INTERAÇÃO FUNGO-BACTÉRIA E BIOCHAR NA PROMOÇÃO DO CRESCIMENTO E NO MANEJO DA PODRIDÃO DO PÉ DO MAMOEIRO

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CAMPOS DOS GOYTACAZES – RJ
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Orientador: Prof. Silvaldo Felipe da Silveira

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Barroso, Laura Mathias; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. Fevereiro de 2022. Interação fungo-bactéria e biochar na promoção do crescimento e no manejo da podridão do pé do mamoeiro. Orientador: Silvaldo Felipe da Silveira. Coorientadora: Luciana Aparecida Rodrigues.

O mamoeiro (*Carica papaya* L.) é uma das frutíferas tropicais de maior importância econômica no Brasil. Porém, diversas doenças afetam a cultura, em especial a podridão do pé causada pelo pseudofungo *Phytophthora palmivora* Butler (Oomycota). Estudos preliminares conduzidos na UENF demonstram que *Trichoderma longibrachiatum* F476, *Herbaspirillum seropedicae* HRC54 e biochar têm potencial para promover o crescimento do mamoeiro, abrindo possibilidade para serem aplicados como agentes de biocontrole (ABCs) de doenças de solo. Com isso, os efeitos de *T. longibrachiatum* F476 e *H. seropedicae* HRC54, isolados ou em combinação com biochar de cama de aviário foram testados sobre a promoção do crescimento e controle da podridão do pé do mamoeiro. No primeiro artigo foram avaliados o crescimento, a qualidade e a nutrição de mudas de mamoeiro cultivadas em casa de vegetação. Para tanto, 10^6 esporos.mL^{-1} de F476 e/ou 10^8 células.mL^{-1} de HRC54 foram adicionados, ou não, com 1% (v/v) de biochar, e incorporados como inóculo ao substrato comercial Basaplant®. Os tratamentos com biochar apresentaram ganhos de 8% na altura, 12% no diâmetro do caule, 12% na área foliar e 40% na biomassa seca da parte aérea, em média,

em comparação aos tratamentos sem biochar. Além disso, o biochar promoveu aumento significativo na biomassa seca total de raízes e raízes finas, bem como ganho significativo no comprimento total, área superficial e volume radicular, e no conteúdo foliar de nutrientes. Os tratamentos com Biochar produziram mudas mais vigorosas, de maior qualidade e mais eficientes na utilização de S, P e Mn. No segundo artigo, dois métodos de controle biológico contra *P. palmivora* Pp13 foram testados, utilizando F476, HRC54 e biochar: i) no primeiro experimento, o substrato de mudas de mamoeiro foi tratado com os ABCs, posteriormente as mudas obtidas foram transplantadas para solo infestado com Pp13 e; ii) no segundo experimento, o solo infestado com o patógeno foi tratado com os ABCs e após 7 d foi realizado o semeio direto nos vasos contendo solo previamente tratado. No primeiro experimento, F476 aplicado isoladamente propiciou 70% de sobrevivência de mudas, seguido de Biochar+F476 e HRC54, com 60,3 e 56,9%, respectivamente, em relação ao controle não tratado. No segundo experimento, F476+HRC54 apresentou a maior porcentagem de sobrevivência, com 66,8%, seguido por Biochar+HRC54 com 60%, em relação ao controle não tratado. O tratamento do substrato de mudas com F476 reduziu em 18,9% a mortalidade de plantas, enquanto o tratamento de solo com F476+HRC54 reduziu em 42,9% a mortalidade das mudas em relação à mortalidade verificada na condição sem tratamento biológico. F476 e HRC54 apresentam potencial para serem utilizados como agentes de biocontrole contra *P. palmivora* Pp13, entretanto as respostas obtidas são dependentes do método de tratamento aplicado. A adição de 1% de biochar não apresentou alterações significativas na sobrevivência de mudas, mas maximizou os efeitos benéficos de F476 e HRC54 no biocontrole da doença, reduzindo a mortalidade de mudas. A partir dos dados obtidos nesta tese, abre-se a possibilidade para desenvolvimento de um bioinoculante contendo fungo-bactéria-biochar, com aplicabilidade na promoção do crescimento e manejo de doenças de solo.

ABSTRACT

Barroso, Laura Mathias; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro. February de 2022. Fungal-bacterial interaction and biochar in plant growth-promotion and control of papaya root rot. Advisor: Silvaldo Felipe da Silveira. Co-supervisor: Luciana Aparecida Rodrigues.

Papaya (*Carica papaya* L.) is one of the most important tropical fruits for the Brazilian economy. However, several diseases affect its crop, mainly root rot, which is caused by pseudofungus *Phytophthora palmivora* Butler (Oomycota). Preliminary studies conducted at UENF have shown that *Trichoderma longibrachiatum* F476, *Herbaspirillum seropedicae* HRC54 and biochar have the potential to promote papaya growth, and it gives these species the possibility of being applied as biocontrol agents (BCAs) for soil diseases. Therefore, the effects of *T. longibrachiatum* F476 and *H. seropedicae* HRC54, either alone or in combination to poultry litter biochar, were tested on growth promotion and control of papaya root rot. The growth, quality and nutrition of papaya seedlings grown in greenhouse were evaluated in the first article. Thus, 10^6 spores.mL^{-1} of F476 and/or 10^8 cells.mL^{-1} of HRC54 were added (or not) with 1% (v/v) biochar and incorporated as inoculum to the commercial substrate Basaplant®. Treatments with biochar showed 8% gain in height; 12%, in stem diameter; 12%, in leaf area; and 40%, in shoot dry biomass, on average, in comparison to treatments without biochar. In addition, biochar promoted significant increase in total root and fine

root dry biomass, as well as significant gain in total length, surface area and root volume and leaf nutrient content. The treatments containing biochar produced more vigorous, higher quality and seedlings more efficient in utilization S, P and Mn. Two biological control methods against *P. palmivora* Pp13 were tested by using F476, HRC54 and biochar in the second article, namely: i) papaya seedlings' substrate was treated with BCAs and subsequently transplanted to soil infested with Pp13, in experiment 1 and; ii) soil infested with the pathogen was treated with BCAs; seven days later, seeds were directly sown in pots containing previously treated soil in experiment 2. The single application of F476 enabled 70% seedling survival in experiment 1; it was followed by Biochar+F476 and HRC54, which recorded 60.3% and 56.9%, respectively, in comparison to the untreated control. F476+HRC54 presented the highest survival rate in experiment 2 (66.8%) in comparison to the untreated control; it was followed by Biochar+HRC54 (60%). The treatment based on seedling substrate added with F476 reduced plant mortality by 18.9%, whereas soil treatment based on the addition of F476+HRC54 reduced seedling mortality by 42.9%, in comparison to the mortality recorded for samples without biological treatment. F476 and HRC54 have the potential to be used as biocontrol agent against *P. palmivora* Pp13; however, the observed responses dependent on the adopted treatment method. The addition of 1% biochar did not show significant changes in seedling survival, but it has maximized the beneficial effects of F476 and HRC54 on disease biocontrol since it reduced seedling mortality. Based on data in the current thesis, there is the possibility of developing a bio-inoculant containing fungus-bacteria-biochar, applicable to plant growth-promotion and management of soil diseases.

1 INTRODUÇÃO

O mamoeiro (*Carica papaya* L.) é uma das frutíferas tropicais de maior importância econômica na produção nacional, com destaque para o Norte do estado do Espírito Santo e Sul da Bahia (IBGE, 2020). Apesar da alta produtividade brasileira e mundial, diversas pragas e doenças afetam essa planta. A podridão-do-pé é uma das principais doenças fúngicas do mamoeiro, ocasionando perdas que podem atingir entre 10 e 60% da produção, especialmente em locais com altos índices pluviométricos e temperaturas elevadas (Silva, 2001). As raízes são mais suscetíveis nos três primeiros meses após a germinação da semente, em que o patógeno ataca as raízes laterais, e se alastra pelo sistema radicular, apodrecendo-o (Silva, 2001).

O principal agente etiológico causador de podridões radiculares em mamoeiro no Brasil é o oomiceto *Phytophthora palmivora* (Butler). Medidas de controle efetivas contra *P. palmivora* no controle da podridão-do-pé do mamoeiro inexistem e não há variedades comerciais de mamoeiro resistentes a essa doença (Oliviera et al., 2014a). Entretanto, fungos do gênero *Trichoderma* e bactérias promotoras do crescimento vegetal já foram descritas no biocontrole de doenças fúngicas, incluindo doenças causadas por *Phytophthora* spp. em inúmeras culturas agrícolas (Lopes e Michereff, 2018). A associação entre esses agentes benéficos promove o crescimento vegetal pela ação direta de microrganismos produtores de fitormônios, solubilizadores de minerais e fixadores

de nitrogênio e diminui ocorrência de doenças devido à competição, antibiose, parasitismo ou predação e indução sistêmica de resistência do hospedeiro (Egamberdieva et al., 2016). Além disso, o biochar destaca-se como um composto capaz de melhorar a qualidade física do solo, aumentar o estoque de carbono, os níveis de nutrientes, aumentar a atividade biológica e diversificar as populações microbianas, em alguns casos, estimulando ou favorecendo o controle biológico a patógenos do solo (Nóbrega, 2011; Biederman et al., 2013; Chaer et al., 2014).

Foi demonstrado, em mamoeiro, que a coinoculação de *T. longibrachiatum* F476 e *H. seropedicae* HRC54 em substrato de mudas aumentou significativamente a massa fresca e seca da parte aérea e da raiz de mudas de mamoeiro (Reis, 2018), aos 25 dias após o semeio. Por sua vez, a adição de 1% (v/v) de biochar em solo, incrementou a biomassa seca de mudas de mamoeiro, avaliada aos 90 e 150 dias após o semeio (Silva, 2019; Barcelos, 2016). Considerando os ganhos isolados de cada aplicação, a coinoculação de bactérias e fungos, e os prováveis benefícios de se adicionar matéria orgânica estabilizada, como o biochar, propôs-se avaliar essas associações, na produção de mudas do mamoeiro e no biocontrole da podridão-do-pé, causada por *P. palmivora.* Esta associação visa o desenvolvimento de um cultivo sustentável e menos dependente de agrotóxicos, a partir da produção de mudas mais sadias e vigorosas, em substratos supressivos.

2 REVISÃO BIBLIOGRÁFICA

2.1 A cultura do mamoeiro

O mamão (*Carica papaya* L.) é um fruto que consiste principalmente de água e carboidratos, rico em minerais e vitaminas, particularmente A e C, além de compostos antioxidantes (Huerta-Ocampo et al., 2012). Também produz papaína e quimopapaína, duas enzimas proteolíticas de importância industrial, encontradas no látex branco leitoso exsudado pelo fruto. O látex serve como um excelente amaciador de carne, também utilizado no tratamento de queimaduras e ainda na fabricação de cosméticos (Ming et al., 2012). O látex de leite de mamão também apresenta propriedades antibacterianas e antifúngicas. Além disso, extratos de frutas e sementes têm pronunciada atividade bactericida (Krishna et al., 2008; De Oliveira, et al., 2011).

Dado a apreciação do fruto *in natura*, suas características nutritivas e aplicações industriais, a produção de mamão tem grande destaque comercial no Brasil e no mundo, sendo uma das frutíferas mais cultivadas e consumidas nas regiões tropicais e subtropicais do mundo (Silva et al., 2007). Segundo a *Food and Agriculture Organization of the United Nations* (FAO), em 2019, a produção mundial de mamão girou em torno de 13,7 milhões de toneladas. Seu cultivo ocorre em quase todos os países das Américas tropicais e também é cultivado na Índia, Sri Lanka, vários países asiáticos, e nos países tropicais da África (Benassi e Cattaneo, 2010). No Brasil, o estado da Bahia é o maior produtor, seguido pelo

estado do Espírito Santo. Juntos, esses estados participam com, aproximadamente, 70% da produção nacional, alcançando aproximadamente 1,17 mi de toneladas no ano de 2019 (IBGE, 2019). O país foi o terceiro maior produtor mundial de mamão, ficando atrás somente da Índia e da República Dominicana (FAO, 2019).

Entretanto, as lavouras de mamoeiro são frequentemente atacadas por doenças bióticas, ocasionando perdas econômicas significativas na produção, venda e exportação dos frutos (Zhu e Jia, 2016). No Brasil, as principais doenças fúngicas que acometem o mamoeiro são a antracnose, a pinta-preta, oídios e a podridão-do-pé e dos frutos (Ventura et al., 2004).

2.2 Podridão do pé do mamoeiro causada por *Phytophthora palmivora*

O agente etiológico da podridão-do-pé do mamoeiro é *Phytophthora palmivora* Butler (*Phytophthoraceae*, Peronosporales, Oomicota, Stramenopili). Esse patógeno hemibiotrófico possui micélio cenocítico, heterotálico (5-8 µm) e produz esporângios terminais e intercalados, caducos e papilados (50-33µm) em abundância (Figura 1A e 1B). Os zoósporos biflagelados (entre 10 e 40) são formados dentro de esporângios maduros. Também produz clamidosporos globosos (35 a 45 µm de diâmetro), estrutura de resistência, que constitui um dos principais inóculos no solo (Figura 1C). A espécie *P. palmivora* é patogênica às diversas plantas cultivadas. Além do mamoeiro, causa doenças em coqueiro e palmáceas, pimentas, cacau, citrus e seringueira, dentre outras (Erwin e Ribeiro, 1996; Drenth e Guest, 2012; Maizatul-Suriza et al., 2019).

No campo, temperaturas entre 25-30 ºC, alta umidade e solos mal drenados favorecem o desenvolvimento da podridão-do-pé do mamoeiro, que pode incidir em qualquer idade da planta. *P. palmivora* pode ser introduzida no pomar via substratos de mudas, plântulas infectadas e água de irrigação. Quando em condições favoráveis, os esporângios liberam zoósporos móveis, que atraídos quimiotaticamente e eletrostaticamente pelos exudados da raíz encistam e germinam. Após a germinação de um zoósporo, um tubo germinativo emerge e cresce em toda a superfície da planta até o desenvolvimento de um apressório, que tem a função de penetrar nas células epidérmicas (Fawke et al., 2015). O patógeno produz um grande número de esporângios na superfície dos tecidos doentes, principalmente quando a temperatura está próxima a 25 ºC. Por outro

lado, a produção de esporângios não ocorre quando a temperatura é inferior a 15 ºC ou superior a 35 ºC. Na estação seca, os clamidosporos permancem dormentes no solo e germinam em condições de alta umidade, coincidindo com o crescimento das raízes e reinicando um novo ciclo infeccioso (Luz, 2001; Oliveira et al., 2014b).

Em plantas jovens de mamoeiro, *P. palmivora* inicia infecção normalmente a partir de raízes laterais e progride para a raiz principal, apodrecendo todos os tecidos parenquimáticos. Todo o sistema radicular fica apodrecido e com odor fétido, pela presença de colonização bacteriana oportunista. Consequentemente a parte aérea colapsa e a planta morre (Figura 1D). Plantas mais velhas resistem mais tempo, mas, com o total anelamento basal, não raramente, a planta perde a sustentação física e tomba. Consequentemente, ocorre também queda prematura de frutos, murcha do topo e morte das plantas adultas. Sintomas semelhantes nas partes aéreas decorrem também da podridão-do-colo, mais caracterizada por uma lesão de tecidos encharcados na região do coleto. Nos frutos, ocorre uma podridão-mole dos tecidos e estes são cobertos pelo micélio branco do fungo, onde são produzidas grandes quantidades de esporângios e clamidosporos (Figura 1E) (Ventura et al., 2004).

