be an effective inoculation technique for *Muscodor* fungi, promoting effective seed colonization without damage to fungus growth. However, some isolates affected negatively the germination, height and the shoot/root ratio of the seedlings. On the other hand, *M. coffeanum* COAD1842 and *M. yucatanensis* CML4016 significantly increased the dry biomass of the roots, in addition to the seedlings presented lower shoot/root ratio than the control. Tomato for industry inoculated with *M. coffeanum* COAD1900 showed moderate resistance to bacterial wilt, besides an increase in fresh fruit yield and total soluble solids content. The observation of radicle colonization suggests the ability of colonization of *Muscodor* fungi in tomato roots. In addition, the fungi *M. coffeanum* COAD1842 and *M. yucatanensis* CML416 showed compatibility with the seed treatment (metalaxyl+deltamethrin). Our results reinforce the potential of using *Muscodor* fungi in the tomato crop, with the development of application strategies in seedling and seed aiming the plant development and control of bacterial wilt.

Keywords: VOCs. Antibacterial activity. Osmoconditioning. *Solanum lycopersicum*. Biological control. Xylariaceae.

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

A associação mutualística entre planta e microrganismos endofíticos, aqueles que colonizam o interior de tecidos vegetais sem danos aparentes, apresenta benefícios adaptativos para ambos os parceiros. O endófito produz metabólitos secundários bioativos importantes ao hospedeiro que, em contrapartida, protege e fornece nutrientes.

Fungos são os microrganismos endofíticos mais frequentemente encontrados. Eles interagem com plantas superiores, podendo desempenhar diversas funções indispensáveis na natureza, tais como promoção do crescimento vegetal, tolerância a estresses bióticos e abióticos, e adaptação ambiental. Vários fungos endofíticos associados a plantas estão ganhando atenção pela resistência conferida ao hospedeiro a agentes patogênicos, sendo fonte de vários estudos aplicados ao controle de doenças de plantas.

Os fungos endofíticos podem combater o desenvolvimento de patógenos por diversos mecanismos, de forma isolada ou pela ação simultânea. Indiretamente, algumas espécies podem, por exemplo, induzir mecanismos de defesa do hospedeiro e também concorrer por espaço e nutrientes. Diretamente, a ação dos endófitos sobre patógenos pode ocorrer pela produção de compostos antimicrobianos e enzimas líticas.

Os fungos endofíticos são produtores de diversos compostos metabólicos, muitos demonstrando atividade antimicrobiana. Destes, fungos do gênero *Muscodor* têm sido descritos por produzirem uma mistura de compostos orgânicos voláteis (COVs) letal a uma ampla variedade de fungos e bactérias patogênicas, além de nematoides e insetos. Diversos trabalhos relatam o potencial de utilização por micofumigação dos voláteis produzidos por estes fungos no controle de fitopatógenos associados ao solo. Entretanto, não há informações na literatura de inoculação visando o estabelecimento endofítico deste grupo de microrganismos, podendo, então, ser uma nova estratégia de aplicação no controle de doenças.

A avaliação dos benefícios da aplicação de fungos *Muscodor* spp. tem como desafio a não esporulação. Assim, o desenvolvimento ou adequação de metodologias para inoculação eficiente destes microrganismos, nas plantas, é necessário. Uma alternativa é a técnica de restrição hídrica, relatada como mais eficaz em comparação a metodologias tradicionais no estudo de fungos fitopatogênicos associados à semente.

Avaliações *in vitro* relatam que a mistura dos COVs produzidos pelos fungos endofíticos *M. coffeanum*, *M. vitigenus* e *M. yucatanensis*, objeto de estudo do presente trabalho, apresentam ação antimicrobiana contra vários fitopatógenos que incidem na cultura do tomate, cultura de grande importância econômica e social no contexto do agronegócio brasileiro. Portanto, a ação direta dos COVs produzidos por estes fungos, combinado com mecanismos indiretos de controle de doenças no hospedeiro, podem revelar importantes agentes de biocontrole, como uma alternativa sustentável no manejo integrado de doenças. Assim, o objetivo do presente trabalho foi avaliar o potencial desses fungos na promoção do crescimento e biocontrole em plantas de tomate.

2 REFERENCIAL TEÓRICO

2.1 Fungos endofíticos

Endofitismo é um fenômeno natural de associação entre microrganismo endofítico e planta, com benefícios adaptativos para ambos os parceiros, quando proveniente de uma associação mutualística. Nesse caso, o endófito produz metabólitos secundários bioativos importantes ao hospedeiro que, em contrapartida, protege e fornece nutrientes (Wani et al., 2015). Tal associação pode ser hospedeiro-específica, no qual uma espécie prefere um determinado hospedeiro, ou não, em que um endófito pode colonizar múltiplos hospedeiros, relacionados taxonomicamente ou não, dentro do mesmo habitat (Selim et al., 2012).

O termo endófito ou endofítico ainda é muito discutido. Em sentido amplo, significa "na planta" (*endo* Gr., dentro; *fito*, planta) e designa microrganismos que colonizam, pelo menos em parte do seu ciclo de vida, os tecidos vegetais sem danos aparentes. Diferentes grupos de bactérias e fungos interagem com plantas superiores podendo desempenhar diversas funções indispensáveis na natureza, tais como crescimento vegetal, tolerância a estresses bióticos e abióticos e adaptação ambiental (Schulz & Boyle, 2005; Hardoim et al., 2015).

Fungos são frequentemente encontrados com endofíticos e compõem um grupo polifilético de grande diversidade, principalmente Ascomicetos. Eles são produtores de diversos compostos metabólicos, muitos demonstrando atividade antimicrobiana, inclusive com implicação na planta hospedeira contra fitopatógenos, embora não haja muitas evidências da produção destes compostos na planta. Tal simbiose parece ocorrer em todas as plantas em ecossistemas naturais com colonização sistêmica ou de tecidos vegetais específicos: raiz, caule ou folha, em espaços intercelulares ou intracelulares (Rodriguez et al., 2009; Aly et al., 2010; Kusari et al., 2012).

Os benefícios da simbiose endófito-planta baseiam-se num equilíbrio perfeito entre as necessidades do invasor e a resposta da planta, sob condições ambientais, controle fisiológico e genético. Há sempre algum grau de virulência do fungo que permite sua infecção, porém, a defesa da planta limita o desenvolvimento do invasor e, consequentemente, de uma posterior doença. Portanto, quando a interação se tornar desequilibrada, o fungo pode deixar de ser endófito e se comportar como parasita, desencadeando uma patologia ou havendo exclusão do microrganismo pelas defesas da planta. No entanto, patógenos latentes parecem representar um pequeno grupo de endofíticos (Schulz & Boyle, 2005; Kogel et al., 2006).

Uma hipótese do mutualismo defensivo proposto por Kusari & Spiteller (2011) é a criação, por parte do endófito, de um perfil químico heterogêneo dentro e entre os tecidos da planta, resultando em variações em termos de palatabilidade para herbívoros e infectividade para patógenos. No entanto, o potencial de defesa fornecido pelos endófitos *in vivo* ainda é pouco conhecido e necessita de mais investigações. O emprego da tecnologia endofítica pode fornecer uma produção eficiente de culturas relevantes e de produtos vegetais importantes (Wani et al., 2015).

Vários fungos endofíticos são relatados com indutores de resistência contra agentes patogênicos e pragas, além de promotores do crescimento de plantas, sendo fonte de vários estudos aplicados ao controle de doenças (Zabalgogeazcoa, 2008; Gao et al., 2010). Esse potencial tem sido demonstrado muitas vezes para fungos microrrízicos (filo Glomeromycota) e fungos endofíticos do filo Ascomycota, como espécies de *Trichoderma* e fungos anamórficos (Andrade-Linares et al., 2011). Nesse contexto, novos grupos de fungos endofíticos vêm sendo explorados, como espécies do gênero *Muscodor*, no qual se destacam pela produção de compostos orgânicos voláteis (COVs) com ação antimicrobiana sobre vários patógenos. Logo, possíveis agentes de biocontrole munidos de um potente mecanismo de ação, a antibiose proporcionada por voláteis. Entretanto, até então, a seleção e inoculação visando à colonização endofítica como estratégia de manutenção de tais fungos no microbioma planta, não foi explorada.

2.1.1 Gênero *Muscodor*

Relacionado molecularmente com o gênero *Xylaria* do filo Ascomycota, fungos do gênero *Muscodor* são caracterizados pela produção de micélio esbranquiçado em diversos meios de cultura e, pela não produção, até então, de estruturas de frutificação ou esporos, também conhecido como micélio estéril. *Muscodor albus* foi a primeira espécie descrita,

caracterizada por produzir uma mistura de compostos voláteis com propriedades antibióticas (Worapong et al., 2001; Strobel et al., 2001).

Este gênero é composto por fungos endofíticos bastante promissores biologicamente, inclusive para a agricultura. A mistura de compostos orgânicos voláteis (COVs) emitida por eles compreende, principalmente, álcoois, ácidos, ésteres, cetonas e lipídios, letais a uma ampla variedade de fungos e bactérias fitopatogênicas, além de nematoide e inseto (Strobel, 2011). Além disso, tais compostos podem desempenhar importantes papéis de sinalização para o fungo em seu ambiente natural, mediando interações ecológicas com o hospedeiro e outros endófitos (Morath et al., 2012).

Os COVs apresentam uma propriedade química extremamente importante, o baixo peso molecular, que facilita sua difusão por ambientes heterogêneos (Morath et al., 2012). Um aspecto fisiológico importante da produção dos COVs microbianos é a síntese tanto no metabolismo primário quanto no secundário, podendo ser explorado desde o início de crescimento, porém, a composição do meio de crescimento exerce grande influência na qualidade e eficácia dos COVs (Ezra & Strobel, 2003; Morath et al., 2012). Testes com misturas artificiais de substâncias identificadas dos voláteis fúngicos mostraram efeito biológico limitado a alguns agentes patogênicos em comparação ao efeito da mistura produzida naturalmente pelo fungo sobre uma vasta gama de patógenos (Strobel, 2011). Isso ressalta a importância não só da prospecção de biomoléculas como também do desenvolvimento de estratégias para utilização do microrganismo, explorando os voláteis emitidos naturalmente.

Porém, não há relatos da inoculação de espécies deste gênero visando o estabelecimento endofítico, podendo, então, ser uma nova estratégia de aplicação deles na agricultura. No entanto, os benefícios da utilização destes microrganismos, tendo como desafio a não esporulação, parte do desenvolvimento de uma metodologia que seja eficaz na colonização endofítica e mais provável de ser praticada pelo agricultor.

2.2 Endofitismo e o controle biológico de doenças de plantas

Endofíticos podem combater o desenvolvimento de doenças por diversos mecanismos, de forma isolada ou simultânea. Algumas espécies endofíticas podem alterar a bioquímica da planta induzindo mecanismos de defesa, outras podem produzir compostos antimicrobianos e enzimas líticas, além de concorrer por espaço e recursos vegetais. Além disso, alguns endofíticos podem apresentar características hiperparasitas. Moléculas do endófito também podem atuar como elicitores, estimulando a planta hospedeira e outros endófitos (fungos e

bactérias) a produzir determinados metabólitos secundários bioativos com possível influência na resistência da planta (Zabalgogeazcoa, 2008; Gao et al., 2010; Kusari et al., 2012).

