

UNIVERSIDADE FEDERAL DE ALFENAS

KAMILLA PACHECO GOVÊA

**CHÁS DE COMPOSTO NO CRESCIMENTO DE PLANTAS DE MILHO E SORGO
EXPOSTOS AO DÉFICIT HÍDRICO E METAIS PESADOS**

ALFENAS/MG

2023

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Tese apresentada como parte dos requisitos para obtenção do título de Doutora em Ciências Ambientais, pela Universidade Federal de Alfenas. Área de concentração: Ciências Ambientais.

Orientador: Prof. Dr. Thiago Corrêa de Souza

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O Presidente da banca examinadora abaixo assina a aprovação da Tese apresentada como parte dos requisitos para obtenção do título de Doutora em Ciências Ambientais pela Universidade Federal de Alfenas. Área de concentração: Ciências Ambientais.

Aprovada em: 29 de setembro de 2023

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Universidade Federal de Alfenas (UNIFAL-MG)

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Dedico este trabalho aos meus pais, minhas raízes;
e ao meu filho, meus frutos.

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Finalizar este Doutorado tendo vivenciado uma pandemia, uma gravidez e um puerpério no meio do processo, não foi fácil. Ter feito boa parte dele durante o governo (2019-2022) mais negacionista e que menos investiu em ciência na história do Brasil, além de ter sucateado o ensino superior público de forma recorde, foi menos fácil ainda. Mas estar aqui hoje é uma vitória com gosto de “tudo valeu a pena”, graças ao afeto de quem me rodeia, e à minha potência interna que – creio que agora eu não precise mais duvidar – pode criar muitas possibilidades apesar das adversidades.

RESUMO

Os chás de composto têm sido relatados como bioestimulantes e/ou biofertilizantes de plantas, melhorando as respostas no crescimento e na tolerância a estresses bióticos e abióticos, sendo os estresses abióticos ainda pouco explorados na literatura. Neste sentido, os objetivos deste trabalho foram: 1) elucidar, através de uma meta-análise, o potencial indutor de tolerância a estresses abióticos que os chás de composto e de vermicomposto podem proporcionar; e 2) verificar os efeitos de dois chás de composto no crescimento inicial de milho e sorgo e na tolerância de ambas as espécies ao déficit hídrico, à presença de chumbo (Pb) e de alumínio (Al). Para isso, foram realizadas duas compostagens que diferiram em relação às fontes de nitrogênio: uma somente à base de plantas, e a outra contendo esterco bovino. Os compostos obtidos serviram como matéria-prima para extração dos chás (respectivamente, PCT: *plants compost tea* e CMCT: *cattle manure composttea*). Foram realizados quatro experimentos para cada espécie, totalizando os seguintes conjuntos de dados: 1) ação dos chás de composto sobre milho e sorgo; 2) ação dos chás de composto sobre milho e sorgo sob déficit hídrico; 3) ação dos chás de composto sobre milho e sorgo sob estresse por Pb; e 4) ação dos chás de composto sobre milho e sorgo sob estresse por Al. A metodologia utilizada e as análises estatísticas estão descritas em detalhes nos artigos contidos nesta Tese. A meta-análise demonstrou que os chás de composto e de vermicomposto podem ser aplicados como indutores de tolerância a estresses abióticos em plantas, e seus mecanismos de ação envolvem a modulação do metabolismo antioxidante enzimático, o aumento do metabolismo fotossintético, a homeostase de potássio e sódio, a estimulação do crescimento e, conseqüentemente, o aumento da produtividade. Além disso, os chás de composto testados apresentaram atividade bioestimulante sobre milho e sorgo, por meio da estimulação do crescimento inicial, da morfologia e arquitetura radicular, e da mitigação do déficit hídrico e dos estresses ocasionados por Pb e Al.

Palavras-chave: Meta-análise; Estresses abióticos; Seca; Chumbo; Alumínio.

ABSTRACT

Compost teas have been reported as plant biostimulants and/or biofertilizers, improving responses in growth and tolerance to biotic and abiotic stresses, with abiotic stresses still little explored in the literature. In this context, the objectives of this work were: 1) to elucidate, through a meta-analysis, the potential to induce tolerance to abiotic stresses that compost and vermicompost teas can provide; and 2) verify the effects of two compost teas on the initial growth of maize and sorghum and on the tolerance of both species to water deficit, the presence of lead (Pb) and aluminum (Al). For this, two composting processes were carried out that differed in relation to nitrogen sources: one completely plant-based, and the other using cattle manure. The composts obtained served as raw material for extracting teas (respectively, PCT: plants compost tea, and CMCT: cattle manure compost tea). Four experiments were carried out for each species, totaling the following data sets: 1) action of compost teas on maize and sorghum; 2) action of compost teas on maize and sorghum under water deficit; 3) action of compost teas on maize and sorghum under Pb stress; and 4) action of compost teas on maize and sorghum under Al stress. The methodology used and the statistical analyzes are detailed in the articles contained in this Thesis. The meta-analysis demonstrated that compost teas and vermicompost teas can be applied as tolerance-inducers to abiotic stresses in plants, and their mechanisms of action involve the modulation of enzymatic antioxidant metabolism, enhancing photosynthetic metabolism, homeostasis of potassium and sodium, growth stimulation and, consequently, the increase in productivity. Furthermore, the compost teas tested showed biostimulant activity on maize and sorghum, by stimulating initial growth, root morphology and architecture, and mitigating water deficit and stress caused by Pb and Al.

Keywords: Meta-analysis; Abiotic stresses; Drought; Lead; Aluminum.

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1 INTRODUÇÃO

A Revolução Verde, que ocorreu em meados dos anos 1960, possibilitou maior produtividade de alimentos, devido à utilização intensiva de agroquímicos. Porém, de acordo com Adhikary (2012), a utilização indiscriminada destes compostos teve alto custo para o meio ambiente e a sociedade, visto que os mesmos têm capacidade de diminuir a fertilidade natural do solo e prejudicar seus organismos benéficos, danificando a tolerância da colheita tornando-a mais suscetível a pragas e doenças; além do fato de alimentos cultivados quimicamente afetarem negativamente a saúde humana. Nesse contexto, a comunidade científica tem buscado uma alternativa economicamente viável, socialmente segura e ecologicamente sustentável aos agroquímicos (ADHIKARY, 2012; GAMAGE *et al.*, 2023).

Desde então, a compostagem e a vermicompostagem têm sido apresentadas como possíveis alternativas ao uso imoderado de fertilizantes químicos. Ambas as técnicas já têm reconhecimento na literatura por promoverem tratamento de resíduos sólidos orgânicos e produzirem compostos fertilizantes ao final do processo, alterando positivamente as características físicas, químicas e biológicas do solo (ENEBE; ERASMUS, 2023; WAQAS *et al.*, 2023). Dessa forma, têm efeito estimulante sobre o crescimento, desenvolvimento e produtividade de culturas agrícolas, além de alguns estudos demonstrarem que as técnicas em questão podem auxiliar na resposta de tolerância das plantas frente a estresses ou aumentar a capacidade de biorremediação (AYILARA *et al.*, 2020; BLOUIN *et al.*, 2019; REHMAN *et al.*, 2023).

Mais recentemente, agricultores que objetivam minimizar a utilização de fertilizantes e pesticidas têm utilizado como alternativa extratos aquosos de composto e de vermicomposto. De acordo com Arancon *et al.* (2007), a vantagem de utilizar tais extratos, chamados de “chás” (*compost tea e vermicompost tea*), é a facilidade de transporte e aplicação nas colheitas em relação aos respectivos materiais sólidos. Os chás de composto e de vermicomposto vêm sendo estudados como bioestimulantes de plantas, devido a sua composição rica em ácidos húmicos e ácidos fúlvicos, e estão associados à fertilização do solo, ao estímulo de processos naturais que possam aumentar ou beneficiar a absorção de nutrientes, ao controle de doenças e pragas, além de induzir respostas de tolerância a diversos estresses (EUDOXIE; MARTIN, 2019; RAMÍREZ-GOTTFRIED *et al.*, 2023; YATOO *et al.*, 2021).

Apesar dos estudos consistentes na área e do aperfeiçoamento da técnica ao longo do tempo, a maior parte da literatura científica que objetivou estudar os efeitos dos chás de

composto ou vermicomposto como indutores de tolerância a estresses abióticos estão relacionados ao estresse salino. Portanto, existem poucos estudos que exploram outros tipos de estresses abióticos, como o estresse hídrico, e os estudos são ainda mais escassos com relação ao estresse ocasionado pela presença de metais pesados (ABDOU *et al.*, 2023; AMER *et al.*, 2020, 2021; ELBAGORY, 2023; EON *et al.*, 2023; HENDAWY *et al.*, 2013; LI *et al.*, 2021). No entanto, cabe salientar que tais estresses abióticos vêm trazendo prejuízos para a agricultura no Brasil e no mundo (ADEJUMO, 2015; BAYOUMY; KHALIFA; ABOELSOU, 2019; NAZIR *et al.*, 2017; PACETTI; CAPORALI; RULLI, 2017), demonstrando a importância de se conhecer alternativas para viabilizar o aumento da produção agrícola de forma sustentável.

Neste contexto, os objetivos deste trabalho foram: 1) elucidar, através de uma meta-análise, o potencial indutor de tolerância a estresses abióticos que os chás de composto e de vermicomposto podem proporcionar; e 2) verificar os efeitos de dois chás de composto no crescimento inicial de milho e sorgo e na tolerância de ambas as espécies ao déficit hídrico, à presença de chumbo (Pb) e de alumínio (Al).

2 REVISÃO DE LITERATURA

A seguir, acompanha revisão de literatura atualizada acerca dos temas abordados nesta Tese, com intuito de gerar embasamento teórico para a análise e discussão dos resultados obtidos para os três artigos.

2.1 BIOESTIMULANTES E BIOFERTILIZANTES

Bioestimulantes de plantas ou bioestimulantes agrícolas são substâncias e/ou microrganismos que promovem aumento do crescimento e desenvolvimento quando aplicados sobre plantas ou na rizosfera, a despeito do conteúdo de nutrientes. Os bioestimulantes são assim chamados por estimularem processos naturais, aumentando ou beneficiando a absorção e a eficiência do uso de nutrientes, a qualidade da colheita, a tolerância a estresses e a melhora da composição mineral dos tecidos (BULGARI *et al.*, 2014; DROBEK; FRĄC; CYBULSKA, 2019; DU JARDIN, 2015; SIBLE; SEEBAUER; BELOW, 2021).

Os bioestimulantes são muito variáveis, podendo ser representados por diversas classes de compostos – desde que não sejam nutrientes, pesticidas ou aditivos de solo –, como substâncias húmicas, substâncias contendo hormônios, substâncias contendo aminoácidos e outros compostos nitrogenados, extratos de algas marinhas, quitosana e outros biopolímeros, poli e oligossacarídeos, vitaminas, compostos inorgânicos benéficos e alguns microrganismos benéficos (BULGARI *et al.*, 2014; DU JARDIN, 2015). Eles podem agir sobre plantas diretamente de diversas maneiras, tendo alguns efeitos relatados na literatura, como estímulo do crescimento de raízes ou de plântulas inteiras, auxílio na regeneração de raízes, aumento do conteúdo de clorofila e da área foliar, aumento da biomassa e da produtividade, aumento da concentração de nutrientes em raízes e caules, proteção contra radiação UV e estresse oxidativo, manutenção da atividade fotossintética sob estresses abióticos e auxílio na resposta contra patógenos (BROWN; SAA, 2015; BULGARI *et al.*, 2014; DROBEK; FRĄC; CYBULSKA, 2019; DU JARDIN, 2015). Porém, também podem agir indiretamente, pela melhora da qualidade do solo com a adição de microrganismos benéficos (BROWN; SAA, 2015; BULGARI *et al.*, 2014).

Dentre os bioestimulantes, as substâncias húmicas são largamente exploradas. De acordo com Canellas *et al.* (2015), o efeito estimulatório das substâncias húmicas sobre o crescimento, desenvolvimento e produtividade de plantas já é bem documentado na literatura, e alguns mecanismos de ação já foram elucidados. Devido ao fato de modificarem o potencial

de membrana das células radiculares ou foliares pela indução da H⁺-ATPase, as substâncias húmicas podem atuar ativando o transporte de íons secundários (aumentando a absorção de nutrientes minerais), promovendo o crescimento radicular pela ativação de vias metabólicas e ativando canais de transporte de Ca²⁺. Isto, por sua vez, desencadeia uma cascata de reações que pode promover o crescimento de raízes em cabeleira, a fosforilação pelas proteínas quinases cálcio-dependentes (*Calcium-dependent protein kinases* – CDPK), ativação de canais de ânions e efeito tampão, o que pode levar à exudação de ácidos orgânicos. As substâncias húmicas, portanto, podem atuar estimulando tanto o metabolismo primário – na fotossíntese, no metabolismo energético e na assimilação de nutrientes – quanto o metabolismo secundário – na via do ácido chiquímico, na produção de fenóis e na defesa de plantas contra estresses (CANELLAS *et al.*, 2015; CONSELVAN *et al.*, 2017).

Diante do exposto, é comum a associação dos chás de composto e de vermicomposto como bioestimulantes de plantas na literatura científica, uma vez que são ricos em nutrientes e microrganismos benéficos, além de poderem conter uma série de outras substâncias favoráveis (EUDOXIE; MARTIN, 2019; RAMÍREZ-GOTTFRIED *et al.*, 2023).

Os biofertilizantes, por sua vez, podem ser compreendidos como fertilizantes não-químicos, isto é, são culturas de microrganismos como bactérias, fungos e microalgas, que quando adicionados ao solo ou à superfície das plantas, lhes oferecem nutrição direta pelo aumento da disponibilidade de nutrientes (BORASTE *et al.*, 2009; VESSEY, 2003). Por isso, podem também ser chamados de inoculantes microbianos, pois consistem em uma preparação contendo células vivas ou latentes de cepas com alta eficiência na fixação de nitrogênio, solubilização de fosfato e/ou degradação de celulose, que aceleram a disponibilização de nutrientes minerais. Deste modo, representam uma fonte renovável de nutrientes vegetais para substituir fertilizantes químicos, apresentando baixo custo e alta eficácia (BERRUTI *et al.*, 2016; BORASTE *et al.*, 2009; MOHAMMADI; SOHRABI, 2012).

De acordo com BrahmaPrakash e Sahu (2012), os biofertilizantes têm como efeito o melhoramento do crescimento de plantas devido ao aumento da absorção de nutrientes e/ou produção de hormônios de crescimento, e Bhardwaj *et al.* (2014) ressaltaram a importância dos biofertilizantes como aceleradores de disponibilização de nutrientes e indutores de tolerância a estresses ambientais. Dentro deste contexto, alguns autores caracterizam os chás de composto e de vermicomposto como biofertilizantes, uma vez que têm a capacidade de melhorar as características microbiológicas do solo (EL-HADDAD *et al.*, 2014; NAIDU *et al.*, 2010; ZEWAİL; AHMED, 2015).

Na Legislação Brasileira, a Instrução Normativa nº 61 de 08 de julho de 2020 (BRASIL, 2020) estabelece as regras sobre definições, exigências, especificações, garantias, tolerâncias, registro, embalagem e rotulagem dos fertilizantes orgânicos e dos biofertilizantes, destinados à agricultura. Seu conteúdo não define as substâncias bioestimulantes, mas caracteriza as substâncias biofertilizantes da seguinte forma:

[...] XXIII - biofertilizante: produto que contém princípio ativo ou agente orgânico, isento de substâncias agrotóxicas, capaz de atuar, direta ou indiretamente, sobre o todo ou parte das plantas cultivadas, elevando a sua produtividade, sem ter em conta o seu valor hormonal ou estimulante, subdivido nos seguintes grupos:

- a) biofertilizante de aminoácidos: produto obtido por fermentação ou hidrólise de materiais orgânicos naturais;
- b) biofertilizante de substâncias húmicas: produto obtido por decomposição e solubilização de materiais orgânicos e posterior oxidação e polimerização, formadas basicamente por ácidos húmicos, ácidos fúlvicos e huminas;
- c) biofertilizante de extratos de algas ou algas processadas: produto obtido por extração e beneficiamento de algas;
- d) biofertilizante de extratos vegetais: produto obtido por extração de compostos orgânicos solúveis da fermentação ou beneficiamento de materiais orgânicos, isentos de contaminação biológica;
- e) biofertilizante composto: produto obtido pela mistura de dois ou mais biofertilizantes dos grupos de aminoácidos, substâncias húmicas, extratos de algas, extratos vegetais e outros princípios ou agentes orgânicos aprovados;
- f) outros biofertilizantes que venham a ser aprovados pela pesquisa brasileira oficial ou credenciada. [...]

É possível perceber, portanto, que a Legislação Brasileira classifica como biofertilizantes uma ampla variedade de substâncias que a literatura científica internacional entende como bioestimulantes. Diante do exposto, pode-se compreender os chás de composto e de vermicomposto como substâncias bioestimulantes e biofertilizantes.

2.2 MATÉRIA ORGÂNICA NO SOLO

A matéria orgânica é o componente orgânico do solo, que pode ser dividida em dois compartimentos: a matéria orgânica viva, composta por raízes, microrganismos e macrofauna; e a matéria orgânica não-vivente, que pode ser matéria orgânica leve ou substâncias húmicas. A matéria orgânica leve compreende restos vegetais, da macrofauna e de microrganismos que ainda não sofreram decomposição e contém substâncias de degradação rápida, como proteínas, lipídeos e carboidratos; e também substâncias de degradação lenta, como celulose, hemicelulose, lignina e tecidos fibrosos. Já as substâncias húmicas são polímeros moleculares, de cor escura, parcialmente aromáticos, quimicamente complexos, de alto peso molecular, geralmente hidrofílicos, cujas estruturas moleculares são variáveis e sobre as quais não existe

um consenso, que são resistentes à degradação química e microbiológica (GMACH *et al.*, 2020; LIANG *et al.*, 2019; PRADO, 2013; WITZGALL *et al.*, 2021).

A matéria orgânica no solo pode atuar de três formas: como fornecedora de nutrientes, condicionadora físico-química e condicionadora biológica do solo. Como fornecedora de nutrientes, a matéria orgânica tem a capacidade de disponibilizar nutrientes minerais para as plantas conforme sua demanda. Como condicionadora físico-química, a matéria orgânica pode promover o aumento da capacidade de troca catiônica (CTC) do solo, a agregação das partículas, a infiltração e a retenção de água, a complexação e imobilização de elementos tóxicos como metais pesados, além de possuir um efeito conhecido como poder tampão, estabilizando o pH do solo e mantendo-o adequado ao desenvolvimento dos organismos que nele vivem. E como condicionadora biológica do solo, a matéria orgânica é capaz de promover a formação de nichos e fornecer fonte de energia para bactérias e fungos, além de atuar como um veículo para adição de microrganismos benéficos ao solo (PRADO, 2013; SIX *et al.*, 2004).

As substâncias húmicas compõem cerca de 60 – 80% da matéria orgânica do solo e são formadas pela decomposição da celulose, hemicelulose e lignina – ou seja, as substâncias de degradação lenta – e sua estrutura complexa faz com que sejam mais dificilmente degradadas do que as substâncias que lhes deram origem. Em função do grau de polimerização, as substâncias húmicas podem ser classificadas em ácidos fúlvicos, ácidos húmicos e humina, respectivamente (CANELLAS *et al.*, 2015; PRADO, 2013). A bioatividade desses compostos é benéfica para as plantas, promovendo aumento da taxa de germinação, do comprimento radicular, do número de raízes, da parte aérea, da biomassa, da eficiência do uso de nutrientes; além de proporcionarem melhora no metabolismo enzimático, no crescimento, na floração e na qualidade dos frutos; auxiliarem na resposta de tolerância a estresses bióticos e abióticos e poderem substituir os reguladores sintéticos de plantas. Assim, são exploradas como bioestimulantes quando extraídas e isoladas, reduzindo a aplicação de fertilizantes químicos (CANELLAS *et al.*, 2015; KLECZEK, 2022; VACCARO *et al.*, 2015).

Algumas técnicas realizadas pelo homem, como por exemplo a compostagem e a vermicompostagem, permitem manipular a produção de matéria orgânica e conseqüentemente obter estes produtos desejáveis à agricultura (ENEBE; ERASMUS, 2023; WAQAS *et al.*, 2023).

2.3 COMPOSTAGEM

A compostagem é um processo de decomposição biológica da matéria orgânica sob condições aeróbicas controladas. Tais condições permitem a ocorrência de temperaturas termofílicas, resultado do calor produzido biologicamente pela ação de microrganismos. Ao final do processo, a compostagem origina um substrato orgânico estável, homogêneo, sem cheiro, de cor escura, rico em húmus, livre de patógenos e sementes de plantas, chamado composto, o qual pode ser aplicado com intuito de melhorar características físicas, químicas e biológicas do solo (HAUG, 2018; LÓPEZ-GONZÁLEZ *et al.*, 2015; WAQAS *et al.*, 2023).