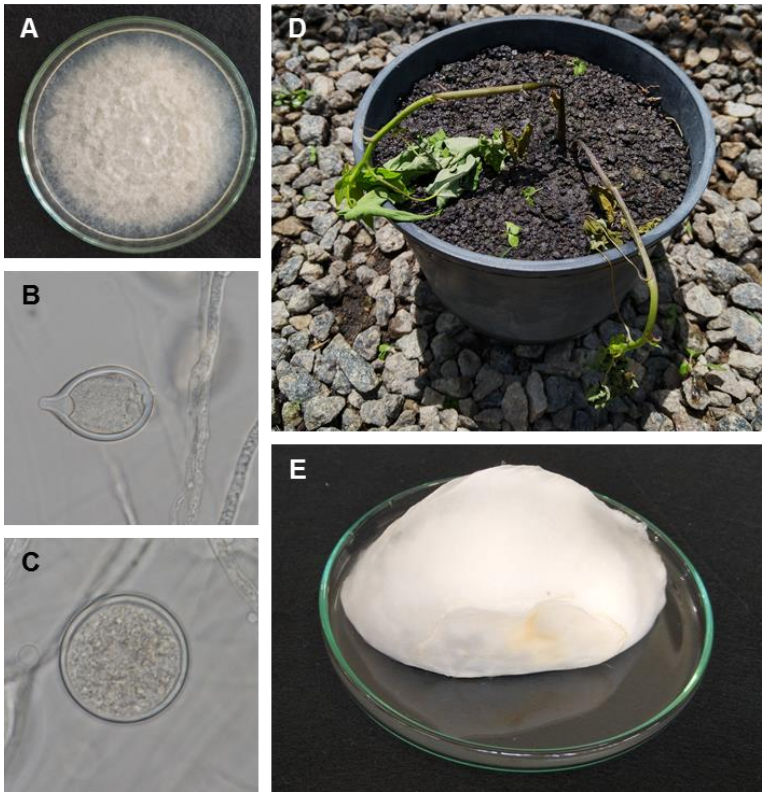

Figura 1: Características culturais e morfológicas de *P. palmivora* Pp13. A) Colônia de Pp13 em meio BDA; B) Esporângio ovoide e papilado; C) clamidósporo isolado; D) Mudas de mamoeiro com sintomas de murcha e podridão basal, após inoculação com *P. palmivora* Pp13; E) fruto de mamão verde com podridão-mole, coberto pelo micélio de Pp13.

Algumas das medidas de controle cultural incluem: evitar o plantio em solos excessivamente argilosos, mal drenados e em regiões com alta pluviosidade; promover o plantio em curvas de nível e camaleões; cultivar em solos onde o patógeno não foi relatado; utilizar substrato esterilizado para mudas; utilizar sementes tratadas com fungicidas; evitar lesões nas plantas durante as capinas; remover plantas doentes e frutos do pomar (Erwin e Ribeiro, 1996).

O patógeno, habitante do solo, não é eficientemente controlado pela aplicação de fungicidas, os quais não têm efeito descendente na planta e mesmo a aplicação no solo, via sementes ou encharcamento, não promovem a proteção das raízes (Santos et al., 2017). Como o controle químico não é medida eficaz para a podridão-do-pé do mamoeiro e uma vez que não existe cultivares comerciais resistentes à *P. palmivora* (Oliviera et al., 2014a), o controle biológico pode ser uma alternativa mais eficiente a ser utilizada na produção de mudas e no tratamento do solo na cultura do mamoeiro.

2.3 Microrganismos promotores do crescimento vegetal e sua aplicação no biocontrole de *Phytophthora* spp.

Segundo Cook e Baker (1983), controle biológico consiste na redução da quantidade de inóculo ou da atividade causadora de doença de um patógeno feita por um ou mais organismos, que não o homem. Contudo, um método efetivo para o controle biológico de *P. palmivora* ainda não foi estabelecido. Tal fato pode ser justificado devido à versatilidade do patógeno, capaz de produzir diferentes estruturas e tipos de inóculo; à sua ampla gama de hospedeiros; e à sua capacidade de sobreviver no solo, mediante crescimento saprofítico ou produzindo esporos de resistência (clamidósporos) (Erwin & Ribeiro, 1996).

Estudos demonstram que o biocontrole vem apresentando resultados promissores em diferentes culturas agrícolas contra *P. palmivora*. Em seringueira, a pulverização foliar de extrato de alga marrom (*Sargassum polycystum*) estimulou a síntese de enzimas antioxidantes e metabólitos de indução de resistência sistêmica, indicando um possível agente de biocontrole contra a queda foliar (Khompatara et al., 2019). Em cacau, *Pseudomonas chlororaphis* CP07 tem potencial para induzir defesa da planta contra a podridão-negra, dependendo do genótipo (Acebo-Guerrero et al., 2015; Miguelez-Sierra et al., 2019). Enquanto que *Pseudomonas aeruginosa* e *Chryseobacterium proteolyticum* reduziram as lesões da podridão-negra em frutos, com 100% de inibição de *P. palmivora* (Alsultan et al., 2019). Fungos endofíticos isolados de frutos de cacau (*Aspergillus* sp., *Fusarium* sp. e *Ramichloridium* sp.) demonstraram atividade antagônica *in vitro* contra *P. palmivora*, embora não foram capazes de suprimir o desenvolvimento da doença no campo (Simamora et al., 2021).

Embora o controle biológico ainda não seja uma prática generalizada para o agronegócio, tampouco para agricultores familiares, há falta de produtos comerciais visando atender a esta necessidade, embora existam exemplos de pesquisas de sucesso com o uso de espécies de *Trichoderma* no biocontrole de *Fusarium*, *Pythium, Rhizoctonia*, *Macrophomina*, *Sclerotinia*, *Sclerotium*, *Botrytis, Crinipellis e Phytophthora*, nas culturas do feijão, soja, algodão, fumo, morango, tomate, cebola, alho, plantas ornamentais, cacau e maçã (Bettiol e Morandi, 2009).

Em relação aos fungos, o gênero *Trichoderma* representa um dos principais agentes de biocontrole de fitopatógenos utilizados na agricultura

(Machado et al., 2012). A aplicação de *Trichoderma* spp. já foi utilizada como estratégia para o controle de podridão negra do cacau (Hanada et al., 2009; Harni et al., 2020), desfolha da seringueira (Promwee et al., 2017) e podridão mole de frutos de mamão (De Oliveira et al., 2018). Porém, até o momento, estudos de controle biológico infestando substratos com *Trichoderma* spp., em períodos anteriores à infestação com *P. palmivora,* contrastam entre si quanto à efetividade do antagonismo no controle da podridão-do-pé do mamoeiro (Tatagiba et al., 2005; Carnaúba, 2006; Tocafundo, 2008; Tavares et al., 2009; Dianese et al., 2012; Sánchez-Rangel et al., 2016; Soesanto et al., 2019).

Além do potencial antagônico direto, devido à produção de diversas classes de antibióticos e parasitismo aos fungos fitopatogênicos, espécies de *Trichoderma* também competem por nicho contra microrganismos patogênicos e atuam na indução de resistência nas plantas. Além disso, esse gênero fúngico é capaz de promover o crescimento vegetal, direta ou indiretamente, mediante solubilização de fósforo e outros nutrientes; produção de fitormônios, que aumentam o crescimento de raízes e a absorção de nutrientes; e melhoria na eficiência de uso do nitrogênio pelas plantas – que de modo geral, estimulam a produção de mudas mais vigorosas e mais resistentes (Harman et al., 2004; Waghunde et al., 2016).

Bactérias promotoras do crescimento vegetal também foram descritas no biocontrole de doenças fúngicas em plantas como tomate, feijão, algodão e batata (Mariano et al., 2004; Berg, 2009), incluindo *Bacillus subtillis* e *Bacillus pumilus* que atuam no controle de *Phytophthora* em maçã, limão e pimentão (Utkhede e Smith, 1991; Amorim e Melo, 2002; Lee et al., 2008). Palmieri et al. (2019) utilizaram separadamente duas cepas de *Photorhabdus* spp., aplicadas uma semana após a infestação com *P. palmivora* no solo, contendo mudas de mamoeiro. Nestas condições, houve uma redução de 40% na incidência de doença, e uma sobrevivência entre 100 a 87%, de acordo com a cepa aplicada, 46 dias pós-semeio.

Porém, a coinoculação de bactéria e fungos pode ser uma estratégia para maximizar as respostas benéficas desses microrganismos no sistema solo-planta, resultando em efeitos biofertilizantes, bioestimulantes, biocontrole, biorremediação e indução de resistência de estresses abióticos (Bashan et al., 2014). Sukhada et al. (2011) demonstram que inóculos prévios de *Glomus*

mosseae, *Trichoderma harzianum* e *Pseudomonas fluorescens* promovem o crescimento de mudas de mamoeiro e reduzem a severidade da doença causada por *Phytophthora parasitica* em comparação ao controle não inoculado, em experimentos de vasos e em condições de campo. A aplicação combinada de metabólitos isolados de *P. fluorescens* e *T. harzianum* também revelou um efeito promissor no controle de *Phytophthora* spp., com a obtenção de 69,2% de folhas sadias se comparado à condição com uso de fungicida Mancozeb em mamoeiro (Soesanto et al., 2019).

Em trabalho desenvolvido por Reis (2018), no Laboratório de Biologia Celular e Tecidual (LBCT) da UENF, a coinoculação de *Trichoderma longibrachiatum* F476 e da bactéria *Herbaspirillum seropedicae* HRC54 aumentou significativamente a massa fresca e seca da parte aérea e da raiz de mudas de mamoeiro. *H. seropedicae* vem sendo amplamente estudada por seus efeitos positivos na promoção do crescimento vegetal, nas culturas de sorgo, arroz, cana-de-açúcar, feijão, milho, tomate e abacaxi (Baldani et al., 1986, Olivares et al., 2017), além do mamoeiro (Reis, 2018). Entretanto, diferentemente de *Trichoderma* spp., até o momento não há relatos sobre seus efeitos no controle de fitopatógenos de solo.

2.4 Biochar

O uso de um inoculante misto, à base de *Trichoderma* spp. e bactérias benéficas, com efeito sinérgico, em uma base orgânica, como por exemplo, o biochar, pode oferecer maiores chances de sucesso, no estabelecimento dos agentes biológicos e no desalojamento do patógeno, podendo ser uma estratégia mais eficiente para o controle da podridão-do-pé mamoeiro.

O biochar é um produto rico em carbono resultante da pirólise de biomassa orgânica natural. A produção de biochar é um processo de decomposição térmica, entre 400 e 800 ºC e ocorre sob limitado suprimento ou na ausência de oxigênio (Bridgwater, 2012; Mukherjee e Zimmerman, 2013). A matéria-prima para a produção de biochar pode ser oriunda de resíduos vegetais ou animais da agroindústria, tornando-o um subproduto que pode seguramente ser utilizado na agricultura. A composição estrutural e química do biochar varia de acordo com a matéria-prima utilizada e o método de processamento. Mas, de modo geral, possuem cor escura, pH neutro a básico, alto conteúdo de C e baixo

de N (Lehmann et al., 2011). O produto obtido tem potencial como condicionante do solo, melhorando suas propriedades físicas, químicas e biológicas (Lone et al., 2015). Além disso, melhora a disponibilidade de nutrientes, devido à presença de cargas negativas na superfície do biochar (CTC), sua aplicação aumenta a retenção e reduz a lixiviação de nutrientes, aumentando a fertilidade do solo (Méndez et al., 2013; Gao et al., 2016).

Apesar da grande diversidade ecológica e funcional, a natureza heterotrófica dos microrganismos que compõem a microbiota edáfica apresenta elevada demanda por substratos orgânicos reduzidos, que servem como fonte de energia, carbono e sais minerais. Além dos benefícios nutricionais às plantas, o biochar (ou biocarvão) pode aumentar a atividade biológica, alterar a microbiota do solo, bem como incrementar o biocontrole de fitopatógenos habitantes do solo. Pelo menos cinco mecanismos diferentes têm sido propostos ao se atribuir ao biochar atividade associada ao controle de doenças e ao crescimento das plantas: (i) indução de resistência sistêmica nas plantas hospedeiras; (ii) aumento da abundância e/ou atividades de microrganismos benéficos; (iii) modificação da qualidade do solo em termos de disponibilidade de nutrientes e de matéria orgânica; (iv) efeito antifúngico direto aos patógenos de solo; (v) sorção de compostos fitotóxicos alelopáticos (Bonanomi et al., 2015).

Vecstaudza et al. (2018) descreveram que a sobrevivência de *Trichoderma* spp. no solo foi consideravelmente melhorada quando aplicado em associação com 5% de biochar, após 35 dias de cultivo de centeio. O uso de *T. asperellum* associado a um composto orgânico, produzido a partir de resíduos de videira e turfa preta, mostrou-se eficaz em reduzir a abundância de *Phytophthora nicotianae* no substrato e consequentemente reduzir os sintomas da podridão em pimenta (Ros et al., 2017). Foi demonstrado também que quando aplicado no solo, o biochar induz a resistência sistêmica aos fungos patogênicos e reduz a taxa de progresso de doenças de solo em aspargos (*Fusarium oxysporum, Fusarium proliferatum),* pepino *(Rhizoctonia solani),* feijão *(Rhizoctonia solani)* e tomate (*Botrytis cinerea*) (Graber et al., 2014; Mehari et al., 2015).

Em mudas de bordo vermelho (*Acer rubrum*) e carvalho americano (*Quercus rubra*), cultivados em casa de vegetação e inoculadas com *Phytophthora cinnamomi e Phytophthora cactorum*, o subtrato foi tratado com doses crescentes de biochar (0, 5, 10 e 20%). A mistura com 5% de biochar

resultou em redução da expansão de lesões e aumento na biomassa do caule, em relação às plantas controle, sem biochar (Zwart e Kim, 2012). Assim, o biochar tem sido considerado um aditivo promissor a ser avaliado no contexto do biocontrole de doenças de plantas, podendo colaborar com o aumento da supressividade aos patógenos do solo, desfavorecendo o estabelecimento destes ou estimulando o controle biológico natural (Graber et al., 2014). No entanto, não há, até o presente momento, estudos que correlacionem os efeitos da interação fungo-bactéria-biochar na promoção do crescimento e/ou no controle da podridão do pé do mamoeiro. Sendo uma cultura severamente afetada por patógenos (Zhu e Jia, 2016), a produção de mudas mais vigorosas pode ter impacto direto na produtividade, com potencial para i) reduzir o tempo de cultivo no viveiro, ii) aumentar a sanidade das mudas, iii) estimular a resistência e tolerância ao estresse e iv) aumentar a sobrevivência das mudas no pós-transplantio.