Algumas espécies de *Trichoderma* são conhecidas por parasitar microrganismos patogênicos, sendo utilizadas e comercializadas como agentes de biocontrole. Os fungos deste gênero vivem frequentemente em associação com raízes de plantas e podem produzir numerosos COVs que, por sua vez, têm demonstrado desempenhar um papel chave no micoparasitismo e também na interação com plantas (Stoppacher et al., 2010). Andrade-Linares e colaboradores (2011) relatam a redução do efeito negativo do patógeno *Verticillium dahliae* no tomateiro, por dois fungos endofíticos mitospóricos (*Leptodontidium orchidicola* e outro de taxonomia não definida), isolados da raiz desta mesma espécie vegetal. Ambos foram inoculados por imersão de raízes de mudas de tomate em suspensão de micélio, além do encharcamento do solo com a mesma suspensão.

O ambiente interno dos tecidos vegetais fornece uma grande vantagem adaptativa aos microrganismos endofíticos em virtude da proteção contra intempéries ambientais (radiação ultravioleta, umidade e temperatura, por exemplo), além da maior disponibilidade de nutriente e menor competição contra microrganismos epifíticos (Silva et al., 2006; Latz et al., 2018). Logo, o desenvolvimento de estratégias de inoculação de endófitos selecionados visando o endofitismo é fundamental para o sucesso da tecnologia. No entanto, a colonização para ser bem-sucedida depende de muitas variáveis, como: tecido vegetal, genótipo da planta e do endofítico, condições bióticas e abióticas, etc. (Hardoim et al., 2015; Wani et al., 2015).

De forma geral, a entrada de microrganismo endofíticos se dá por aberturas naturais (estômatos e hidatódios), ferimentos e, principalmente, áreas de emergência de raízes secundárias (Azevedo, 1998). A penetração também pode ocorrer na semente, passivamente, via ferimentos causados por danos físicos e, no caso de fungos, ativamente (Silva et al., 2006). Portanto, a inoculação via semente possibilitando a colonização da plântula nos primeiros estágios de crescimento parece ser uma importante e promissora técnica de inoculação de fungos endofíticos, principalmente aqueles que não esporulam.

É de interesse generalizado o entendimento da melhor estratégia de inoculação de microrganismos endofíticos, depois de selecionados, para fins agronômicos. A técnica de inoculação pode interferir na colonização de diferentes partes da planta e também na persistência durante o ciclo da cultura. Logo, a efetiva colonização na planta reflete diretamente no potencial dos endófitos em promover maior tolerância a doenças e pragas, e maior desenvolvimento vegetativo. Neste contexto, o tratamento de sementes fornece uma

alternativa ambientalmente amigável e mais provável de ser adotada pelo produtor, devido à facilidade de utilização (Muvea et al., 2014; Parsa et al., 2018).

Muvea et al. (2014) testaram a colonização de plantas de cebola por isolados de fungos endofíticos usando dois métodos de inoculação com suspensão de conídios, na muda e semente, e depois avaliaram os efeitos da colonização na biologia do *Thrips tabaci*, principal praga da cebola. Tanto por inoculação na muda como na semente, os fungos conseguiram colonizar endofiticamente a planta. No entanto, a inoculação na semente resultou em 1,47 vezes maior percentagem de recuperação dos fungos inoculados. Em plantas inoculadas com alguns endofíticos, por inoculação na semente, foi observado menos thrips, menos punções de alimentação e menor número de ovos depositados, em comparação ao tratamento controle. Outra informação importante do trabalho supracitado foi a capacidade de colonização em plantas de cebola, de dois fungos isolados da parte aérea de sorgo e milho, além daqueles isolados da própria planta. O estabelecimento desses fungos foi observado na folha, no caule e, principalmente, na raiz, com a inoculação de um destes se destacando entre os melhores resultados contra a praga. Portanto, estes resultados demonstram que fungos endofíticos podem não só colonizar hospedeiros alternativos como também promover maior tolerância da planta a herbivoria. A colonização de várias plantas hospedeiras, mesmo não relacionadas taxonomicamente, sugere uma adaptação dos endófitos em superar diferentes tipos de defesas do hospedeiro (Selim et al., 2012). Isso demonstra que a inoculação de endofíticos em plantas não-hospedeira pode render resultados ainda melhores em relação aqueles nativos.

Em estudos com fungos endofíticos e patogênicos por infecção em semente, a imersão em suspensão de esporos é um dos métodos de inoculação mais tradicionais. No entanto, esta metodologia apresenta baixa eficiência não assegurando a infecção, além de não poder ser aplicada a fungos que não esporulam. Uma alternativa a esse grupo peculiar é a técnica de restrição hídrica em meio BDA com diferentes potenciais osmóticos (0,0 a -1,2 MPa) por adição de um soluto (sacarose, manitol, KCl, etc.), com eficácia já comprovada na inoculação de vários fungos patogênicos. A metodologia consiste em colocar sementes em contato com o micélio do fungo cultivado em meio BDA ajustado osmoticamente. O condicionamento osmótico retarda a germinação da semente permitindo maior tempo de exposição ao inóculo para que ocorra a colonização (Machado et al., 2001). No entanto, para maior efetividade da técnica, é necessário avaliar o efeito de diferentes potenciais osmóticos no crescimento do fungo a ser inoculado e na qualidade fisiológica da semente após o condicionamento

osmótico, pois podem ser afetados negativamente (Machado et al., 2001; 2004; Costa et al., 2003; Pedroso et al, 2010; Reis et al., 2014).

Portanto, considerando a característica de não produção de esporos dos fungos endofíticos do gênero *Muscodor* que serão objeto de estudo no presente trabalho, o contato das sementes com o micélio do fungo por um longo período, torna a técnica de restrição hídrica bastante promissora como estratégia de inoculação para colonização endofítica deste grupo de microrganismos.

2.3 Promoção do crescimento de plantas por fungos endofíticos

Diretamente, fungos endofíticos podem produzir compostos bioativos (fitormônios), como: ácido indol-3-acético (AIA); giberelinas (GAs) e citocininas. Estes compostos agem como sinalizadores que controlam o crescimento e o desenvolvimento, além de modular respostas das plantas a mudanças ambientais. No entanto, também podem atuar indiretamente na estimulação do crescimento vegetal, pelo antagonismo a fitopatógenos, disponibilização de nutrientes e indução da produção de hormônio no hospedeiro (Harllen & Bettiol, 2009; Selim et al., 2012; Khan et al., 2015).

Suwannarach e colaboradores (2015) estudando uma espécie fúngica do gênero *Muscodor*, *M. cinnamomi*, constatou a sua capacidade de produção do fitormônio AIA. Além disso, o extrato bruto do fungo induziu o alongamento do coleóptilo de aveia e arroz, aumentou a germinação e o comprimento da raiz de plantas de feijão preto e milho. O estudo ainda mostrou que a inoculação de 30 g de inóculo de *M. cinnamomi* em 500g de solo promoveu incremento no comprimento da raiz, peso seco da parte aérea e da raiz de plantas de tomate. Adicionalmente, o fungo foi capaz de solubilizar e tolerar metais tóxicos, tolerar herbicida (2,4-D, glifosato e paraquat) e inseticida (metomil). Esse relato demonstra o potencial bioestimulante de um fungo *Muscodor*, além dos COVs antimicrobianos comumente reportados para várias espécies deste gênero.

Andrade-Linares et al. (2011) avaliaram o impacto da colonização de três fungos endofíticos anamórficos em plantas de tomate, todos promoveram aumento da biomassa de plantas jovens em 10 e 20%, e um destes aumentou o diâmetro da raiz de plantas com 24 semanas de idade. Além disso, um endofítico aumentou a biomassa e o teor de glicose (17%) dos frutos.

2.4 A cultura do tomate e doenças bacterianas

De grande importância na civilização humana, principalmente como fonte alimentar há milhares de anos, a família Solanaceae é composta por 3000-4000 espécies classificadas em aproximadamente 90 gêneros. Dos membros com importância destaca-se o gênero *Lycopersicon*, composto por nove espécies de tomate, agora incluídos no gênero *Solanum* com base em taxonomia molecular (Gebhardt, 2016). O tomate cultivado (*Solanum lycopersicum* L., anteriormente *Lycopersicon esculentum* Miller) tem distribuição mundial e é, hoje, a segunda mais importante planta cultivada das Solanáceas depois da batata (Gebhardt, 2016).

Em 2016 a produção mundial de tomate superou 177 milhões de toneladas, o Brasil contribuiu com quase 2%. O valor da produção neste ano atingiu aproximadamente 5,5 bilhões de reais para o Brasil, tornando o tomate uma das principais hortaliças, em termos de valor nacional de produção. Em 2018 a região sudeste foi responsável por quase metade da produção nacional de tomate e o estado de Minas Gerais, terceiro maior produtor nacional, participou com 17,4%, destaque para as regiões central e sul de minas (FAOSTAT, 2016; IBGE, 2018).

Diversas doenças incidem na cultura do tomate, as fitobactérias são responsáveis pelas maiores perdas, como: murcha bacteriana, causada pela *Ralstonia solanacearum*; pinta bacteriana, cujo agente causal é a *Pseudomonas syringae* pv. *tomato*; e mancha bacteriana, **c**ausada pelo complexo *Xanthomonas* spp., tendo como agentes várias espécies: *X. euvesicatoria*, *X. vesicatoria*, *X. perforans* e *X. gardneri* (Jones et al., 2004). De acordo com 458 bacteriologistas da comunidade internacional, associados à revista *Molecular Plant Pathology*, levando em consideração a importância científica e econômica, estes patógenos estão no Top 5 da lista das mais importantes bactérias patogênicas de plantas, encabeçando a lista a *P. syringae* patovares e *R. solanacearum* (Mansfield et al., 2012).

A *R. solanacearum* é o agente causal da murcha bacteriana em mais de 200 espécies de plantas em 54 famílias botânicas diferentes, é caracterizada por alta diversidade genética e patogênica sendo considerada um dos patógenos vegetais mais importante (Rodrigues et al., 2011). A murcha bacteriana é uma das doenças mais importante do tomateiro, favorecida por temperatura e umidade altas. O patógeno é transmitido pelo solo podendo sobreviver por longos períodos e sua infecção na planta de dá por feridas, pontas de raiz ou rachaduras nos locais de emergência das raízes. Os sintomas característicos são: murcha repentina da planta, de cima para baixo, escurecimento vascular de cor marrom na base do caule e também pode

haver a formação de raízes adventícias. O manejo da doença permanece limitado e é dificultado pela sobrevivência por anos, tanto no solo como em detritos de plantas, ou em plantas daninhas assintomáticas (Mansfield et al., 2012; Alvarenga, 2013). O uso de porta-enxertos comerciais tem sido a principal medida de controle e propicia resistência intermediária. Entretanto, em condições ambientais favoráveis ao patógeno e alta virulência do isolado, esse sistema proporciona níveis de controle insatisfatório exigindo medidas complementares e preventivas de controle (Lopes et al., 2015).

A mancha bacteriana, causada pelo complexo *Xanthomonas* spp., também conhecida por pústula bacteriana, é uma das doenças mais destrutivas do tomateiro, causando perdas significativas. Ocorre predominantemente em lavouras durante o período chuvoso e/ou sob irrigação por aspersão convencional ou pivô central. Os prejuízos decorrentes da doença são devidos à perda da superfície fotossintética e queda das flores e frutos em formação, reduzindo a produtividade. O controle da doença é difícil, principalmente, devido a pouca disponibilidade de cultivares resistentes, ausência de agrotóxicos eficazes em condições ótimas à doença, e a não adoção de algumas práticas culturais por parte do agricultor (Quezado-Duval & Lopes, 2010).