De acordo com Diaz e Bertoldi (2007), a necessidade de gestão de resíduos orgânicos acompanha as sociedades humanas desde o estabelecimento dos primeiros assentamentos coletivos, nos quais era comum a utilização de “poços de resíduos” construídos fora das casas. Nesses poços, os resíduos orgânicos produzidos eram armazenados com intuito de posterior aplicação nos campos agrícolas. O indício mais antigo de um poço de resíduos foi encontrado nas cidades sumérias, datando de cerca de 6000 anos atrás, e em sociedades primitivas da América do Sul, Índia, China e Japão, bem como na Antiga Grécia e Antiga Roma. Os autores ressaltaram que uma das técnicas antigas mais precisas sobre o processo de compostagem foi realizada e documentada pelos Cavaleiros Templários, no século XIII.

A partir do século XX, foram conduzidos ao redor do mundo estudos científicos mais aprofundados e técnicos, com intuito de estabelecer metodologias aplicáveis de compostagem com os mais diversos resíduos orgânicos (DIAZ; BERTOLDI, 2007; WAQAS *et al.*, 2023). Isto surge em um contexto que se estende até a atualidade, no qual as áreas urbanas vêm crescendo consideravelmente, fazendo com que a quantidade de resíduos sólidos gerados diariamente alcance magnitudes significativas e a destinação adequada seja essencial para manter boas condições de higiene na coletividade (ATALIA *et al.*, 2015; COSTA *et al.*, 2016). Assim, a compostagem passa a ser aplicada para reduzir a quantidade de resíduos orgânicos no meio ambiente, utilizando como matérias-primas resíduos e subprodutos agrícolas ou industriais, que podem ser urbanos e municipais, resíduos verdes, esterco, resíduos de culturas agrícolas, e demais restos orgânicos que possam ser compostados (CAVAGNARO, 2015; WAQAS *et al.*, 2023).

Além de ser compreendida como técnica que redireciona os resíduos orgânicos, impedindo que cheguem aos aterros sanitários, a compostagem gera produto – o composto – a custo relativamente baixo, que é adequado para fins agrícolas (ATALIA *et al.*, 2015; LÓPEZ-GONZÁLEZ *et al.*, 2015). Isto é um fator importante quando consideramos os problemas ambientais ocasionados pela utilização indiscriminada e inadequada de agroquímicos – fertilizantes químicos, herbicidas e pesticidas.

Desde a Revolução Verde na década de 1960, grandes quantidades de fertilizantes químicos foram aplicados amplamente para promover o crescimento das culturas agrícolas (LEITA *et al.*, 1999). No entanto, a aplicação em excesso de fertilizantes químicos desencadeia vários efeitos negativos no ecossistema, como degradação do solo, mudanças na diversidade genética de culturas, alterações na comunidade microbiana do solo, contaminação de lençóis freáticos, poluição atmosférica e redução da qualidade do solo devido à perda de conteúdo de matéria orgânica (SELVAKUMAR *et al.*, 2018; VALARINI *et al.*, 2002; ZHEN *et al.*, 2014). Assim, os compostos representam uma alternativa importante a ser considerada para substituir os fertilizantes químicos no meio agrícola, visto que há uma preocupação crescente com alimentos saudáveis e redução da poluição ambiental (LIGUORI *et al.*, 2015; WAQAS *et al.*, 2023).

As características presentes nos compostos devem-se ao processo de decomposição microbiana, que, de acordo com Haug (2018), pode ser realizada de forma aeróbica (na presença de oxigênio) ou anaeróbica (na ausência de oxigênio). A compostagem anaeróbica é menos eficiente do que a aeróbica em termos metabólicos, além de ter maior potencial de causar odores indesejáveis devido à natureza de muitos metabólitos intermediários liberados durante o processo. Por esses motivos, a maior parte dos estudos com compostagem privilegia sistemas aeróbicos (HAUG, 2018; KALEMELAWA *et al.*, 2012; WAQAS *et al.*, 2023).

Durante a compostagem, há desprendimento de dióxido de carbono, energia e vapor de água devido à ação dos microrganismos, os quais utilizam parte da energia para seu crescimento e liberam o restante na forma de calor. Como resultado, os resíduos orgânicos que estão sendo compostados se aquecem, atingem uma temperatura termofílica, posteriormente resfriam-se e atingem estágio de maturação. Após a maturação, o composto estará pronto, sendo constituído de partes resistentes dos resíduos orgânicos, produtos decompostos e microrganismos mortos e vivos (GAMAGE *et al.*, 2023; SOUZA *et al.*, 2001; WAQAS *et al.*, 2023).

Diante do exposto, pode-se considerar a compostagem como uma forma de estabilização de resíduos orgânicos, que requer condições especiais de umidade e aeração para produzir temperaturas termofílicas, geralmente acima de 45°, as quais são essenciais para a eliminação de patógenos e sementes de plantas daninhas. Os três principais fatores que devem ser controlados durante o processo de compostagem são: aeração, temperatura e umidade (HAUG, 2018; WAQAS *et al.*, 2023).

Para Cotta *et al.* (2015), o predomínio de algumas espécies de microrganismos e sua respectiva atividade metabólica determinam a fase em que o processo de compostagem se encontra. De acordo com os autores, a primeira fase, chamada mesófila, compreende o início

da decomposição da matéria orgânica, onde a predominância de bactérias que realizam a quebra inicial dos resíduos orgânicos promove a liberação de calor, aquecendo a leira, e alguns fungos produzem ácidos que degradam substâncias como proteínas e açúcares. Quando a leira atinge temperaturas superiores a 45°C, a comunidade microbiológica se altera, dando início à segunda fase, chamada de termofílica, na qual há predominância de fungos e bactérias termófilas, que promovem a máxima decomposição dos resíduos orgânicos. À medida que a matéria vai sendo decomposta, a temperatura da leira gradualmente se iguala à temperatura ambiente, fazendo com que uma nova comunidade de microrganismos mesófilos se instale, promovendo a humificação dos compostos em processo conhecido como estabilização ou maturação.

Com relação ao resíduo escolhido para compostagem, Leal *et al.* (2013a) atentaram para o fato de que os resíduos orgânicos possuem diferentes graus de contaminação química e/ou biológica, dependendo de sua origem. Assim, Reyes-Torres *et al.* (2018) elencaram os resíduos verdes como vantajosos, pelo fato de frequentemente apresentarem baixos conteúdos de micropoluentes. Entende-se por resíduo verde aqueles de origem vegetal.

Neste contexto, Leal *et al.* (2013b) elucidaram que o processo de compostagem requer uma combinação de materiais com altas relações C:N – como palhadas e serragem como fonte de carbono, e esterco e tortas como fonte de nitrogênio. Por este motivo, identificam os resíduos verdes – mais especificamente a biomassa de capim elefante e a torta de mamona – como materiais de origem vegetal propícios à utilização como matérias-primas para compostagem, devido ao fato de serem resíduos abundantes, de custo competitivo e com baixos níveis de contaminação química e/ou biológica, características que viabilizam economicamente a compostagem para obtenção de adubos orgânicos e substratos (LEAL *et al.*, 2007). A utilização destes materiais possibilita a obtenção de um substrato orgânico estabilizado, com alto teor de nitrogênio, sem necessidade de utilização de inoculantes ou aditivos (LEAL *et al.*, 2013b).

Um composto é considerado de boa qualidade quando é formado por uma textura fina, é úmido e livre de patógenos, contém grandes quantidades de microrganismos benéficos, nutrientes minerais solúveis, substâncias húmicas e fitohormônios, e quantidades irrisórias de ácidos orgânicos fitotóxicos e metais pesados (PANT *et al.*, 2012). Os compostos são amplamente relatados na literatura por promover inúmeros benefícios, como o fornecimento de nutrientes para as plantas e microrganismos do solo, a curto e a longo prazo; adição de matéria orgânica, macro e micronutrientes ao solo; liberação de ácidos húmicos e fúlvicos; melhorias na estrutura, na capacidade de troca catiônica e de retenção hídrica do solo; supressão do desenvolvimento de patógenos; aumento da estabilidade e aeração do substrato; mudanças na

mobilidade e biodisponibilidade de metais; e estimulação do crescimento, desenvolvimento e produtividade de culturas agrícolas (ADUGNA, 2016; ANASTOPOULOS; KYZAS, 2015; GAMAGE *et al.*, 2023; HAUG, 2018; LIGUORI *et al.*, 2015). Além disso, alguns estudos demonstram que os compostos podem ainda auxiliar na resposta tolerante de plantas a possíveis estresses bióticos e abióticos (AZIM, 2019; GIMÉNEZ *et al.*, 2019; JUNG *et al.*, 2016; KHAN *et al.*, 2019; MBARKI *et al.*, 2018; MEHTA *et al.*, 2012; TAROURA; YOUSSEF, 2011).

Vale ressaltar que a compostagem é uma técnica já difundida e utilizada em vários países como tratamento de resíduos sólidos, de forma que a variedade de compostos produzidos mundialmente representa vasta oferta de matérias-primas para obtenção de extratos aquosos (chás), sendo uma vantagem econômica e ambiental.

2.4 VERMICOMPOSTAGEM

A vermicompostagem é um processo de compostagem que utiliza algumas espécies de minhocas para aprimorar a conversão de resíduos e melhorar a qualidade do produto gerado (ADHIKARY, 2012; ENEBE; ERASMUS, 2023). Na vermicompostagem, embora os microrganismos sejam os responsáveis pela decomposição da matéria orgânica, a presença das minhocas influencia o processo físico e bioquímico, auxiliando na fase de estabilização da matéria orgânica quando se alimentam, transformando-a em um composto de maior qualidade do que aqueles produzidos pela compostagem tradicional (COTTA *et al.*, 2015; ENEBE; ERASMUS, 2023).

A vermicompostagem, portanto, é um processo não-termofílico no qual as minhocas fragmentam os resíduos ao se alimentarem, aumentando a atividade microbiana e acelerando as taxas de decomposição, levando a um processo de humificação que estabiliza a matéria orgânica e a transforma em composto, chamado de vermicomposto (ENEBE; ERASMUS, 2023). Assim como na compostagem tradicional, na vermicompostagem podem ser utilizados como matérias-primas inúmeros resíduos sólidos orgânicos, como resíduos verdes, lodos têxteis e industriais, esterco, resíduos urbanos e municipais, bem como oriundos da agricultura e pecuária (ADHIKARY, 2012; ALI *et al.*, 2015; ENEBE; ERASMUS, 2023; MAKKAR *et al.*, 2023). A técnica de vermicompostagem pode, inclusive, ser aplicada como biorremediadora de alguns resíduos (LEE *et al.*, 2018; SWATI; HAIT, 2017).

As vantagens da utilização da vermicompostagem se devem a alguns fatores, como baixo custo de investimento e de operação, simplicidade de ação e uma eficiência relativamente alta (COTTA *et al.*, 2015). Além disso, a utilização de resíduos verdes é vantajosa, por reduzir

a possibilidade de desenvolvimento de organismos prejudiciais às plantas e à saúde humana. (REYES-TORRES *et al.*, 2018).

Um fator imprescindível no desenvolvimento da técnica é a escolha da espécie adequada de minhoca, sendo a *Ensenia foetida*, conhecida popularmente como minhoca californiana ou minhoca vermelha da Califórnia, a mais utilizada. Isto deve-se a algumas características que esta espécie apresenta, como ampla distribuição geográfica, tolerância a largas faixas de variação de temperatura, estabelecimento em resíduos orgânicos com diferentes níveis de umidade, resistência ao manuseio, elevada capacidade reprodutiva e crescimento rápido (AQUINO; ALMEIDA; SILVA, 1992; COTTA *et al.*, 2015; PEREIRA *et al.*, 2005). Além da espécie utilizada, outros fatores são importantes no desenvolvimento da vermicompostagem de maneira adequada, como a matéria-prima a ser utilizada para alimentação das minhocas, pH, temperatura, umidade, densidade populacional de minhocas e relação C:N (ALI *et al.*, 2015).

Quando bem empregada a técnica, o vermicomposto é rico em elementos essenciais para as plantas, como nitrogênio, fósforo, magnésio, enxofre e potássio, e apresenta bactérias fixadoras de nitrogênio e reguladores de crescimento de plantas (ADHIKARY, 2012; MAKKAR *et al.*, 2023; PEREIRA *et al.*, 2005). Além disso, modula a estrutura do solo, aumentando sua capacidade de retenção hídrica, porosidade e estabilidade, bem como pode influenciar a capacidade de biorremediação (ADHIKARY, 2012; SWATI; HAIT, 2017).

Por isso, os vermicompostos são amplamente utilizados como fertilizantes, devido à sua capacidade de disponibilizar maiores quantidades de nutrientes para as plantas e melhorar as características físicas, químicas e microbiológicas do solo, proporcionando maior crescimento, desenvolvimento e produtividade de plantas cultivadas (BLOUIN *et al.*, 2019; ENEBE; ERASMUS, 2023; JOSHI; SINGH; VIG, 2015). Complementarmente, alguns estudos têm verificado a possibilidade da utilização de vermicompostos como indutores de tolerância a estresses bióticos e abióticos (GARCÍA *et al.*, 2012; HOSSEINZADEH; AMIRI; ISMAILI, 2016; SALLAKU *et al.*, 2009; XIAO *et al.*, 2016).

2.5 CHÁ DE COMPOSTO (*COMPOST TEA*)

O chá de composto é definido como um extrato aquoso obtido pela mistura de composto e água por períodos definidos de tempo, durante o qual pode haver aeração ou não, e acréscimo ou não de aditivos que aumentam a densidade populacional microbiana durante a produção (EUDOXIE; MARTIN, 2019).

O chá de composto evoluiu de práticas históricas na horticultura, tendo início com a maceração de esterco ou plantas na água, com o intuito de aplicar o líquido produzido nas culturas com finalidades nutricionais e de proteção contra patógenos (SCHEUERELL; MAHAFFEE, 2004). Existem evidências históricas do uso de chá de composto em sociedades antigas, como a romana e a egípcia, além de ser conhecidamente produzido e utilizado na Europa há centenas de anos (CARBALLO *et al.*, 2008; HARGREAVES; ADL; WARMAN, 2009). A partir do século XX, o interesse em chás de composto diminuiu devido ao início da utilização de agroquímicos, e mais recentemente, os estudos com chás de composto vêm sendo retomados, devido às preocupações crescentes com o meio ambiente e a saúde humana relacionadas à utilização de pesticidas, herbicidas e fertilizantes químicos (EUDOXIE; MARTIN, 2019; ISLAM *et al.*, 2016).

A obtenção do chá de composto é proposta por diversas metodologias na literatura, que diferem umas das outras pela presença ou ausência de aeração durante o processo, pela adição ou não de nutrientes que promovam crescimento de microrganismos e pelos tipos de compostos utilizados (EUDOXIE; MARTIN, 2019; SAMET *et al.*, 2019). Islam *et al.* (2016) salientaram que a qualidade do chá de composto varia consideravelmente em função dos procedimentos utilizados na preparação dos extratos, da matéria-prima, composição, qualidade e maturidade do composto, tempo de armazenamento e possivelmente outros fatores.

A presença ou ausência de aeração durante a obtenção dos chás é a principal característica de produção que os separa em dois grandes grupos: aerados e não-aerados. Nos métodos aerados, que variam de 12 – 36 horas, adiciona-se ar continuamente à mistura com intuito de aumentar a oxigenação durante o processo e geralmente acrescentam-se nutrientes para intensificar o crescimento microbiano; enquanto nos métodos não-aerados, que variam de alguns dias a algumas semanas, procura-se interferir o mínimo possível depois da homogeneização inicial, produzindo um extrato cuja concentração de oxigênio dissolvido caia abaixo de 5,5 ppm ou 70%, e geralmente não há acréscimo de nutrientes (CARBALLO *et al.*, 2008; EUDOXIE; MARTIN, 2019; INGHAM, 2005; SCHEUERELL; MAHAFFEE, 2004, 2002). Os métodos aerados são os mais populares e recomendados, devido ao fato de proporcionarem um nível de oxigenação que mantém os nutrientes disponíveis e suprime os organismos causadores de doenças. A presença de um conjunto de microrganismos aeróbicos que competem entre si é necessária para evitar o crescimento de patógenos (EUDOXIE; MARTIN, 2019; HARGREAVES; ADL; WARMAN, 2009; INGHAM, 2005).

Outra importante característica a ser considerada na obtenção dos chás é a maturidade do composto, que apresenta interferência direta na qualidade do chá produzido. Compostos

maduros geralmente apresentam quantidades maiores de nutrientes minerais solúveis e baixos níveis de ácidos orgânicos fitotóxicos e metais pesados, quando comparados a compostos imaturos (PANT *et al.*, 2012). Deve-se, portanto, utilizar compostos maduros para produção dos chás, com aproximadamente 2 – 6 meses, considerando que o produto gerado apresentará maior qualidade (EUDOXIE; MARTIN, 2019; ISLAM *et al.*, 2016).

Outro fator que merece atenção na produção do chá de composto é a utilização ou não de aditivos, que são nutrientes acrescentados durante o processo de extração, com intuito de aumentar e sustentar a população de microrganismos, bem como de amplificar o potencial supressor de patógenos (NAIDU *et al.*, 2010; SAMET *et al.*, 2019). Este efeito inibitório sobre o desenvolvimento de patógenos está relacionado à competição existente entre os organismos benéficos contidos nos chás e/ou produção de antibióticos por tais organismos, tornando claras as vantagens de se obter chá de composto com considerável diversidade microbiana (SAMET *et al.*, 2019; SIDDIQUI *et al.*, 2009).

O chá de composto, quando bem produzido, possui em sua composição nutrientes e uma ampla variedade de organismos, podendo ser aplicado sobre o solo ou pulverizado sobre as plantas (CARBALLO *et al.*, 2008; EUDOXIE; MARTIN, 2019). Sua aplicação tem intuito de conferir resistência a doenças, fornecer microrganismos benéficos às plantas e ao solo e fornecer nutrientes essenciais às plantas (EUDOXIE; MARTIN, 2019; SAMET *et al.*, 2019; SCHEUERELL; MAHAFFEE, 2002). Isto se deve à capacidade de proporcionar biomassa microbiana, matéria orgânica particulada fina, ácidos orgânicos benéficos – como ácidos húmicos e ácidos fúlvicos –, substâncias reguladoras do crescimento de plantas, fitohormônios e nutrientes minerais solúveis, bem como de aumentar a capacidade de retenção hídrica do solo (EUDOXIE; MARTIN, 2019; PANT *et al.*, 2012; TAHA *et al.*, 2016).

Inúmeros trabalhos indicam que a aplicação de chás de composto melhoram a saúde das plantas, sua qualidade nutricional e produtividade (EUDOXIE; MARTIN, 2019; FOUDA; NIEL, 2021). Também já é amplamente reconhecido seu potencial de auxiliar na resposta das plantas a estresses bióticos, como doenças e pragas (RAMÍREZ-GOTTFRIED *et al.*, 2023). Porém, cabe ressaltar que o campo de estudos que elucidam os efeitos do chá de composto sobre plantas expostas a estresses abióticos é bem menos explorado, sendo, em sua maioria, relacionados ao estresse salino (ADEJUMO, 2015; AMER *et al.*, 2020, 2021; BAYOUMY; KHALIFA; ABOELSOU, 2019; EON *et al.*, 2023; HEBA; IBRAHIM; SHERIF, 2014; LI *et al.*, 2021; SELEIMAN *et al.*, 2021).

2.6 CHÁ DE VERMICOMPOSTO (*VERMICOMPOST TEA*)

Os chás de vermicompostos são extratos aquosos que extraem as excelentes propriedades bioquímicas dos vermicompostos sólidos, e têm sido utilizados como uma alternativa – ainda que igualmente eficaz em relação aos vermicompostos – para o crescimento e rendimento das plantas e supressão de algumas doenças (ARANCON; OWENS; CONVERSE, 2019; GÓMEZ-BRANDON *et al.*, 2014; YATOO *et al.*, 2021).

A principal vantagem de utilização dos chás em relação aos vermicompostos sólidos deve-se à maior facilidade de transporte e aplicação nas colheitas, permitindo sua pulverização, e já existem estudos que verificam a possibilidade de aplicação dos chás de vermicomposto como solução nutritiva no cultivo hidropônico (ARANCON *et al.*, 2007; ARANCON; OWENS; CONVERSE, 2019; MANTHEI, 2021).