3 TRABALHOS

3.1 BIOCHAR AND PLANT GROWTH-PROMOTING MICROORGANISMS INCREASE BIOMASS AND QUALITY OF PAPAYA SEEDLINGS

RESUMO

Herbaspirillum seropedicae e *Trichoderma longibrachiatum* vêm sendo testados em substratos de mudas para culturas economicamente importantes devido às suas caracteristicas promotoras do crescimento de plantas. Analisamos o efeito de *H. seropedicae* HRC54 e *T. longibrachiatum* F476, isolados ou em combinação com biochar de cama de aviário, sobre o crescimento, a qualidade e a nutrição de mudas de mamoeiro cultivadas em casa de vegetação. 10^8 células.mL^{-1} de HRC54 e/ou 10^6 esporos.mL^{-1} de F476 foram adicionados, ou não, com 1% (v/v) de biochar, e foram incorporados como inóculo ao substrato comercial Basaplant®. Sementes de mamão (cv. Calimosa) foram semeadas nesses substratos tratados (tubos de plástico de 290 cm^3); após 45 d do semeio as mudas foram transplantadas para vasos de 6 L cheios de solo, sem reposição de inóculo ou de biochar, e mantidas em casa de vegetação (30% de

sombreamento). A população bacteriana e fúngica total, o teor de nutrientes nas folhas e as medidas morfométricas da parte aérea e das raízes das plantas foram avaliadas 90 d após o semeio. A análise de variância (ANOVA) seguida do teste de Tukey (P≤0,05) foram adotadas para comparar os dados quantitativos. Não houve diferença significativa na população bacteriana total, mas a inoculação microbiana do substrato aumentou significativamente a população fúngica. Os tratamentos com biochar apresentaram ganhos de 8% na altura, 12% no diâmetro do caule, 12% na área foliar e 40% na biomassa seca da parte aérea, em média, em comparação aos tratamentos sem biochar. Além disso, o biochar promoveu aumento significativo na biomassa seca total de raízes e raízes finas, bem como ganho significativo no comprimento total, área superficial e volume radicular. Tais efeitos foram mais evidentes nas combinações de *H. seropedicae* HRC54 + Biochar e *T. longibrachiatum* F476 + Biochar, uma vez que ambos os tratamentos produziram mudas mais vigorosas, de maior qualidade e mais eficientes na utilização de S, P, K e Mn.

Palavras-chave: *Carica papaya* L., *Herbaspirillum seropedicae* HRC54, *Trichoderma longibrachiatum* F476, Índice de qualidade de Dickson.

ABSTRACT

Herbaspirillum seropedicae and *Trichoderma longibrachiatum* have been tested in seedling substrates of economically important crops due to their plant–growth promotion feature. We analyzed the effect of *H. seropedicae* HRC54 and *T. longibrachiatum* F476, isolated or in combination with aviary bedding biochar, on the growth, quality and nutrition of papaya seedlings grown under shade house conditions. 1 mL of suspension containing 10^8 cells.mL^{-1} of HRC54 and/or 1 mL of suspension containing 10^6 spores.mL^{-1} of F476 were added, or not, with 1% (v/v) of biochar, and were incorporated as inoculum to commercial Basaplant® substrate in plastic tubes of 290 cm³. Papaya (cv. Calimosa) was sown in these treated substrates; 45–day old seedlings were transplanted to 6-L pots filled with soil,

without inoculum or biochar replacement, and maintained in 30% shade cloth greenhouse for growth purposes. Total bacterial and fungal population, leaf nutrient content and the morphometric measures of plant shoots and roots were evaluated 90 days after sowing. Variance analysis (ANOVA) followed by Tukey test (P≤0.05) were adopted to compare quantitative data. There was no significant difference in total bacterial population, but the microbial inoculation of seedling substrate has significantly increased the fungal population. Treatments with biochar has shown gains by 8% in height, 12% in stem diameter, 12% in leaf area and 40% in shoot dry biomass, on average, in comparison to treatments without biochar. In addition, biochar promoted significant increase in the total dry biomass recorded for roots and fine roots, as well as significant gain in total length, surface area and root volume, and leaf nutrient content. The treatments containing biochar produced more vigorous, higher quality and seedlings more efficient in utilization S, P and Mn.

Keywords: *Carica papaya* L., *Herbaspirillum seropedicae* HRC54, *Trichoderma longibrachiatum* F476, Dickson quality index.

INTRODUCTION

In 2019, Brazil produced 1.16 million tons of papaya, it is the third-largest papaya producer in the world, behind India and the Dominican Republic (FAOSTAT, 2021), with U$ 203 million turnover. The European Union accounted for buying 88% of Brazilian papaya fruits export destinations (IBGE, 2020). Given the economic and expansion potential of papaya crops in the tropics and the growing demand for sustainable agricultural practices, using beneficial microorganisms and soil conditioners can stimulate papaya seedlings' growth, reduce costs with fertilizers, promote seedlings' escape to soil pathogens, as well as induce resistance to pests and diseases and minimize environmental impacts by reducing the need of chemicals.

Different microbial species, mainly diazotrophic bacteria, such as *Herbaspirillum seropedicae,* have been assessed as an attempt to accomplish crop yield gains given their ability to plant growth-promoting (Glick, 2012). This bacterium is acknowledged by its positive effects on sorghum, rice, sugarcane, beans, corn, tomato and pineapple growth (Baldani et al., 1992; Baldani et al., 2000; Monteiro et al., 2012; Olivares et al., 2017; Dos Santos et al., 2019). Similarly to bacteria, fungal species such as *Trichoderma* spp. also indirectly or directly stimulate plant growth, because they are capable of producing several bioactive compounds that have antibiosis effect on pathogens; and as well as produce phytohormones, solubilize phosphorus, increase uptake of nutrients and water by the roots, increase the emission of secondary roots and improve the root system architecture (Harman et al., 2004; Chacón et al., 2007; Schuster and Schmoll, 2010; Pandya et al., 2011).

In parallel, biochar stands out as soil conditioner by improving its physical, chemical and biological properties (Najar et al., 2015). Applying biochar to soil increases nutrient retention, reduces leaching and increases fertility due to negative charges on biochar's large specific surface. This process favors cation exchange capacity in the soil-plant system (Méndez et al., 2013; Gao et al., 2016). In addition to influencing plant growth and development, biochar also changes soil microbial activity and composition (Lehmann et al., 2011). High compost porosity and the proportion of pore spaces filled with water - where microorganisms are protected from desiccation, pH changes, competition and predation – are among the main factors influencing microorganism inocula survival in the soil due to using biochar as conditioner (Hale et al., 2015; Głodowska et al., 2017).

The co-inoculation of *H. seropedicae* HRC54 and *T. longibrachiatum* F476 has significantly increased papaya seedlings' shoot and root fresh and dry mass (Reis, 2018) 25 days after sowing. The addition of 1% (v/v) biochar to the soil increased papaya seedlings' dry biomass 90 and 150 days after sowing (Silva, 2019; Barcelos, 2016). Thus, bacteria and fungi co-inoculation in organic basis, such as biochar, can be an interesting strategy to maximize soil-plant system responses if one takes into account the gains of each application, in separate. Furthermore, papaya crops are severely affected by pathogens (Zhu and Jia, 2014), and the production of more vigorous seedlings may have direct impact on the crop with the potential to i) reduce cultivation time in the nursery, ii) improve

seedlings' health, iii) stimulate resistance and tolerance to stress, and iv) increase seedlings post-transplantation survival rates. Accordingly, we analyzed *H. seropedicae* HRC54 and *T. longibrachiatum* F476 effect on the growth, quality and nutrition of papaya seedlings, in separate or in combination with biochar.

MATERIALS AND METHODS

Experimental conditions

The experiments were carried out in greenhouse with 30% shade cloth at the Laboratory of Entomology and Phytopathology of Darcy Ribeiro State University of North Fluminense (Campos dos Goytacazes, Rio de Janeiro), between June 5 and October 20, 2019. Mean maximum temperature reached 22.7 ± 4 ºC and mean minimum temperature reached 21.6 ± 3.7 ºC; total rainfall in the analyzed period was 191.7 mm (INMET, 2019).

Obtaining, multiplying and calibrating microorganisms inoculum

Bacterium species *H. seropedicae* HRC54 (Baldani et al., 1996) was multiplied in liquid DIGS medium (Döbereiner et al., 1999) under constant agitation for 48 h, at 30 °C; inoculum concentration was adjusted based on optical density equivalent to the suspension of 10^8 cells.mL^{-1}. *T. longibrachiatum* F476 (Reis et al., 2020) was cultivated in commercial potato-dextrose-agar (PDA) medium for seven days, at 28 ºC; the inoculum consisted of conidial suspension with adjusted density of 10^6 spores.mL^{-1}, after counting in Neubauer hemacytometer.

Biochar obtainment, characteristics and concentration

Biochar was produced with poultry bed pyrolysis at 400 ºC, as described by Lin et al. (2012), SP – Pesquisa e Tecnologia Ltda. (SPPT), Mogi-Mirim (São Paulo, Brazil). The biochar concentration applied to the papaya substrate was 1% (v/v); it corresponded to 2.76 g.dm^{-3} (5.5 t.ha^{-1}) (Silva et al., 2019). The generated product presented the following chemical composition (g.kg^{-1}): N total = 31.80;

P=29.40; K = 47.20; Ca = 48.30; Mg = 14.60; S = 10.00; A1 = 15.50; Na = 7.30; Fe = 8.50; Zn = 1.07; Cu = 0.6; B = 0.48; Mn = 0.65; C total = 429.00; pH =8.9.

H. seropedicae HRC54 and T. longibrachiatum F476 survival in substrate with or without biochar

The test was carried out without plants, based on randomized block design (DBC), with eight treatments (1 - Control; 2 - HRC54; 3 - F476; 4 - HRC54 + F476; 5 - Biochar; 6 - HRC54 + Biochar; 7 - F476 + Biochar; 8 - HRC54 + F476 + Biochar), and three replications to confirm microorganisms' survival in the inoculated substrate (Basaplant®), over time - each experimental plot consisted of three 100-mL plastic cups. Chemical substrate analysis followed the description by Claessen et al. (1997) it was carried out at the North Fluminense Regional Development Foundation (FUNDENOR): pH (water) = 5.2; S-SO_4 = 1170 mg.dm^3 (monocalcium phosphate); P = 588 mg.dm^3 and K = 11.40 $mmol_c$.dm^3 (Mehlich 1 extractor); Ca = 173.5 $mmol_c$.dm^3, Mg = 52 $mmol_c$.dm^3 and Al = 1.4 $mmol_c$.dm^3 (extractor: KCl + 1 mol L^{-1}); H+Al = 61.10 $mmol_c$.dm^3 (calcium acetate 0.5 mol L^{-1} pH 7); OM = 129.3 g.dm^3 (potassium dichromate); CEC 302 $mmol_c$.dm^3; V = 80%; m = 1%; Fe = 69.35 mg.dm^3, Cu = 0.75 mg.dm^3, Zn = 25.45 mg.dm^3, Mn = 70.28 mg.dm^3 (Mehlich 1 extractor) and B = 1.03 mg.dm^3 (extractor: hot water).

Suspensions containing 1 mL (10^8 cells.mL^{-1}) of bacterium *H. seropedicae* HRC54 and 1 mL of (10^6 cells.mL^{-1}) fungus *T. longibrachiatum* F476 were added to the substrate (Basaplant®), as well as biochar. Containers filled with each treatment were stored in protected environment for 7, 14, 28, and 56 days. The substrate was irrigated daily in order to maintain humidity. At the end of each evaluation time, 100-mg substrate samples were collected and serially diluted in sterile distilled water to assess microorganisms' survival in the substrate. The Most Probable Number of *Herbaspirillum* spp. was established by using the McCrady table in JNFb selective semi-solid medium, as described by Döbereiner et al. (1995). *Trichoderma* spp. presence was evaluated in TSM selective medium (*Trichoderma* selective medium g.L^{-1}: $MgSO_4$ $7H_2O$ - 0.2; K_2HPO_4 - 0.9; KCl - 0.15; NH_4NO_3 - 1.0; glucose - 3.0; chloramphenicol - 0.25; pentachloronitrobenzene – 0.2; rose bengal – 0.15; agar – 20.0) (Elad et al., 1981).

H. seropedicae HRC54, T. longibrachiatum F476 and biochar effect on soil microbiology, papaya growth promotion and mineral nutrition

H. seropedicae HRC54, *T. longibrachiatum* F476 and biochar were evaluated in order to check their ability to stimulate papaya seedlings' growth and their effect on mineral nutrition. The experiment was conducted in DBC, at 2x4 factorial arrangement with eight treatments (1 - Control; 2 - HRC54; 3 - F476; 4 - HRC54 + F476; 5 - Biochar; 6 - HRC54 + Biochar; 7 - F476 + Biochar; 8 - HRC54 + F476 + Biochar), and four replicates - composed of two pots, with two seedlings, each. Papaya seeds (*Carica papaya* L.) (UENF-Caliman 01 hybrid, known as Calimosa) were donated by Caliman Agrícola S/A.

Based on microorganisms' survival results, substrate inoculation (Basaplant®) was performed seven days before sowing in order to overlap the bacterial population reestablishment period - papaya seedlings' emerged 15 days after sowing, on average. The substrate was added with a suspension of 1 mL of (10^8 cells.mL^{-1}) *H. seropedicae* HRC54, a suspension of 1 mL of (10^6 cells.mL^{-1}) of *T. longibrachiatum* F476 and 1% of biochar, simultaneously, in plastic tubes of 290 cm³. Three papaya seeds were planted per tube, 2 cm deep, seven days after inoculation; thinning was carried out 21 days after sowing - two seedlings were kept per tube. Transplantation was carried out 45 days after sowing; seedlings were taken to 6-L pots filled with soil, without inoculum or biochar replacement. Chemical soil analysis was performed prior to conducting the experiment, without addition microbial inoculum and biochar – following the description by Claessen et al. (1997) it was carried out at the North Fluminense Regional Development Foundation (FUNDENOR): pH (water) = 4.8; S-SO_4 = 18 mg.dm^3 (monocalcium phosphate); P = 50 mg.dm^3 and K = 5 mmol$_c$.dm^3 (Mehlich 1 extractor); Ca = 49.6 mmol$_c$.dm^3, Mg = 37.5 mmol$_c$.dm^3 and Al = 5.4 mmol$_c$.dm^3 (extractor: KCl + 1 mol L^{-1}); H+Al = 110.4 mmol$_c$.dm^3 (calcium acetate 0.5 mol L$^-$1 pH 7); OM = 86.2 g.dm^3 (potassium dichromate); CEC 207.9 mmol$_c$.dm^3; V = 47%; m = 5%; Fe = 38.4 mg.dm^3, Cu = 2.27 mg.dm^3, Zn = 2.6 mg.dm^3, Mn = 23.18 mg.dm^3 (Mehlich 1 extractor) and B = 0.29 mg.dm^3 (extractor: hot water). Irrigation was performed on a daily basis; any invasive plants were manually removed.

Four individual samples were collected from the 0 to 10 cm layer in the rhizosphere region 90 days after sowing in order to determine total bacterial and fungal populations. The simple samples were mixed and homogenized to

compose one complex sample, which was diluted in 0.85% sterile saline and serialized up to the dilution of 10^{-6} in order to evaluate microbial population size. Martin medium (Martin, 1950) was used for fungal growth and nutrient agar was adopted for bacterial growth purposes; plates were incubated at 28 ºC, for 5 days, for fungi, and for 48 h, for bacteria. The number of microorganisms per gram of soil was estimated through the equation: Number of microorganisms/g of soil = [number of colonies x (1/dilution) x (1/aliquot)]/soil dry mass.

Non-destructive measurements were taken in all plants, 90 days after sowing, namely: shoot height in centimeters (millimeter ruler); stem basal diameter in millimeters (digital caliper) and leaf area in cm² (LI-3100C Area Meter - LI-COR Biosciences). All tissues were collected and dried in forced air circulation oven at 65 ºC for 72 h to determine shoot dry biomass (g). Roots from plants in one of the pots were manually removed, washed in running water and stored in 70% alcohol solution. Root length (cm), surface area (cm²) and volume (cm³) were determined in WinRHIZO image analysis system (Regent Instrument Inc., Quebec, Canada). Roots with diameter greater than 3,000 mm were discarded in order not to overestimate analysis values. All root biomass (g) was dried in forced air circulation oven at 65 ºC, for 72 h and weighed. Total plant biomass and Dickson Quality Index [total biomass/ ((shoot dry biomass/root dry biomass) + (height/diameter))] (Dickson, Leaf, Hosner, 1960) were measured based on collected data. The shoot was macerated and subjected to nitric-perchloric and sulfuric digestion to determine N, P, K, S, Ca, Mg, Fe, Cu, Zn, Mn and B levels ($g.kg^{-1}$). Data were transformed into nutrient content per plant ($g.kg^{-1}$ for macronutrient and $mg.g^{-1}$ for micronutrient) and nutrient utilization efficiency (shoot dry biomass/nutrient content) ($g.g^{-1}$) (Moll et al., 1982).