Outra doença também economicamente importante na cultura do tomateiro, sob condições de baixa temperatura e alta umidade, é a pinta bacteriana, causada pela bactéria *P. syringae* pv. *tomato*. Os sintomas necróticos e cloróticos são ocasionalmente confundidos com a mancha bacteriana. Também infectam frutos verdes causando manchas necróticas e maturação tardia, comprometendo sua qualidade e valor comercial. O patógeno penetra na planta por espaços intercelulares das folhas através de aberturas naturais (estômatos, por exemplo) e ferimentos, onde se multiplicam internamente antes do desenvolvimento dos sintomas (Preston, 2000). Apesar do controle da doença ter limitações quanto à disponibilidade de produtos químicos, o controle biológico vem recebendo bastante destaque, no qual bactérias endofíticas têm se mostrado agentes eficientes de controle (Silva et al., 2008).

As bacterioses são de difícil controle, pois o controle depende mais do manejo da cultura do que propriamente de produtos químicos, que por sua vez não apresentam efetividade de controle (Marcuzzo et al., 2015). Portanto, a pouca disponibilidade de produtos eficazes tem propiciado a busca por novas alternativas de controle, e também alternativas que contribuam para a prática de uma agricultura mais saudável para o homem e meio ambiente.

3 CONSIDERAÇÕES GERAIS

A crescente demanda mundial por alimentos exerce grande pressão sobre a agricultura, acarretando no uso intensivo de produtos químicos para o controle de doenças e pragas que, sob utilização deliberada e demasiada, implica em diversos problemas, principalmente ambiental. Em virtude disso, a preocupação da sociedade vem alterando o cenário agrícola através da busca por defensivos sustentáveis. Dentre as alternativas o controle biológico é uma das mais importantes.

Os microrganismos endofíticos mutualistas, principalmente bactérias e fungos, que colonizam a planta sem danos aparentes, apresentam grande potencial como agentes de biocontrole. Essa associação visando o controle biológico ainda é muito pouco entendida devido à complexidade de interações. No entanto, pode estimular o crescimento das plantas, aumentar a adaptação e resistência a patógenos e pragas. Assim, nas últimas décadas, intensificaram-se trabalhos buscando microrganismos, dentre eles os endofíticos mutualistas, com potencial para utilização na agricultura, com capacidade de melhorar o desenvolvimento vegetativo e atuarem como agentes de biocontrole de fitopatógenos e pragas em culturas de interesse agrícola.

Portanto, visando o desenvolvimento de novas soluções fitossanitárias para a cultura do tomate, de grande importância econômica e social no contexto do agronegócio brasileiro, esse trabalho abordou o estudo de fungos endofíticos do gênero *Muscodor*, produtores de compostos orgânicos voláteis bioativos, no biocontrole de fitobactérias patogênicas e na aptidão de plantas de tomate. Logo, a prospecção de biomoléculas e seleção de novos agentes de biocontrole efetivos pode representar uma alternativa sustentável de manejo.

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SEGUNDA PARTE – ARTIGOS

ARTIGO 1

Endophytic species of *Muscodor*: inoculation in seedling and seed of tomato and effect on plant performance in semi-hydroponic system

ABSTRACT - Endophytic fungi of the *Muscodor* genus are well-known for their bioactive volatile organic compounds (VOCs) against phytopathogens, but also have potential for promote plant growth. Therefore, we examined the potential as growth promoter on tomato plants of nine isolates of *Muscodor* fungi belonging to three species (*M. vitigenus*, *M. coffeanum* and *M. yucatanensis*). Two inoculation strategies were used, mycelial suspension on seedlings, evaluated in two independent experiments, and the water restriction technique on seed, with adaptations aiming to maximize colonization. We also evaluated the compatibility of the most promising fungi with seed treatment. The inoculation with mycelial suspension of five fungi increased the yield of fresh fruits of tomato in the two experiments, with the highest gains promoted by the fungi *M. coffeanum* COAD1842 (20%), *M. vitigenus* CML4015 (19%) and *M. vitigenus* CML4014 (15%). Dry biomass gains of root and shoot were observed only in the second experiment, carried out in winter. Osmoconditioning proved to be an effective inoculation technique for *Muscodor* fungi, promoting effective seed colonization without damage to fungus growth. However, some isolates affected negatively the germination, height and the shoot/root ratio of the seedlings. On the other hand, *M. coffeanum* COAD1842 and *M. yucatanensis* CML4016 significantly increased the dry biomass of the roots, in addition to the seedlings presented lower shoot/root ratio than the control. The observation of radicle colonization suggests the ability of colonization of *Muscodor* fungi in tomato roots. In addition, the fungi *M. coffeanum* COAD1842 and *M. yucatanensis* CML416 showed compatibility with the seed treatment (metalaxyl+deltamethrin). Our results reinforce the potential of using *Muscodor* fungi in the tomato crop, with the development of application strategies in seedling and seed aiming the plant development.

Index terms: Water restriction technique; mycelial suspension; endophytic fungi; growth promoting; seed treatment.

Introduction

Molecularly related to the Xylaria genus of the Ascomycota phylum, fungi of the genus *Muscodor* are characterized by the production of a whitish mycelium in various culture media and by the non-production until then of fruiting structures or spores, also known as mycelium sterile. *Muscodor albus* was the first species described, characterized by producing a mixture of volatile compounds with antibiotic properties (Worapong, 2001; Strobel et al.,

2001). These VOCs play important roles in signaling fungi in their natural environments, mediating ecological interactions with the host and other endophytes (Morath et al., 2012).

Aimed efficacy inoculation with these endophytes, host tissue must be colonized successful. In this sense, the inoculation technique influences colonization and persistence during the crop cycle reflecting directly on the promoting growth (Muvea et al., 2014; Parsa et al., 2016). When it comes specifically of fungi non-producing of spores like those of the genus *Muscodor*, becomes even more relevant. However, the water restriction technique has been reported as an effective infection method in the inoculation of pathogenic fungi in seeds (Machado et al., 2001; 2004; Costa et al., 2003; Pedroso et al, 2010; Reis et al., 2014) and probably can be implemented for non-producing spore fungi..

Suwannarach et al. (2015) reported the potential of endophytic fungus *M. cinnamomi* in the production of phytohormone indole-3-acetic acid (AIA). The study also showed that the inoculation of 30 g of inoculum of *M. cinnamomi* in 500 g of soil promoted increase in root length, dry weight of shoot and roots of tomato plants in relation to the control. Andrade-Linares et al. (2011) evaluating the impact of the colonization of three endophytic fungi on tomato plants, observed that all of them promoted increase of the biomass of young plants in 10 and 20%, and one of them increased the root diameter of 24 week old plants. In addition, an endophytic increased the biomass and glucose content of fruits in 17%, demonstrating the potential for improvement of others agronomic attributes. Indirectly, endophytic fungi can stimulate plant growth by increasing the availability of nutrients, action of extracellular enzymes or soil pH regulation, and even by inducing the production of hormones in the host.

Considered the above-mentioned, we examined the potential a growth promoter in tomato plants, under semi-hydroponic cultivation in protected environment, by nine *Muscodor* endophytic fungi belonging to three species (*M. vitigenus*, *M. coffeanum* and *M. yucatanensis*). Two methods of inoculation were tested in tomato, in seedling, in two independent experiments, with mycelium suspension, and seed, through adaptations in the water restriction technique, in which growth promotion was evaluated. In addition, compatibility endophytically with the seed treatment was verified.

Materials and Methods

Origin and storage of *Muscodor* species

Nine isolates of three species of endophytic fungi *Muscodor* isolated from organic coffee plants (*Coffea arabica*) and identified by Monteiro et al. (2017) were used: *M. yucatanensis*, isolates CML4016 and CML4017; *M. coffeanum*, isolates COAD1842,

COAD1899 and COAD1900; *M. vitigenus*, isolates CML4012, CML4013, CML4014 and CML4015. The fungi were preserved through methods of Castellani (Castellani, 1974) and cryogenics at -80ºC in CML, the mycological culture collection of Lavras (CML/UFLA).

Inoculation of *Muscodor* fungi on tomato seedlings and effect on growth

Fungi were grown in 500 mL Erlenmeyer flasks by inoculation of six 5 mm mycelial agar plugs from a 14 days old culture (grown on PDA at 25ºC) into 200 mL sterilized Potato Dextrose Broth (pH 6.0) and incubated at 25 ºC, on a rotary shaker, at 125 rpm for 14 days, with three replicates and a control without fungus. After cultivation, the inoculant was prepared by fragmenting in blender of 2 g of fresh mycelium for each 100 mL of distilled water. Tomato seedlings, italian group and hybrid Aguamiel (Vilmorin Brazil™), coming from a commercial seedling production system, were transplanted to 5L pot filled with commercial substrate Tropstrato HT Hortaliças™ 21 days after seedling emergence. The micelial suspension (50 mL per plant) was applied soon after transplanting and 30 days later. Plants were sprayed till run-off and the surface of the substrate moistened using a hand held sprayer. Before and after the first inoculation, the seedlings were kept in a humidity chamber for 24 hours. The plants were tutored with single stem in greenhouse in semi-hydroponic system fertigated with nutritive solution daily (supplementary material). The fertigation (200 mL per pot) was intercalated with automatic drip irrigation, four times a day, with water volume beyond pot capacity keeping electrical conductivity below 3 dS cm^{-1}. Experimental design was compound by six replicates and ten treatments. The experiment was performed twice, December 2015 to February 2016 and June to Augusto 2016. After 90 days of cultivation we measured root and shoot dry mass (oven dried at 65ºC for four days) and yield of fresh fruit.

Inoculation of seeds by water restriction technique and seedling development

An alternative for inoculation of *Muscodor* fungi in tomato seeds was developed from adaptation of the methodology proposed by Machados et al. (2001), in which mannitol was the osmotic solute used. Before, was evaluated the effect of water restriction at potentials of -0.6 (18.40 g L^{-1}); -0.8 (33.12 g L^{-1}); -1.0 (47.84 g L^{-1}) and -1.2 MPa (62.57 g L^{-1}) in PDA medium on the mycelial growth of the fungi, as the objective of selecting the highest osmotic potential without negative effect on fungal growth and tomato seed germination. PDA medium without mannitol was the control whose osmotic potential is equivalent to -0.35 MPa (Machado et al., 2007). Calculation of the concentration of mannitol to adjust the osmotic potential was performed based on the Van't Hoff equation using the following parameters: i = 1 mol l^{-1}; R = 0.0083 MPa mol^{-1} $ºK^{-1}$; T = 298.15 ºK; C = 182.17 g mol^{-1} L^{-1}. The evaluation

was performed every two days for 20 days, using a digital caliper, measuring on the back of the dish radial growth of the colony. After this period, the Micelial Growth Velocity Index (MGVI) was calculated.

Ten seeds (hybrid N-901, BASF - Nunhems^{TM} Brazil) superficially disinfested were arranged equidistant from each other and 1 cm from the fungal mycelial disk in PDA medium adjusted osmotically with selected potential -1.2 MPa in 15-cm diameter Petri dish (Figure 5A). The seeds were placed slightly after inoculation of the fungus in the culture medium and both incubated at 25ºC for 10 days in the dark. Tomato seeds (hybrid N-901) inoculated were sown in expanded polystyrene tray 5 x 10 cells with cell size of 3.5 x 3.5 cm^2 filled with Tropstrato™, two seed by cell, and grown in greenhouse for 30 days, with four replicates. The experiment had 11 treatments, nine fungi and seeds without the fungus, with and without priming. The nutrients were supplied by the addition of nutrient solution (supplementary material) and the fertigation was intercalated with potable water. The percentage of germination was evaluated and, after 30 days, shoot and root dry mass, height of seedlings and percentage of normal and abnormal plants were analyzed.