Os mesmos fatores que influenciam a produção dos chás de compostos também merecem destaque na produção de chás de vermicompostos, como presença ou ausência de aeração, tipo de resíduo utilizado no vermicomposto e adição ou não de nutrientes que aumentem a diversidade microbiana (GÓMEZ-BRANDON *et al.*, 2014). Portanto, aqui também são válidas as observações feitas anteriormente, onde destacam-se como vantajosas a utilização de resíduos verdes como matérias-primas para produção dos vermicompostos, a utilização de vermicompostos maduros e a aeração para obtenção dos chás, devido ao fato de reduzirem a possibilidade de desenvolvimento de organismos patogênicos.

Diversos autores relatam o aumento do crescimento, desenvolvimento e produtividade de plantas expostas aos chás de vermicompostos, relacionados ao aumento da eficiência de absorção de nutrientes, da atividade microbiana, de fitohormônios, de substâncias húmicas e fúlvicas, e do conteúdo total de nutrientes nos extratos (CHAICHI *et al.*, 2018; EDWARDS; ARANCON; GREYTAK, 2006; FRITZ *et al.*, 2012; GÓMEZ-BRANDON *et al.*, 2014). Também é amplamente relatada na literatura a utilização de chás de vermicompostos para supressão de doenças em plantas (MISHRA *et al.*, 2017; MORALES-CORTS; PÉREZ-SÁNCHEZ; GÓMEZ-SÁNCHEZ, 2018; YATOO *et al.*, 2021).

Por fim, já são encontrados alguns estudos que demonstram que o chá de vermicomposto pode ser aplicado como indutor de respostas de tolerância a alguns estresses abióticos – como os estresses salino, hídrico, por temperatura e metais pesados –, porém tais estudos são encontrados em menor número (BENAZZOUK *et al.*, 2020; BEYKKHORMIZI *et al.*, 2018; CHINSAMY; KULKARNI; VAN STADEN, 2014; MARCELIS; LUTTS, 2019).

2.7 COMPOSIÇÃO DOS CHÁS DE (VERMI)COMPOSTOS

A composição dos chás de composto e vermicomposto é um fator importante a ser considerado em trabalhos que avaliam seus efeitos, visto que, com as informações sobre as características físicas, químicas e microbiológicas dos chás, é possível associar o conteúdo aos efeitos verificados.

Os trabalhos envolvendo a produção de chá de (vermi)composto demonstram algumas análises importantes, sendo as principais encontradas na literatura: pH, condutividade elétrica, oxigênio dissolvido, conteúdo de nitrogênio total, concentração de amônia, carbono total, carbono oxidável, relação C:N, estabilidade ou atividade microbiana (medidas pela análise da taxa respiratória), conteúdo de nutrientes totais, teor de metais pesados, densidade da população microbiana (bactérias, fungos e actinomicetos), quantificação de ácidos húmicos e fúlvicos, quantificação de substâncias orgânicas, conteúdo de fitohormônios (via HPLC), e presença de microrganismos patogênicos (AMOS, 2017; CARBALLO *et al.*, 2008; EUDOXIE; MARTIN, 2019; GONZÁLEZ-HERNÁNDEZ *et al.*, 2022; ISLAM *et al.*, 2016; YATOO *et al.*, 2021).

É importante salientar que a composição física, química e microbiológica dos chás é altamente variável em função do (vermi)composto utilizado, da matéria-prima do (vermi)composto, do método de extração, do tempo de extração, do tempo de armazenamento e da concentração do extrato. Assim, não é possível observar um padrão na composição dos chás, porém algumas observações podem ser destacadas: 1) o composto utilizado como matéria-prima para obtenção dos chás deve ser estável e maduro; 2) a extração aerada permite obtenção de chás de melhor qualidade e menor incidência de organismos patogênicos; 3) não são necessários tempos de extração superiores a 24 horas; 4) os chás obtidos devem ser utilizados frescos para realização dos experimentos com plantas; 5) algumas análises demandam que os chás sejam liofilizados; e 6) não é interessante armazenar os chás para uso posterior, mas caso haja necessidade, estes devem ser armazenados por no máximo 4 semanas, sob risco de alteração na composição física, química e microbiológica (CARBALLO *et al.*, 2008; EUDOXIE; MARTIN, 2019; GÓMEZ-BRANDON *et al.*, 2014; ISLAM *et al.*, 2016; KHAN *et al.*, 2007; PANT *et al.*, 2012).

2.8 ESTRESSES ABIÓTICOS EM PLANTAS

As plantas em seu ambiente natural ou em áreas agricultáveis estão sujeitas a condições externas que podem afetar seu crescimento, desenvolvimento e/ou produtividade, isto é, estão

sujeitas a possíveis estresses ambientais. Tais estresses podem ser bióticos, quando causados por outros organismos vivos, ou abióticos, quando provocados por excesso ou déficit de algum componente químico ou físico (SHINOZAKI *et al.*, 2015). Dentre os diversos estresses abióticos, podemos citar o estresse hídrico (déficit hídrico e alagamento), o estresse salino e a presença de metais pesados.

O déficit hídrico pode ser compreendido como o estresse causado em uma planta quando a qualidade ou quantidade de água disponíveis são incapazes de suprir as necessidades metabólicas básicas, isto é, quando a disponibilidade hídrica é insuficiente (MUKARRAM *et al.*, 2021; SELEIMAN *et al.*, 2021). Pode-se citar, como efeitos primários do déficit hídrico, a redução do potencial hídrico, a desidratação celular e a resistência hidráulica; e como efeitos secundários a redução da expansão celular/foliar, redução das atividades celulares e metabólicas, o fechamento estomático, a inibição da fotossíntese, a abscisão foliar, alteração na partição do carbono, citorrise, cavitação, desestabilização de membranas e de proteínas, produção de espécies reativas de oxigênio (EROs), citotoxicidade iônica e morte celular (BLUMWALD; MITTLER, 2017).

O estresse por déficit hídrico é desencadeado tanto nas áreas naturais quanto nas agrícolas por períodos de estiagem, ou seja, períodos intermitentes ou contínuos sem precipitação (BLUMWALD; MITTLER, 2017), e no Brasil alguns impactos negativos podem ser relacionados aos períodos de estiagem – os quais estão cada vez mais frequentes e evidentes, causando redução ou inibição da expansão da agricultura (SOUZA; SOUZA; MAGALHÃES, 2018).

O alagamento, por sua vez, é caracterizado pela baixa disponibilidade de oxigênio no solo (hipoxia), cujos poros estão preenchidos com o excesso de água (DE SOUZA *et al.*, 2009; JIA *et al.*, 2021). Em condições de alagamento, a respiração aeróbica nas raízes é suprimida devido à baixa disponibilidade de oxigênio, aumentando a fermentação, levando a um estresse anaeróbico que pode provocar esgotamento de energia, acidificação do citosol e toxicidade pelo acúmulo de etanol (BLUMWALD; MITTLER, 2017). Além da diminuição da taxa respiratória das raízes, as respostas das plantas às inundações representam mecanismos que combinam alterações anatômicas, morfológicas e fisiológicas, como a redução da eficiência fotossintética, clorose, murcha prematura, redução do potencial hídrico e da concentração de nutrientes, diminuição do crescimento e da produtividade, formação de aerênquima, formação de barreiras à perda radial de oxigênio, acúmulo de EROs e estresse oxidativo (JIA *et al.*, 2021).

O estresse por alagamento pode ocorrer em áreas naturais ou agricultáveis. No entanto, nas regiões tropicais, a maioria das espécies cultivadas frequentemente sofre alagamentos

durante a estação chuvosa do verão, devido ao excesso de irrigação, tempestades, má drenagem do solo ou transbordamento dos rios (LONE *et al.*, 2016; PROMKHAMBUT *et al.*, 2010). No Brasil, o alagamento proporciona consideráveis limitações agrícolas nas planícies de inundação ou em áreas de várzeas, nas quais as inundações temporárias ou a longo prazo restringem a agricultura, com exceção do arroz inundado (SILVA *et al.*, 2007).

O estresse salino ocorre em locais cujo solo possui alta concentração de sais, que podem ser separados em dois grandes grupos de acordo com Szabolcs e Fink (1974): (1) os solos salinos, que são capazes de prejudicar o crescimento da grande maioria das culturas agrícolas e que contêm principalmente NaCl e Na₂SO₄ como sais solúveis, além de poderem apresentar também íons Cl⁻ e SO₄⁻ de sais com Ca²⁺ e Mg²⁺; e (2) os solos sódicos, que contêm sais de Na⁺ como o Na₂CO₃, capazes de ocasionar hidrólise alcalina.

A presença em excesso desses minerais no solo cria um habitat salino que dificulta que as raízes das plantas extraiam água do ambiente, visto que o potencial hídrico do solo é influenciado pela presença de sais ionizados. Isso significa que o estresse salino pode ocasionar um estresse por déficit hídrico (estresse osmótico), mesmo que a disponibilidade de água não seja um problema. Além disso, a absorção desses minerais pelas plantas – que pode ocorrer com a diferença de potencial hídrico decorrente da transpiração – é capaz de lesionar as células das folhas transpirantes, prejudicando ainda mais o crescimento e desenvolvimento; efeito da salinidade conhecido como sal-específico ou de excesso de íons (KUMAR *et al.*, 2020; PARIHAR *et al.*, 2015).

De maneira geral, o estresse salino afeta todos os principais processos metabólicos de plantas – germinação, crescimento, fotossíntese e relações hídricas. São relatados como efeitos do estresse salino sobre plantas: desequilíbrio osmótico, desequilíbrio de nutrientes, toxicidade iônica, redução do crescimento inicial, fechamento estomático, inibição da expansão foliar, diminuição ou limitação da produtividade, estresse oxidativo, senescência e morte (KUMAR *et al.*, 2020; NEGRÃO; SCHMÖCKEL; TESTER, 2017; PARIHAR *et al.*, 2015; TUTEJA, 2007).

O estresse salino é um dos mais importantes fatores limitantes para o crescimento e produtividade das culturas agrícolas ao redor do mundo, que pode ocorrer tanto naturalmente, oriundo dos processos de gênese dos solos, como por influência antrópica – com desmatamento para aumento da fronteira agrícola e práticas de drenagem e irrigação excessiva de solos cujas condições ambientais propiciam o acúmulo de sais, como em climas (semi)áridos ou com drenagem do solo deficiente (FLORES *et al.*, 2002; PARIHAR *et al.*, 2015). Hoje, cerca de 20% da área agricultável do mundo é afetada pela salinidade, e espera-se que até 2050, essa

taxa aumente para cerca de 50% (KUMAR *et al.*, 2020). No Brasil, a estimativa é de que a salinidade atinja 2% do território nacional, principalmente nas regiões semi-áridas do Nordeste e de Minas Gerais, e alguns locais do Centro-Oeste (FLORES *et al.*, 2002).

Os metais pesados são elementos metálicos que possuem alta densidade encontrados naturalmente em formações rochosas de forma dispersa, porém processos antrópicos como a mineração, fundição, industrialização, geração de resíduos, aplicações de fertilizantes, dentre outros, contribuíram com o aumento dos metais pesados na biosfera, principalmente nos solos e ecossistemas aquáticos devido à lixiviação. Incluem chumbo (Pb), cádmio (Cd), alumínio (Al), mercúrio (Hg), manganês (Mn), níquel (Ni), cobalto (Co), ferro (Fe), zinco (Zn), cromo (Cr), arsênio (As), prata (Ag) e os elementos do grupo da platina; e embora alguns deles sejam considerados micronutrientes de plantas e tenham participação em processos metabólicos agindo como cofatores enzimáticos, outros não apresentam nenhum benefício e todos podem ser tóxicos quando excedem certos limites de concentração, devido a suas propriedades químicas (BERNI *et al.*, 2019; GHORI *et al.*, 2019; GILL, 2014; JALMI *et al.*, 2018).

O estresse ocasionado pela presença de metais pesados no solo tem efeitos adversos notáveis no crescimento e produtividade das culturas agrícolas, variando de acordo com a espécie, com o metal, a concentração, a forma química e as propriedades físico-químicas do solo, desencadeando desde respostas bioquímicas até diminuição do rendimento da colheita (GILL, 2014). É um estresse abiótico notável e importante, devido ao fato de os metais pesados não serem biodegradáveis, ou seja, não poderem ser eliminados do ambiente por processos naturais, e conseqüentemente poderem ser bioacumulados nos organismos e/ou biomagnificados na cadeia trófica (BERNI *et al.*, 2019; GHORI *et al.*, 2019; MISHRA; SINGH; ARORA, 2017).

Nas plantas, a nível celular, os metais pesados provocam danos por uma série de mecanismos, sendo o mais comum a produção de EROs e o conseqüente estresse oxidativo, mas também podem ser citados a inativação de biomoléculas pela afinidade com os sítios de ligação de enzimas, o bloqueio de grupos funcionais essenciais e danos ao material genético (GHORI *et al.*, 2019; JALMI *et al.*, 2018). A nível de organismo, podemos verificar como efeitos o dano às membranas e paredes celulares, inibição da germinação, diminuição da absorção de nutrientes, limitações ao crescimento e desenvolvimento, alterações na produção de metabólitos secundários, senescência, clorose, necrose, perda de turgor, danos ao aparelho fotossintético e até a morte (BERNI *et al.*, 2019; GHORI *et al.*, 2019; GILL, 2014; SHAHID *et al.*, 2015).

Dentre os metais pesados, o chumbo (Pb) é um dos principais contaminantes do solo, resultante de atividades antrópicas como mineração e fundição, bem como de processos climáticos naturais, que se acumula nas raízes, pecíolos e folhas (GHORI *et al.*, 2019). São relatados na literatura diversos sintomas de toxicidade ao Pb em plantas, como clorose, atrofia ou reduções de crescimento, redução do comprimento das raízes, alteração da permeabilidade de membranas, alterações hormonais, inibição da atividade de várias enzimas, redução no conteúdo de água, diminuição da capacidade de nutrição mineral, inibição da germinação, danos à fotossíntese e à respiração celular e estresse oxidativo (ALI; NAS, 2018; GHORI *et al.*, 2019; MITRA *et al.*, 2020).

O alumínio (Al) é outro metal pesado contaminante do solo que possui uma particularidade: é um elemento não-tóxico em locais onde o solo não apresenta acidez, porém ocasiona toxicidade em locais que apresentam solo ácido – pH entre 4,5 e 5,5. Em solos em condições normais (não-ácidos), o Al está presente na forma de silicatos, fosfatos, sulfetos e óxidos; enquanto em solos ácidos, o Al passa a ser solubilizado e ficar disponível para absorção das plantas em formas químicas tóxicas, como $[Al(H_2O)_6]^{3+}$, $AlOH_2^+$, $Al(OH)_3$ e $Al(OH)_4$ (CHOWRA *et al.*, 2017; RAHMAN; UPADHYAYA, 2021).

Os efeitos do Al, que podem ser benéficos ou tóxicos nas plantas, dependem de inúmeros fatores como pH do solo, espécie química na qual está disponível, genótipo da planta e suas respectivas condições de crescimento. O estresse ocasionado pelo Al desencadeia uma série alterações morfológicas, fisiológicas, bioquímicas e moleculares, podendo reduzir o crescimento, desenvolvimento e produtividade de culturas agrícolas (RAHMAN; UPADHYAYA, 2021). Os efeitos tóxicos do Al comumente relatados na literatura incluem redução da absorção de água e nutrientes, inibição do transporte intercelular pelos plasmodesmos, produção de EROs, redução da taxa fotossintética, estresse oxidativo e danos às membranas, inibição do crescimento e do diâmetro das raízes, descoloração das raízes, inibição do crescimento da parte aérea, clorose e necrose de folhas, impacto no metabolismo de proteínas e na atividade enzimática, e danos ao material genético (GUPTA; GAURAV; KUMAR, 2013; RAHMAN; UPADHYAYA, 2021; ROUT; SAMANTARAY; DAS, 2001).

A produtividade de lavouras importantes para a economia brasileira, como o milho e o sorgo, pode ser afetada por alguns dos estresses aqui citados. A germinação, o crescimento e o desenvolvimento das culturas em questão são prejudicados pela baixa disponibilidade hídrica, e o rendimento do milho e do sorgo são variáveis, devido principalmente às alterações na disponibilidade hídrica provocadas pela instabilidade no regime pluviométrico (BERGAMASCHI *et al.*, 2006; SANTOS *et al.*, 2014). Neste contexto, Souza, Souza e

Magalhães (2018) ressaltaram que o sorgo apresenta maior tolerância ao déficit hídrico do que o milho. Além disso, ambas as culturas agrícolas são afetadas pela presença de Al em solos ácidos, que são comuns em regiões tropicais como o Brasil, e pela presença de Pb em solos contaminados por atividades antrópicas, ocasionando danos a processos fisiológicos, bioquímicos e metabólicos que resultam na diminuição do crescimento, desenvolvimento e/ou da produtividade (HAMVUMBA; MATAA; MWEETWA, 2014; MAZZOCATO *et al.*, 2002; PEIXOTO; PIMENTA; CAMBRAIA, 2007; RATHIKA *et al.*, 2020; ZUBA JUNIO *et al.*, 2011).

2.9 META-ANÁLISE

Pode-se definir meta-análise como um conjunto de métodos estatísticos que combinam a magnitude dos resultados – isto é, os tamanhos de efeito – de diferentes estudos que buscaram responder à mesma pergunta através de testes e experimentos. Justamente por isso, geralmente, utiliza-se a meta-análise em artigos de revisão da literatura, pois tal ferramenta fornece um resultado estatístico para sintetizar e comparar os resultados de estudos cujas hipóteses apresentam similaridade (CROMBIE; DAVIES, 2009; GUZZO; JACKSON; KATZELL, 1987; HARRISON, 2011).

De acordo com Harrison (2011), a meta-análise proporciona ferramentas quantitativas para realizar duas aplicações: (1) fornecer um método para calcular o efeito médio da variável independente – normalmente representada por alguma forma de manipulação experimental, como tratamento *versus* grupo controle ou uma variável contínua que represente o nível de tratamento –, em todas as tentativas, caso uma série de tentativas tenham sido feitas para medir o efeito de uma variável sobre a outra; e (2) medir a quantidade de mudança induzida experimentalmente na variável dependente entre os estudos, buscando explicar esta variabilidade usando variáveis moderadoras definidas, as quais podem ser diferenças filogenéticas, ecológicas ou metodológicas entre os grupos de estudo.

Mengist, Soromessa e Legese (2020) salientaram a importância da revisão sistemática da literatura – que representa o primeiro passo da meta-análise – quando comparadas à revisão narrativa tradicional, visto que apresenta uma produção replicável, científica e transparente. Isso ocorre porque tal metodologia ajuda a coletar todas as publicações que se enquadram nos critérios de inclusão predefinidos para responder a uma pergunta de pesquisa específica, utilizando procedimentos sistemáticos que minimizam a ocorrência de viés durante todo o processo. Além disso, os autores também propõem uma metodologia detalhada em seis passos

para conduzir uma meta-análise em pesquisas na área de ciências ambientais: (1) elaboração de um protocolo de pesquisa para revisão sistemática de literatura; (2) busca e pesquisa dos estudos a serem utilizados na revisão sistemática da literatura; (3) avaliação sistemática dos estudos encontrados e seleção daqueles que se encaixam nos critérios de inclusão; (4) síntese, extração e classificação de dados importantes dos artigos selecionados; (5) análise dos dados através de meta-análise; e (6) divulgação dos resultados obtidos.

A meta-análise é uma ferramenta poderosa e informativa que pode ser utilizada tanto em pesquisas básicas quanto aplicadas. Para a ciência aplicada, tal ferramenta pode fornecer respostas com robustez estatística a questões de importância ecológica (HARRISON, 2011; MENGIST; SOROMESSA; LEGESE, 2020). Este é o caso da indução de tolerância a estresses abióticos proporcionada pelos chás de composto e de vermicomposto.

3 ARTIGO I: COMPOST TEAS AND VERMICOMPOST TEAS AS TOLERANCE-INDUCERS TO ABIOTIC STRESSES IN PLANTS: A META-ANALYSIS

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COMPOST TEAS AND VERMICOMPOST TEAS AS TOLERANCE-INDUCERS TO ABIOTIC STRESSES IN PLANTS: A META-ANALYSIS

ABSTRACT

Compost teas and vermicompost teas are aqueous extracts that extract the excellent biochemical properties of their respective solid materials and have been used as an alternative to enhance plant growth and yield, suppress plant diseases, and assist in responding to stresses. Among these, the abiotic stresses must be highlighted, as they represent barriers to the productivity of agricultural crops. The review aimed to analyze compost teas and vermicompost teas as tolerance-inducers to abiotic stresses in plants. To this end, a systematic search was carried out on the main indexing platforms for scientific studies published in the last three years (2021–2023), which were subjected to a meta-analysis. Were calculated the global effect size, and the effect by moderators (abiotic stresses), with the aim of verifying the effectiveness of teas in protecting plants and improving their growth, yield, physiology and/or morphology, despite stressful conditions. The results elucidate that compost teas or vermicompost teas application is beneficial for plants exposed to abiotic stresses, alleviating the stresses, and helping plants to tolerate them, enhancing survival and productivity.