Statistical analysis

Results were subjected to normality and homogeneity tests, followed by two-way analysis of variance (ANOVA) to assess soil microbiology, growth promotion and mineral nutrition - in DBC, at 2x4 factorial arrangement (with or without biochar vs. microbial inoculations). Treatment variation sources were split into factor analysis followed by Tukey test to compare the means, at 5% probability level, when significant two-way ANOVA effect was recorded. Statistical analyses and graph plotting were carried out in GraphPad Prism 5.0 software.

RESULTS

H. seropedicae HRC54 and T. longibrachiatum F476 survival in substrate with and without biochar

The first evaluation, which was carried out at the 7th substrate inoculation day, revealed *H. seropedicae* HRC54 population decrease in all evaluated treatments in comparison to the initial inoculated concentration: 10^8 cells.mL^{-1}. However, 14 and 28 days after inoculation, this bacterial population increased to numbers similar to those recorded at the first evaluation - numbers remained stable until the last evaluation, at 56 days (Table 1).

Table 1. *H. seropedicae* HRC54 population density (cells.mL^{-1}) estimate 56 days after 100-mL commercial substrate inoculation, with or without biochar and *T. longibrachiatum* F476 (n = 3).

	Control	**HRC54**	**HRC54 + ‡F476**	**HRC54 + Biochar**	**HRC54 + F476 + Biochar**
Initial inoculum	0	$1.0x10^8$	$1.0x10^8$	$1.0x10^8$	$1.0x10^8$
7 days	$1.1x10^4$	$1.1x10^4$	$3.0x10^4$	$6.5x10^4$	$3.0x10^4$
14 days	$4.0x10^3$	$4.5x10^5$	$2.0x10^5$	$4.5x10^5$	$1.2x10^5$
28 days	$6.0x10^3$	$1.4x10^6$	$1.4x10^6$	$1.4x10^6$	$1.4x10^6$
56 days	$6.0x10^3$	$1.4x10^6$	$1.4x10^6$	$1.4x10^6$	$1.4x10^6$

†HRC54 – *H. seropedicae*; ‡F476 – *T. longibrachiatum.*

It was expected that diazotrophic bacteria from the control condition (untreated) would recover in nitrogen-free medium since the used substrate was not sterilized. Despite such an expectation, population density in this treatment was 50% lower than that inoculated with *H. seropedicae* HRC54.

Trichoderma colonies characteristic of this genus were observed in all treatments until the last evaluation. However, it was not possible distinguishing the *T. longibrachiatum* F476 isolate from other *Trichoderma* species under the tested conditions, because the used culture medium was not selective for the applied species.

H. seropedicae HRC54, T. longibrachiatum F476 and biochar change total fungal population in the soil

Substrate inoculation and biochar addition did not stimulate significant changes in total bacterial population in the soil at the end of the experiment (Figure 1A). However, the treatment with F476 showed significant increase in total fungal population in comparison to the control treatment (untreated), regardless of biochar addition (Figure 1B).

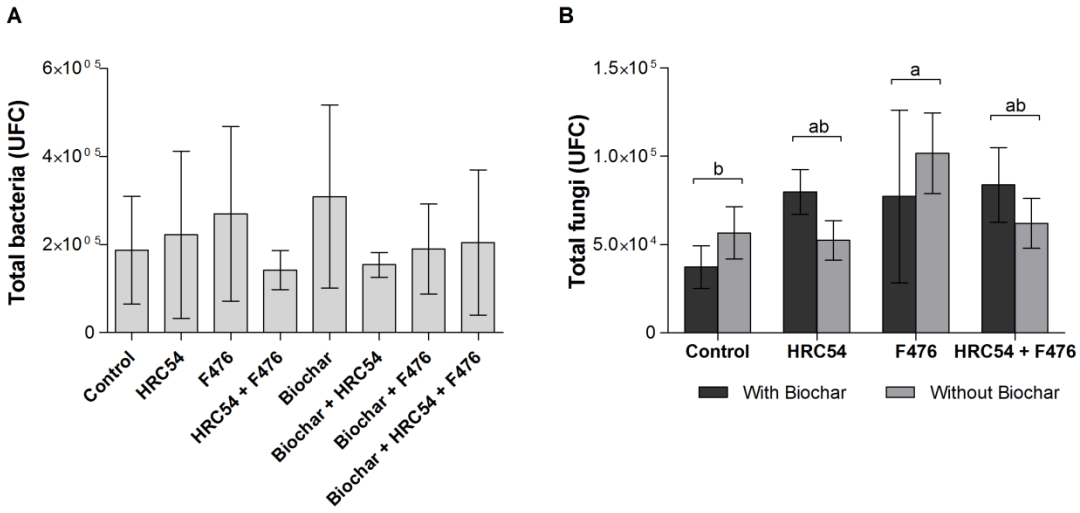

Figure. 1. Total bacterial (A) and fungal (B) population in the soil, 45 days after the transplantation of seedlings previously treated with *H. seropedicae* HRC54, *T. longibrachiatum* F476, and biochar. Means (n = 4) ± standard deviation followed by equal letters did not differ from each other in the Tukey test (P ≤ 0.05). HRC54 – *H. seropedicae*; F476 – *T. longibrachiatum*.

Co-inoculation and biochar effect on papaya seedlings' growth and mineral nutrition

H. seropedicae HRC54, *T. longibrachiatum* F476 and biochar addition to the substrate did not significantly affect seedlings' average height and leaf area under the tested conditions. However, treatments with biochar addition showed gains by 8% in height, 12% in stem diameter and 12% in leaf area (Table 2). Biochar addition promoted significant gains in the shoot dry biomass, in comparison to the control (untreated) (Figure 2).

Table 2. Mean papaya seedlings' height, basal stem diameter and leaf area 90 days after sowing and grown in substrate previously treated with *H. seropedicae* HRC54, *T. longibrachiatum* F476 and biochar.

	Average height (cm)	*Basal stem diameter (mm)	Leaf area (cm²)
Control	32.0 ± 1.6	9.54 ± 1.39b	564.9 ± 92.1
†HRC54	33.3 ± 2.1	9.54 ± 1.61b	586.4 ± 107.4
‡F476	32.3 ± 2.4	9.52 ± 0.46b	529.9 ± 110.2
HRC54 + F476	33.4 ± 4.7	9.89 ± 1.02b	588.2 ± 65.5
Biochar	36.1 ± 2.8	10.67 ± 0.82a	633.4 ± 119.7
Biochar + HRC54	34.2 ± 4.7	10.64 ± 1.03a	676.2 ± 162.6
Biochar + F476	33.2 ± 2.6	10.76 ± 0.55a	595.5 ± 34.9
Biochar + HRC54 + F476	34.7 ± 4.1	10.81 ± 0.58a	636.3 ± 160.1

*Column means (n = 4) ± standard deviation followed by equal letters did not differ from each other in the Tukey test (P ≤ 0.05). †HRC54 – *H. seropedicae*; ‡F476 – *T. longibrachiatum.*

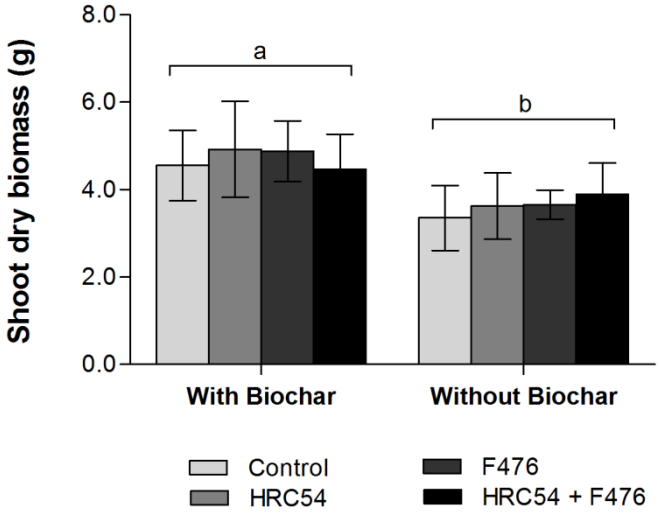

Figure. 2. Papaya seedlings' shoot dry biomass 90 days after sowing, and grown in substrate previously treated with *H. seropedicae* HRC54, *T. longibrachiatum* F476, with or without biochar. Means (n = 4) ± standard deviation followed by equal letters did not differ from each other in the Tukey test (P ≤ 0.05).

The effect of biochar addition led to gains in total root dry biomass (Figure 3A), taproot dry biomass (Figure 3B) and increase in fine roots (Figure 3C). In addition, there was increase in total root length (Figure 3D), surface area (Figure 3E) and volume (Figure 3F) in comparison to treatments without biochar, regardless of microbial inoculation. There was no significant difference because of microbial inoculation; therefore, there was no interaction between biochar factors and inoculation.

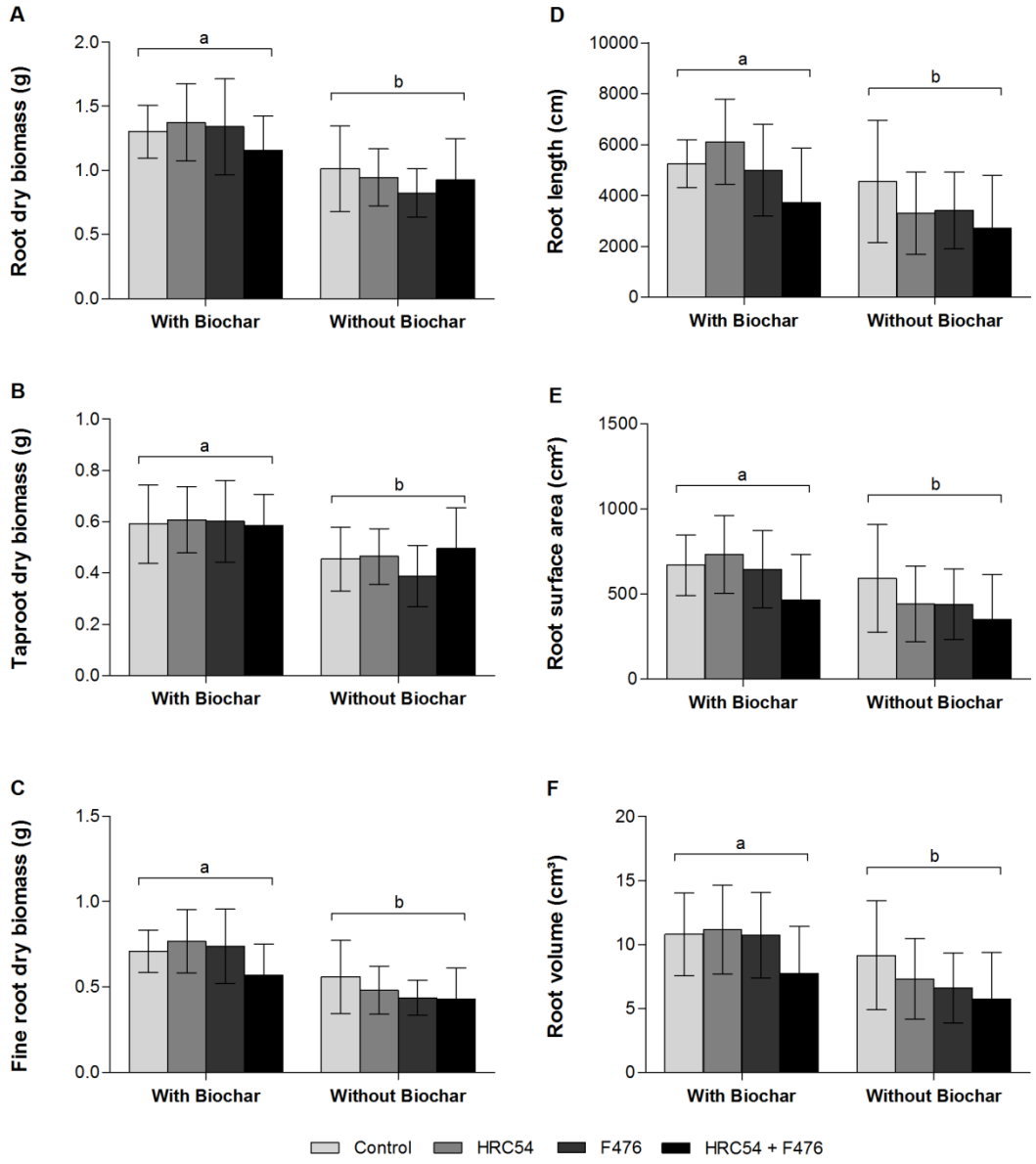

Figure. 3. Papaya seedlings' root growth 90 days after sowing and grown in substrate previously treated with *H. seropedicae* HRC54, *T. longibrachiatum* F476, and biochar. A) Root dry biomass (g), B) taproot dry biomass (g), C) fine root dry biomass (g), D) root length (cm), E) root surface area (cm²), and F) root volume (cm³). Means (n = 4) ± standard deviation followed by equal letters did not differ from each other in the Tukey test (P ≤ 0.05). HRC54 – *H. seropedicae*; F476 – *T. longibrachiatum*.

Biochar addition to the substrate also promoted significant gains in total shoot and root biomass in comparison to substrates without it - there was relative increase in total biomass by 44% due to HRC54 + Biochar and by 42.4% due to F476 + Biochar, in comparison to the control (untreated) condition (Figure 4A). Similar results were recorded for the Dickson Quality Index (DQI), which showed

significant gains due to treatments with biochar addition and relative increase by 50.2% due to HRC54 + Biochar and by 42.3% due to F476 + Biochar, in comparison to the control condition (untreated) (Figure 4B).

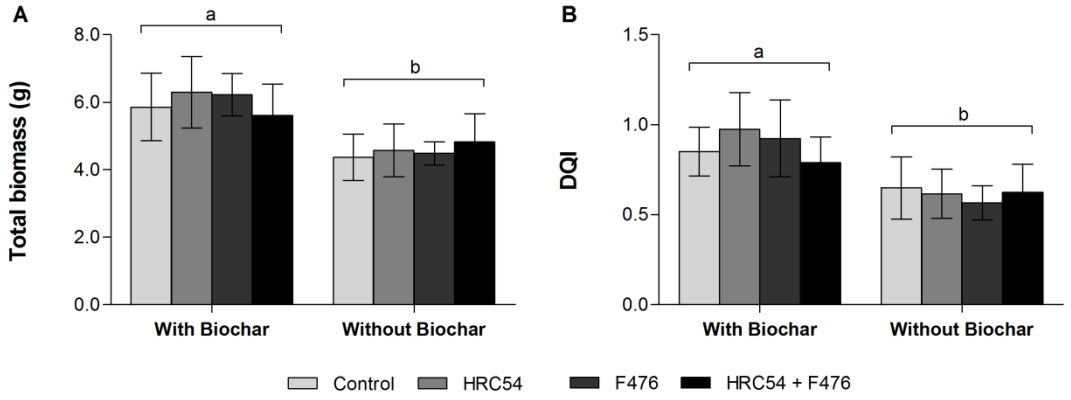

Figure. 4. Total papaya seedling biomass (g) (A) and Dickson Quality Index (DQI) (B) 90 days after sowing and grown in substrate previously treated with *H. seropedicae* HRC54, *T. longibrachiatum* F476 and biochar. Means (n = 4) ± standard deviation followed by equal letters did not differ from each other in the Tukey test (P ≤ 0.05). HRC54 – *H. seropedicae*; F476 – *T. longibrachiatum*.