Muscodor fungi compatibility with the seed treatment

A fungal mycelial disk from a 14-day pre-culture was inoculated into osmotically adjusted PDA medium (-1.2MPa) in 9-cm Petri dish. Subsequently, three tomato seeds treated with metalaxyl and deltamethrin (hybrid N-901) and disinfested were spreaded equidistant on surface of the PDA medium around the mycelial disc. For disinfestation, the seeds were exposed to direct UV radiation in the dark, for one hour, and kept in the dark overnight. The assay was performed in triplicate and, after 20 days, the compatibility was confirmed by non-inhibitory halo formation.

Statistical analyze

For all assays, the experimental design was a completely randomized and, for the assay of water restriction technique on fungal growth, used a factorial scheme being the osmotic potentials the levels. The data were submitted the ANOVA and the means were compared with the Scott-Knott test (p = 5 %) in the statistical software SISVAR version 5.6.

Results

Inoculation of *Muscodor* fungi on tomato seedlings and effect on growth

There was no significant difference for all analyzed variables of the plants inoculated in the summer in relation to the control (Figure 1). However, most of the fungi improved the

fresh fruit mass, especially the fungi *M. coffeanum* COAD1842, *M. vitigenus* CML4013 and *M. vitigenus* CML4014 that promoted gains of 21.9%, 19.9% and 18.8%, respectably. It was possible to observe that all the inoculated plants presented lower root dry mass compared with the control, in which the inoculation with *M. coffeanum* (COAD1842) resulted in the lowest root and shoot dry mass (Figure 1A,B). However, such reductions did not reflect negatively on the productivity.

In the second experiment, the performance of the inoculated plants was better when compared to the first, all variables showed an increase. Highlight for the fungi *M. coffeanum* COAD1842, *M. vitigenus* CML4015 and *M. vitigenus* CML4014, with yield increased of fresh tomato fruits in the two experiments, with productivity of 20%, 19 % and 15%, respectively. Dry biomass gains of root and shoot were observed only in the second experiment, carried out in winter.

Inoculation of seeds by water restriction technique and seedling development

In general, the osmotic adjustment through increasing addition to the potential -1.2MPa (mannitol 62.57 g L^{-1}), compared to the PDA medium (-0.35 MPa), did not affect fungal growth (Figure 1A). *Muscodor* species had negative variation in mycelial growth index in the osmotic potential -1.0 MPa, but never inferior to the control. In contrast, most fungi grew significantly better and more uniform with each other at -1.2MPa potential (Figure 2B), which justified their choice for subsequent test.

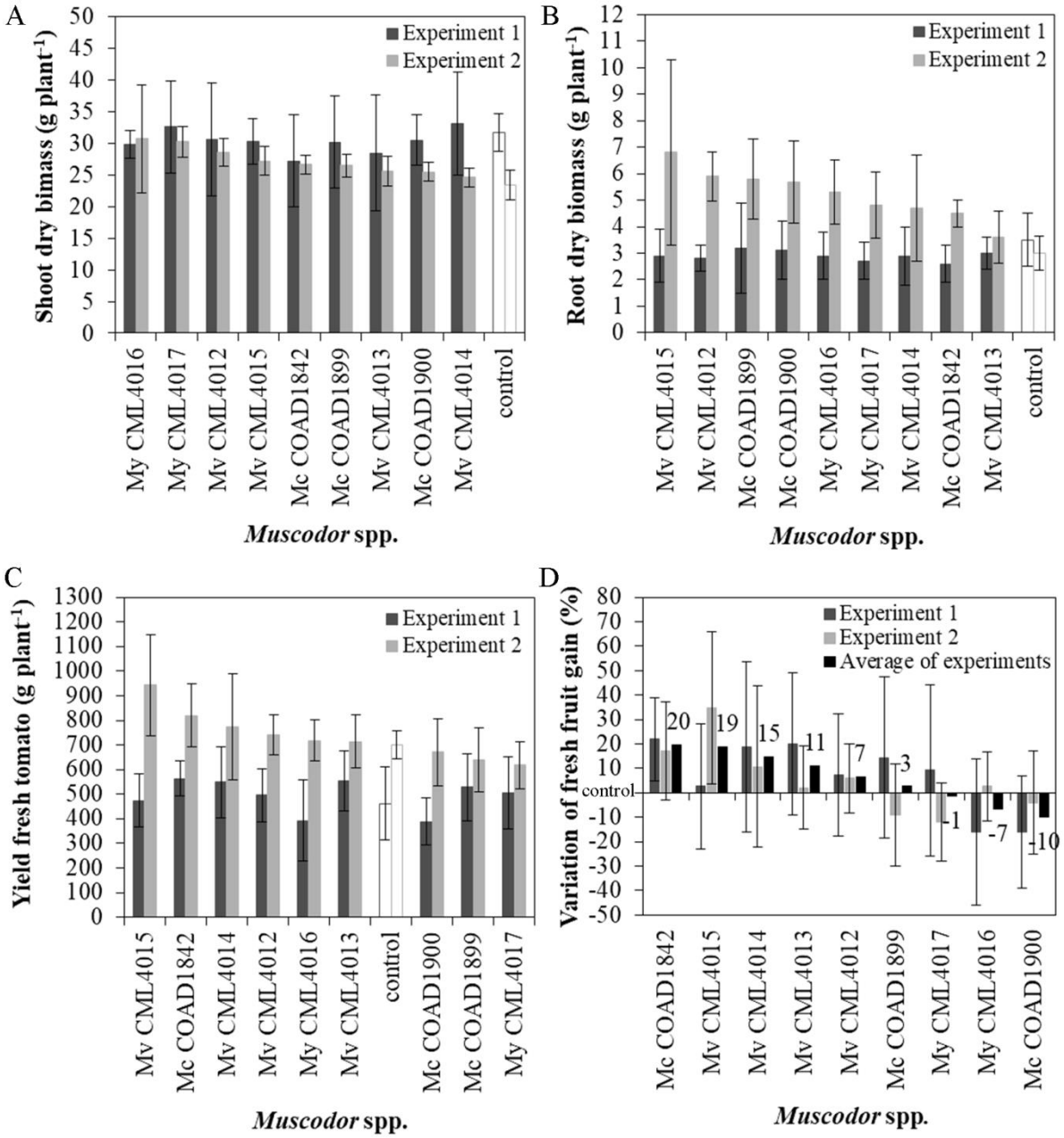

Figure 1. Performance of tomato plants (italian group, hybrid Aguamiel) inoculated with nine endophytic fungi of the genus *Muscodor* in two experiments: the first in December 2015 to February 2016, and the second in June 2016 to August 2016. (A) Shoot and (B) root dry biomass, (C and D) yield of fresh tomato and variation of fresh fruit gain. Values indicate means (n=6), error bars the standard deviation and asterisks indicate significant difference (ANOVA, Scott-Knott test at 5%). The abbreviations Mv, My and Mc represented *M. vitigenus*, *M. yucatanensis* and *M. coffeanum*, respectively.

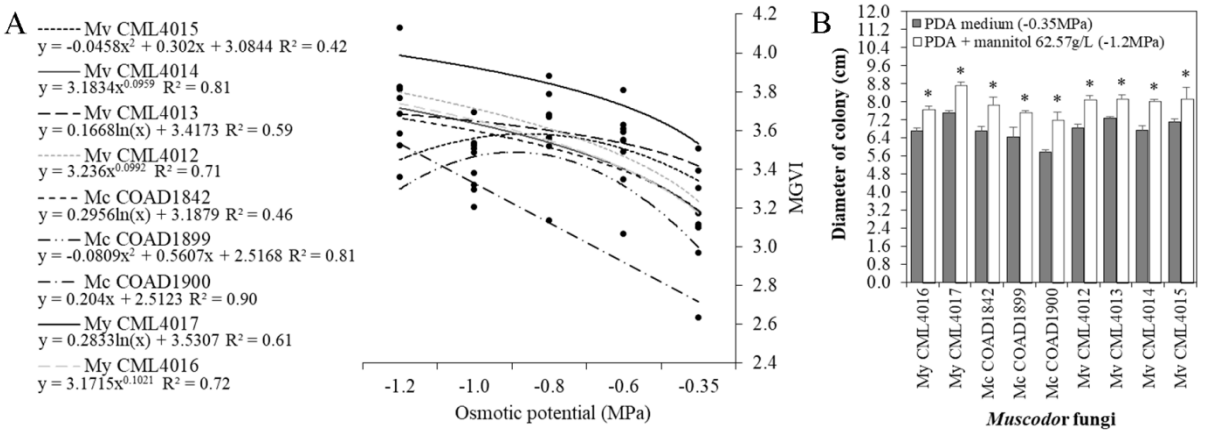

Figure 2. Growth of *Muscodor* endophytic fungi in osmotically adjusted PDA medium. (A) Mycelial Growth Velocity Index (MGVI) and (B) colony growth in common PDA medium relative to PDA at -1.2MPA, after 20 days of growth. Values indicate means and error bars the standard deviation. Asterisks indicate significant difference (ANOVA, Scott-Knott test at 5% of probability). The abbreviations Mv, My and Mc represented *M. vitigenus*, *M. yucatanensis* and *M. coffeanum*, respectively.

Regarding the percentage of germination of tomato seeds (hybrid N-901), four of the ten fungi significantly reduced and long exposure period to the priming did not reduce affect germination (Figure 2A). The inoculation with some fungi resulted in abnormal plants, however in percentages below 5% (Figure 2A). It was also observed a significant reduction of seedling height when inoculated with some fungi, in the number of leaves there was no significant variation (Figure 2B).

Although not significant to differ, seed inoculation of *Muscodor* species by osmoconditioning increased dry and shoot biomass. Significant increases of root dry biomass were observed for some fungi. *M. coffeanum* COAD1842 promoted the best result, approximately 35% of dry shoot and 56.1% of dry root. In addition, seedlings inoculated with this fungus presented lower shot/root ratio (2.3), desired parameter in seedlings production. The fungi *M. yucatanensis* CML4016 had the lowest ratio 1.8 compared to 2.5 of the control. In the present study, osmoconditioning proved to be an efficient inoculation technique for *Muscodor* fungi, promoting the effective colonization of the seeds without damage to fungus growth.