Keywords: Plant growth; plant physiology; stress-tolerant; photosynthetic metabolism; antioxidant metabolism; crop yield.

1 INTRODUCTION

Compost teas and vermicompost teas are defined as aqueous extracts obtained by mixing compost or vermicompost and water for defined periods of time, to extract the chemical and microbiological properties of the solid materials with the aim of application in plants to enhance growth, yield, and diseases-suppression (Eudoxie and Martin, 2019; Ramírez-Gottfried *et al.*, 2023; Rehman *et al.*, 2023). More recently, the teas have been also explored as tolerance-inducers to abiotic stresses (Amer *et al.*, 2021; Benazzouk *et al.*, 2018; Eon *et al.*, 2023; Seleiman *et al.*, 2021).

The abiotic stresses are environmental stresses caused by an excess or deficit of some chemical or physical component. Among the various abiotic stresses, can be mentioned water stress (water deficit or flooding), saline stress and the presence of heavy metals.

Water deficit, water restriction or drought can be understood as the stress caused to a plant when the water availability is insufficient to supply basic metabolic needs, affecting various morpho-physiological phenomena causing restriction to plant growth, development, and productivity (Mukarram *et al.*, 2021). In front of water deficit, plants present a series of biochemical, physiological, and morphological changes in their performance, among which the principal: decrease in stomatal conductance and in chlorophyll contents, decline in photochemical efficiency, photosynthesis inhibition, decrease in cellular and metabolic activities, reactive oxygen species (ROS) production triggering oxidative stress, proline accumulation, changes in cell wall integrity, leaf number and leaf area reduction, and decline in shoot growth and plant height (Seleiman *et al.*, 2021).

Flooding or waterlogging, in turn, is characterized by low oxygen availability in the soil (hypoxia), whose pores are filled with excess water. The oxygen deficiency in soil triggers some plant responses that restricts its growth, resulting in decline of crop yield (Jia *et al.*, 2021). Under flooded conditions, aerobic respiration in roots is suppressed due to low oxygen availability, increasing anaerobic respiration, leading to stress that can cause energy depletion, acidification of the cytosol and toxicity from ethanol accumulation (Blumwald and Mittler, 2017). Plant responses to flooding represents mechanisms that combine anatomical, morphological, and physiological changes, such as aerenchyma formation, formation of barriers to radial oxygen loss (ROL), ROS accumulation and oxidative stress, limited photosynthetic rates, and reduction in growth and development (Jia *et al.*, 2021).

Saline stress occurs in places where the soil has a high concentration of salts, which makes it difficult for plant roots to extract water from the environment leading to osmotic stress; besides, the absorption of these minerals by plants damages the transpiring leaf cells, further impairing growth and development (Kumar *et al.*, 2020). In general, salt stress affects all the main metabolic processes

of plants – germination, growth, photosynthesis, and water relations. The effects of saline stress on plants are osmotic imbalance, nutrient imbalance, ionic toxicity, reduction of initial growth, stomatal closure, inhibition of leaf expansion, decrease or limitation of productivity, oxidative stress, senescence, and death (Kumar *et al.*, 2020; Negrão *et al.*, 2017).

Heavy metals, in turn, are metallic elements that have a high density found naturally in rock formations in a dispersed form, but anthropogenic processes have contributed to its increase in the biosphere (Berni *et al.*, 2019; Ghori *et al.*, 2019). The stress caused by heavy metals in the soil has notable adverse effects on the growth and productivity of agricultural crops, varying according to the species, metal, concentration, chemical form, and physical-chemical properties of the soil, triggering from biochemical responses to decreased crop yield (Gill, 2014). In plants, heavy metals cause damage through a series of mechanisms, the most common being ROS accumulation and the consequent oxidative stress, inactivation of biomolecules, damage to genetic material, damage to cell membranes and walls, inhibition of germination, decreased absorption of nutrients, damage to the photosynthetic system, limitations to growth and development, changes in the production of secondary metabolites, senescence, chlorosis, necrosis, loss of turgor, and even death (Berni *et al.*, 2019; Ghori *et al.*, 2019; Jalmi *et al.*, 2018).

Along with climate changes, as water deficit as flooding have been causing damage to agriculture around the world, both triggering severe crop reduction in both yield and quality, representing serious threats to world food security (Abobatta, 2019; Jia *et al.*, 2021). Besides, today, around 20% of the world's arable land is affected by salinity, and this rate is expected to increase to around 50% by 2050 (Kumar *et al.*, 2020). In this context, it is noteworthy the importance of studies that elucidate the potential of compost teas and vermicompost teas to promote stress-tolerance effects, since they represent the possibility of enhancing crop productivity in front of the cited abiotic stresses. In addition, in front of the pollutant anthropogenic processes which contribute to heavy metal bioavailability (Ghori *et al.*, 2019), the importance is also notable, as compost teas and vermicompost teas can offer a solution to promote plants survival and growth despite of contaminated areas. Finally, compost teas and vermicompost teas are techniques that contribute to reducing organic residues in the environment, and value compost products, contributing to circular economy (Bakan *et al.*, 2022).

In view of the above, the objective of this study was to perform a meta-analysis to verify if compost teas and vermicompost teas are significantly beneficial to promote plants stress-tolerance under abiotic stresses.

2 MATERIAL AND METHODS

To conduct the meta-analysis, studies published in the last three years (2021–2023), year-by-year, were selected through a systematic search on the principal indexing platforms: Web of Science, Scopus, and Google Scholar. The systematic search was performed with the following keywords: "compost tea" OR "vermicompost tea" AND "heavy metal" OR "trace element" OR "water stress" OR "flooding" OR "drought" OR "salinity" OR "salt stress".

For each indexing platform, the first 10 search pages were evaluated. As inclusion criteria for the case studies, were selected those articles that: a) contained the previously determined keywords, b) represented the results as means, and c) presented the associated standard error. As exclusion criteria, the studies that did not fit these requirements were disregarded, besides those that presented compost teas or vermicompost teas associated with other substances and/or additives.

Was defined as only moderator the abiotic stresses, and the parameters evaluated were separated in four classes:

- 1) Growth parameters: shoot length, shoot fresh biomass, shoot dry biomass, number of leaves, root length, root fresh biomass, and root dry biomass;
- 2) Photosynthetic analyses: stomatal conductance, photosystem II efficiency (Fv/Fm), total chlorophyll, carotenoids, and total soluble sugars;
- 3) Antioxidant system analyses: proline, hydrogen peroxide (H₂O₂), lipid peroxidation, catalase (CAT) activity, and peroxidases activities;
- 4) Agronomic analyses: potassium (K) content, sodium (Na) content, K/Na ratio, grain yield, straw yield, biological yield, and harvest index.

A test of heterogeneity was performed on the cases obtained, with the aim to the type of effect (fixed or random) to be used in calculating the overall effect size and effect by moderators (da Cunha Neto *et al.*, 2023).

The overall effect size was calculated for each case by performing the standardized mean difference (d) obtained from equation 1, as follows:

$$(Eq\ 1)\ d = \frac{(X1-X2)}{S_{within}},$$

where X1 is the mean of the treatment (compost tea or vermicompost tea), X2 is the mean of the control (abiotic stress), and S_{within} is the standard deviation parameter, which considers the values of the standard deviation and the number of replicates in the control and treatment.

The variance (Vd) of d was calculated by equation 2, as follows:

$$(Eq\ 2)\ Vd = \left[\frac{(n1+n2)}{(n1.n2)} \right] + \left[\frac{d^2}{2.(n1+n2)} \right],$$

where n1 is the treatment error and n2 is the control error.

It was performed for each parameter which number of cases was ≥ 10 .

For most parameters, positive values of d in each case study indicate that the treatments were beneficial in promoting stress-tolerance effects, while negative values indicate the opposite. The exceptions are proline, H_2O_2 , lipid peroxidation, and Na content, in which negative values of d indicate that the treatments were beneficial in promoting stress-tolerance effects, while positive values indicate the opposite. Minimum and maximum values were obtained for each case to indicate if the study was significant.

The described methodology was performed using Launch Open Meta Analyst software, with 5% significance level.

3 RESULTS

After carrying out the systematic search and applying the inclusion and exclusion criteria, were selected 17 articles, from which the case numbers were extracted for each parameter. The parameters excluded from the meta-analysis because they did not present the minimum number of cases ($n = 10$) were: number of leaves ($n = 8$), root fresh biomass ($n = 6$), straw yield ($n = 8$), and harvest index ($n = 8$). For the further parameters, the meta-analysis was conducted, and for all of them, the heterogeneity test performed demonstrated that type of effect was random.

Considering the growth parameters (Table 1), the meta-analysis demonstrated that the use of compost tea or vermicompost tea was significant and beneficial, providing positive values for overall size effect in all parameters analyzed: shoot length, shoot fresh biomass, shoot dry biomass, root length, and root dry biomass.

When evaluating the moderators, the same positive effect of compost teas and vermicompost teas application is verified, as the teas promoted increase in all growth parameters analyzed in the face of stresses assessed individually (Table 1). For shoot length, shoot dry biomass and root length, were possible to analyze as moderators the saline stress and water deficit; while for root dry biomass, it was possible to obtain a response only for the water deficit moderator. For shoot fresh biomass, was applied only the overall size effect, once the moderators' numbers of cases were insufficient ($n < 10$).

Table 1. Overall effect sizes and effects by moderators for growth parameters of plants exposed to abiotic stresses with compost tea or vermicompost tea application.

Parameter	Overall effect				Effect by moderators				
	Effect size	Minimum limit	Maximum limit	I ²	Moderator	Effect size	Minimum limit	Maximum limit	I ²
Shoot length	6533	5540	7525	99.65%	Saline stress	7844	1286	14401	99.80%
					Water deficit	6469	5938	6999	98.01%
Shoot fresh biomass	7341	5470	9213	99.91%	n < 10				
Shoot dry biomass	2518	1667	3369	99.76%	Saline stress	1466	0.640	2293	99.48%
					Water deficit	3609	2529	4689	99.62%
Root length	3378	2406	4351	93.66%	Saline stress	2913	1967	3858	89.54%
					Water deficit	3990	2007	5974	95.46%
Root dry biomass	0.936	0.548	1324	98.19%	Water deficit	1205	0.631	1780	98.59%

Were represented only the significant data.

Regarding the photosynthetic analyses, the overall effect was also significant and beneficial for all, promoting increase in stomatal conductance, photosystem II efficiency (Fv/Fm), total chlorophyll, carotenoids, and total soluble sugars (Table 2).

For the moderators, only water deficit presented sufficient case numbers in the analyzed parameters, except for Fv/Fm (n < 10). The results demonstrated that compost teas and vermicompost teas application also presented positive effects, enhancing the photosynthetic parameters in plants exposed to water deficit.

Table 2. Overall effect sizes and effects by moderators for photosynthetic analyses of plants exposed to abiotic stresses with compost tea or vermicompost tea application.

Parameter	Overall effect				Effect by moderators				
	Effect size	Minimum limit	Maximum limit	I ²	Moderator	Effect size	Minimum limit	Maximum limit	I ²
Stomatal conductance	8139	5437	10841	100%	Water deficit	14503	11499	17508	100%
Fv/Fm	0.075	0.055	0.095	99.94%	n < 10				
Total chlorophyll	0.350	0.058	0.642	99.97%	Water deficit	1087	0.795	1380	99.96%
Carotenoids	2660	2189	3131	99.91%	Water deficit	2736	2227	3245	99.93%
Total soluble sugars	9719	8684	10754	99.70%	Water deficit	11198	9959	12436	99.79%

Were represented only the significant data. Fv/Fm: photosystem II efficiency.

When analyzing parameters related to the antioxidant system (Table 3), for the parameters hydrogen peroxide (H₂O₂), lipid peroxidation, catalase (CAT) and peroxidases activities, the overall effect sizes and effect by moderators were significant. However, for proline, the overall effect size

was not significant, only for the moderator. Only water deficit as moderator presented enough cases for the cited parameters.

For the antioxidant system, the meta-analysis confirms that compost tea or vermicompost tea application promotes abiotic-stress-tolerance effects in general and under water deficit individually, by increasing CAT and peroxidases activities, and decreasing H₂O₂ and lipid peroxidation. In addition, considering water deficit, is also possible verify the stress-tolerance effect by decreasing proline (Table 3).

Table 3. Overall effect sizes and effects by moderators for antioxidant system parameters of plants exposed to abiotic stresses with compost tea or vermicompost tea application.

Parameter	Overall effect				Moderator	Effect by moderators			
	Effect size	Minimum limit	Maximum limit	I ²		Effect size	Minimum limit	Maximum limit	I ²
Proline					Water deficit	-1380	-1908	-0.851	99.50%
H ₂ O ₂	-1168	-1222	-1115	99.83%	Water deficit	-4985	-6110	-3860	99.79%
Lipid peroxidation	-5345	-6667	-4022	99.99%	Water deficit	-3427	-4316	-2538	97.56%
CAT activity	0.346	0.272	0.420	97.62%	Water deficit	0.279	0.208	0.349	97.48%
Peroxidases activity	0.489	0.457	0.521	99.90%	Water deficit	0.221	0.198	0.244	99.82%

Were represented only the significant data. H₂O₂: hydrogen peroxide; CAT: catalase.

Considering the agronomic parameters, compost tea or vermicompost tea application as tolerance-inducers to abiotic stresses is also significant and beneficial, according to overall effect size, promoting increase in K content, K/Na ratio, grain yield, and biological yield, besides decreasing Na content (Table 4).

For the moderators, the only parameter with enough number of cases was K content, for which were analyzed as moderators the saline stress and water deficit. In both, compost tea or vermicompost tea application for stress alleviation was significant and beneficial, enhancing the values.

Table 4. Overall effect sizes and effects by moderators for agronomic analyses parameters of plants exposed to abiotic stresses with compost tea or vermicompost tea application.

Parameter	Overall effect				Moderator	Effect by moderators			
	Effect size	Minimum limit	Maximum limit	I ²		Effect size	Minimum limit	Maximum limit	I ²
K content	4697	3445	5949	100%	Saline stress	2059	0.804	3314	99.99%
					Water deficit	0.276	0.106	0.446	98.66%
Na content	-0.861	-0.994	-0.728	99.08%	n < 10				
K/Na ratio	0.185	0.084	0.286	98.58%	n < 10				
Grain yield	1618	1383	1852	99.91%	n < 10				
Biological yield	32909	11406	54411	100%	n < 10				

Were represented only the significant data. K: potassium; Na: sodium.

It was possible to verify through the meta-analysis that the toleration mechanisms to abiotic stresses promoted by compost teas and vermicompost teas application is due to combined responses in plants morphology, physiology, and biochemical metabolism.

4 DISCUSSION

Plants under stressful conditions evolved a wide range of mechanisms to make it possible to survive. One of the most observed changes is the alteration of photosynthetic metabolism, which is rapidly reduced or even inhibited in plants exposed to abiotic stresses (Sharma *et al.*, 2020). Once photosynthesis is the metabolic process that makes it possible to plants synthesize carbohydrates for energetic metabolism use, reduction in photosynthetic rates is directly correlated to reduction in plants growth and productivity, and photosynthesis is strongly influenced by environmental conditions (Ehleringer and Sandquist, 2017).

Among the factors that can be analyzed to verify photosynthesis efficiency, can be cited the photosynthetic parameters considered in the present study. Evaluating stomatal conductance allows checking the efficiency of gas exchange and transpiration, that is, the influx of carbon dioxide (CO₂) as essential substrate for photosynthesis reactions, and the release/loss of water vapor into the atmosphere (Zeiger, 2017). By controlling the exchange of water and CO₂ between plants and the atmosphere, stomata play a central role in the regulation of photosynthesis, leaf and plant water status and transport, and drought sensitivity and tolerance (Buckley, 2017). A drop in stomatal conductance is a common response of plants to water deficit and saline stress (Abobatta, 2019; Seleiman *et al.*, 2021), as an attempt to reduce water loss through transpiration, which leads to reduction in CO₂ assimilation and ultimately affects photosynthesis (Sharma *et al.*, 2020).

Evaluating the photosystem II efficiency (Fv/Fm), as well as quantifying photosynthetic pigments (chlorophyll and carotenoids) and total soluble sugars, also contributes to photosynthesis comprehension. Photosystem II (PS II) is a chief component of the electron transport mechanism that occurs in light reactions of photosynthesis, responsible for converting light energy in chemical energy for further use in carbon fixation. The reaction center of PS II has two types of pigment–protein complexes forming the antenna, inside which are the photosynthetic pigments, responsible for absorbing and transferring light energy to PSII as well as dissipate excess of energy, avoiding photo-oxidative damage (Sharma *et al.*, 2020; Son *et al.*, 2020). According to Sharma *et al.* (2020), abiotic stresses negatively affect photosystems (PS I and PS II), photosynthetic electron transport, and chlorophyll biosynthesis. So, damage to photosynthetic apparatus can be verified by reduction in Fv/Fm, total chlorophyll and carotenoids, and total soluble sugars. The latter, in turn, offer information about carbon fixation, since soluble sugars represent a product arising from

photosynthetic reactions which are used in cell respiration to produce chemical energy, spent on common metabolic processes such as growing, developing and reproduction (Buchanan and Wolosiuk, 2017). In this line of reasoning, decrease in photosynthetic rates are strongly related to decrease in plants' growth and yield.

Osman *et al.* (2022) demonstrated that compost tea increased stomatal conductance, chlorophyll content, and total soluble sugars in sugar beet exposed to moderate drought under saline soil conditions, correlating the positive effect to its direct contribution to cell respiration, photosynthesis, protein polymerization and other enzymatic reactions. Elbagory (2023) and Omara *et al.* (2022) associates the improvement provided by compost teas application in plant's photosynthetic system by increasing the number of leaves and their area, reducing osmotic stress in soils affected by salt and drought, and promoting ATPase synthesis in wheat leaves.

The abiotic stresses also cause damage in plants by inducing reactive oxygen species (ROS) production and accumulation, such as singlet oxygen ($^1\text{O}_2$), hydroxyl radicals ($\text{HO}\bullet$), superoxide anion radicals ($\text{O}_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which can react quickly with various cellular constituents and oxidize them, triggering oxidative stress (Blumwald and Mittler, 2017; Sandmann, 2019). The plant cells will be under oxidative stress if the ROS quantity is more than the inside defense mechanisms (Xie *et al.*, 2019). The oxidative stress is represented by damage caused by ROS to several cell constituents, biochemical and physiological processes, such as the disruption of plasma membrane via carbohydrate deoxidation, lipid peroxidation, protein denaturation, and the destruction of genetic material, enzymes, and pigments (Chaki *et al.*, 2020; Xie *et al.*, 2019). Thus, in the face of such severe stress, the photosynthetic rate is also reduced, leading to a reduction in plant growth, development, and productivity (Sharma *et al.*, 2020).

Plants evolved mechanisms to respond to oxidative stress, by activating enzymatic- and/or non-enzymatic antioxidant metabolism. According to Xie *et al.* (2019), enzymatic antioxidants are represented by enzymes among which can be cited catalase (CAT) and various peroxidases, such as guaiacol peroxidase (GPX) and ascorbate peroxidase (APX); while the non-enzymatic antioxidants are molecules among which are carotenoids and proline. Considering the antioxidant enzymes, CAT and the peroxidases in general are responsible for scavenging H_2O_2 and other peroxides, consequently reducing its oxidative damage (de Oliveira *et al.*, 2021; Liu *et al.*, 2022). On the other hand, the antioxidant activity of carotenoids is related to their photoprotective function against photo-oxidative damages caused by ROS, by four different mechanisms: dissipation of excess energy by heat, peroxy radical ($\text{RO}_2\bullet$) scavenging, $^1\text{O}_2$ quenching, and by preventing the formation of $^1\text{O}_2$ by deactivating photosensitizers such as triplet-state chlorophyll (Coulombier *et al.*, 2020; Sandmann, 2019). And proline, in turn, is an amino acid known as one of the most effective osmoregulatory and signaling molecules in plants, which accumulation is associated to improving stress tolerance. Proline performs

multiple functions such as improving protein stability and protecting membranes integrity by binding to hydrogen bonds, protect cells by increasing water uptake potential and facilitating activation of enzymes, acting as an antioxidant defense molecule by ROS scavenging, and contributing to sodium (Na^+) and potassium (K^+) homeostasis (Hosseinifard *et al.*, 2022).