With respect to seedlings' mineral nutrition, there was significant increase in N, S, P, K, Ca, Mg, and Mn content when trated with biochar (Table 3). Treatments with biochar were significantly more efficient in S, P and Mn use (Figure 5).

Table 3. Leaf nutrient content (g.kg^{-1} and mg.g^{-1}) in papaya seedlings 90 days after sowing and grown in substrate previously treated with *H. seropedicae* HRC54, *T. longibrachiatum* F476 and biochar.

	N*	**S***	**P***	**K***	**Ca***	**Mg***	**Fe**	**Cu**	**Zn**	**Mn***	**B**
	g.kg^{-1}						mg.g^{-1}				
Control	0.069±0.02b	0.024±0.00b	0.011±0.00b	0.087±0.02b	0.027±0.01b	0.031±0.01b	0.788±0.67	0.041±0.03	0.086±0.09	0.418±0.05b	0.136±0.03
†HRC54	0.076±0.01b	0.027±0.00b	0.011±0.00b	0.093±0.02b	0.028±0.01b	0.034±0.00b	0.535±0.21	0.013±0.01	0.056±0.02	0.467±0.09b	0.147±0.03
‡F476	0.077±0.01b	0.026±0.00b	0.012±0.00b	0.092±0.01b	0.027±0.00b	0.033±0.00b	0.520±0.12	0.034±0.03	0.042±0.01	0.412±0.04b	0.160±0.03
HRC54 + F476	0.086±0.01b	0.027±0.01b	0.012±0.00b	0.095±0.02b	0.031±0.00b	0.035±0.01b	0.469±0.15	0.043±0.03	0.047±0.01	0.521±0.16b	0.169±0.02
Biochar	0.091±0.02a	0.032±0.00a	0.013±0.00a	0.119±0.02a	0.035±0.00a	0.041±0.01a	1.351±0.85	0.046±0.04	0.068±0.03	0.598±0.11a	0.197±0.06
Biochar + HRC54	0.107±0.01a	0.029±0.01a	0.014±0.00a	0.119±0.03a	0.039±0.01a	0.043±0.01a	0.654±0.07	0.087±0.08	0.070±0.01	0.516±0.09a	0.202±0.03
Biochar + F476	0.093±0.02a	0.029±0.00a	0.013±0.00a	0.113±0.02a	0.039±0.01a	0.045±0.01a	1.349±1.33	0.082±0.08	0.058±0.01	0.551±0.05a	0.205±0.04
Biochar + HRC54 + F476	0.090±0.02a	0.032±0.01a	0.013±0.00a	0.104±0.02a	0.035±0.01a	0.041±0.01a	0.616±0.17	0.045±0.02	0.049±0.01	0.548±0.09a	0.211±0.04

*Column means (n = 4) ± standard deviation followed by equal letters did not differ from each other in the Tukey test (P ≤ 0.05). †HRC54 – *H. seropedicae*; ‡F476 – *T. longibrachiatum*.

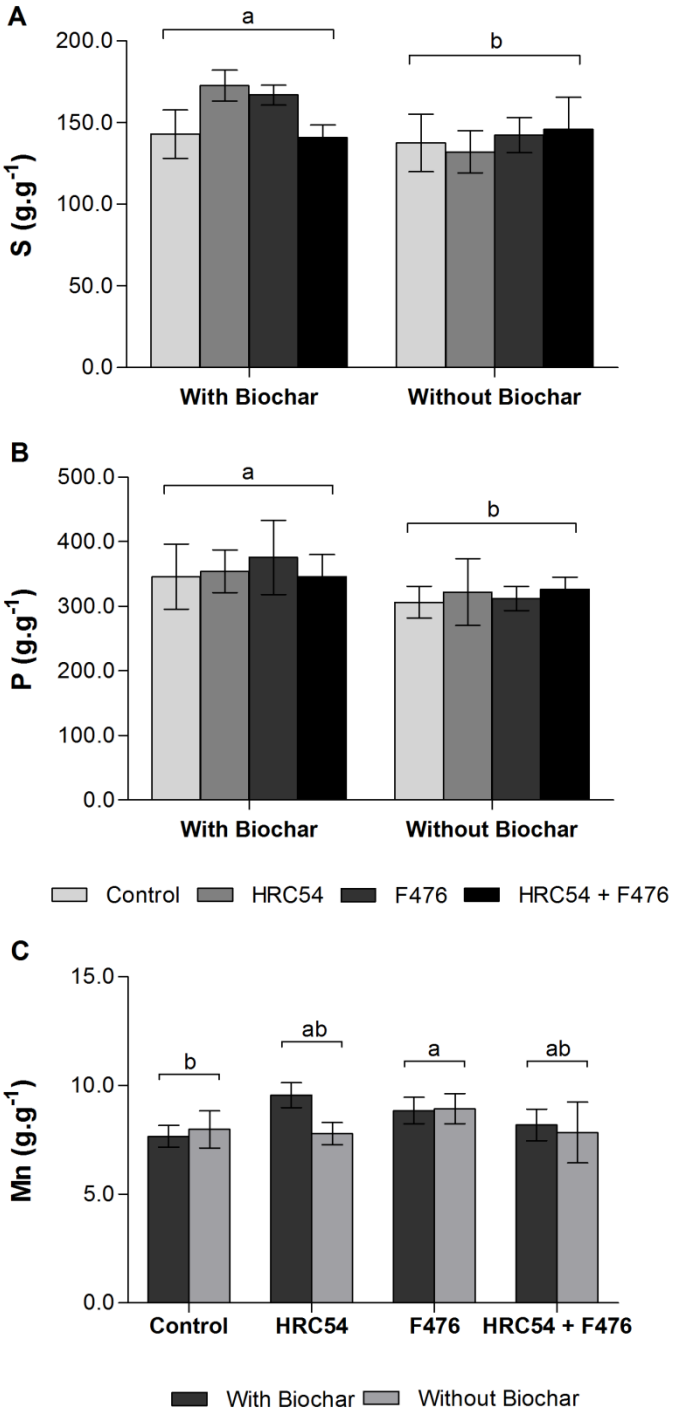

Figure. 5. S (A), P (B) and Mn (C) ($g.g^{-1}$) utilization efficiency of papaya seedlings at 90 days after sowing and grown in substrate previously treated with *H. seropedicae* HRC54, *T. longibrachiatum* F476 and biochar. Means (n = 4) ± standard deviation followed by equal letters did not differ from each other in the Tukey test (P ≤ 0.05). HRC54 – *H. seropedicae*; F476 – *T. longibrachiatum*.

DISCUSSION

Environmental biotic and abiotic conditions must be favorable for inoculant survival and root colonization in order to achieve plant growth through microbial inoculation (Ajeng et al., 2020). Previous studies have shown that *H. seropedicae* can form biofilm on *T. longibrachiatum* hyphae surface and increase their survival in the soil, even in the absence of host plants (Oliveira, 2015; Reis, 2018). Furthermore, biochar addition also provides the favorable microenvironment for inoculated microorganisms' survival (Lehmann et al., 2011). Biochar also changes nutrient availability and helps its prompt assimilation (Yang et al., 2016) or the modulation of cation exchange capacity in soil solution (Zheng et al., 2019), which favors microbial survival, mainly bacteria.

Despite biochar benefits for inoculated microorganisms' survival, no differences in the population of *H. seropedicae* HRC54 and *T. longibrachiatum* F476 were identified 56 days after inoculation – the first evaluation was carried out without host plant - with or without this compound. The total bacterial population in soil planted with previously treated seedlings also did not show any difference. But, the final bacterial population was 233 times larger in inoculated treatments than in the control, regardless of biochar and/or *T. longibrachiatum* F476 addition. On the other hand, there was fungal population increase in treatments that have received the inoculants.

According to Rékási et al. (2019), biochar (3 $t.ha^{-1}$ from grain husk and paper fiber sludge) did not affect microbial biomass in acidic soil; however, Kari et al. (2021) recorded increased relative abundance of diazotrophic bacteria inoculated with biochar (15 and 30 $t.ha^{-1}$ from grain husk and paper fiber sludge) in acidic soil. On the other hand, *Trichoderma harzianum* + biochar (0.6 $t.ha^{-1}$ from wood residue) + 50% of NPK application increased the relative abundance of total bacteria and fungi (Sani et al., 2020), whereas Hu et al. (2014) reported 14.5% increase in the *Trichoderma* spp. population due to the application of 5% biochar (from forest residue). In this sense, different responses can be associated with different experimental conditions, microbial population stabilization at the end of the evaluation period, used raw material influence on biochar production and used

concentration. Furthermore, increase in relative abundance may not represent soil microbial diversity, since the use of this compound tends to increase microbial diversity and the metabolic potential of the rhizosphere, and, consequently, stimulate plant growth (Han et al., 2017; Kolton et al., 2017).

H. seropedicae, *Trichoderma* spp. and biochar, in separate, have recognized stimulating effects on the growth and nutrition of different plant cultures assessed in experiments carried out under controlled conditions (Chagas et al., 2017; Rouphael et al., 2017; Xiang et al., 2017; Dos Santos et al., 2019; Ramos et al., 2020). Mean gains with treatments added with biochar were higher than those recorded for the control treatment (untreated), regardless of microbial inoculation. Altogether, these results contributed to significant increase in DQI, according to which, the production of more vigorous and higher quality seedlings is closely related to greater chances of post-transplant establishment and agricultural yield (Grossnickle and MacDonald, 2018).

Nevertheless, biochar effects on aboveground papaya tree growth can be variable. Silva et al., 2019found significant increase in height, stem diameter and shoot dry mass 90 days after sowing, in comparison to the control (without biochar) in an experiment conducted with 1% (v/v) aviary bedding biochar in papaya, using the Golden cultivar. Control (untreated) seedlings reached 11.6 cm in height, 5.54 mm in diameter and 0.38 g of shoot dry biomass (Silva et al., 2019). However, hybrid Calimosa seeds with greater vigor and yield under field conditions than the Golden cultivar (Dias et al., 2011) - whose control seedlings reached 32 cm in height, 9.54 mm in stem diameter and 3.3547 g of shoot dry biomass, 90 days after sowing, with 1% biochar (v/v) – were herein used. It allowed observing that the recorded results depended on the host plant cultivated in soil added with biochar, rather than just on soil physicochemical properties. In addition, biochar effects are correlated to plant tissue, since it is benefited by the raw material used to produce the compost, and by the compost production process (pyrolysis time and temperature) (Vaccari et al., 2015; Xiang et al., 2017; Kavitha et al., 2018; Gonzaga et al., 2018).

No studies associated *Herbaspirillum* spp. inoculation with biochar sources, so far. However, recent studies have shown the beneficial effects of other growth-promoting bacteria associated with biochar. *Bradyrhizobium japonicum* and *Pseudomonas putida* co-inoculation and 3% biochar in soybeans have significantly

improved plant growth and nutrient content, as well as nutrient and enzyme levels in the soil (Jabborova et al., 2020). *Bacillus thuringiensis*, *P. putida* and *Klebsiella varicola,* in combination with biochar, increased plant height by 13% and the number of leaves by 53%, in comparison to the control in cowpea crops (Taiwo et al., 2018). Biochar addition or *P. fluorescens* inoculation in rosemary significantly increased shoot and root height and fresh biomass. However, gains with combined inoculation were even more expressive when they were compared to the control (Sadegh et al., 2019), as herein observed.

Several studies have investigated the effects of *Trichoderma* spp. association with biochar. The *T. viride*/biochar combination increased maize germination and the attainment of taller plants (Muter et al., 2017). Araujo et al. (2019) observed the synergistic effect of sewage sludge biochar and *T. harzianum* on soybean production; they recorded 70% increase in fresh and dry mass of plants in response to their combined application. Biochar from ground coffee, inoculated with *T. aureoviride* added to the soil increased shoot length and dry biomass of watermelon and melon plants in comparison to the control (Medeiros et al., 2020a and 2020b).

On the other hand, biochar application straight on the soil, without microorganisms' addition, also increased root biomass, length, surface area and volume, as well as the number of root nodules, without changing the shoot:root ratio (Xiang et al., 2017). The addition of 1% biochar, under the herein tested conditions, also resulted in significant increase in root biomass and growth, which was similar to data recorded for the meta-analysis carried out by Xiang et al. (2017). Furthermore, gains in the root system had positive impact on total plant biomass, and this finding suggests that biomass allocation is not compartmentalized. Its direct impact on root growth is attributed to biochar's porous structure, which makes it easy for roots to proliferate, mainly fine roots (Sani et al., 2020).

All treatments containing biochar, with or without microorganisms, showed significant gain in N, S, P K, Mg, Ca and Mn contents. Furthermore, treatments with biochar presented higher fine root biomass than the control (untreated) and were more efficient in using S, P and Mn – nutrients with relevant effect on papaya yield. Nutrient use efficiency is plants' ability to convert acquired nutrients into

biomass allocation, showing that treatments with biochar (mainly HRC54 + Biochar and F476 + Biochar) better used mineral resources than other treatments.

Sulfur acts in papain's chemical composition, increase fruit production and improves its quality. Phosphorus is the most important nutrient at the initial papaya root development stage (Oliveira et al., 2004). Biochar application, with or without inoculants, can be used as strategy to manage papaya crops and reduce the need of fertilizers, if one takes into account P as limiting nutrient (Manning, 2010; Maranguit et al., 2017); consequently, this process minimizes environmental impacts and reduces production costs.

Several mechanisms can contribute to a more efficient nutrient uptake, mainly fine roots length and surface area increase (Xiang et al., 2017). However, fine roots' increase is not directly proportional to nutrient content increase, since it is not identical for all nutrients. Rice inoculated with *H. seropedicae* HRC54 shows significant increase in S, P, K and Zn in comparison to non-inoculated plants, but this outcome was not observed for other nutrients (Ramos et al., 2020). Likewise, biochar addition, alone, may not affect nutrient content in both shoot and root (Xiang et al., 2017). The addition of 1% eucalyptus bark biochar did not significantly change the nutrient content of treated tomato plants in comparison to untreated plants, after 12 weeks (Kolton et al., 2017). In rosemary, biochar + *P. fluorescens* increased N, P, K, Fe, Zn, Cu and Mn content in comparison to the control and to treatments with isolated inoculation (Sadegh et al., 2019). In corn plants, Saranya et al. (2011) observed increased plant growth, N, P and K uptake, and ear yield due to biochar + *Azospirillum* sp. application. Biochar presents large specific surface area and exposed negative charges where its particles can absorb more soil nutrients and exchange cations with soil solution in order to make it easier for plants to absorb minerals (Sun et al., 2015). New tests are needed to evaluate whether the beneficial effects of biochar addition are associated with substrates microbial inoculation at the seedling stage, and whether it has positive impact on fruit yield and quality, at the harvest stage.

CONCLUSION

Substrate treatment with *H. seropedicae* HRC54, *T. longibrachiatum* F476 and biochar did not have any impact on the bacterial population, but it increased the total fungal population 45 days after seedling transplantation. However, shoot growth gains, the significant increase in root biomass and leaf nutrient content of papaya seedlings were associated with biochar addition to the substrate. The treatments containing biochar produced more vigorous, higher quality and seedlings more efficient in utilization S, P and Mn.