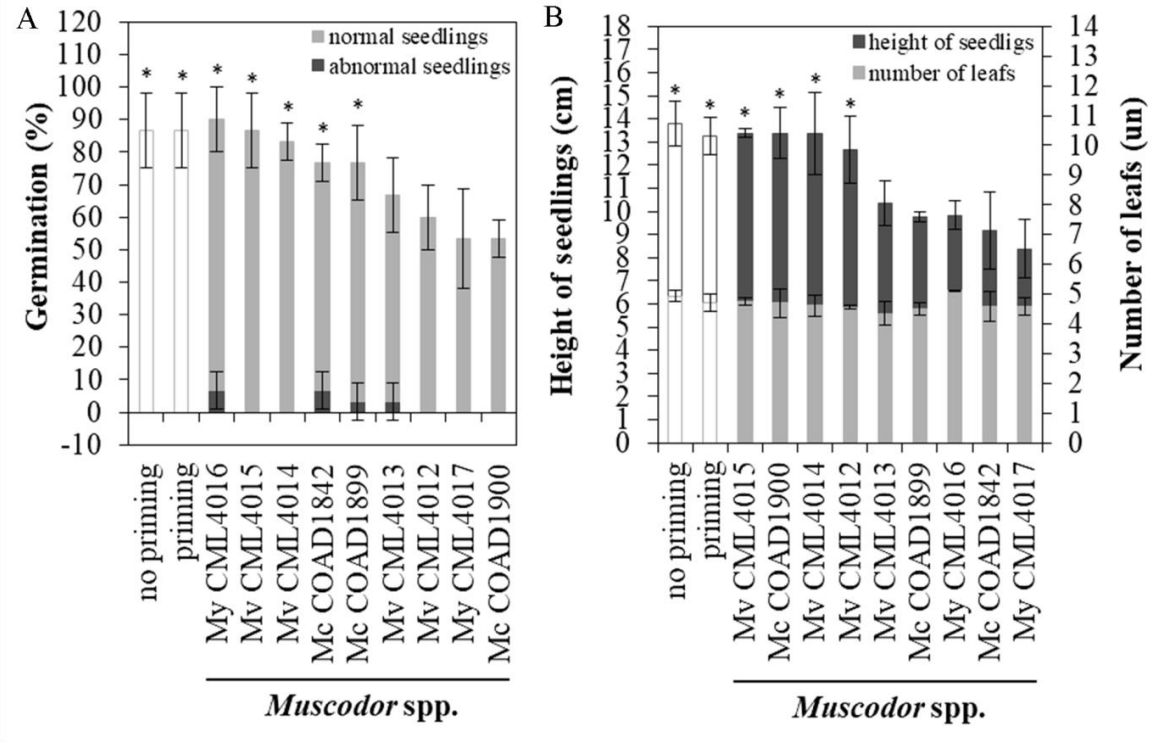

Figure 3. Performance of seedlings inoculated via seed. (A) Germination percentage and (B) height of seedlings and number of leafs after 30 days of growth. Values indicate means and error bars the standard deviation. Asterisks indicate significant difference (ANOVA, Scott-Knott test at 5% of probability). Asterisks indicate significant difference at 5% of probability (ANOVA, Scott-Knott test). The abbreviations Mv, My and Mc represented *M. vitigenu*s, *M. yucatanensis* and *M. coffeanum*, respectively.

Muscodor fungi compatibility with the seed treatment

Of the three evaluated fungi, only *M. vitigenus* CML4012 showed sensitivity to seed treatment, evidenced by the formation of inhibition halo around the seed, while *M. coffeanum* COAD1842 and *M. yucatanensis* CML4016 were compatible (Figure 4).

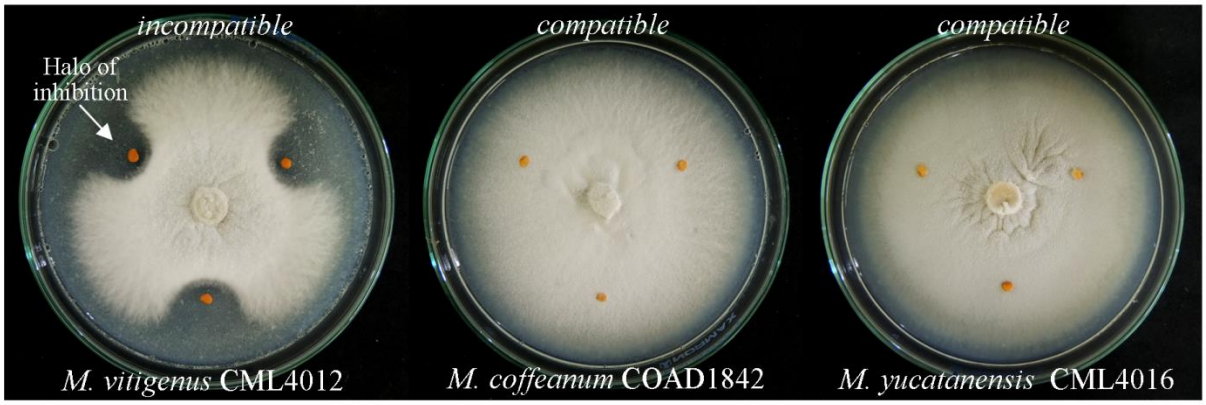

Figure 4. Compatibility assay of *Muscodor* fungi with the fungicide seed treated (metalaxyl and deltamethrin, Nunhems™ Brazil), in which the fungus *M. vitigenus* CML4012 was incompatible and *M. coffeanum* COAD1842 e *M. yucatanensis* CML4016 were compatible.

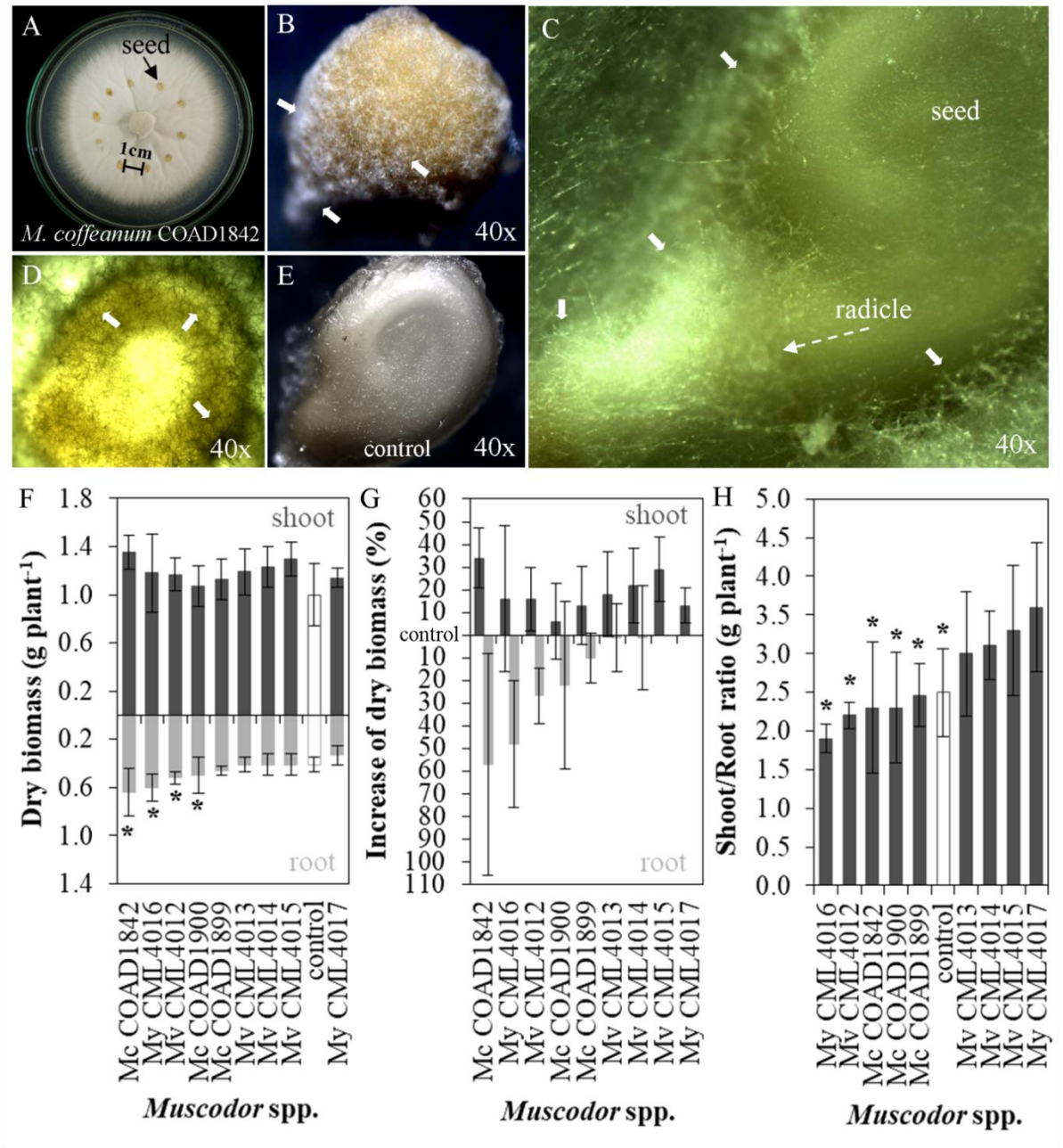

Figure 5. Performance of tomato plant inoculated via seed. (A) Tomato seed inoculated by water restriction technique with hyphae colonizing your surface and colonizing of tomato radicle by *M. coffeanum* COAD1842 and (E) control. Colonized seeds images were recorded using Lumenera^{TM} microscopy camera and Infinity Capture software version 6.5.4. (C) Total solid soluble of fruits in complete maturation stage and (D) yield of fresh tomato of plants under infection of pathogen. Values indicate means and error bars standard deviation. Values indicate means, error bars the standard deviation and asterisks indicate significant difference (ANOVA, Scott-Knott test at 5%).

Discussion

The inoculation with mycelial suspension of some fungi promoted an increase in the fresh fruit yield in the two evaluated experiments. This result highlights not only the potential of the fungus as biostimulant of tomato plants, but also the repeatability of the effect. In the

first experiment, performed at the time of year with milder temperatures, the inoculation with the fungi yielded higher dry biomass production of both root and shoot.

In our work, tomato seeds exposed to the osmotic conditioning showed normal germination. However, when inoculated with some fungus of the genus *Muscodor*, there was reduction germination and height of the plants. Macías-Rubalcava et al. (2010) also reported *in vitro* phytoinhibitory activity of VOCs from *M. yucatanensis* on the root elongation of tomato, as well as the organic extracts from the culture medium and mycelium and also Siri-Udom et al. (2017) from *M. heveae*. The fungi presented higher growth as the mannitol solute concentration increased, probably due to the metabolism of the solute (Machado et al., 2007).

Tomato plants inoculated with the fungus *M. coffeanum* COAD1842 presented a considerable increment of productivity in the two experiments. Suwannarach et al. (2015) reported the potential of endophytic fungus *M. cinnamomi* in the production of phytohormone indole-3-acetic acid (AIA). In addition, the crude extract of the fungus induced the elongation of the coleoptile of oats and rice and increased the germination and root length of common bean and maize plants, proving to be biologically active in the growth promotion. The study also showed that the inoculation of 30 g of inoculum of *M. cinnamomi* in 500 g of soil promoted increase in root length, dry weight of shoot and root of tomato plants and controlled *Rhizoctonia* root rot. Additionally, the fungus was able to solubilize and tolerate toxic metals, tolerate herbicide (2,4-D, glyphosate and paraquat) and insecticide (methomyl). Andrade-Linares et al. (2011) evaluating the impact of the colonization of three endophytic fungi on tomato plants, observed that all of them promoted increase of the biomass of young plants in 10 and 20%, and one of them increased the root diameter of 24 week old plants. In addition, an endophytic increased the biomass and glucose content of fruits in 17%, demonstrating the potential for improvement of others agronomic attributes.

Indirectly, endophytic fungi can stimulate plant growth by increasing the availability of nutrients, action of extracellular enzymes or soil pH regulation, and even by inducing the production of hormones in the host. However, they may act directly by producing bioactive compounds (phytohormones), such as indole-3-acetic acid (AIA), gibberellins (GAs), and cytokinins (Shoresh et al., 2010; Kanchiswamy et al., 2015; Suwannarach et al., 2015). These compounds act as signals that control plant growth and development, as well as modulate plant responses to environmental changes (Harllen and Bettiol, 2009, Selim et al., 2012, Khan et al., 2015).

The inoculation with mycelial suspension of five fungi increased the yield of fresh fruits of tomato in the two experiments, with the highest. The water restriction showed to be an effective technique for inoculating these fungi in tomato seeds resulting in increased dry shoot and root biomass, and also low shoot-root ratio of seedling. The observation of radicle colonization suggests the ability of colonization of *Muscodor* fungi in tomato roots. In addition, the fungi *M. coffeanum* COAD1842 and *M. yucatanensis* CML416 showed compatibility with the seed treatment (metalaxyl+deltamethrin).