In this context, it is clear the importance of evaluating the antioxidant system parameters in this study, once H_2O_2 is one of the ROS produced that cause oxidative stress by inducing destabilization of proteins and membranes, leading to lipid peroxidation (Chaki *et al.*, 2020; Liu *et al.*, 2022). The values for H_2O_2 and lipid peroxidation indicate oxidative stress; while antioxidant enzymes activity indicate activation of enzymatic antioxidant metabolism, and carotenoids and proline quantification indicate activation of non-enzymatic antioxidant metabolism (Xie *et al.*, 2019). Besides, due to proline action as osmolyte, its accumulation in the plant cells uptakes water by osmotic adjustment, playing an important role in plant's defense system against stress, mostly under saline and water deficit stresses (Abobatta, 2019; Hosseinifard *et al.*, 2022; Seleiman *et al.*, 2021).

Osman *et al.* (2022) founded increase in antioxidant enzymes activity and decrease in proline content promoted by compost tea application in sugar beet under moderate drought in saline soil. The authors corelated the increase of antioxidant enzymes activity due to compost teas potential in enhancing the electron transport chain and the biostimulant effect in protecting the plant cells from oxidative damage through its direct or indirect impact on osmotic regulation, protein stabilization and antioxidants equilibrium; besides corelated proline decrease to a compensation by directing the plant to synthesize more soluble sugars to ensure osmotic adjustments and maintain plant development. Marcelis and Lutts (2019) verified that vermicompost tea application decreased proline content in both leaves and roots under arsenic stress and boron excess in *Atriplex atacamensis*, and the same was verified by Omara *et al.* (2022) in rice under drought stress with compost tea application. In addition, Elbagory (2023) highlights that antioxidant enzymes are the most effective way to combat the negative impact of salt stress on the permeability of the cell membrane, minimizing lipid peroxidation and guarding against oxidative damage.

Equally important, the homeostasis of K^+ and Na^+ in plants provides valuable information about abiotic stresses in plants. K^+ is an essential macronutrient for plants, and has important functions such as osmoregulation, membrane potential regulation, cotransport of sugars, stress adaption, growth promotion, and activation of several enzymes (Johnson *et al.*, 2022). It performs as regulator in several biochemical processes related to protein synthesis, carbohydrate metabolism and enzyme activation; is directly associated to photosynthesis, since it controls stomatal opening; and provides abiotic stress tolerance to water deficit and saline stress due to control both the stomatal conductance and the osmotic balance (Johnson *et al.*, 2022; Kumar *et al.*, 2020). Na^+ , in turn, is not considered an essential nutrient, but can be very useful at low levels, particularly in low K^+ conditions,

by fulfilling some metabolic functions of K^+ . However, Na^+ excess is harmful to most plants, characterizing salt stress (Maathuis, 2014). According to Adams and Shin (2014), high concentrations of Na^+ in plants often cause K^+ -deficiency symptoms, interrupting various physiological processes mediated by K^+ ; therefore, adequate K/Na ratios are essential for plant survival under abiotic stresses.

Amer (2016) demonstrated that compost tea application in maize and wheat increased several parameters under saline stress, among which can be cited grain yield and K^+ uptake, and Amer *et al.* (2021) also verified increase in grain yield of rice under saline-sodic soils, and both studies correlated this effect to compost teas' capacity to increase the tolerance of plants to salinity at physiological growth stages and improve some soil proprieties. Osman *et al.* (2022) also demonstrated that compost tea caused improvement in the uptake of K^+ in sugar beet leaves, leading to an increase in the K/Na ratio and mitigating the harmful impact of salinity and water stresses combined, associating this effect to compost tea capacity to reestablish the ionic equilibrium by alleviating excessive Na^+ absorption and augmenting other ions levels, including K^+ , providing suitable nutrition. It corroborates with results founded by Elbagory (2023) to wheat under saline stress and compost teas application, as well as by Omara *et al.* (2022) to rice under drought conditions, and their respective explanations.

According to the meta-analysis and considering the points discussed in this study, compost teas and vermicompost teas application in plants under abiotic stresses further stress-toleration by increasing enzymatic and non-enzymatic antioxidant metabolism, promoting K^+ and Na^+ homeostasis, enhancing photosynthetic metabolism, promoting growth in shoot and root, and ultimately increasing yield. It demonstrates that the teas enhance the mechanisms by which plants commonly respond to abiotic stresses (Blumwald and Mittler, 2017), that is, can be applied as tolerance-inducers to mitigate the stress and increase crop productivity.

Increase in growth and/or yield of various crops under abiotic stresses by compost teas and vermicompost teas have been reported, being a common response and demonstrating stress-toleration (Amer, 2016; Amer *et al.*, 2021; Elbagory, 2023; El-Maaz and Ismail, 2016; Eon *et al.*, 2023; Omara *et al.*, 2022; Osman *et al.*, 2022; Pibars *et al.*, 2018). It might be ascribed to teas composition: providing microorganisms, biostimulant substances and growth-promoting compounds, which improve plants metabolisms (Osman *et al.*, 2022), and to its effects in nutrient- and water-transporting cell transport systems (Elbagory, 2023). Under heavy metals stress, Eon *et al.* (2023) correlate this effects to compost tea ability to mobilize copper by humic substances biding capacity and nutrient content.

Toleration responses are also verified for the stresses assessed individually, as it was possible to demonstrate through meta-analysis that, under water deficit stress, the teas application increases enzymatic and non-enzymatic antioxidant metabolism, enhances photosynthetic metabolism, and promotes K^+ and Na^+ homeostasis, osmoregulation, and plant growth. Plants evolved a wide of

complex resistance and adaptation mechanisms to cope with water deficit, among which can be cited the activation of metabolic and physiological processes presented before (Mukarram *et al.*, 2021; Seleiman *et al.*, 2021) that were enhanced by compost teas and vermicompost teas application.

In the same way, under saline stress, meta-analysis demonstrated that the teas promote growth in shoot and root, and increased K^+ uptake. It is associated to common mechanisms of saline-stress alleviation (Abobatta, 2019; Elbagory, 2023). Therefore, compost teas and vermicompost teas can be applied with the aim to alleviate both stresses, sustaining plant survival and growth.

5 CONCLUSION

Compost teas and vermicompost teas can be applied to plants as tolerance-inducers to abiotic stresses, as the teas are capable of mitigate the stresses and promote plant growth and development despite the stressful conditions. The toleration mechanisms promoted by the teas to abiotic stresses are associated to increasing enzymatic and non-enzymatic antioxidant metabolism, contributing to K^+ and Na^+ homeostasis, and enhancing photosynthetic metabolism, consequently increasing growth and ultimately increasing yield of crop species.

When considered the abiotic stresses individually, was possible to verify that the teas were beneficial for both water deficit and saline stress. For water deficit, the teas application increases growth, enhances photosynthetic metabolism, activates enzymatic and non-enzymatic antioxidant metabolism, and promotes K^+ and Na^+ homeostasis and osmoregulation. For saline stress, the teas enhance growth and increase K^+ uptake.

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4 ARTIGO II: INFLUENCE OF TWO COMPOST TEAS ON INITIAL GROWTH AND TOLERANCE TO WATER DEFICIT IN MAIZE AND SORGHUM

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INFLUENCE OF TWO COMPOST TEAS ON INITIAL GROWTH AND TOLERANCE TO WATER DEFICIT IN MAIZE AND SORGHUM

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ABSTRACT

Once compost teas can improve plants growth and its tolerance to stresses, and considering that water deficit is still little explored among abiotic stresses, the objective of this work was to verify the effects of two compost teas, plants compost tea (PCT) and cattle manure compost tea (CMCT), on the initial growth of maize and sorghum and on their tolerance to water deficit simulated by mannitol. We produced and physical-chemically and microbiologically analyzed the two compost teas, then applied them to maize and sorghum, evaluating their effects on germination, early growth, root morphology/architecture, antioxidant enzyme activity and lipid peroxidation. The same analyzes were repeated in maize and sorghum under water deficit stress. Our results demonstrate that CMCT was more beneficial than PCT, but both treatments presented biostimulant action according to 1) their effects on maize and sorghum initial growth and root morphology/architecture, and 2) their toleration to water deficit effects. The biostimulant action was due to chemical and microbiological composition and was more effective in diluted concentrations.

Statement of Novelty

This work was carried out with the aim of understanding the effect of two compost teas on maize and sorghum, as well as verifying whether compost teas can help both species to respond better to the stress caused by drought (water deficit). This study is relevant since few studies were found in the scientific literature on the effects of compost teas on maize, in addition to the lack of studies with sorghum. Furthermore, there are even fewer studies on the effects of compost teas as tolerance-inducers to drought in plants, regardless of the target species studied.

Keywords: Biostimulant; Root morphology; Antioxidant enzymes; Drought; *Zea mays*; *Sorghum bicolor*.

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1 INTRODUCTION

Composting is a biological decomposition process of organic matter under controlled aerobic conditions. Such conditions provide thermophilic temperatures that result from heat biologically produced by microorganisms' action. At the end of the process, composting originate a stable organic substrate called compost, which is homogeneous, odorless, dark in color, rich in humus, free from pathogens and plant seeds, which can be applied as a fertilizer in order to improve physical, chemical and biological soil characteristics (López-González *et al.* 2015; Haug 2018). Composting is applied to reduce the amount of waste in the environment, using agricultural or industrial by-products as raw materials, which can be urban, green waste, animal manure, agricultural crop wastes and other residues that can be composted (Cavagnaro 2015). In addition, it has importance within the scope of circular economy, as represents a possibility of valuing residues (Bakan *et al.* 2022; Zhou *et al.* 2022).

More recently, farmers who aim to minimize the use of fertilizers and pesticides have used aqueous compost extracts as an alternative. Such extracts, called compost teas, have the advantage of being more easily transported and applied to crops than their respective solid materials (Arancon *et al.* 2007). Compost tea is defined as an aqueous extract obtained by mixing compost and water for defined periods of time, during which there may or may not be aeration and inoculation or not of additives for enhance microbial population density during production (Eudoxie and Martin 2019). When well produced, compost tea is composed by nutrients and a wide variety of organisms and can be applied to the soil or sprayed on plants (Carballo *et al.* 2008; Islam *et al.* 2016). Its application is intended to confer resistance and provide essential nutrients to plants, as well as provide beneficial microorganisms to plants and/or soil (Hargreaves *et al.* 2009; Eudoxie and Martin 2019).

Numerous scientific studies indicate that application of compost tea improves plants' health, their nutritional quality and productivity (Pant *et al.* 2012; Canellas *et al.* 2015; Liguori *et al.* 2015; Amos 2017; Otero *et al.* 2019; Fouda and Niel 2021; González-Hernández *et al.* 2022), as well as it helps plants respond to biotic (Pane *et al.* 2012; st. Martin and Brathwaite 2012; On *et al.* 2015; Siddiqui *et al.* 2015; Amos 2017; González-Hernández *et al.* 2021) and abiotic stresses. It is noteworthy that studies that elucidate compost tea's effects on plants exposed to abiotic stresses are mostly related to salt stress, with few studies on water deficit (Hendawy *et al.* 2013; Heba *et al.* 2014; Bayoumy *et al.* 2019; Amer *et al.* 2020, 2021; Osman *et al.* 2022; Elbagory 2023).

Among abiotic stresses, water deficit can be understood as the stress caused to a plant when the quality or quantity of available water is unable to meet basic metabolic needs, that is, when water availability is insufficient (Bergamaschi *et al.* 2006; Abobatta 2019; Seleiman *et al.* 2021). Water deficit can cause morphological, physiological and biochemical changes in plants: as primary effects of water deficit, can be mentioned the reduction of water potential, cellular dehydration and hydraulic resistance; and as secondary effects the reduction of cellular/leaf expansion, reduction of cellular and metabolic activities, stomatal closure, inhibition of photosynthesis, leaf abscission, alteration in carbon partition, cytorrhisis, cavitation, destabilization of membranes and proteins, production of reactive oxygen species (ROS), ionic cytotoxicity and cell death (Blumwald and Mittler 2017; Abobatta 2019; Seleiman *et al.* 2021).

Stress due to water deficit is triggered both in natural and agricultural areas by periods of drought, that is, intermittent or continuous periods without precipitation that has been aggravated by climate changing (Abobatta 2019). In Brazil, some negative impacts can be related to periods of drought – which are increasingly frequent and evident –, by reducing or inhibiting the agriculture expansion (Souza *et al.* 2018).

The literature still does not present many studies that correlate the use of compost teas as tolerance inducers to water deficit (Pibars *et al.* 2018; Hussein *et al.* 2019; Osman *et al.* 2022; Omara *et al.* 2022), which have been causing

damage to agriculture around the world since drought is one of the most grave environmental stresses affecting crop productivity, and considered by some authors as the most serious threat to world food security (Abobatta 2019; Seleiman *et al.* 2021). So, it is noteworthy the importance of studies that elucidate compost teas' chemical and microbiological composition and their effects as tolerance inducers to water deficit, especially because of the drought aggravation due to climate changing.

In this context, maize (*Zea mays*) and sorghum (*Sorghum bicolor*) are crop species of great economic importance, being used for human and animal food, and cultivated in various regions of the planet, on which there is few studies with compost teas effects – or similar substances as compost leachates or water-soluble fractions of compost – on their growth (Gutiérrez-Miceli *et al.* 2008; Vaccaro *et al.* 2009; Ou-Zine *et al.* 2022). In addition, there is no studies that correlate compost teas as tolerance inducers to water deficit in sorghum, and only one study in maize (Hussein *et al.* 2019) Our hypothesis is that compost teas can improve the initial growth of maize and sorghum, as well as provide a better response to water deficit.

Therefore, the objective of this work was to verify the effects of two compost teas on the initial growth of maize and sorghum, and on their tolerance to the presence of mannitol, which can be applied as a simulator of water deficit stress.

2 MATERIALS AND METHODS

2.1 Composts obtaining and characterization

The composting was carried out in the greenhouse at Federal University of Alfenas, Alfenas city (Minas Gerais state, Brazil, geographic coordinates: 21°25'08.2"S 45°56'52.4"W). Windrows of size 2x1x0.5 m were placed on a canvas on the ground, consisting of two treatments: plants compost and cattle manure compost. For plants compost production, 10 L of castor bean pie dry mass – a residue of castor bean (*Ricinus communis*) processing – was mixed to 990 L of elephant grass (*Pennisetum purpureum*) fresh mass. For cattle manure compost production, 32 L of cattle manure dry mass was mixed to 968 L of elephant grass fresh mass. These proportions were obtained based on a spreadsheet made available by Embrapa Agrobiologia, which determines the appropriate quantities of raw materials aiming at a C:N ratio close to 30 of the compost produced (Leal *et al.* 2013).

The windrows were moistened daily, in order to maintain the moisture content suitable for composting – between 40 to 60%. Every three days, windrows temperatures were measured with a skewer-type thermometer (Delta OHM HD2307.0, Italy) at four different points to check the efficiency of the thermophilic phase. The windrows were turned every 30 days to promote aeration of the compost, in order to eliminate possible pathogens. Treatments were maintained for 90 days, from February 2020 to May 2020.

After 90 days, the two treatments were homogenized and samples were collected (plants compost and cattle manure compost), which were analyzed by physical, chemical, and general microbiological analysis to characterize them, such as: macro and micronutrient content, organic carbon content, heavy metal content, C:N ratio, presence of pathogenic organisms, microbial population density and presence of pathogenic microorganisms, pH and humidity.

For macro and micronutrient content, total organic carbon content, C:N ratio, pH and humidity, data were obtained according to normative instruction no. 37 (Brasil 2017) by a laboratory specialized in the analysis of fertilizers, soils and plants, as follows: total N contents were obtained by Raney alloy macromethod; total P₂O₅ were obtained by nitroperchloric digestion and posterior determination by Quimociac gravimetric method; P₂O₅ ammonium citrate + H₂O

were obtained by Quimociac gravimetric method; water soluble K_2O were obtained by sodium tetraphenylborate volumetric method; total Ca, Mg, Cu, Fe, Mn and Zn were obtained by nitroperchloric digestion and determination by atomic absorption spectrophotometry; total S were obtained by barium sulfate gravimetric method; total B were obtained by azomethine-H spectrophotometric method. For total organic carbon contents, potassium dichromate volumetric method was used.

For C:N ratio, obtention occurred by division of the results in percentage in mass obtained for organic carbon and nitrogen, both referred to the sample on a dry basis. For pH, the samples were suspended in 0.01 mol.L^{-1} $CaCl_2$ solution and carried out the measurement of the pH using a potentiometer with thermocompensator and combined electrode.

Humidity data were obtained by reducing the samples by careful quartering to approximately 60 g; after, the mass of the sample “*in natura*” (M1) were weighed, taken to drying in an oven at $105 \pm 5 \text{ }^\circ\text{C}$ until constant weight, and after cooling in a desiccator, were determined the mass of the dried sample (M2). These data were used in the calculation of the humidity (H), as follows:

$$H = \frac{100(M1 - M2)}{M1}$$

Heavy metal content data was obtained by the same laboratory cited before, according to United States Environmental Protection Agency methods, in which samples were submitted to acid digestion and posterior determination of As, Cd, Hg, Ni, Pb, Se and hexavalent Cr by high sensitivity atomic emission spectrometry (Agilent Technologies MP-AES 4200, USA).

The microbial population density was obtained by simple analyses to count colony forming units (CFU). Were used seven microbial culture media, five for fungi, one for bacteria, and one for fungi and bacteria, respectively: Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar, Dichloran Rose Bengal Chloramphenicol (DRBC) Agar Base, Czapek-Dox Agar, Malt Extract Agar Base, Levine EMB Agar and Nutrient Agar. The culture media were prepared according to the instructions on each package: weighed, dissolved in distilled water, heated with a boiler until the agar melted, autoclaved, and later poured still hot into Petri dishes until solidification. Antibiotics were added to media intended for fungal growth.

For inoculation, 10 g of sample (plants compost or cattle manure compost) were mixed in 90 mL of 0.55% saline solution, characterizing the dilution factor 10^{-1} , from which the other dilution factors (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) were obtained. Were inoculated 0.1 mL of each dilution factor and used three repetitions per dilution factor. The inoculated Petri dishes were placed in B.O.D. germinations chambers (Solab SL-224/300, Brazil) at 24°C for 2 to 3 days in the case of bacteria, and for 4 to 5 days in the case of fungi, after which the CFU were counted by standard plate count, considering for calculation the dilution factor that presented $30 < \text{CFU} < 300$. The results were obtained as follows:

$$\text{CFU} \cdot \text{g}^{-1} = \frac{\text{CFU} \cdot \text{df}^{-1}}{a}$$

where df^{-1} = inverted dilution factor, and a = aliquot = 0.1 mL.

In addition to the characterization analyses, the composts generated in the two treatments were used to extract the compost teas.

2.2 Compost teas obtaining and characterization

Plants compost and cattle manure compost served as raw material to obtain the compost teas, which consisted of the following treatments: plants compost tea (PCT) and cattle manure compost tea (CMCT). For each treatment, were

obtained stock solutions of concentration 1:1 (volume of compost : volume of distilled water), by aerated aqueous extraction method using an aquarium pump, for 24 hours. After brewing, the two teas were filtered through filter paper with the aid of an air pump.

Both compost teas in liquid state was used to verify microbial population density and presence of pathogenic microorganisms, pH, electrical conductivity, dissolved oxygen and the following phytohormones content: trans-zeatin-riboside, gibberellic acid, indoleacetic acid, salicylic acid, abscisic acid, acid indole-3-butyric acid, jasmonic acid, gibberellin A4 and 6-(γ , γ -dimethylallylamino) riboside purine.

For microbial population density and presence of pathogenic microorganisms, an experiment similar to the described in item 2.1 was carried out, with the difference of using 10 mL of 1:1 concentration of the compost tea (PCT or CMCT) as a sample instead of 10 g of compost, in order to obtain the 10^{-1} dilution factor. The results were obtained by the same formula described in 2.1 item and expressed as CFU.mL⁻¹. Electrical conductivity and dissolved oxygen data was obtained by using a Multiparameter Water Quality Monitor (Horiba U-50 series, Japan).

Phytohormones content was obtained by Trapp *et al.* (2014) method, on which the compost teas were frozen in liquid nitrogen (N₂) (-80 °C) immediately after collection and kept during transport to the laboratory. Samples were removed from liquid N₂ and added to 10 mL of extraction solution (acetonitrile: Mili-Q water 1:1). After the addition, the tubes containing the solutions were briefly vortexed, kept under agitation for 30 minutes on a shaker and then centrifuged at 16,000 rpm and at 4 °C for 5 minutes. The supernatant was transferred to a new centrifuge tube (1.5 mL) and dried at VCA speed. After drying, 100 μ L of methyl alcohol were added to each of the samples, centrifuged again at 16,000 rpm and at 4 °C for 10 minutes. The supernatant was analyzed by High Performance Liquid Chromatography coupled to a Mass Spectrometer (HPLC/MS) (Agilent Technologies 6490 Triple Quad LC/MS, USA).