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3.2 FUNGAL-BACTERIAL INTERACTION AND BIOCHAR IN PAPAYA ROOT ROT CONTROL

RESUMO

A podridão do pé é uma das principais doenças fúngicas do mamoeiro, causada por *Phytophthora palmivora* Butler. Apesar dos benefícios isolados, não há, até o presente momento, estudos que correlacionem os efeitos da interação entre *Trichoderma*, *Herbaspirillum* e biochar no tratamento de mudas e de solo, visando o controle biológico dessa doença. Por isso, objetivou-se neste trabalho avaliar dois métodos de controle contra *P. palmivora* Pp13, utilizando *T. longibrachiatum* F476, *H. seropedicae* HRC54 e biochar. Inicialmente o potencial antagônico dos isolados foi testado *in vitro*. F476 hiperparasitou Pp13 em meio de cultura e em frutos de mamão verde, bem como em microcultura. Observou-se adesão de esporos, germinação de hifas, enovelamento de hifas ao redor e invasão das hifas sobre as estruturas de Pp13. Já HRC54 não afetou o crescimento e esporulação de Pp13. Entretanto, quando se associou HRC54 e F476, apesar da maior abundância de esporos de F476 associados às hifas do patógeno, não se observou a invasão das hifas ou hiperparasitimo a Pp13, em microcultura mista. Sob condições de viveiro-telado, foram conduzidos dois experimentos de biocontrole de doença em mudas de mamoeiro. No primeiro experimento, o

substrato de mudas foi tratado com F476, HRC54 e biochar, e posteriormente as mudas obtidas foram transplantadas para solo infestado com Pp13. No segundo experimento, o solo infestado com o patógeno foi tratado F476, HRC54 e biochar, e após 7 d foi realizado o semeio direto nos vasos com solo previamente tratado. Em relação ao tratamento do substrato de mudas (experimento 1) F476 aplicado isoladamente propiciou 70% de sobrevivência de mudas, seguido de Biochar+F476 e HRC54, com 60,3 e 56,9%, respectivamente, em relação ao controle não-tratado. No tratamento de solo (experimento 2), HRC54+F476 apresentou a maior porcentagem de sobrevivência, com 66,8%, seguido por Biochar+HRC54 com 60%, em relação ao controle não-tratado. O tratamento do substrato de mudas com F476 reduziu em 18,9% a mortalidade de plantas, enquanto o tratamento de solo com HRC54+F476 reduziu em 42,9% a mortalidade das mudas, em relação à mortalidade verificada na condição sem tratamento. F476 e HRC54 apresentam potencial para serem utilizados como agentes de biocontrole contra *P. palmivora* Pp13, entretanto as respostas obtidas são dependentes do método de tratamento aplicado. A adição de 1% de biochar não apresentou alterações significativas na sobrevivência de mudas, mas maximizou os efeitos benéficos de F476 e HRC54 no biocontrole da doença, reduzindo a mortalidade de mudas.

Palavras-chave: *Phytophthora palmivora*, *Carica papaya* L., *Herbaspirillum seropedicae* HRC54, *Trichoderma longibrachiatum* F476.

ABSTRACT

Root rot is one of the main papaya fungal diseases caused by *Phytophthora palmivora* Butler. To be best of our knowledge, there are no studies correlating the effects of interaction among *Trichoderma*, *Herbaspirillum* and biochar on seedling and soil's treatment to biologically control this disease, despite their isolated benefits. Therefore, the objective of the current study is to evaluate two control methods against *P. palmivora* Pp13 by using T*. longibrachiatum* F476,

H. seropedicae HRC54 and biochar. The antagonistic potential of isolates was tested *in vitro*. F476 hyperparasitized Pp13 in culture medium and in green papaya fruits, as well as in microculture. Spore adhesion of F476, hyphae germination, hyphae folding around and hyphal invasion in Pp13 structures were observed. On the other hand, HRC54 did not affect Pp13 growth and sporulation. However, when HRC54 and F476 were associated there was no Pp13 hyphal invasion or hyperparasitism in mixed microcultures, despite the greater abundance of F476 spores associated with the pathogen's hyphae. Two disease biocontrol experiments were carried out with papaya seedlings under screened nursery conditions. Seedling substrate was treated with F476, HRC54 and biochar in the first experiment; subsequently seedlings were transplanted to soil infested with Pp13. Soil infested with the pathogen was treated with F476, HRC54 and biochar in the second experiment; seven days later, sowing was directly carried out in pots with previously treated soil. With respect to seedling substrate treatment (experiment 1), F476 applied alone led to 70% seedling survival; it was followed by Biochar+F476 and HRC54, which recorded 60.3% and 56.9%, respectively, in comparison to the untreated control. HRC54+F476 showed the highest survival rate for soil treatment (experiment 2) (66.8%) in comparison to the untreated control; it was followed by Biochar+HRC54 (60%). Seedling substrate treated with F476 reduced plant mortality by 18.9%, whereas soil treatment with HRC54+F476 reduced seedling mortality by 42.9% in comparison to the mortality observed for untreated samples. F476 and HRC54 have the potential to be used as biocontrol agent against *P. palmivora* Pp13; however, the recorded responses depend on the adopted treatment method. The addition of 1% biochar did not show significant changes in seedling survival, but it has maximized the beneficial effects of F476 and HRC54 on disease biocontrol, since it has reduced seedling mortality.

Keywords: *Phytophthora palmivora*, *Carica papaya* L., *Herbaspirillum seropedicae* HRC54, *Trichoderma longibrachiatum* F476.

INTRODUCTION

Root rot is one of the main fungal diseases affecting papaya crops. Pseudofungus *Phytophthora palmivora* Butler is the main etiologic agent causing this disease. This pathogen causes economic loss due to the high mortality of plants in the field, at any age, in rainy season (Tavares et al., 2016). Such a process accounts for severe production losses and for lack of resistant commercial cultivars (Oliveira et al., 2014). In addition, *P. palmivora* is not efficiently controlled by fungicide application on plants or in the soil (Santos et al., 2017). Thus, biological control emerges as safer and more sustainable alternative for seedlings' production and soil treatment to control root rot in papaya crops.

Fungi belonging to genus *Trichoderma* represent the main agents for the biocontrol of phytopathogens used in agriculture, including diseases caused by *Phytophthora* spp. (Machado et al., 2012; Lopes and Sami, 2018). *Trichoderma* spp. application was the adopted strategy to control black pod rot in cacao (Hanada et al., 2009; Harni et al., 2020), rubber tree defoliation (Promwee et al., 2017) and papaya soft fruit rot (De Oliveira et al., 2018), which are diseases caused by *Phytophthora* spp. However, so far, biological control studies based on infesting substrates with *Trichoderma* spp. contrast to each other when it comes to antagonism effectiveness in controlling papaya root rot (Tatagiba et al., 2005; Carnaúba, 2006; Tocafundo, 2008; Tavares et al., 2009; Dianese et al., 2012; Sánchez-Rangel et al., 2012; Sánchez-Rangel et al. al., 2016; Soesanto et al., 2019). Similarly, plant growth-promoting bacteria have also been described for the biocontrol of fungal diseases in plants. *Pseudomonas chlororaphis* CP07 has the potential to induce plant defense against black pod rot in cocoa crops, depending on the plant genotype (Acebo-Guerrero et al., 2015; Miguelez-Sierra et al., 2019). *P. aeruginosa* and *Chryseobacterium proteolyticum,* in their turn, reduce black pod rot lesion in fruits, with 100% *P. palmivora* inhibition (Alsultan et al., 2019). *Bacillus subtilis* and *B. pumilus* have been used to control *Phytophthora* in apple, lemon and pepper plants (Utkhede and Smith, 1991; Amorim and Melo, 2002; Lee et al., 2008).

If one takes into consideration the isolated effects of fungi and bacteria, it is possible stating that the co-inoculation of these microorganisms can be used as strategy to maximize the beneficial responses in soil-plant system and induce the biofertilization, biocontrol, bioremediation and resistance of abiotic stress effects

(Bashan et al. al., 2014). On the other hand, using a mixed inoculant in organic base, such as biochar, can lead to greater chances of achieving successful establishment and survival of associated biological agents and of reducing the pathogen population by increasing the chances of controlling papaya root rot. At least five different mechanisms have been attributed to biochar activity associated with disease control and plant growth: (i) systemic resistance induction in host plants; (ii) increased abundance and/or activity of beneficial microorganisms; (iii) changes in nutrient quality and organic matter availability; (iv) direct antifungal effect on soil pathogens and; (v) sorption of allelopathic phytotoxic compounds (Bonanomi and Scala, 2015).

We have shown that 1% poultry litter biochar can increase papaya seedlings' biomass and vigor, mainly when it is associated with *T. longibrachiatum* F476 and *H. seropedicae* HRC54 (Barroso et al., 2022). These results allow hypothesizing that such an association would also have beneficial effects on papaya root rot control and would help the development of an environmentally sustainable agricultural crop. Therefore, two biological control methods against *P. palmivora* Pp13 were tested in protected cultivation based on the use of *T. longibrachiatum* F476, *H. seropedicae* HRC54 and biochar as biocontrol agent (BACs): i) papaya seedlings' substrate was treated with BACs in experiment 1 and, subsequently the seedlings were transplanted to soil infested with Pp13; ii) soil infested with Pp13 was treated with BACs in experiment 2 and then sowing was directly carried out in pots with previously treated soil.

MATERIALS AND METHODS

Experiment conduction and environmental monitoring

Experiments were carried out in the Laboratory of Entomology and Phytopathology (LEF) and in the greenhouse with 30% shade cloth of Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), between June 6 and September 20, 2020. Mean temperature ranged from 22.6 °C ± 4 ºC

maximum and 21.4 °C ± 3.7 °C minimum; total rainfall in the assessed period was 73 mm (INMET, 2021).

Microbial inoculum obtainment, growth and production

Fungus *T. longibrachiatum* F476 and bacterium *H. seropedicae* HRC54 belonged to the culture collection of Laboratory of Cell and Tissue Biology (LBCT/UENF). *T. longibrachiatum* F476 was cultivated in commercial potato-dextrose-agar (PDA) medium for 7 days, at 28 ºC; inoculum concentration was adjusted to suspension of 10^6 spores.mL^{-1}. Bacterium *H. seropedicae* HRC54 was grown in DIGS liquid medium (Döbereiner et al., 1999) under constant agitation for 48 h, at 30 °C; inoculum concentration was adjusted to suspension of 10^8 cells.mL^{-1}.

The *Phytophthora palmivora* Pp13 isolate belonged to LEF's culture collection and was obtained from plant samples presenting papaya root rot symptoms - they were collected at Caliman Agrícola S/A (Linhares, Espírito Santo). The pathogen was cultivated in commercial potato-dextrose-agar (PDA) medium for 10 days, at 28 ºC. A 1-cm disc of culture deriving from PDA was inserted into a hole made in the peel of papaya green-fruits, which were pesticide-free and harvested in domestic orchard, to obtain the spores. The fruits were sanitized in detergent and water, immersed for 1 min in 2% sodium hypochlorite (500 mg.L^{-1} Cl_2-active), washed in running water and dried at room temperature, under UV light, for 2 min, in laminar flow. Inoculated fruits were kept in humid chamber for 10 days, at room temperature. Mycelium found on the fruit surface was washed in distilled water and homogenized in order to recover the spores. Inoculum concentration was adjusted to 10^3 chlamydospores.dm^{-3} in Fuchs-Rosental counting chamber.

Biochar obtainment and concentration

Biochar from the Soil Laboratory (LSOL) was made of poultry litter pyrolysis at 400 ºC, as described by Lin et al. (2012). It was used at concentration of 1% (v/v) substrate or soil, which corresponds to 2.76 g dm³. Biochar chemical analysis was: N_{total} = 39.81 g.kg^{-1}; P_2O_5 = 27.62 g.kg^{-1}; K_2O = 61.00 g.kg^{-1}; S=5.1 g.kg^{-1}; Ca = 56.51 g.kg^{-1}; Mg = 11.42 g.kg^{-1}; C_{total} = 345.4 mg.kg^{-1}; Fe = 1620 mg.kg^{-1}; Cu = 583 mg.kg^{-1}; Zn = 588 mg.kg^{-1}; Mn = 677 mg.kg^{-1}; pH = 8.9.

T. longibrachiatum F476 and H. seropedicae HRC54 antagonism in vitro against P. palmivora Pp13

T. longibrachiatum F476 and *H. seropedicae* HRC54 to *P. palmivora* Pp13 parasitism was determined through culture pairing. The experiment followed a completely randomized design with six treatments (1- F476; 2- HRC54; 3- Pp13; 4-F476 vs. Pp13; 5- HRC54 vs. Pp13; 6- F476 + HRC54 vs. Pp13) and three repetitions. Accordingly, a disk (0.5 cm in diameter) of a *T. longibrachiatum* F476 colony and 100 uL of suspension (10^8 cells.mL^{-1} of *H. seropedicae* HRC54) were placed 4 cm away from a disk (0.5 cm in diameter) of a *P. palmivora* Pp13 colony in a Petri dish filled with PDA medium. The plates were incubated for 7 days, at 25 ºC, under 12h photoperiod; subsequently, antagonism of BCAs was measured according to Bell et al. (1982): 0 (>95% of the plate covered by *Trichoderma* mycelium/spores); 1 (>95% of the plate covered by *Trichoderma*, but low sporulation or no sporulation); 2 (51% to 95% of the plate covered by *Trichoderma* mycelium); 3 (up to 50% of the plate covered by *Trichoderma* mycelium); 4 (2/3 of the plate covered by the pathogen); 5 (100% of the plate covered by the pathogen). A disk (0.5 cm in diameter) was removed from the parasitized region and deposited on fragments of papaya green-fruit to confirm the antagonistic effect of the isolates against the pathogen - the fragments were kept in humid chamber for 7 days, at room temperature, to observe whether, or not, the mycelial growth of *P. palmivora* Pp13 would take place. The experiment was carried out based on the same design adopted for the pairing in plates.

The aliquot of 1 mL of fondant PDA was deposited under the surface of sterilized microscope slides to describe hyperparasitism in microcultures. The experiment has followed a completely randomized design with six treatments (1- F476; 2- HRC54; 3- Pp13; 4- F476 vs. Pp13; 5- HRC54 vs. Pp13; 6- F476 + HRC54 vs. Pp13;) and three repetitions. The *T. longibrachiatum* F476 inoculum comprised 20 uL suspension with 10^6 spores.mL^{-1} and the *H. seropedicae* HRC54 inoculum encompassed 20 uL suspension with 10^8 cells.mL^{-1}. Suspensions were applied to the culture medium 3 cm away from the *P. palmivora* Pp13 inoculum, which was composed of 20 uL suspension with 10^4 spores.mL^{-1}. The slides were incubated in humid chamber at 25 ºC; for 24, 48 and 72 h; and observed in optical microscope. Fungi mycelial growth, spores production and interactions between

microorganisms (independent, associated, coiled growth, cell wall degradation, and hyphal and spore invasion of the pathogen) were evaluated.

T. longibrachiatum F476, H. seropedicae HRC54 and biochar effect on seedling survival against P. palmivora Pp13

Two methods were tested in independent experiments, based on a randomized block design, with eight treatments and an absolute control untrated (1- Pp13; 2- Pp13 vs. F476; 3- Pp13 vs. HRC54; 4- Pp13 vs. F476+HRC54; 5- Pp13 vs. Biochar; 6- Pp13 vs. Biochar+F476; 7- Pp13 vs. Biochar+HRC54; 8- Pp13 vs. Biochar+F476+HRC54). Five replications were used, and the experimental plot consisted of three pots with three seedlings, each. Papaya seeds (UENF-Caliman 01 hybrid, known as Calimosa), without chemical treatment, were provided by Caliman Agrícola S/A (Linhares, Espírito Santo).