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Supplementary material

Square 1. Nutritive solution adapted from Furlani et al. (1998) by Valdemar Faquin (personal communication) and substrate characterization used in experiments

Fertilizer for fertigation	Characteristics				
	Composition	Concentration and nutrients supplied			Stock solution [1]
Maxsol MX-21 Jaraguá™	macro and micronutrients	38% K_2O, 11% P_2O_5, 8% N, 2.9% S, 1.6% Mg, 0.2% Fe, 0.04% Mn, 0.02% B, 0.02% Zn, 0.004% Cu and 0.004% Mo.			96g L^{-1}
Calcinit Yara™	calcium nitrate	19% Ca and 15.5% N			72g L^{-1}
Ferrilene Valagro™	Fe-EDDHA chelate	Fe 6%			4g L^{-1}
Substrate	Characteristics*				
	pH (5:1, v:v)	EC[2] mS cm^{-1}	Dry density Kg m^{-3}	WRC[3] %	Raw material
Tropstrato HT Hortaliças™	5.8±0.3	0.5±0.3	200	130	Pinus bark, peat, vermiculite, ammonium nitrate and potassium

[1]Preparation of nutrient solution for electrical conductivity of 2.0 dS cm^{-1} from mix of 100 mL of each stock solution into 10 L potable water, [2]Electric conductivity (5:1, water:substrate), [3]Water Retention Capacity. *The information detailed in this square is described on the manufacturer's packaging.

ARTIGO 2

Antibacterial activity, allelopathy and characterization of volatiles from *Muscodor* fungi and their potential for biocontrol of bacterial wilt

ABSTRACT - Tomato is one of the most economically important crops in Brazil. Several diseases limit their production, in which the phytobacteria are responsible for the greatest losses. Endophytic fungi of the genus *Muscodor* produce bioactive volatile organic compounds (VOCs) against phytobacteria, showing to be potential biological control agents. Therefore, we examined the antimicrobial activity of VOCs against three important tomato pathogenic bacteria (*Ralstonia solanacearum* race 3, *Xanthomonas vesicatoria* and *Pseudomonas syringae* pv. *tomato*) in addition to the allelopathic effect on tomato radicles by nine endophytic fungi of *Muscodor* belonging to three species (*M. vitigenus*, *M. coffeanum* and *M. yucatanensis*). The identification of VOCs from the most representative fungus was carried by gas chromatography-mass spectrometry (GC-MS). For biocontrol assay, the plants were inoculated in seed of tomato for industry by osmoconditioning, in which the fitness of plant was evaluated as well as plant resistance to bacterial wilt caused by *R. solanacearum* race 3. Several isolates of different species produced volatiles with antibacterial and allelopathic activity, especially the VOCs of the fungus *M. coffeanum* COAD1900, with a lethal effect against all phytobacteria, except *P. syringae* that grew partially, and phytoinhibition from the second day of growth. After eight days of growth, 18 VOCs were characterized, among them: acids; esters; alcohols; terpenes; aldehyde and amide. Tomato for industry inoculated with *M. coffeanum* COAD1900 showed moderate resistance to bacterial wilt, besides an increase in fresh fruit yield and total soluble solids content. Our results indicate fungi of the genus *Muscodor* with potential to control the bacterial wilt associated with increased tomato plants development.

Index terms: Volatile organic compounds; GC-MS technique; antibacterial activity; *Ralstonia solanacearum*; *Xanthomonas vesicatoria*; *Pseudomonas syringae* pv. *tomato*.

Introduction

Endophytic fungi of the *Muscodor* genus are well-known by its bioactive volatile organic compounds (VOCs) and commonly reported inhibiting plant pathogens showing up BCAs potentially useful to agriculture (Strobel, 2011). They produces a mixture of volatile organic compounds (VOCs) consisting mainly of alcohols, acids, esters, ketones and lipids, lethal to a wide variety of phytopathogenic fungi and bacteria, as well as nematode and insect (Strobel, 2011).

This genus is composed of endophytic fungi very promising biologically, including for agriculture (Strobel, 2011). This potential suggests its use in agriculture for the biological

control of diseases and pests, biostimulant and source of biomolecules. Suwannarach et al. (2015) reported *M. cinnamomi* controlling *R. solani* and, by soil mycofumigation, *M. albus* controlled root-knot of tomato caused by *Meloidogyne incognita* (Grimme et al., 2007). Also by biofumigation, *M. albus* presented bactericidal activity against *Erwinia carotovora* pv. *Carotovora* (Schotsmans et al., 2008). These volatile compounds, due to the small molecular weight, are able to diffuse through heterogeneous environments, a great advantage over the soluble compounds (Morath et al., 2012). Therefore, this microorganism could be an important complementary and preventive measure of the development of the pathogen in the soil environment, besides the possible biostimulation of the plant growth.

A crop very affected by diseases is tomato (*Solanum lycopersicum*), economically important crop in Brazil, country in the top 10 world producers (FAOSTAT, 2016) with production value, in 2016, of R$ 5.5 billion (IBGE, 2018), where the southeast region is the largest producer (IBGE, 2018). The emergence of new technologies and solutions, aimed at phytosanitary management, can generate significant financial gains. Phytobacteria are responsible for the greatest damage, such as bacterial wilt, caused by *Ralstonia solanacearum*; bacterial speck, caused by *Pseudomonas syringae* pv. *tomato*; and bacterial spot caused by *Xanthomonas* spp. complex, having as causal agents several species: *X. vesicatoria*, *X. perforans* and *X. gardneri* (JONES et al., 2004).

Bacterial wilt caused by *Ralstonia solanacearum*, a soil-borne disease, is one of the most relevant pathosystem to the tomato crop, mainly in rainy summers and protected cropping, while in the north and northeast regions it is a problem most of the year (Lopes, 2009). Bacterial wilt has become a serious threat to tomatoes also in south and southeast of Brazil after the expansion of protected cultivation (Lopes et al., 2015). *R. solanacearum* ranks second among the top 10 bacterial plant pathogens with significant economic losses worldwide (Mansfield et al., 2012). It induces rapid and fatal wilt, causing losses in tomato production of up to 91% (Yuliar et al., 2015) and, after its manifestation in the field, the control is difficult due persistent in the soil for long periods, which makes integrated preventive control always recommended (Lopes, 2009). Several control measures can be used in integrated management against bacterial wilt caused by *R. solanacearum*. The use of commercial rootstocks has been the main measure of control and promise intermediate resistance, however, under favorable environments and high virulence of the isolate, this system provides unsatisfactory levels of control (Lopes et al., 2015). A promising control

measure has been the biological control due, beside other things, to the diversified suppression mechanism employed by biological control agents (BCAs) (Yuliar et al., 2015).

Antimicrobial activity *in vitro* of *Muscodor* species *M. coffeanum*, *M. vitigenus* and *M yucatanensis* against tomato pathogens was reported by Monteiro et al. (2017), in which verified antimicrobial VOCs against *Rhizoctonia solani*, *Phoma* sp., *Fusarium oxysporum*, *Fusarium verticillioides* and *Botrytis cinerea*. In the soil mycofumigation *M. cinnamomi* was reported controlling *R. solani* (Suwannarach et al., 2015) and *M. albus* controlled root-knot of tomato caused by *Meloidogyne incognita* (Grimme et al., 2007). However, studies on the potential of *Muscodor* fungi in the control of pathogenic bacteria associated with tomato culture are still unpublished. Allelopathy of VOCs produced by fungi of this genus on tomato roots also is reported (Macías-Rubalcava et al., 2010; Siri-Udom et al., 2017), an important and negative effect. Nevertheless, considering the application in tomato plants, fungi reported as producers of this compound may represent a real problem in the plant development?

Therefore, aimed preventive/eradicative biological control by these endophytes we examined the antimicrobial activity of VOCs against three important tomato pathogenic bacteria and allelopathic effect on tomato radicles by nine endophytic fungi of *Muscodor* belonging to three species (*M. vitigenus*, *M. coffeanum* and *M. yucatanensis*).

Materials and Methods

Origin and storage of *Muscodor* fungi and phytobacteria

Nine isolates of three species of endophytic fungi *Muscodor* isolated from organic coffee plants (*Coffea arabica*) and identified by Monteiro et al. (2017) were used (Table 1). The fungi were stored by different techniques: They were preserved through methods of Castellani (Castellani, 1974) and cryogenics at -80ºC in CML, the mycological culture collection of Lavras (CML/UFLA).

The phytobacteria, provided by the vegetal bacteriology laboratory of Federal University of Lavras (UFLA) was obtained from infected tomato plants. Storage was done in mineral water sterile (electric conductivity of 159.6 µS cm^{-1} and pH 7.37) and potassium sodium phosphate buffer solution (with fungicide and pH 6.98 at 25ºC, Sigma-Aldrich™), both in ambient temperature.

Table 1. Endophytic fungi species of Muscodor genus used in this study

Muscodor specie	Code		Origin tissue	No. GenBank	Phytopathogens of tomato plants sensible to the VOCs *in vitro*[1]
	Current	Others			
Muscodor coffeanum	COAD1900	COAD1900	Leaf	KP862879	*Bc*; *Fo*; *Fv*; *P*; *Rs*.
	COAD1899	COAD1899	Leaf	KM514681	*Bc*; *Fs*; *Fv*; *P*; *Rs*.
	COAD1842	COAD1842	Leaf	KM514680	*Bc*; *Fv*; *P*; *Rs*.
Muscodor vitigenus	CML4012	HZM41	Stem	KU094055	*Bc*; *Fo*; *P*; *Rs*.
	CML4013	HZM39	Stem	KU094054	*Bc*; *P*; *Pl*; *Rs*.
	CML4014	HZM10	Stem	KU094053	*Bc*; *Fo*; *P*; *Rs*.
	CML4015	C20	Stem	KU094049	*Bc*; *Fv*; *P*; *Rs*.
Muscodor yucatanensis	CML4016	HZM64	Leaf	KU094052	*Bc*; *Fv*; *P*; *Rs*.
	CML4017	HZM60	Leaf	KU094056	*Bc*; *Fs*; *Fv*; *P*; *Rs*.

Bc = Botrytis cinerea; *Fo = Fusarium oxysporum*; *Fs = Fusarium solani*; *Fv = Fusarium verticillioides*; *P = Phoma* sp. and *Rs = Rhizoctonia solani*.[1]Monteiro et al. (2017).

Antibacterial activity *in vitro* of VOCs emitted by *Muscodor* species against *Ralstonia solanacearum, Xanthomonas vesicatoria* and *Pseudomonas syringae* pv. *tomato*

In 9-cm bipartite Petri dishes, on one side, each fungus was grown for 7 days in PDA medium at 25ºC. Posteriorly, on other side, were inoculated 100 µL of a bacterial suspension ($OD_{600\ nm}$ = 0.1 ≈ 10^8 UFC ml^{-1} of *R. solanacearum* and 0.3 ≈ 5 x 10^8 UFC ml^{-1} of *X. vesicatoria* and *P. solanacearum*) thereabout 523 solid medium (Kado and Heskett, 1970) followed of incubation for 72 hours, with four replicates. The bacterial suspensions were obtained from grown for 24h (120 rpm at 28ºC) and spreaded onto the surface of the culture medium, poured into 9 cm diameter Petri bipartite dishes. Less colony emergence compared to control, without the fungus, was considered partial inhibition and, total inhibition, when there were no colonies of phytobacteria. To confirm the bactericidal effect, the fungus was removed leaving only the pathogen for another 72 hours of incubation.