Furthermore, the compost teas were submitted to lyophilization (LioTop L101, USA) and chemical characterization. Were analyzed macro and micronutrient content and heavy metal content, conducted by a laboratory specialized in the analysis of fertilizers, soils and plants, based on recommendations of Malavolta (1997): N was determined by sulfur digestion followed by the Kjeldahl distillation; Ca, Mg, K, Cu, Fe, Zn, Mn, Cd and Pb were determined by high sensitivity atomic emission spectrometry (Agilent Technologies MP-AES 4200, USA); P, S and B were determined by spectrophotometry (FEMTO 600S, Brazil). And to obtain data for Cr and Ni, were used IAC method (Raij *et al.* 2001) by extraction in DTPA solution (pH 7.3) and posterior determination by flame atomic absorption spectrophotometry (Varian SpectrAA-400 Plus Atomic Absorption Spectrometer, USA). All data reported are based on the dry weight of lyophilized samples.

2.3 Analyzes of maize and sorghum initial growth, root morphology, antioxidant enzymes activity and lipid peroxidation under compost teas influence

For the initial growth analysis of maize (*Zea mays* – Pioneer® P30F53 hybrid) and sorghum (*Sorghum bicolor* – BRS 332), a growth chamber experiment was carried out with two treatments: plants compost tea (PCT) and cattle manure compost tea (CMCT). For each treatment, four concentrations were used: 1:10, 1:5, 1:2.5 and 1:1 (v:v), in addition to distilled water as a negative control and 100% Hoagland solution – nutritional solution world-recognized – as a positive control. Four repetitions were used to each concentration.

The seeds were placed to germinate in rolls with three sheets of Germitest® paper, two of which were used as a base and one to close the rolls, with each roll representing a repetition. The three sheets were weighed to determine the volume (mL) to be used for imbibition with the concentrations used, in the proportion of 2.5 mL x paper weight. After, the rolls were soaked with the concentrations, containing 50 seeds each and placed in plastic bags for autoclave. These

were kept for four days in a B.O.D. germination chamber (Ethik Technology 411FPD, Brazil), with 30 °C temperature and 12 hours photoperiod. The period of four days was determined in pilot experiments that elucidated as the necessary time to maize and sorghum growth without causing damage due to the mechanical shock caused by the autoclave bags in which the rolls were packed.

This experiment was repeated in order to collect samples for the analyzes of root morphology, antioxidant enzymes activity and lipid peroxidation.

For initial growth analysis, after four days, the following parameters were evaluated: germination percentage at 24 hours and on the 4th day (%), Germination Speed Index (GSI), fresh biomass (g), dry biomass (g) and shoot length (mm).

GSI was obtained as proposed by Chiapusio *et al.* (1997), as follows:

$$GSI = \frac{N_1}{1} + \dots + \frac{(N_n - N_{n-1})}{n}$$

where N_1 , N_n , and N_{n-1} correspond to the number of germinated seeds in the first, n , $n-1$ evaluations, respectively, and n is the evaluation number. Four evaluations were performed, every 24 hours.

Fresh and dry biomasses were measured using a precision balance with four decimal places (Shimadzu Marte AY220, Japan), and dry biomass were obtained after drying in an oven with forced air circulation at 40 °C (Ethik Technology 410/3ND, Brazil). For shoot length, the 10 visibly larger seedlings were selected and measured using a digital caliper (Digimess 100.170, Brazil).

For the other analyzes, after repeating the experiment, on the fourth day 10 seedlings were randomly collected from each repetition for root morphology analysis. The roots were fixed in 70% alcohol for further analysis in WinRhizo Pro 2007a (Regent Instruments, Canada). The parameters evaluated were total root length (cm), root surface area (cm²), average root diameter (mm), root volume (cm³), number of root tips, number of root forks and root fineness (cm/cm³). Root fineness were measured according to Souza *et al.* (2012). In addition, also were evaluated the parameters root length, root surface area and root volume according to root diameter classes: very thin (0 – 0.5 mm), thin (0.5 – 2.5 mm), thick (2.5 – 4.5 mm) and very thick (>4.5 mm). Roots classified as very thick was not present in sorghum's data so was not analyzed for this species.

An observation is necessary here: due to the pandemic, some repetitions could not be analyzed, due to the long storage time between the fixation in 70% alcohol and the analysis in WinRhizo. For most of them, were used a mean of the other repetitions to represent those repetitions that could not be analyzed, in order to maintain $n = 4$. Although, for 1:5 concentration of PCT treatment, in maize, all the four repetitions could not be analyzed in WinRhizo. Therefore, data for this concentration are not shown for any of the root morphology parameters in maize under PCT influence, nor were they considered in the statistical analysis.

For the analyzes of antioxidant enzymes activity and lipid peroxidation, after the fourth day of the experiment, the remaining fresh biomass – which was not collected for root morphology analysis – was collected and stored in liquid N₂ for further extraction.

For the extraction of antioxidant enzymes, 200 mg of seedlings were macerated in liquid N₂ with 50% PVPP and homogenized in 1.5 mL of extraction buffer containing: 375 µL of 400 mM potassium phosphate (pH 7.8), 15 µL of 10 mM EDTA and 75 µL of 200 mM ascorbic acid. The homogenates were centrifuged at 13,000 rpm for 10 minutes at 4°C and the supernatants were collected for enzymatic analyzes of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), quantified by spectrophotometry in ELISA reader (Biochrom Anthos Zenyth 200 RT, England). The final reaction volume for reading the enzymes was 2 mL, in a visible plate. All readings were performed in triplicate.

The quantification of SOD activity was performed according to the method proposed by Giannopolitis and Ries (1977), at 560nm, and a unit of SOD activity is defined as the amount of enzyme that inhibits the photoreduction of NBT by 50%. To quantify the CAT activity, the method of Havir and McHale (1987) was used, at 240 nm, every 15 seconds for 3 minutes, and a unit of CAT activity is defined as the amount of enzyme that catalyzes the decomposition of 1 $\mu\text{mol}\cdot\text{min}^{-1}$ of H_2O_2 . APX activity was quantified according to the method of Nakano and Asada (1981), at 290 nm, every 15 seconds for 3 minutes, and an APX activity unit is defined as the amount of enzyme that oxidizes 1 $\mu\text{mol}\cdot\text{min}^{-1}$ of ascorbate.

For extraction of lipid peroxidation, 200 mg of seedlings were macerated in liquid N_2 with PVPP and homogenized in 1.5 mL of 0.1% trichloroacetic acid (TCA). The homogenates were centrifuged at 12,000 rpm for 15 minutes at 4°C, collecting the supernatants. Lipid peroxidation was determined by quantifying thiobarbituric acid reactive species, as described by Buege and Aust (1978), at 535 and 600 nm, quantified by spectrophotometry in an ELISA reader (Biochrom Anthos Zenyth 200 RT, England). The final reaction volume was 2 mL, in a visible plate. All readings were performed in triplicate.

2.4 Analyzes of maize and sorghum initial growth, root morphology, antioxidant enzymes activity and lipid peroxidation under influence of mannitol and compost teas association

For water deficit stress associated with compost teas, growth chamber experiments were carried out with mannitol (-2.5 MPa) as a stressful solution. The osmotic pressure of mannitol was defined in a pilot experiment as stressful for maize and sorghum as it altered their initial growth.

For the experiments, were used the stressing solution (mannitol -2.5 MPa) mixed to four concentrations of compost tea (1:10, 1:5, 1:2.5 and 1:1), in addition to two controls: stressing solution and distilled water. Four repetitions were used to each concentration.

The experimental conditions, methodology and analyzed parameters were the same as described in item 2.3. However, in initial growth analysis, was added the parameter number of anomalous seedlings. Were considered as anomalous seedlings the visually defective seedlings, as twisted and/or blackened roots or shoots.

2.5 Experimental design and statistical analysis

For statistical analysis, a completely randomized design (CRD) was used with a 2 x 6 factorial (2 treatments x 6 concentrations) and 4 repetitions. The data obtained were submitted to analysis of variance (ANOVA) to verify difference between the two treatments (PCT and CMCT) and the occurrence of interaction between treatments and concentrations.

For difference between the two treatments, the means were compared by the Scott-Knott test at 5% significance. Regarding the interaction treatment*concentrations, was applied a regression model using the control with distilled water as zero, for the experiments that verify the effect of the two compost teas; and using the control with stressful solution (mannitol -2.5 MPa) as zero, for the experiments that verify the effect of mannitol and compost teas association. For data that did not fit the regression model, the means were compared by the Scott-Knott test at 5% significance. All data analyzes cited were performed using the Sisvar program version 5.8.

In addition, were applied the Dunnett's test at 5% significance using the Action extension for Excel, to compare the concentrations means with the other controls means: for the experiments that verify the effect of the compost teas, were compared to Hoagland solution; and for the experiments that verify the effect of mannitol and compost teas association, were compared to distilled water.

3 RESULTS

3.1 Composts characterization

The relative humidity and ambient temperature conditions to which the windrows were subjected during the 90 days are shown in Supplementary File (Fig. 1), as well as the temperature of the windrows. The average relative humidity during the 90 days was 61.9% and average ambient temperature was 26.5 °C. The thermophilic phase of the windrows started on the 7th day – from 59.8 °C in plants compost windrow and 54.3 °C in cattle manure compost windrow – and lasted for 13 days to plants composting and 10 days for cattle manure composting – to 42.7 °C and 40 °C, respectively. The average temperatures of thermophilic phase were 48.7 °C for plants composting and 47.7 °C for cattle manure composting. The temperatures gradually reduced, until they reached ambient temperature around the 40th day – 22.2 °C in plants compost windrow and 23.5 °C in cattle manure compost windrow –, defining the start of maturation process. At the end of 90 days, the windrows originated dark, homogeneous, and odorless composts with reduced volume in relation to the original volume (Supplementary File, Fig. 2).

Regarding the composts characterization, the results obtained for the physicochemical parameters can be seen in the Supplementary File (Table 1). In general, the plants compost presented higher amounts of macro and micronutrients and higher total organic carbon than the cattle manure compost, besides having slightly higher moisture. In the other hand, the cattle manure compost presented higher C:N ratio and pH in relation to plants compost since the plant compost had a pH close to neutrality and the cattle manure compost had a slightly basic pH. In addition, both composts presented negligible amounts of heavy metals (As for plants compost and Cd and Pb for both composts).

No pathogenic organisms of any species were found in the plants compost, while in the cattle manure compost some ruminant's pathogenic organisms were found, as *Haemonchus spp.*, *Cooperia spp.*, *Oesophagostomum spp.*, *Strongyloides spp.*, *Trichostrongylus spp.* and some coccidial oocysts (Supplementary File, Fig. 3). Although were not found any parasites with potential to infect humans.

Regarding microbial population density (Supplementary File, Table 2), both composts did not present pathogenic microorganisms such as dermatophytic fungi, *Escherichia coli*, *Klebsiella pneumoniae* nor *Salmonella enteritidis*, but presented similar density of fungi in general. Plants compost presented more slow-growing fungi and saprophytic fungi than cattle manure compost; while cattle manure compost presented more undemanding bacteria and fungi, fungi that grow in high concentration of carbohydrates, and fungi that use sodium nitrate as a nitrogen source, when compared to plants compost.

3.2 Compost teas characterization

The results obtained for the physicochemical analyzes of the compost teas (macro and micronutrients contents, heavy metal content, pH, electrical conductivity, and dissolved oxygen) are shown in Table 1. In general, the amounts of macro and micronutrients of both compost teas were proximal, with some nutrients (as N, P, Ca, Mg, B, Cu and Mn) presenting higher values in plants compost tea (PCT) and other nutrients (as K, S, Fe and Zn) presenting higher values in cattle manure compost tea (CMCT). The extraction of compost teas enhanced amounts of Cu, Fe and Mn in relation to solid composts, and both compost teas presented insignificant amounts of Cr and relevant amounts of Pb – highlighting PCT. The pH values demonstrated that PCT is slightly acid while CMCT is slightly basic, but both compost teas are proximal from neutrality. PCT presented higher values for dissolved oxygen than CMCT, while CMCT presented higher electrical conductivity.

Both compost teas had negative results for the presence of the phytohormones analyzed and therefore such data are not demonstrated.

Table 1. Physicochemical characterization of plants compost tea (PCT) and cattle manure compost tea (CMCT).

Parameters	PCT	CMCT	Unit
N	29.74	25.12	g.kg ⁻¹
P	12.15	11.96	g.kg ⁻¹
K	61.69	71.14	g.kg ⁻¹
Ca	15.37	14.01	g.kg ⁻¹
Mg	9.83	9.10	g.kg ⁻¹
S	3.56	3.59	g.kg ⁻¹
B	6.39	3.36	mg.kg ⁻¹
Cu	21.83	19.13	mg.kg ⁻¹
Fe	630.22	1088.01	mg.kg ⁻¹
Mn	232.97	196.72	mg.kg ⁻¹
Zn	80.43	83.15	mg.kg ⁻¹
Cr	0.80	0.10	mg/dm ³
Ni	N/D	N/D	mg/dm ³
Cd	N/D	N/D	mg.kg ⁻¹
Pb	13.60	6.52	mg.kg ⁻¹
pH	6.29	7.35	-
Dissolved oxygen	5.29	4.26	mg.L ⁻¹
Electrical conductivity	16.00	19.90	mS.cm ⁻¹

N/D = Not detected

Regarding microbial population density and presence of pathogenic microorganisms (Supplementary File, Table 2), as well as in solid composts, both treatments did not present pathogenic microorganisms – dermatophytic fungi, *E. coli*, *K. pneumoniae* nor *S. enteritidis*. The PCT extraction was able to maintain similar values of microbial population density in relation to plants compost for fungi in general and fungi that grow in high concentration of carbohydrates; but increased by approximately four times the population density of fungi that use sodium nitrate as a nitrogen source, while decreased the population densities of undemanding bacteria and fungi, slow-growing fungi, and saprophytic fungi. The CMCT extraction, on the other hand, increased the population densities of fungi that grow in high concentration of carbohydrates and saprophytic fungi when compared to cattle manure compost, while reduced the population densities of undemanding bacteria and fungi, fungi in general, fungi that use sodium nitrate as a nitrogen source, and slow-growing fungi.

Comparing the treatments, CMCT presented higher population densities of undemanding bacteria and fungi, fungi that grow in high concentration of carbohydrates, slow-growing fungi, and saprophytic fungi; while PCT just presented higher population densities of fungi in general and fungi that use sodium nitrate as a nitrogen source.

3.3 Analyzes of maize and sorghum under compost teas influence

When compared the compost teas treatments, for both species, cattle manure compost tea (CMCT) was more beneficial than plants compost tea (PCT) (Table 2).

Considering maize, CMCT presented higher values for germination percentage at 24h and Germination Speed Index (GSI) than PCT. The CMCT treatment increased total root length and root surface area compared to PCT, besides promoted higher values for length of very thin and thin roots, for surface area of thin roots and for volume of thin roots. The PCT treatment promoted higher superoxide dismutase (SOD) and catalase (CAT) activities, while CMCT presented higher activity for ascorbate peroxidase (APX) (Table 2). There was no difference between treatments ($p>0.05$) for the other parameters analyzed: germination percentage on the 4th day, fresh and dry biomasses, shoot length, average root diameter, root volume, numbers of root tips and forks, root fineness, the parameters considering the other root diameter classes and lipid peroxidation (Supplementary File, Table 3).

For sorghum, CMCT promoted higher values for length, surface area and volume in very thin roots when compared to PCT, while PCT presented greater shoot length than CMCT. Besides, CMCT demonstrated higher activity of CAT than PCT (Table 2). The other parameters analyzed (germination percentage at 24h and on the 4th day, GSI, fresh and dry biomasses, all root morphology parameters that do not consider the root diameter classes and those that consider the other two classes, APX and SOD activities, and lipid peroxidation) showed no difference ($p>0.05$) between the two treatments for sorghum (Supplementary File, Table 3).

Table 2. Comparison between means of plants compost tea (PCT) and cattle manure compost tea (CMCT) treatments for initial growth, root morphology and biochemical parameters of maize and sorghum \pm standard error; $n = 4$.

Parameters	MAIZE				SORGHUM			
	PCT		CMCT		PCT		CMCT	
Germination percentage at 24h (%)	36.17 \pm 3.54	b	52.75 \pm 3.14	a				
GSI	33.13 \pm 0.90	b	37.36 \pm 0.78	a				
Shoot length (mm)					69.98 \pm 1.47	a	66.94 \pm 1.20	b
Total root length (cm)	5.39x10 ⁵ \pm 7.42x10 ⁴	b	7.41x10 ⁵ \pm 1.13x10 ⁵	a				
Root surface area (cm ²)	2.13x10 ⁵ \pm 3.16x10 ⁴	b	2.70x10 ⁵ \pm 4.08x10 ⁴	a				
Length of very thin roots (cm)	4.23x10 ⁴ \pm 4.67x10 ³	b	8.18x10 ⁴ \pm 2.21x10 ⁴	a	1.37x10 ⁶ \pm 1.04x10 ⁵	b	1.56x10 ⁶ \pm 1.15x10 ⁵	a
Length of thin roots (cm)	4.73x10 ⁵ \pm 6.58x10 ⁴	b	6.27x10 ⁵ \pm 9.96x10 ⁴	a				
Surface area of very thin roots (cm ²)					1.27x10 ⁵ \pm 7.30x10 ³	b	1.49x10 ⁵ \pm 9.58x10 ³	a
Surface area of thin roots (cm ²)	1.66x10 ⁵ \pm 2.28x10 ⁴	b	2.06x10 ⁵ \pm 2.99x10 ⁴	a				
Volume of very thin roots (cm ³)					0.1117 \pm 0.0054	b	0.1299 \pm 0.0077	a
Volume of thin roots (cm ³)	0.5364 \pm 0.0682	b	0.6070 \pm 0.0796	a				
SOD (U min ⁻¹ g ⁻¹)	21.19 \pm 4.33	a	15.87 \pm 3.24	b				
APX (μ M AsA min ⁻¹ g ⁻¹)	23.37 \pm 4.77	b	26.69 \pm 5.45	a				
CAT (μ M H ₂ O ₂ min ⁻¹ g ⁻¹)	2.72 \pm 0.56	a	1.19 \pm 0.24	b	1.61 \pm 0.21	b	3.11 \pm 0.44	a

Inside each species, means followed by different letters, in the same row, differ statistically by the Scott-Knott test at 5% significance. Were represented only the data which statistical difference was significant. Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm.