Experiment 1: seedling substrate treatment before transplantation

The following elements were added to the seedling substrate (Basaplant®) in each tube (0.28 dm^3): 1 mL of suspension with 10^6 spores.mL^{-1} of *T. longibrachiatum* F476, 1 mL of suspension with 10^8 cells.mL^{-1} of *H. seropedicae* HRC54 and 1% (v/ v) biochar. Three seeds were sown per tube, at 2 cm depth, 7 days later. Seedlings were transplanted to 1.7 L pots filled with soil at 45 days, without inoculum and biochar replacement. The soil in the pots was previously infested with the suspension of 10^3 chlamydospores.dm^{-3} of *P. palmivora* Pp13, which was isolated 24 hours before the transplantation. The final seedling survival assessment was performed 30 days after transplantation.

Experiment 2: soil treatment before sowing

Pots (1.7 L in volume) were filled with soil infested with suspension of 10^3 chlamydospores.dm^{-3} of *P. palmivora* Pp13. The aliquot of 1 mL of suspension with 10^6 spores.mL^{-1} of *T. longibrachiatum* F476, 1 mL of suspension with 10^8 cells.mL^{-1} of *H. seropedicae* HRC54, and 1% (v/v) biochar were incorporated to soil surface layer (equivalent to 0.3 dm^3) 24 hours later. Three seeds were sown per pot, 7 days later, at 2 cm depth, without inoculum and biochar replacement. The final seedling survival assessment was performed 75 days after sowing.

Statistical analysis

Data of the survival analyses were subjected to normality and homogeneity test, which was followed by analysis of variance (ANOVA) and Tukey test to compare means at 5% probability level – whenever a significant effect was observed. The absolute control (without both BACs and Pp13) did not receive any treatment; it was only used for experimental control purposes. Statistical analyses were performed in GraphPad Prism software version 5.0.

RESULTS

Antagonism in vitro against P. palmivora Pp13

T. longibrachiatum F476 showed signs of antagonistic activity *in vitro* against *P. palmivora* Pp13 (Figure 1D) - >95% of the plate surface was covered by the colony with mycelium and spores of *T. longibrachiatum* F476 on the *P. palmivora* Pp13 colony, according to the Bell scale (Bell et al., 1982). The same profile was observed when *T. longibrachiatum* F476 and *H. seropedicae* HRC54 were co-cultivated (Figure 1F). On the other hand, bacterium *H. seropedicae* HRC54 seemed not to show signs of antagonism, antibiosis or hyperparasitism against Pp13, except for a slight growth inhibition due to competition for nutrients, when it was paired in PDA culture medium (Figure 1 E).

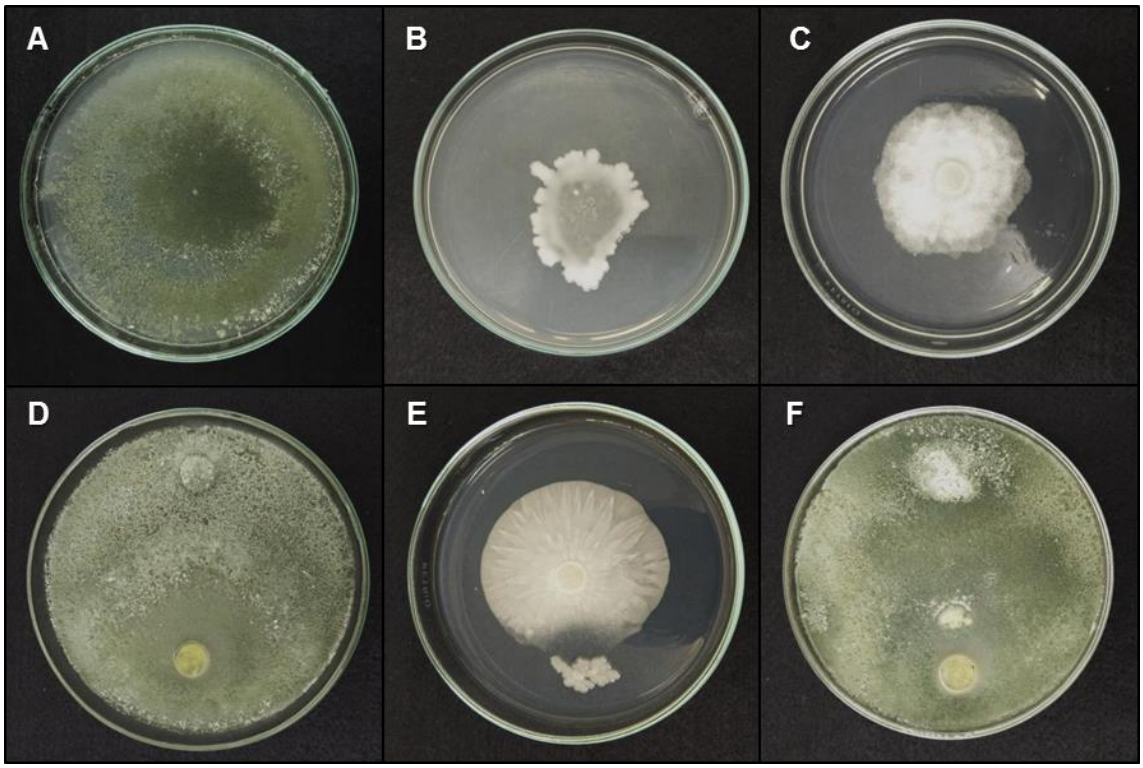

Figure 1. Effect of pairing *T. longibrachiatum* F476 and *H. seropedicae* HRC54 in plate on *P. palmivora* Pp13, seven days after the culture was paired. A) *T. longibrachiatum* F476; B) *H. seropedicae* HRC54; C) *P. palmivora* Pp13; D) Pp13 vs. F476; E) Pp13 vs. HRC54; F) Pp13 vs. F476+HRC54.

P. palmivora Pp13 virulence was confirmed in papaya green-fruit fragments used as host tissue (Figure 2C), whereas *T. longibrachiatum* F476 and *H. seropedicae* HRC54 showed no pathogenic action in fruits (Figures 2A and 2B). *H. seropedicae* HRC54 did not inhibit *P. palmivora* Pp13 growth in fruits, as observed in the plate (Figure 2E). On the other hand, *T. longibrachiatum* F476 was effective in inhibiting *P. palmivora* Pp13 growth in the plate; there was no symptom of soft rot in the inoculated fruit (Figure 2D). However, *T. longibrachiatum* F476 did not inhibit pathogenesis when it was associated with *H. seropedicae* HRC54, *in vitro;* furthermore, extensive *P. palmivora* Pp13 mycelial growth was observed, and it was associated with internal tissue rotting in the inoculated fruit fragments (Figure 2F).

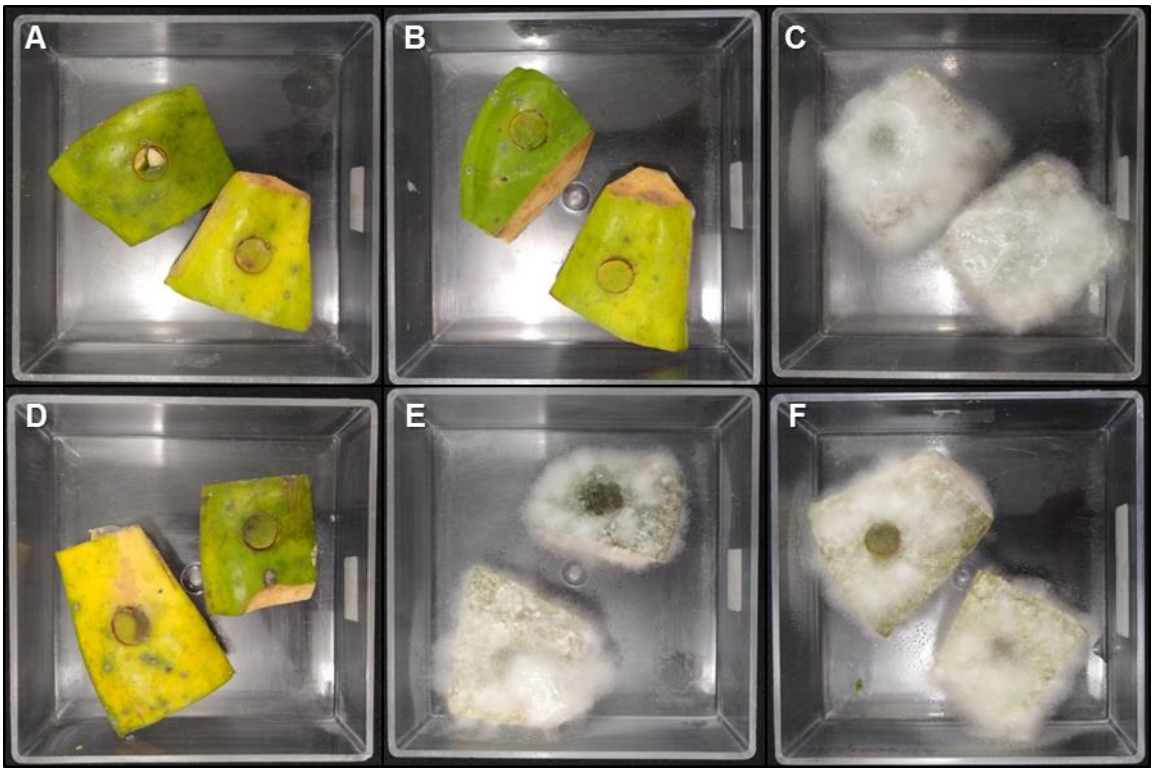

Figure 2. Fragments of green papaya fruit 7 days after culture medium disc inoculation for culture pairing. A) *T. longibrachiatum* F476; B) *H. seropedicae* HRC54; C) *P. palmivora* Pp13; D) Pp13 vs. F476; E) Pp13 vs. HRC54; F) Pp13 vs. F476+HRC54.

It was possible observing that *H. seropedicae* HRC54 did not affect *P. palmivora* Pp13 growth and sporulation 72 hours after microculture cultivation (Figure 3E). *T. longibrachiatum* F476 produced a large amount of hyphae and spores; it was possible observing *Trichoderma* (Figure 3F) hyperparasitism due to its hyphae coiling on the pathogen's hyphae and spores, as well as to spores' hyphae and germ tube invasion in *P. palmivora* Pp13 spores. However, *H. seropedicae* HRC54 and *T. longibrachiatum* F476, altogether, did not hyperparasitize *P. palmivora* Pp13 structures before 72-h incubation, despite the abundance of *T. longibrachiatum* F476 spores.

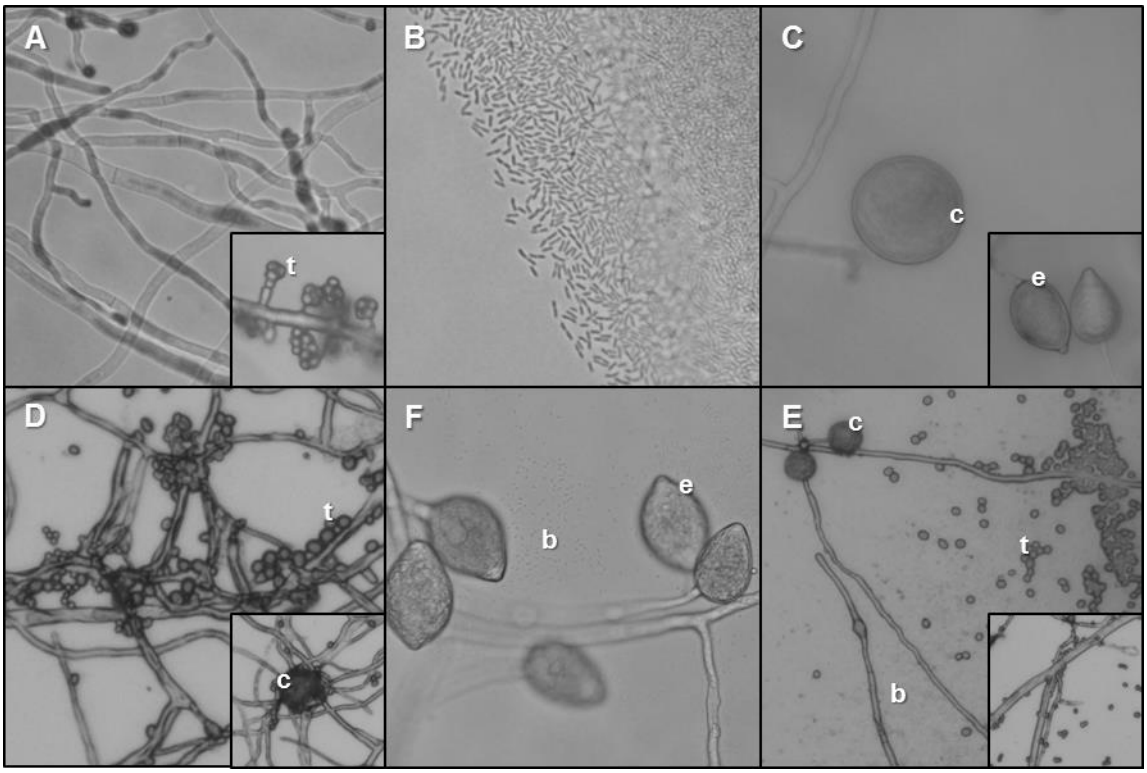

Figure 3. Image of *T. longibrachiatum* F476 hyphae and spores associated (or not) with *H. seropedicae* HRC54, in co-culture with *P. palmivora* Pp13, 72 hours after incubation, *in vitro*. A) hyphae and spores (t) of *T. longibrachiatum* F476; B) cells of *H. seropedicae* HRC54; C) chlamydospore (c) and sporangia (e) of *P. palmivora* Pp13; D) tangled of hyphae and spores (t) of F476 in hyphae and spores of Pp13; E) sporangia (e) of Pp13 without indications of hyperparasitism by the cells (b) of HRC54; F) chlamydospore (c) of hyphae of *P. palmivora* Pp13 intact even with an abundance of spores (t) of F476 and cells (b) of HRC54.

Papaya root rot biocontrol

Rot symptoms started 5 days after treated seedlings' transplantation – experiment 1. There was no significant difference between treatments under experimental conditions. F476 accounted for 70% inoculated seedling survival; it was followed by Biochar+F476 and HRC54, which recorded 60.3% and 56.9%, respectively (Figure 4). Treatments with F476+HRC54, Biochar+HRC54 and Biochar showed the lowest survival rates in comparison to treatments with BCAs.

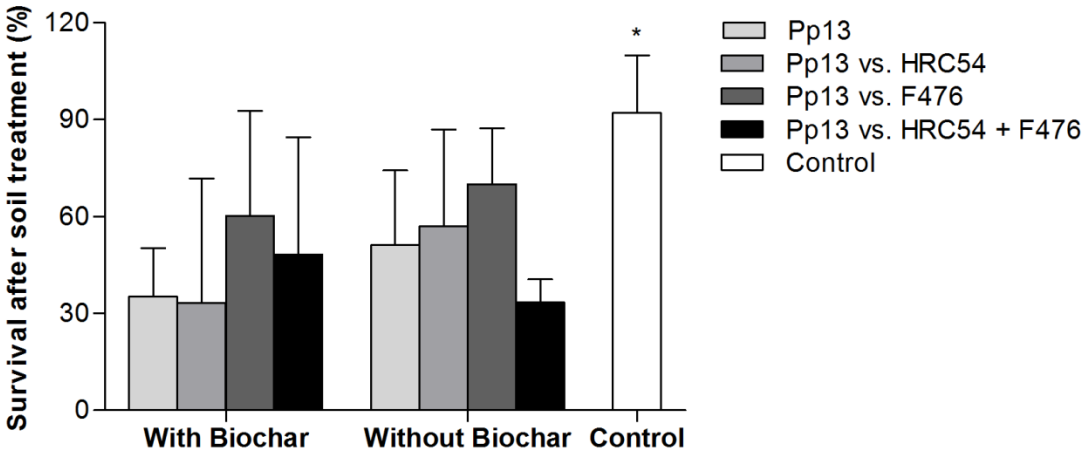

Figure 4. Mean seedling survival (% ± standard deviation) after substrate treatment with *T. longibrachiatum* F476, *H. seropedicae* HRC54 and biochar, followed by transplantation to soil infested with *P. palmivora* Pp13. *The control (without both BCAs and Pp13) did not receive treatment for disease control, but it recorded seedlings' survival without pathogen (Pp13) presence. N = 45 seedlings per repetition.