Development of tomato radicle exposed to the volatiles from *Muscodor* spp.

The methodology used was proposed by Minerdi et al. (2011), with modifications. Seeds of tomato for processing (hybrid N-901, Nunhems Brazil™ Bayer Crop Science) were superficially disinfested (70% ethanol for 5 min and 2% NaClO for 15 min), rinsed in sterile distilled water for three times (15 seconds), both under agitation at 120 rpm, and placed on Petri dishes containing sterile distilled water for pre-germination for 3 days. After emergence of radicles, four seeds were placed on 9-cm diameter Petri dishes containing 0.8% agar-water. In the top layer contained the fungus cultured in PDA medium (20 mL per dish), the plantlets were exposure to the VOCs of fungi in two moments: from first day until eighth day and from

ninth day until sixteenth day of fungal growth. The dishes were incubated 45º inclined in growth cabinet to 12-h light/12-h dark at 25°C and 50% relative humidity for 8 days, with five replicates. For control, plantlets were exposed to PDA medium without the fungi. Root growth was measured every two days with electronic digital caliper.

GC-MS analysis of VOCs

The analyzes were conducted at the Center for Chemical Analysis and Prospecting – CAPQ, Chemistry Department of the Federal University of Lavras (DQI/UFLA). A single fungus was selected to identify its VOCs, the one that presented the most relevant results of antibacterial activity and allelopathy. The volatiles produced between 0 to 8 days of fungus growth were characterized after cultivated in PDA medium into 20 mL SPME vials, in which solid-phase microextraction in the headspace mode (HS-SPME) was used (Arthur and Pawlisyn, 1990). A SPME fiber DVB/CAR/PDMS (Divinylbenzene, Carboxen, and Polydimethylsiloxane) was used, under extraction temperature of 55°C, sample stirring at 250 rpm during 35 min, with further 2 min of desorption in the GC injector. The separation and identification of the VOCs was performed in a GC-MS QP-2010 Ultra (Shimadzu, Kyoto, Japan) gas chromatograph coupled with a mass spectrometer equipped with an AOC-5000 (Shimadzu, Kyoto, Japan) automatic injector for liquids and gases, and an HP-5 (5% phenyl and 95% dimethyl siloxane) 30 m × 0.25 mm × 0.25 µm column. The injector, interface and ion detector temperatures were 250°C, 240°C and 200°C, respectively, in which the injector was operated in the splitless mode. The carrier gas was He 5.0 with a flow of 1.0 mL min^{-1}. The GC oven temperature was increased at a rate of 3°C min^{-1} from 40°C to 160°C and then at 10°C min^{-1} to 240°C.

Chemical identification of VOCs was confirmed by comparison of mass spectra obtained from the Automated Mass Spectral Deconvolution and Identification System (AMDIS) software version 2.63 with the NIST database using the Mass Spectral Search Program software version 1.7 (NIST, Washington DC, USA). *Muscodor* sp. VOCs were identified by retention indices (RI), in which the experimental retention indices (RI Exp.) were obtained by injecting a homologous series of alkanes and comparing to those reported in the literature (RI Lit.) (Adams, 2007; NIST, 2013). For comparing of the volatile compounds was considered mass spectrum with similarity greater than 80%.

Biological control of bacterial wilt and improvement of agronomic attributes in tomato plants

Ten tomato seeds (hybrid N-901) superficially disinfested were arranged equidistant from each other and 1 cm from the fungal mycelial disk in PDA medium (-1.2MPa) (Figure

3B). The seeds were placed slightly after inoculation of the fungus in the culture medium and both incubated at 25ºC for 10 days in the dark. Seeds inoculated were sown in expanded polystyrene tray 5 x 10 cells with cell size of 3.5 x 3.5 cm^2 filled with Tropstrato™, two seeds per cell, and grown in greenhouse for 30 days, with four replicates. Posteriorly were transplanted and after 15 days, with 45 days, were infected via soil, after injury to the root, irrigation with 30 ml of *R. solanacearum* bacterial suspension ($OD_{600\ nm} = 0.1 \approx 10^8$ UFC ml^{-1}). The plants were kept in greenhouse with controlled humidity (≈65%) and temperature (≈27ºC). They were cultivated in semi-hydroponic system fertigated with nutritive solution daily (supplementary material) and tutored with single stem. The fertigation (100 mL per pot) was intercalated with manual localized irrigation to pot capacity and electrical conductivity below 3 dS cm^{-1}.

The visual evaluation of disease severity was performed at 15 days infection with the pathogen, using the Nielsen and Haynes (1960) disease index scale, which: 1, healthy plant; 2, plant with 1/3 of wilted leaves; 3, plant with 2/3 of wilted leaves; 4, totally wilted plant; and 5, dead plant. Scale readings were converted on Bacterial Wilt Index (BWI) proposed by Emping et al. (1962). BWI = Σ (CxP)/N, which: C = assigned grade in each class of symptom; P = number of plants in each symptom class and N = total number of infected plants. The plants were grown in 4L pot according to seedling inoculation experiment and classified for reaction to pathogen: resistant, 1.0-2.0; moderately resistant, 2.1-3.0; moderately susceptible, 3.1-4.0 and susceptible 4.1-5.0 (Morgado et al., 1992). The infected plants were also evaluated for fresh fruit yield and total soluble solids content (°Brix). The °Brix was evaluated after the natural ripening of the fruits, through the pulp collection of each fruit produced, followed by the digital refractometer reading.

Statistical analyze

For all assays, the experimental design was a completely randomized, the data submitted the ANOVA and means were compared with the Scott-Knott test at 5 % of probability in the statistical software SISVAR version 5.6.

Results

Antibacterial activity *in vitro* of VOCs emitted by *Muscodor* species against *Ralstonia solanacearum, Xanthomonas vesicatoria* and *Pseudomonas syringae* pv. *tomato*

Volatiles emitted by six fungi had some inhibitory effect on at least one phytobacter, with representatives of all *Muscodor* species evaluated (Figure 1). VOCs from *M. coffeanum* COAD1842, *M. coffeanum* COAD1900 and *M. vitigenus* CML4014 presented broad spectrum

of action against the three pathogenic bacteria, in which *X. vesicatoria* proved to be the most sensitive to volatiles with total inhibition of its growth. The fungus *M. coffeanum* COAD1900 produced a potent blend of volatile organic compounds with lethal activity on the phytobacteria and, for *X. vesicatoria* and *R. solanacearum*, bactericidal activity (Figure 1).

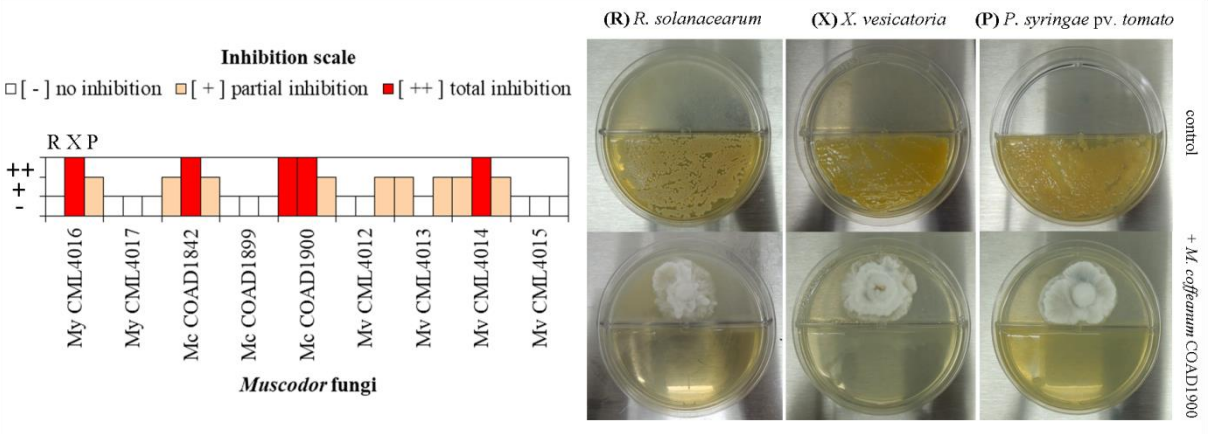


Figure 1. Antibacterial activity *in vitro* of *Muscodor* spp. VOCs against the phytobacteria *R. solanacearum*, *X. vesicatoria* and *Pseudomonas syringae* pv. *tomato*. At the right, effect of exposure to the VOCs of *M. coffeanum* COAD1900 on the phytobacteria growth.

Development of tomato radicle exposed to the volatiles from *Muscodor* spp.

VOCs produced by the fungi demonstrated allelopathic effects on tomato radicles growth, especially from the eighth day of growth (Figure 2).

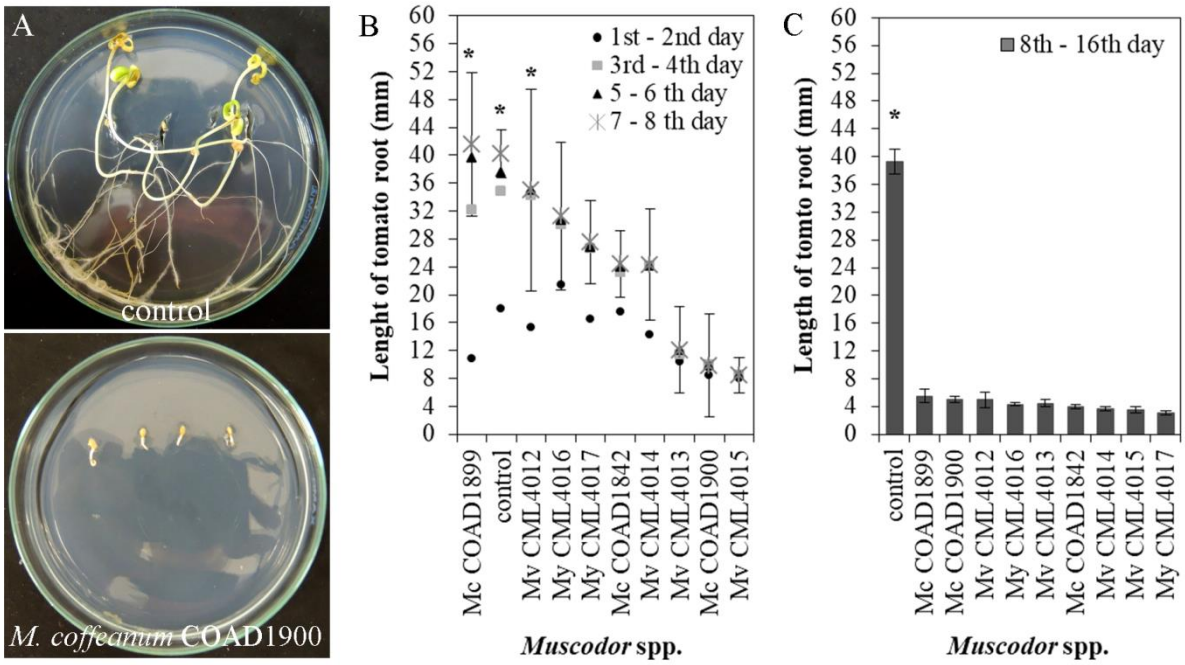


Figure 2. Volatile-exposure assay. (A) Tomato plantlets without exposure to VOCs and after contact with VOCs of *M. coffeanum* COAD1900. (B) Root length of tomato plantlets

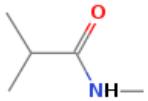
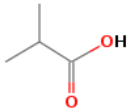
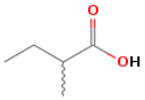
exposure to the VOCs of 0th to 8th *Muscodor* species growth day and (C) from 8th to 16th day. Values indicate means and error bars the standard deviation. Asterisks indicate significant difference at 5% of probability (ANOVA, Scott-Knott test).