When considered the interaction between treatments and concentrations for maize, PCT promoted a dose-response decrease in germination percentage at 24h (Fig. 1a), GSI (Fig. 1b) and fresh biomass (Fig. 1c). For germination percentage at 24h and GSI, the highest concentration (1:1) was the only one among the concentrations tested which was lower than Hoagland solution, while maize's fresh biomass was increased in relation to Hoagland solution at 1:10 and 1:2.5 concentrations as well as distilled water. The shoot length of maize under PCT action (Fig. 1d) was decreased by

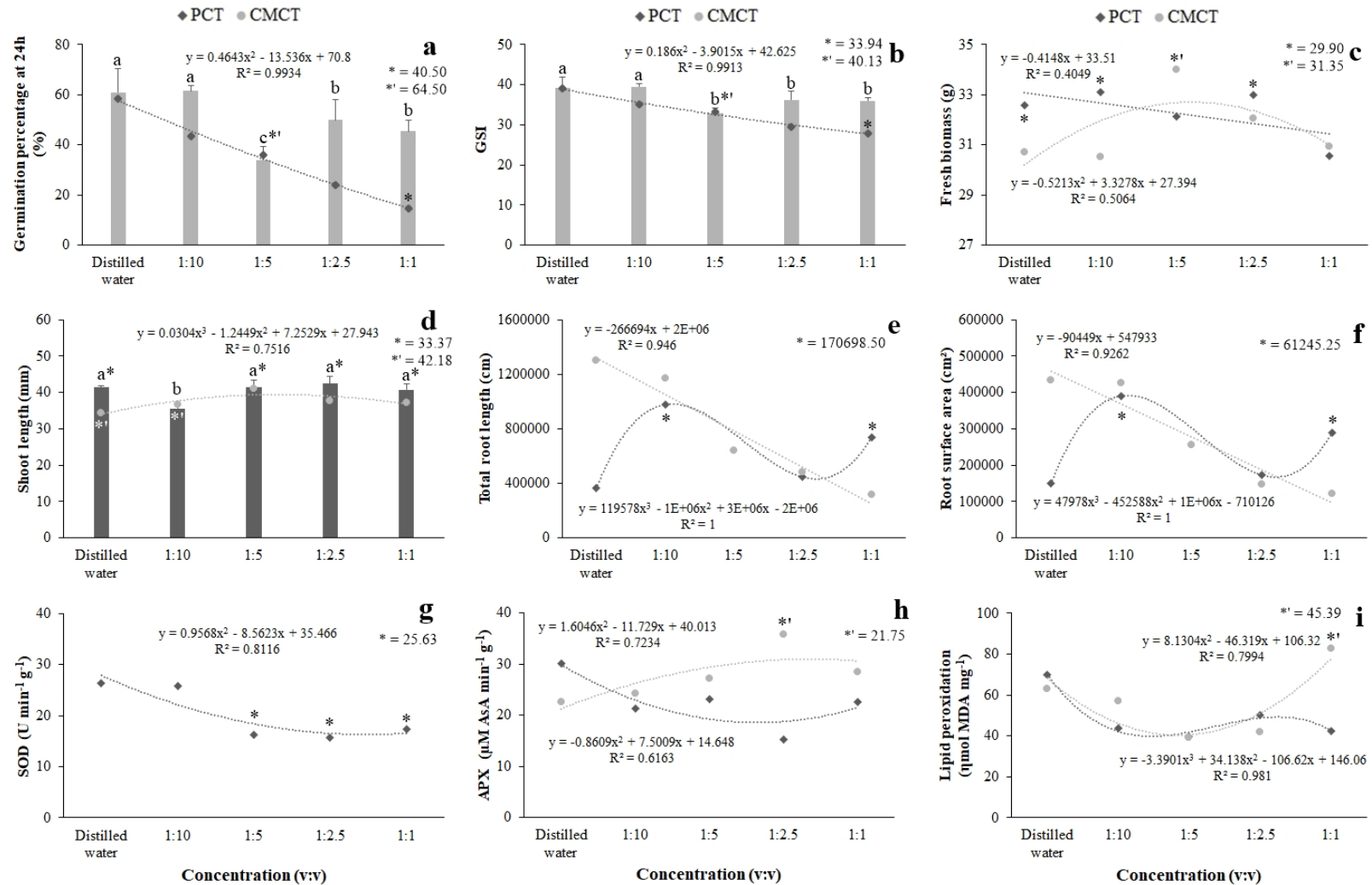


Fig. 1 Initial growth, root morphology and biochemical parameters of maize exposed to plants compost tea (PCT) and cattle manure compost tea (CMCT) (a) Germination percentage at 24h (%) (b) Germination Speed Index (GSI) (c) Fresh biomass (g) (d) Shoot length (mm) (e) Total root length (cm) (f) Root surface area (cm²) (g) Superoxide dismutase (SOD) (U min⁻¹ g⁻¹) (h) Ascorbate peroxidase (APX) (μM AsA min⁻¹ g⁻¹) (i) Lipid peroxidation (ηmol MDA mg⁻¹); Columns and values demonstrated by points indicate means, n = 4; Bars: standard error; Means associated to asterisks differ statistically from the mean of Hoagland solution (n = 4) according to Dunnett's test at 5% significance: *=PCT and *'=CMCT; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScape program

the lowest concentration tested (1:10) in relation to distilled water and all other concentrations, although it was statistically equal to Hoagland solution. For total root length (Fig. 1e) and root surface area (Fig. 1f), the concentrations of PCT presented a tendency of increase these parameters in the lowest (1:10) and highest (1:1) concentrations tested, which even surpassed Hoagland solution effect under maize roots. PCT also decreased SOD (Fig. 1g) and APX (Fig. 1h) activities, as well as lipid peroxidation (Fig. 1i), with a slightly stabilization among the concentrations tested. In addition, SOD activity was reduced in relation to Hoagland solution by concentrations 1:5, 1:2.5 and 1:1.

For CMCT, all concentrations except the lowest (1:10) decreased germination percentage at 24h (Fig. 1a) and GSI (Fig. 1b) in maize compared to distilled water, and 1:5 concentration promoted a reduction even lower than Hoagland solution. On the other hand, maize's fresh biomass (Fig. 1c) was increased by CMCT, highlighting intermediate concentrations with 1:5 concentration even surpassing Hoagland solution, after which occurred a decrease in this effect as the concentration increases. Shoot length also demonstrated increase under CMCT influence (Fig. 1d) with most concentrations tested (1:5, 1:2.5 and 1:1) statistically equaling Hoagland solution. Maize's root length (Fig. 1e) and root surface area (Fig. 1f) demonstrated a dose-response reduction for CMCT data, while APX activity (Fig. 1h) demonstrated a dose-response increase with stabilization in higher concentrations and with 1:2.5 concentration surpassing Hoagland solution. In addition, CMCT decreased lipid peroxidation (Fig. 1i) in maize, highlighting the intermediate concentrations, with a subsequent increase as the concentration increases, being the highest concentration tested (1:1) higher than Hoagland solution.

The further parameters analyzed (germination percentage on the 4th day, dry biomass, average root diameter, root volume, number of root tips, number of root forks, root fineness and CAT activity) showed no interaction ($p>0.05$) between treatments and concentrations in maize (Supplementary File, Table 3). Besides, SOD activity in maize also showed no interaction ($p>0.05$) between the CMCT treatment and the concentrations.

Considering interaction between treatments and concentrations for sorghum, PCT concentrations tended to increase fresh biomass and this effect was even superior to Hoagland solution in intermediate concentrations (1:5 and 1:2.5), after which a drop is observed (Fig. 2a). The PCT concentrations demonstrated a slightly tendency to reduction in total root length (Fig. 2b) and root surface area (Fig. 2c), although presented the tendency of increase the number of tips (Fig. 2d), number of forks (Fig. 2e) and root fineness (Fig. 2f) in intermediate concentrations with posterior decrease in the values as the concentration increases. Besides, the 1:5 concentration was the only capable of increase the root morphology parameters statistically equaling Hoagland solution. The PCT treatment also presented a reduction of SOD activity (Fig. 2g) in a dose-response relation, being the highest concentration (1:1) even lower than Hoagland solution, besides decreased APX activity (Fig. 2h) in a dose-response relation, but making the 1:5, 1:2.5 and 1:1 concentrations lowers than Hoagland solution (Fig. 2i).

CMCT promoted an increase in sorghum fresh biomass and this effect was even superior to Hoagland solution in the highest concentration (1:1) (Fig. 2a). The intermediate concentrations of CMCT tended to increase the total root length (Fig. 2b), root surface area (Fig. 2c), numbers of tips (Fig. 2d) and forks (Fig. 2e) and root fineness (Fig. 2f) of sorghum, statistically equaling Hoagland solution in 1:5 and 1:2.5 concentrations, with posterior decrease in the highest concentration (1:1). In addition, CMCT increased SOD activity and showed a stabilization from 1:5 to 1:1 concentrations (Fig. 2h).

The further parameters analyzed (dry biomass, shoot length, average root diameter, root volume, CAT activity and lipid peroxidation) showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations in sorghum (Supplementary File, Table 3). Besides, APX activity in sorghum also showed no interaction ($p>0.05$) between the CMCT treatment and the concentrations.

Analyzing the root diameter classes (Supplementary File, Fig. 4), for maize, 1:10 concentration of PCT increased the length of very thin roots in relation to Hoagland solution (Supplementary File, Fig. 4a1), although this treatment did

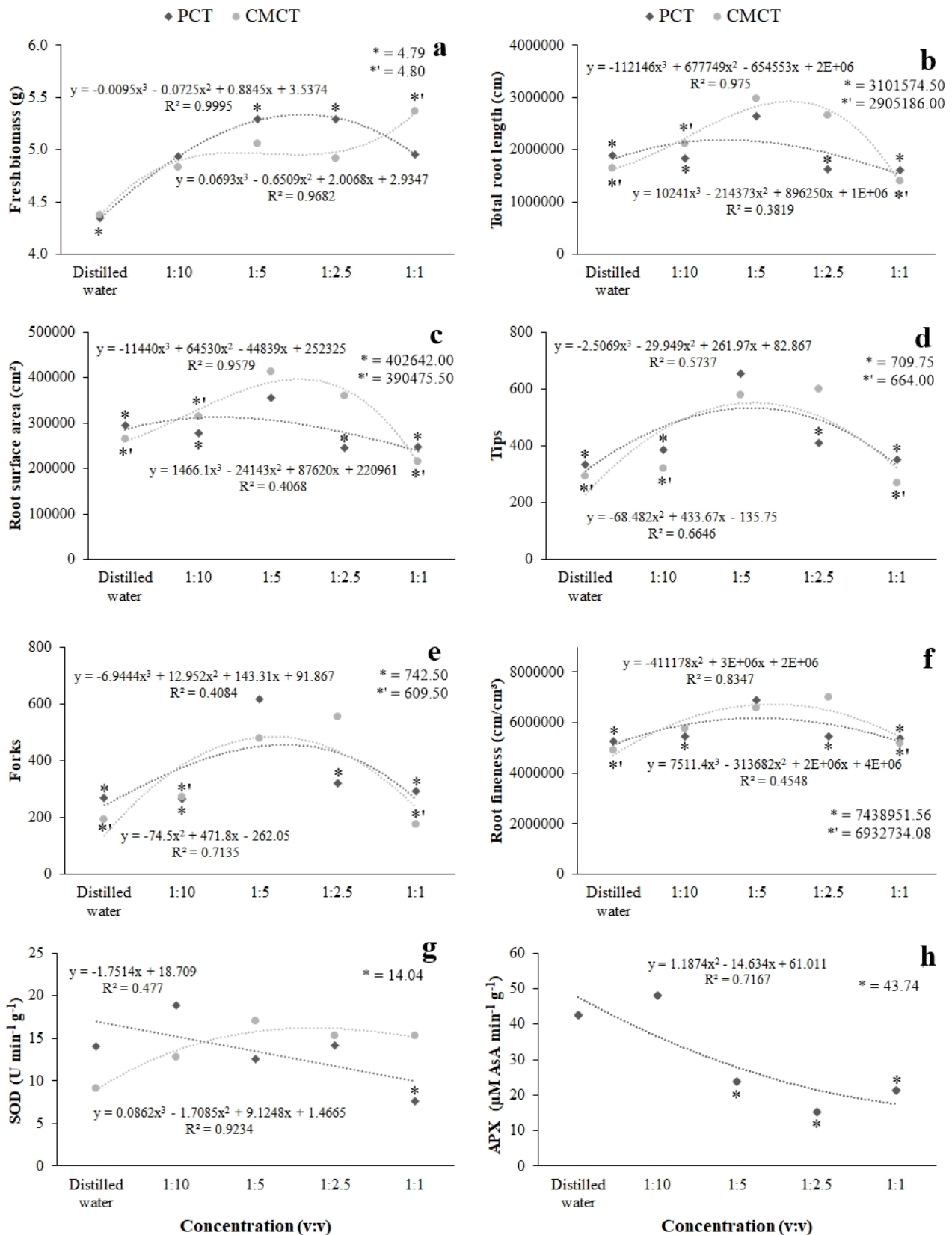


Fig. 2 Initial growth, root morphology and biochemical parameters of sorghum exposed to plants compost tea (PCT) and cattle manure compost tea (CMCT) (a) Fresh biomass (g) (b) Total root length (cm) (c) Root surface area (cm²) (d) Number of root tips (e) Number of root forks (f) Root fineness (cm/cm³) (g) Superoxide dismutase (SOD) (U min⁻¹ g⁻¹) (h) Ascorbate peroxidase (APX) (μM AsA min⁻¹ g⁻¹); Values demonstrated by points indicate means, n = 4; Means associated to asterisks differ statistically from the mean of Hoagland solution (n = 4) according to Dunnett's test at 5% significance: *=PCT and *'=CMCT; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScape program

not present interaction with the concentrations ($p>0.05$) for this class of diameter (Supplementary File, Table 3). The concentrations 1:10 and 1:1 of PCT also increased the length (Supplementary File, Fig. 4a1), surface area (Supplementary File, Fig. 4b1) and volume (Supplementary File, Fig. 4c1) of thin roots in relation to distilled water and the other concentration analyzed (1:2.5), being even superior to Hoagland solution. The other classes of diameter (thick and very thick roots) showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations (Supplementary File, Table 3).

Still about maize, all concentrations of CMCT except the lowest (1:10) reduced the length (Supplementary File, Fig. 4a2), surface area (Supplementary File, Fig. 4b2) and volume (Supplementary File, Fig. 4c2) of thin roots in relation to distilled water. Besides, 1:10 concentration increased the length of very thick roots (Supplementary File, Fig. 4a2) in relation to all other concentrations and both controls. The very thin and thick roots presented no interaction between CMCT treatment and the concentrations ($p>0.05$) for any of the parameters analyzed, as well as root surface area and root volume of very thick roots (Supplementary File, Table 2).

For sorghum, 1:5 concentration of PCT increased length (Supplementary File, Fig. 4d1), surface area (Supplementary File, Fig. 4e1) and root volume (Supplementary File, Fig. 4f1) of very thin roots in relation to distilled water control and all other concentrations, being statistically equal to Hoagland control, besides 1:2.5 concentration enhanced root volume of thin roots in relation to all other concentrations and controls (Supplementary File, Fig. 4f1). The data for thin roots of other both parameters and all data for thick roots showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations (Supplementary File, Table 3).

The CMCT treatment increased sorghum's length (Supplementary File, Fig. 4d2), surface area (Supplementary File, Fig. 4e2) and volume (Supplementary File, Fig. 4f2) of very thin roots in all concentrations except 1:1 compared to distilled water control, and this increase is even greater in 1:5 and 1:2.5 concentrations as well as Hoagland solution. Although, CMCT demonstrated no interaction between treatments and concentrations ($p>0.05$) for data of thin or thick roots of sorghum (Supplementary File, Table 3).

3.4 Analyzes of maize and sorghum under mannitol and compost teas association

Under water deficit induced by mannitol, cattle manure compost tea (CMCT) was more beneficial than plants compost tea (PCT) for maize and sorghum (Table 3).

Regarding maize, CMCT presented higher values for germination percentage at 24h and Germination Speed Index (GSI) than PCT. The CMCT treatment also increased fresh biomass, shoot length, total root length, root surface area, root volume and catalase (CAT) activity, besides reduced lipid peroxidation in relation to PCT. Considering the root diameter classes, CMCT promoted higher values compared to PCT for length of thin roots, surface area of thin and thick roots, and volume of thin, thick, and very thick roots. On the other hand, PCT increased the number of anomalous seedlings and the length of thick roots when compared to CMCT (Table 3). The other parameters analyzed (germination percentage on the 4th day, dry biomass, average root diameter, number of root tips, number of root forks, root fineness, length of very thin and very thick roots, surface area of very thin and very thick roots, volume of very thin roots and superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities) presented no statistical difference ($p>0.05$) between the two treatments for maize (Supplementary File, Table 4).

For sorghum, CMCT presented higher values than PCT for germination percentage at 24h and on the 4th day, GSI, fresh biomass, shoot length, total root length, root surface area and root volume, although presented lower APX activity and higher lipid peroxidation. Considering the root diameter classes, CMCT increased the length and surface area of thin roots compared to PCT (Table 3). The sorghum data presented no statistical difference ($p>0.05$) between treatments for the other parameters analyzed: number of anomalous seedlings, dry biomass, average root diameter, number of root

tips, number of root forks, root fineness, length of very thin and thick roots, surface area of very thin and thick roots, volume of all root diameter classes, and SOD and CAT activities (Supplementary File, Table 4).

When considered the interaction between treatments and concentrations for maize, PCT increased the number of anomalous seedlings as increasing the concentrations, with a slightly reduction in the highest (1:1) concentration (Fig. 3a). The concentration 1:10 of PCT also promoted an increase in fresh biomass (Fig. 3Bb), equaling to distilled water,

Table 3. Comparison between means of plants compost tea (PCT) and cattle manure compost tea (CMCT) mixed to mannitol (-2.5 MPa) treatments for initial growth, root morphology and biochemical parameters of maize and sorghum \pm standard error; n = 4.

Parameters	MAIZE				SORGHUM			
	PCT		CMCT		PCT		CMCT	
Germination percentage at 24h (%)	59.33 \pm 1.80	b	76.25 \pm 1.36	a	76.33 \pm 2.09	b	82.33 \pm 1.47	a
Germination percentage on the 4 th day (%)					83.42 \pm 1.33	b	87.00 \pm 0.95	a
GSI	38.22 \pm 0.49	b	42.30 \pm 0.42	a	39.85 \pm 0.80	b	42.19 \pm 0.53	a
Number of anomalous seedlings	3.92 \pm 0.53	a	2.58 \pm 0.41	b				
Fresh biomass (g)	25.06 \pm 0.39	b	26.34 \pm 0.25	a	4.21 \pm 0.11	b	4.85 \pm 0.10	a
Shoot length (mm)	20.41 \pm 1.45	b	23.67 \pm 0.53	a	66.87 \pm 2.59	b	79.74 \pm 1.90	a
Total root length (cm)	2.79x10 ⁵ \pm 3.49x10 ⁴	b	5.41x10 ⁵ \pm 8.15x10 ⁴	a	1.41x10 ⁶ \pm 1.18x10 ⁵	b	1.61x10 ⁶ \pm 1.30x10 ⁵	a
Root surface area (cm ²)	9.80x10 ⁴ \pm 1.37x10 ⁴	b	2.06x10 ⁵ \pm 3.09x10 ⁴	a	2.21x10 ⁵ \pm 1.70x10 ⁴	b	2.57x10 ⁵ \pm 1.73x10 ⁴	a
Root volume (cm ³)	0.2868 \pm 0.0425	b	253.75 \pm 119.89	a	0.2769 \pm 0.0565	b	0.3305 \pm 0.0184	a
Length of thin roots (cm)	4.81x10 ⁴ \pm 1.02x10 ⁴	b	4.54x10 ⁵ \pm 6.35x10 ⁴	a	6.24x10 ⁵ \pm 5.10x10 ⁴	b	7.54x10 ⁵ \pm 4.38x10 ⁴	a
Length of thick roots (cm)	2.23x10 ⁵ \pm 3.75x10 ⁴	a	1.54x10 ⁴ \pm 6.62x10 ³	b				
Surface area of thin roots (cm ²)	7.32x10 ⁴ \pm 1.46x10 ⁴	b	1.60x10 ⁵ \pm 2.17x10 ⁴	a	1.21x10 ⁵ \pm 9.35x10 ³	b	1.47x10 ⁵ \pm 8.17x10 ³	a
Surface area of thick roots (cm ²)	1.01x10 ³ \pm 7.00x10 ²	b	1.52x10 ⁴ \pm 6.80x10 ³	a				
Volume of thin roots (cm ³)	0.2683 \pm 0.0421	b	0.5195 \pm 0.0664	a				
Volume of thick roots (cm ³)	0.0264 \pm 0.0077	b	0.1671 \pm 0.0558	a				
Volume of very thick roots (cm ³)	0.00 \pm 0.00	b	0.0222 \pm 0.0115	a				
APX (μ M AsA min ⁻¹ g ⁻¹)					57.02 \pm 11.64	a	43.42 \pm 8.86	b
CAT (μ M H ₂ O ₂ min ⁻¹ g ⁻¹)	2.15 \pm 0.22	b	5.62 \pm 0.32	a				
Lipid peroxidation (η mol MDA mg ⁻¹)	52.36 \pm 3.09	a	44.83 \pm 3.12	b	65.92 \pm 13.46	b	70.38 \pm 14.37	a

Inside each species, means followed by different letters, in the same row, differ statistically by the Scott-Knott test at 5% significance. Were represented only the data which statistical difference was significant. Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm.

after which there was a gradual decrease as the concentrations increased. The same is verified for shoot length (Fig. 3c), but with 1:10 concentration surpassing distilled water, while 1:2.5 and 1:1 concentrations presented lower values than distilled water. In addition, PCT increased APX activity (Fig. 3g) until the intermediate concentrations, after which is noted a drop to the highest (1:1) concentration tested, with mannitol and the highest concentration being lower than distilled water. The PCT treatment also reduced lipid peroxidation (Fig. 3h), highlighting the intermediate concentrations, after which is noted a slightly increase to the highest concentration tested, and with mannitol and the lowest (1:10) concentration being higher than distilled water.