Initial rot symptoms were identified at 45 days in experiment 2, approximately 30 days after seedling emergence in soil infested with *P. palmivora* Pp13 and treated with BCAs. There was significant difference between treatments under this condition, according to which, F476+HRC54 showed the highest survival rate of plants (66.8%) (Figure 5). Biochar+F476+HRC54 showed the lowest survival rate among treatments with BCAs - only 32.2% of plants were still alive at the end of the experiment.

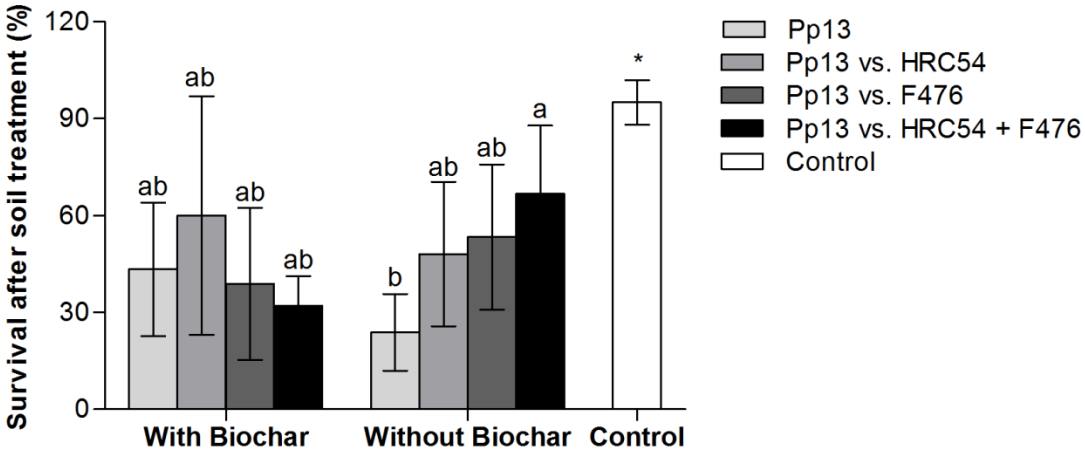

Figure 5. Mean seedling survival (% ± standard deviation) in soil infested with *P. palmivora* Pp13, treated with *T. longibrachiatum* F476, *H. seropedicae* HRC54 and biochar. Means followed by the same letters did not differ from each other in the

Tukey's test (P ≤ 0.05). *The control (without both BCAs and Pp13) was not treated for disease control, but it showed seedlings' survival when the pathogen (Pp13) was not present. N = 45 seedlings per repetition.

Mean seedling survival reached 48.6% in experiment 1 and 45.8% in experiment 2, if one takes into consideration all treatments with *P. palmivora* Pp13. However, treatment form influenced the herein recorded outcomes. Treating seedlings with F476 prior to their transplantation to soil infested with *P. palmivora* Pp13 reduced plant mortality by 18.9%, whereas treating soil infested with *P. palmivora* Pp13 by using F476+HRC54 reduced seedling mortality by 42.9% in comparison to the experiment with pathogen infestation – except for the treatment with BCAs (Pp13).

DISCUSSION

Trichoderma spp. are a fast-growing fungi and an aggressive competitors; are widely used as biocontrol agent against soil disease (Hewavitharana et al., 2018), since it can produce several antibiotic compounds and secondary metabolites that perform mycoparasitism and induce host plant resistance (Waghunde et al., 2016). Accordingly, lytic enzymes, proteases and cellulases - synthesized by *Trichoderma* species – make hydrolysis and pathogens' cell wall degradation easier, when composed of chitin, β-glucans or cellulose, a fact that favors the attack by this fungus to other pathogenic fungi (Blaszczyk et al., 2014). Oomycetes, in particular, have cellulose as the main component of their cell wall, which has little or no chitin and is easily hydrolyzed by proteins secreted by *Trichoderma* (Fawke et al., 2015; Cheng et al., 2019) - members of the *Longibrachiatum* clade are mostly known as cellulolytics (Samuels et al., 2012). Such characteristics reinforce the observation of *P. palmivora* Pp13 hyphae and spores' parasitism by *T. longibrachiatum* F476.

However, *H. seropedicae* HRC54 showed slight competition effect *in vitro* and reduced *T. longibrachiatum* F476 parasitism on *P. palmivora* Pp13, up to 72 hour of microculture. Endophytic microorganisms may not synthesize secondary

metabolites and compounds that act in plant resistance induction, in antibiosis against competing microorganisms or even present disease biocontrol effect in the absence of host plants and under culture conditions *in vitro* (Carneiro Jr. et al. al., 2021). In addition, microorganisms' introduction into the soil comes up against the need for adaptation, environment complexity (Savazzini et al., 2009), competition with natural microbiota and, in the case of disease biocontrol, the greater ability of phytopathogens to multiply in different hosts (Hanada et al., 2009). Beneficial microorganisms compete with a vast microbiota that is adapted to plants and that lives in the rhizosphere and roots, where they interact at multiple temporal and spatial scales (a fact that significantly changes disease symptoms and the involved biocontrol mechanisms), even when they are isolated from the target host plant (Kumar and Verma, 2019; Latha et al., 2019).

T. longibrachiatum F476 and/or *H. seropedicae* HRC54 showed biocontrol effects on root rot in both experiments conducted with papaya seedlings in the current study. If one takes into consideration that *Trichoderma* has direct antagonistic effect against *P. palmivora*, as observed in assessments *in vitro*, it is possible stating that its presence in seedling substrate may have accounted for the direct *P. palmivora* Pp13 parasitism in the soil, a fact that had effect on seedling survival, as observed in other studies. Dianese et al. (2012) used different *Trichoderma* spp. isolates in soil previously infested with *P. palmivora*; they recorded different seedling mortality outcomes depending on the used isolate: rates ranged from 15% to 75%, on average. Sánchez-Rangel et al. (2016) recorded 13.7% reduction in damping-off severity in papaya seedlings by using *Trichoderma* spp. (SP6) isolated from the rhizosphere of healthy plants - evaluations were carried out 40 days after inoculation. *T. longibrachiatum* 4088 was efficient in reducing the size of lesion caused by *P. palmivora* in papaya fruits after harvest – it was applied 24 hours before pathogen infestation (Oliveira et al, 2018).

Despite the outcomes *in vitro*, *H. seropedicae* HRC54 showed promising responses to control rot and higher seedling survival rates in some treatments. Plant growth-promoting bacteria can decrease or prevent damage caused by phytopathogens due to antagonism, systemic resistance induction against pathogens and competition for iron (Perez-Montano et al. 2014). *H. seropedicae* has at least 27 genes involved in iron transport and metabolism (Pedrosa et al.,

2011); thus, siderophore production contributes to bacteria's competitive performance in the host plant (Rosconi et al., 2013; Rosconi et al., 2016); moreover, it has the potential to modulate plant defense responses (Brusamarello-Santos et al., 2012; Do Amaral et al., 2014). *Herbaspirillum* sp. presence in banana seedlings is associated with decrease in wilt severity caused by *Fusarium oxysporum* f. sp. *cubense* (Weber et al., 2007). Seedlings treated with *H. rubrisubalbicans* showed low incidence of seropositive *Leifsonia xyli* subsp. *Xyli* stems in sugarcane; these bacteria cause ratoon stunting disease. Seedlings treated with *H. rubrisubalbicans* showed better agronomic yield levels. These authors attributed the antagonistic effect of *H. rubrisubalbicans* on xylem colonization to the pathogen in variety Co 421 (Carneiro Jr. et al., 2021). The growth of seedlings treated with diazotrophic bacteria was associated with plant physiology modulation and growth stimulation in other treatments where the incidence of seropositive stems in the presence of Lxx was high. It may have happened because of the hormonal effect or of biological nitrogen fixation promoted by *H. rubrisubalbicans*, which induce tolerance to rickets. There was necrotic tissue size reduction and decrease in the number of wilted leaves of papaya seedlings infected by *Phytophthora* sp. and treated with *Phothorhabdus* spp. up to 2 weeks after infection. However, plant mortality decrease can range from 0% to 13%, 42 days after the treatment has started (Palmeri et al., 2019). Pre-treatment of cocoa plants with *Pseudomonas chlororaphis* CP07 had the potential to reduce black pod rot caused by *P. palmivora*, but seedling survival changed depending on the assessed genotype (Miguelez-Sierra et al., 2019).

On the other hand, for two decades now, integrated strategies have been suggested to more effectively manage plant diseases because, overall, only one biocontrol agent may not be able to play this role, on its own (Sahni et al. 2008). The combined application of Bio P60 (gross secondary metabolites of *Pseudomonas fluorescens* P60) and Bio T10 (crude secondary metabolites of *Trichoderma harzianum* T10) reduced leaf wilting in papaya due to *P. palmivora* by 20% (Soesanto et al., 2019). The combined application of *Glomus mosseae*, *T. harzianum* and *P. fluorescens* has increased the height and yield of papaya plants, as well as reduced papaya root rot severity caused by *P. parasitica* var. *nicotianae* (Sukhada et al., 2011). These authors have shown that BCAs caused significant reduction in the *Phytophthora* population in plants' root system, under controlled

conditions and cultivation in sterile soil. The application of 10 tons/ha Tricho-compost fertilizer + 2 tons/ha of rice husk biochar significantly reduced the intensity of late blight in potato plants caused by *P. infestans* and increased their yield by 28.6% (Meilin et al., 2020).

Evidence has shown biochar effectiveness in controlling phytopathogens (Zwart and Kim, 2012, Bonanomi et al., 2015; Poveda et al., 2021). The application of 1.33% biochar on pepper plants inhibited *P. capsici* growth and suppressed root rot incidence. However, biochar application right before planting led to significantly better results for *P. capsici* rot biocontrol in pepper plants than its application 20 days before planting (Wang et al., 2019, 2020). Therefore, the addition of 1% (v/v) of this compound to the treatments was expected to increase seedling survival rate in both experiments. However, it was not possible distinguishing such an isolated effect on *P. palmivora* Pp13 control under the tested conditions. Nevertheless, its association with F476 in experiment 1 and with HRC54 in experiment 2 resulted in higher seedling survival rates. Wang et al. (2020, 2019) showed that biochar-mediated disease suppression is closely correlated to the proliferation of fungi that have intense pathogen-antagonistic activity, such as *Aspergillus* spp., *Penicillium* spp. and *Trichoderma* spp., and plant-growth promoting bacteria like *Bacillus* spp., *Pseudomonas* spp., *Sphingomonas* spp. and *Streptomyces* spp. Assumingly, poultry litter biochar addition has herein maximized the antagonistic effects of *T. longibrachiatum* F476 and *H. seropedicae* HRC54, in separate, against *P. palmivora* Pp13. However, the combined and simultaneous use of *T. longibrachiatum* F476, *H. seropedicae* and biochar did not result in disease biocontrol under the tested experimental conditions. Overall, effects of BACs using depend on treatment method, environmental factors, host plant genotype, and on the beneficial microorganism's effectiveness against the pathogen (Rahman et al., 2018).

With respect to experiment 1, 45 days elapsed after BCAs application in the substrate; there was decline in, and stabilization of, the added microbiota (Barroso et al., 2022). The microbiota present in the seedling substrate underwent a new stabilization process when it got in contact with the soil at post-transplantation. On the other hand, *P. palmivora* Pp13 got adapted to the environment when seedlings that were previously produced under controlled conditions were transplanted to the planting area; this condition was similar to what was observed in the field. This

process can reduce the antagonistic effect of BCAs against the pathogen. In parallel (Barroso et al., 2022), Biochar incorporation to the substrate, in presence or not of *T. longibrachiatum* F476 and *H. seropedicae* HRC54, prior to transplantation, stimulated seedling growth and the development of papaya plants' root system; assumingly, it also increased the production of root exudates that attract *P. palmivora* Pp13 zoospores. In addition, increase in the root system also increases infection sites available for zoosporic infection (Ribeiro, 2013). On the other hand, BCAs incorporation took place 24 hours after soil infestation with Pp13 in experiment 2; it was followed by 7-day incubation prior to sowing. In addition, seed germination (with the emission of the first pair of leaves and rootlets) was observed at approximately 15 days after sowing. Thus, the combined incubation period of 21 days may have been enough to stabilize the microbiota added to the soil and the antagonistic effect of BCAs on *P. palmivora* Pp13 - all treatments showed higher mean survival rates than the inoculated control, which was not treated.

Biological control of soil pathogens aims at reducing the amount of initial inoculum or pathogen activity in the environment. These pathogens are rarely fully ruled out, but population reduction can affect their ability to make hosts' sick. Thus, if one takes into consideration that root rot caused by *P. palmivora* in papaya can affect all plant development stages and reduce by 20% to 40% plant mortality at the initial seedling production stage means significant reduction in crop losses. Further studies are needed to determine whether the application of the tested BCAs has direct effect on the pathogen reduction in the soil and on papaya root rot control under field conditions. It is also important assessing the biochar application impact on microbiota associated with roots and potential stimulus to resistance induction in host plants.

CONCLUSIONS

T. longibrachiatum F476 and *H. seropedicae* HRC54 have the potential to be used as biocontrol agents against *P. palmivora* Pp13; however, the recorded

responses depend on the applied treatment method. Seedling treatment with F476 reduced plant mortality by 18.9%, whereas soil treatment with F476+HRC54 reduced it by 42.1% in comparison to the control, which was inoculated with the pathogen but that was not treated with BCAs. The addition of 1% of biochar into treatments did not cause significant changes in seedling survival rates, but it maximized the effects of F476 on seedling substrate treatment and that of HRC54 on soil treatment.

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4 RESUMO E CONCLUSÕES

As principais conclusões obtidas nesta tese foram:

i) A adição de 1% de biochar no substrato de mudas promoveu ganhos de 8% na altura, 12% no diâmetro do caule, 12% na área foliar e 40% na biomassa seca da parte aérea, em média, em comparação aos tratamentos sem biochar. Esse composto foi responsável por aumentar significativamente a biomassa seca total de raízes e raízes finas, comprimento total, área superficial e volume radicular.

ii) Os efeitos estimuladores de crescimento da adição do biochar ao substrato das mudas de mamoeiro foram mais evidentes nas combinações separadas de Biochar com HRC54 ou de Biochar com F476. Ambas as combinações produziram mudas mais vigorosas, de maior qualidade e mais eficientes na utilização de S, P, e Mn, o que pode ser aplicado em condições de produção comercial de mudas em viveiros.

iii) Em relação ao controle biológico da podridão do pé do mamoeiro, F476 e HRC54 apresentaram potencial para serem utilizados como agentes de biocontrole contra *P. palmivora* Pp13, entretanto, as respostas obtidas foram dependentes do método de tratamento aplicado. O tratamento de mudas com F476 reduziu em 18,9% a mortalidade de plantas, enquanto o tratamento de solo com HRC54+F476 reduziu em 42,1%, em relação à condição não tratada.

iv) A adição de 1% de biochar não apresentou alterações significativas na sobrevivência de mudas, mas maximizou os efeitos de F476 no tratamento de mudas e de HRC54 no tratamento de solo.

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