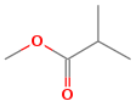
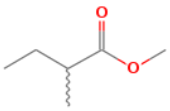
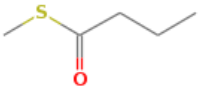
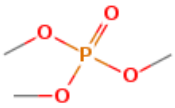
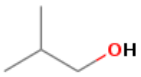
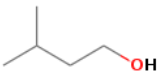
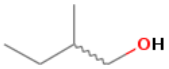
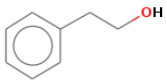
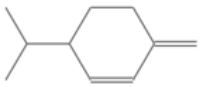
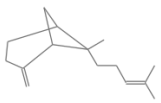
However, in the evaluation until the eighth day of cultivation, some isolates did not showed effect. *M. vitigenus* CML4015 was the fungus that produced allelopathic volatiles earlier, on the second day, with 79% reduction. The others inhibited radicle growth from the fourth day, with the highest intensity for the fungi *M. vitigenus* CML4013 e *M. coffeanum* COAD1900, with 69.9% and 75.5% of reduction, respectively (Figure 2A, B). The VOCs produced by all *Muscodor* species from the eighth day to sixteenth day significantly inhibited growth of tomato radicle, with percentage of inhibition around 89% (Figure 2C).

GC-MS analysis of volatile organic compound

The SPME combined with GC-MS technique revealed the production of 18 compound organic volatiles by the fungi *M. coffeanum* COAD1900, among them, acids, esters, alcohols, terpenes, aldehyde and amide (Table 2). Mass spectral peaks have also been identified whose correct structure of the compounds could not be identified, however, such spectral behavior reveals a standard fragmentation of non-oxygenated sesquiterpenes.

Table 2. GC-MS analysis of the VOCs emitted by *M. coffeanum* COAD1900

Compound	[1]Chemical structure	IR Exp.	IR Lit.
Aldehyde			
Hexanal		801	800
Amide			
Propanamide, N,2-dimethyl-		951	-
Acids			
Propanoic acid, 2-methyl-		823	793
Butanoic acid, 2-methyl-		889	884

Esters			
Propanoic acid, 2-methyl-, methyl ester		680	685
Butanoic acid, 2-methyl-, methyl ester		772	771
Butyric acid, thio-, S-methyl ester		846	-
Methyl phosphate		946	-
Alcohols			
Ethyl alcohol		482	-
1-Propanol, 2-methyl-		624	622
1-Butanol, 3-methyl-		735	734
1-Butanol, 2-methyl-		739	738
Phenylethyl alcohol		1114	1114
Terpenes			
Beta Phellandrene		1030	1025
Alpha Bergamotene		1438	1436
Sesquiterpene	-	1478	-
non-oxygenated sesquiterpene	-	1488	-
non-oxygenated sesquiterpene	-	1657	-

[1]National Institute of Standards and Technology – NIST Standard Reference Database 69: *NIST Chemistry WebBook, 2018.*

Biological control of bacterial wilt and improvement of agronomic attributes in tomato plants

Plants inoculated with *M. coffeanum* COAD1900 have become moderately resistance to bacterial wilt. *M. coffeanum* COAD1842 and *M. vitigenus* CML4012 reduced the susceptibility of tomato plants to moderately susceptible (Figure 2A). In addition, these three fungi increased the fruit yield and total soluble solids of the inoculated plants. *M. coffeanum* COAD1900 was highlighted by the increase of fruit yield in 26.8% and total soluble solids (ºBrix) in 24% (Figure 3C). *M. coffeanum* COAD1842 was the second and *M. vitigenus* CML4012 the third in among those who promoted higher productivity, with 17.3% and 17.1%.

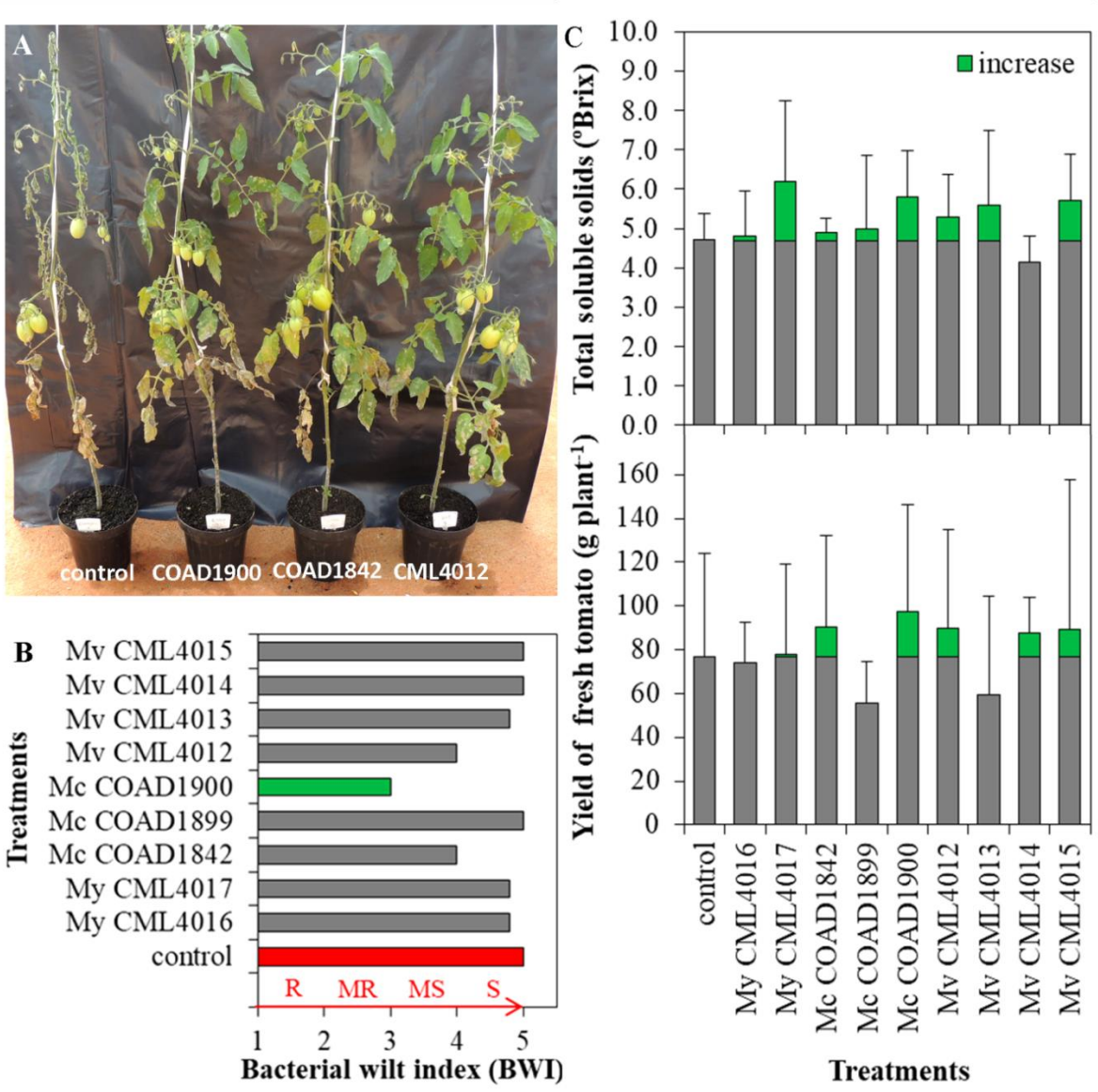


Figure 3. Suppression of bacterial wilt by *Muscodor* species and improvement of agronomic attributes in tomato plants (hybrid N-901). (A) Healthy plants 15 days after *R. solanacearum*

infection. (B) Bacterial Wilt Index (BWI) of plants regarding the reaction to the pathogen: (R) resistant, 1.0-2.0; (MR) moderately resistant, 2.1-3.0; (MS) moderately susceptible, 3.1-4.0 and (S) susceptible 4.1-5.0. (D) Total solid soluble of fruits in complete maturation stage and yield of fresh tomato of plants under infection of pathogen. Values indicate means, error bars the standard deviation (ANOVA, Scott-Knott test at 5%). The averages presented no significant difference.

Discussion

Antibacterial activity against *R. solanacearum*, *X. vesicatoria* and *P. syringae* pv. *tomato* was found for almost all fungi studied. The volatiles emitted by *M. coffeanum* COAD1900 showed, except for *P. syringae*, with partial inhibition, a lethal effect on all phytobacteria. Raza et al. (2016) studying the responses of the tomato wilt pathogen to the VOCs produced by a biocontrol strain *Bacillus amyloliquefaciens*, observed significant inhibited of motility characteristics, production of antioxidants, enzymes and exopolysacharides, biofilm formation and tomato root colonization. *M. albus* presented bactericidal activity against *Erwinia carotovora* pv. *Carotovora* by fumigation *in vitro* (Schotsmans et al., 2008). Monteiro et al. (2017) reported the *in vitro* antimicrobial activity of VOCs emitted by the *M. coffeanum* COAD1900 also against *Botrytis cinerea*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Phoma* sp. and *Rhizoctonia solani*. These results reinforces how promising these compounds are in the biocontrol of the bacterial and fungi phytopathogens.

VOCs from *Muscodor* fungi showed significantly allelopathic activity against tomato radicle, particularly from eighth day of cultivation confirming the possible deleterious effect of volatiles reported for this fungus genus. Macías-Rubalcava et al. (2010) and Siri-Udom et al. (2017) reported allelopaty againsts seed of tomato. However, some species did not produce allelopathic volatiles from 0 to 8 days of growth. The negative impact may be associated with interrupting the plant plasma and organelle membranes or interfering with enzyme activity, besides the oxidative stress related to biotic or abiotic stress responses (Li et al., 2016). However, as observed in inoculation in tomato seedlings, the presence of these metabolites did not significantly interfere with plant growth. Therefore, the effect observed *in vitro* may not represent the true *in vivo* effect.

The fungus *M. coffeanum* COAD1900 produced volatile compounds with both allelopathic and antibacterial activities. Among the compounds, butanoic and propanoic acids are identified, such acids derived from endophytic fungus have already been reported with antifungal and antibacterial activity (Strobel et al., 2001; Metwaly et al., 2016). This effect

was also reported for the compounds hexanal and alcohols (Mari et al., 2016), volatiles produced by the fungus studied in this work.

Tomato plants inoculated with *M. coffeanum* showed moderate resistance against bacterial wilt. In addition to the antibiosis of antibacterial VOCs, molecular patters as chitin and enzymes of the entophytic fungi can act as elicitors inducing plant resistance and stimulating other endophytes (fungi and bacteria) to produce bioactive secondary metabolites, with possible influence on host protection (Zabalgogeazcoa, 2008; Gao et al., 2010; Kusari et al., 2012). Endophytes may prevent the development of the pathogen by several mechanisms, alone or simultaneously. The competition for space and nutrients in the root is another possible mechanism of biocontrol used by the *Muscodor* fungi (Zabalgogiez et al., 2010; Kusari et al. 2012; Li et al., 2017).

Some fungi promoted higher yield in tomato plants, in addition to increasing of ºBrix of fruits, agronomic attributes extremely relevant and high economic return of the tomato for processing. Our results indicate fungi of the genus *Muscodor* with potential to control the bacterial wilt associated with increased tomato plants development.

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