The CMCT treatment, on the other hand, reduced maize's number of anomalous seedlings highlighting the intermediate concentrations – and 1:5 concentration being lower than distilled water –, with a slightly increase in the highest concentration (Fig. 3a). The CMCT treatment also provided a slight increase in fresh biomass (Fig. 3b), but without major variations between concentrations neither being able to mitigate the harmful effect of mannitol on sorghum,

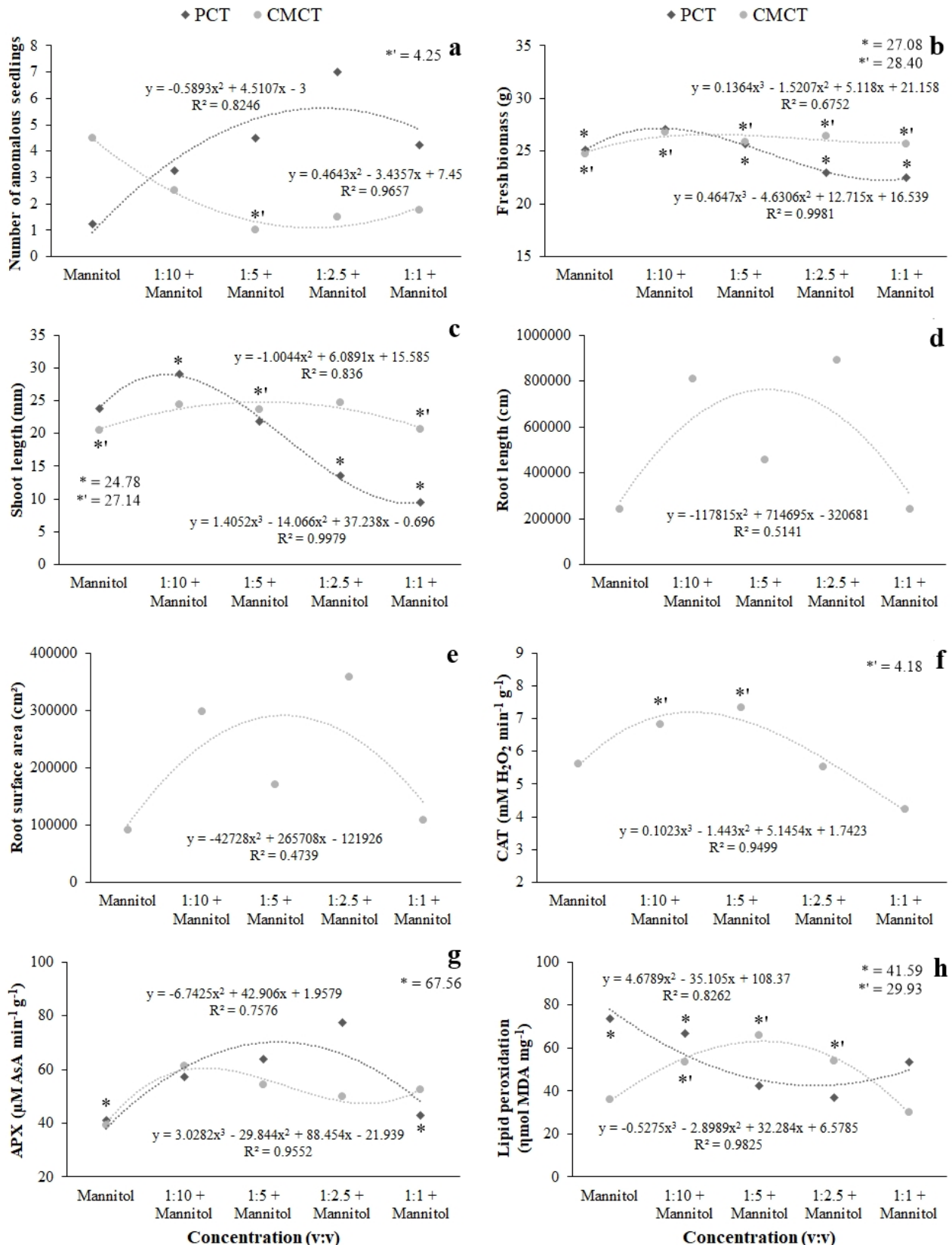


Fig. 3 Initial growth, root morphology and biochemical parameters of maize exposed to water deficit induced by mannitol (-2.5 MPa) associated to plants compost tea (PCT) and cattle manure compost tea (CMCT) (a) Number of anomalous seedlings (b) Fresh biomass (g) (c) Shoot length (mm) (d) Total root length (cm) (e) Root surface area (cm²) (f) Catalase (CAT) (μM H₂O₂ min⁻¹ g⁻¹) (g) Ascorbate peroxidase (APX) (μM AsA min⁻¹ g⁻¹) (h) Lipid peroxidation (ηmol MDA mg⁻¹); Values demonstrated by points indicate means, n = 4; Means associated to asterisks differ statistically from the mean of distilled water (n = 4) according to Dunnett's test at 5% significance: * = PCT and *' = CMCT; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScape program

since all concentrations showed lower values than distilled water. Maize's shoot length (Fig. 3c), total root length (Fig. 3d), root surface area (Fig. 3e) and lipid peroxidation (Fig. 3h) were increased by CMCT as the concentrations increased, reaching the highest values at intermediate concentrations, after which there is a drop to the highest concentration. Besides, for shoot length, the concentrations 1:10 and 1:2.5 mitigated the effect of mannitol, equaling to distilled water; while for lipid peroxidation, all concentrations except 1:1 were higher than distilled water. In addition, CAT (Fig. 3f) and APX (Fig. 3g) activities were enhanced by the lowest concentration of CMCT, after which a drop is verified as the concentrations increased – and with 1:10 and 1:5 concentrations promoting higher values than distilled water for CAT activity.

The other parameters analyzed (germination percentage at 24h, germination percentage on the 4th day, GSI, dry biomass, average root diameter, root volume, number of root tips, number of root forks, root fineness and SOD activity) showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations for maize (Supplementary File, Table 4). In addition, there was no statistical difference ($p>0.05$) for interaction between the PCT treatment and the concentrations, in maize, for the parameters: total root length, root surface area and CAT activity.

Regarding sorghum, the results for interaction between treatments and concentrations demonstrate that PCT decreased germination percentage at 24h (Fig. 4a), GSI (Fig. 4b), total root length (Fig. 4f), root surface area (Fig. 4g) and root volume (Fig. 4i) in a dose-response effect, with the higher concentrations (1:2.5 and 1:1) being lower than distilled water in the three root morphology parameters cited. The number of anomalous seedlings (Fig. 4c), fresh biomass (Fig. 4d), shoot length (Fig. 4e), number of root tips (Fig. 4j), number of root forks (Fig. 4k) and root fineness (Fig. 4l) of sorghum were enhanced in lower concentrations (1:10, and 1:5 in some parameters), after which a drop is verified as the concentrations increased. For all these parameters, except for root fineness, the higher concentrations (1:1, and 1:2.5 in some parameters) were lower than distilled water; while for shoot length and root fineness, 1:5 concentration surpassed distilled water values. Average root diameter (Fig. 4h) was slightly reduced by intermediate concentrations of PCT followed by an increase in the highest concentration, with 1:5 concentration lower than distilled water. The APX activity (Fig. 4m) was increased in the lowest concentration, after gradually decreased as the concentrations increased and presenting a slight increase in the highest concentration; while CAT activity (Fig. 4n) presented the opposite effect: reduction in lowest concentration, gradual increase until 1:2.5 concentration, and after a decrease in the highest concentration. For APX activity, mannitol was lower than distilled water and all concentrations mitigated mannitol effect, being equal (1:2.5 and 1:1) or even superior (1:10 and 1:5) than distilled water. Lipid peroxidation (Fig. 4o) was gradually reduced until intermediate concentrations, after which is noted an increase as the concentrations increased; with mannitol and the highest concentration presenting higher values than distilled water, and the further concentrations (1:10, 1:5 and 1:2.5) reducing lipid peroxidation in relation to distilled water.

The results for CMCT treatment in sorghum for interaction between treatments and concentrations demonstrated a reduction in the number of anomalous seedlings (Fig. 4c) until 1:5 concentration, from which is verified a stabilization, with the highest concentration being lower than distilled water. The CMCT treatment also increased fresh biomass (Fig. 4d) and shoot length (Fig. 4e) as the concentrations increased, and in both cases, most of the concentrations tested (1:5, 1:2.5 and 1:1) mitigated the reduction action of mannitol. The CMCT treatment also increased all the root morphology parameters except average root diameter (Fig. 4f-4l, except 4h), in most cases reaching the apex in intermediate concentrations – with 1:5 concentration being higher than distilled water –, from which a decrease is verified until the highest concentration tested. The average root diameter (Fig. 4h) was slightly reduced until 1:5 concentration and posteriorly increased as the concentrations increased, with the concentrations 1:10, 1:5 and 1:2.5 being lower than distilled water. In addition, CMCT treatment also increased APX activity (Fig. 4m) up to intermediate concentrations, with a subsequent decrease until the highest concentration tested; besides all concentrations mitigated mannitol effect since were superior to distilled water while mannitol was lower. The CAT activity was reduced in a dose-response relation (Fig. 4n),

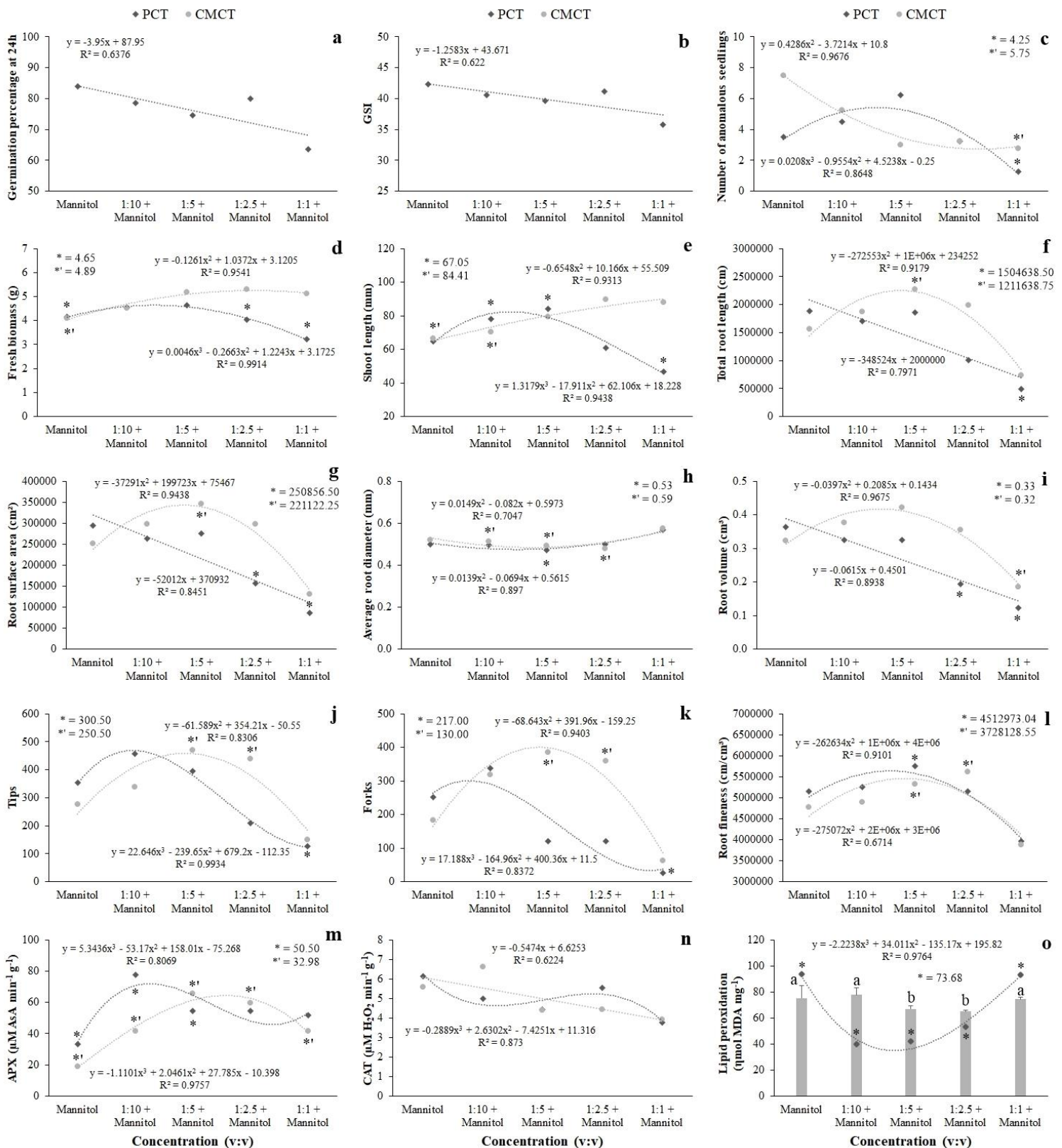


Fig. 4 Initial growth, root morphology and biochemical parameters of sorghum exposed to water deficit induced by mannitol (-2.5 MPa) associated to plants compost tea (PCT) and cattle manure compost tea (CMCT) (a) Germination percentage at 24h (%) (b) Germination Speed Index (GSI) (c) Number of anomalous seedlings (d) Fresh biomass (g) (e) Shoot length (mm) (f) Total root length (cm) (g) Root surface area (cm²) (h) Average root diameter (mm) (i) Root volume (cm³) (j) Number of root tips (k) Number of root forks (l) Root fineness (cm/cm³) (m) Ascorbate peroxidase (APX) ($\mu\text{M AsA min}^{-1} \text{g}^{-1}$) (n) Catalase (CAT) ($\mu\text{M H}_2\text{O}_2 \text{ min}^{-1} \text{g}^{-1}$) (o) Lipid peroxidation ($\eta\text{mol MDA mg}^{-1}$); Columns and values demonstrated by points indicate means, n = 4; Bars: standard error; Means associated to asterisks differ statistically from the mean of distilled water (n = 4) according to Dunnett's test at 5% significance: * = PCT and *' = CMCT; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScape program

and the concentrations 1:5 and 1:2.5 reduced lipid peroxidation (Fig. 4o) in relation to mannitol and the other two concentrations (1:10 and 1:1).

There was no statistical difference ($p>0.05$) for interaction between treatments and concentrations for the further parameters analyzed in sorghum (germination percentage on the 4th day, dry biomass and SOD activity) (Supplementary File, Table 4). Besides, there was no interaction ($p>0.05$) between CMCT and the concentrations for germination percentage at 24h and GSI.

Considering the root diameter classes, for maize, 1:5 concentration of PCT treatment reduced the length, surface area and volume of thin roots in relation to distilled water, although the concentrations did not differ ($p>0.05$) among themselves nor in relation to mannitol, for any of the diameter classes (Fig. 5a1, 5b1 and 5c1). Regarding CMCT treatment, all concentrations except the highest increased the length, surface area and volume of thin roots compared to mannitol, highlighting the concentrations 1:10 and 1:2.5 (Fig. 5a2, 5b2 and 5c2). There was no statistical difference ($p>0.05$) for the other three root diameter classes (Supplementary File, Table 4).

For sorghum, the higher concentrations (1:2.5 and 1:1) of PCT treatment reduced the length and surface area of both very thin and thin roots, besides reduced the volume of very thin roots in relation to mannitol and the lower concentrations (1:10 and 1:5), which did not differ among themselves (Fig. 5d1, 5e1 and 5f1). In addition, 1:5 concentration of PCT increased the length of very thin roots in relation to distilled water, and mannitol increased the surface area and volume of very thin roots in relation to distilled water; while 1:2.5 and 1:1 concentrations reduced the length and surface area of thin roots in relation to distilled water (Fig. 5d1, 5e1 and 5f1).

The CMCT treatment, on the other hand, increased the length of very thin roots in relation to mannitol at most concentrations (1:10, 1:5 and 1:2.5), with 1:5 and 1:2.5 concentrations even surpassing distilled water (Fig. 5d2). Although, the highest concentration (1:1) reduced the length of both very thin and thin roots in relation to mannitol and all other concentrations, and even reduced it in relation to distilled water for thin roots (Fig. 5D2). The concentration 1:5 of CMCT also increased the surface area (Fig. 5e2) and volume (Fig. 5f2) of very thin roots in relation to all other concentrations and mannitol, even surpassing distilled water along with 1:10 and 1:2.5 concentrations (Fig. 5e2 and 5f2); while 1:1 concentration reduced the surface area of both very thin and thin roots in relation to mannitol and all other concentrations, and reduced it in relation to distilled water for thin roots (Fig. 5e2).

In mannitol experiment, there was no data for thick roots of sorghum, once the species presented no roots which fit in this class of diameter. The same is verified for all classes of diameter for root volume, except very thin roots (Supplementary File, Table 4).

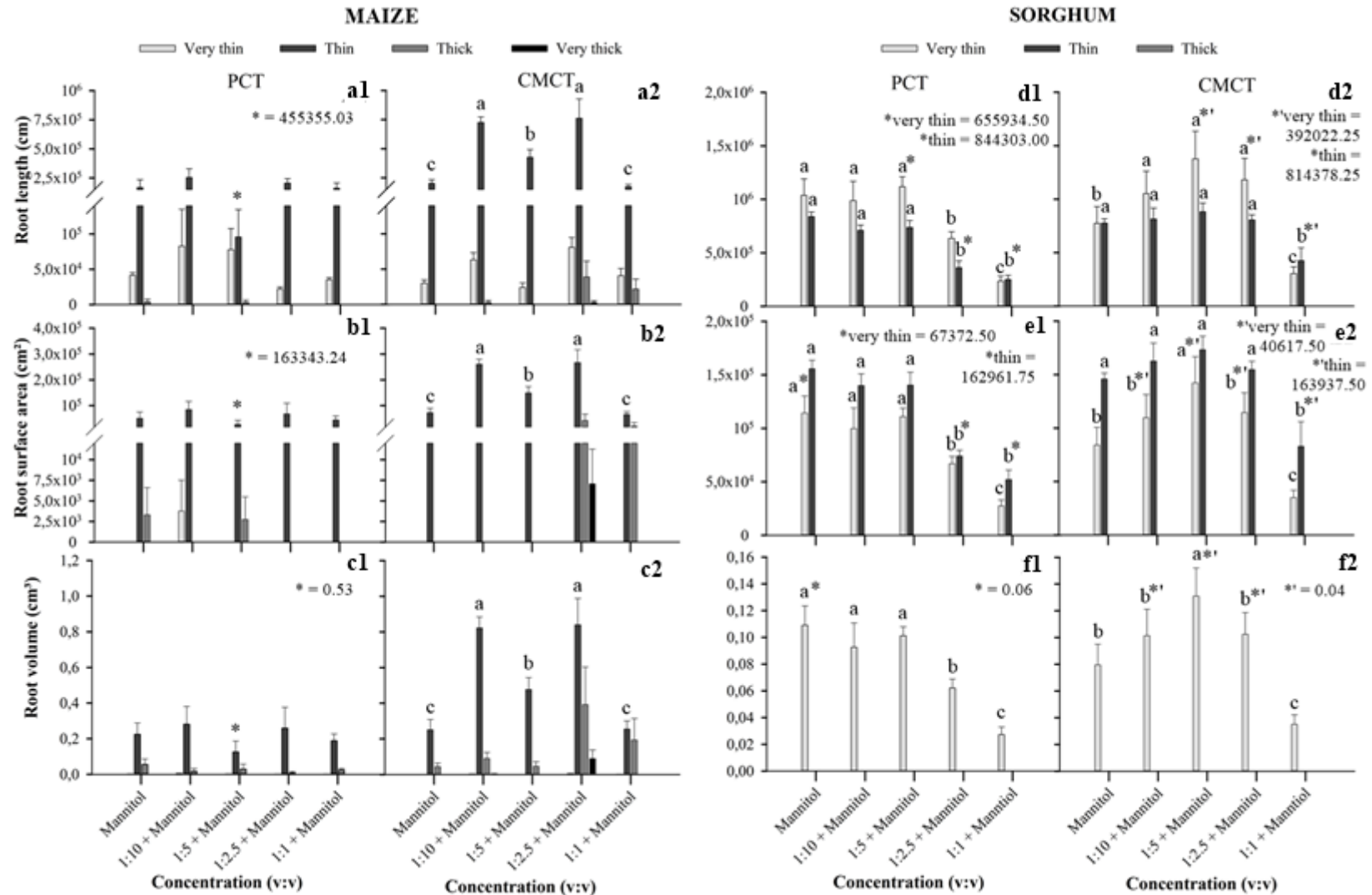


Fig. 5 Root morphology parameters of maize and sorghum under influence of mannitol (-2.5 MPa) associated with compost teas, according to root diameter classes (a) Root length (cm) of maize (b) Root surface area (cm²) of maize (c) Root volume (cm³) of maize (d) Root length (cm) of sorghum (e) Root surface area (cm²) of sorghum (f) Root volume (cm³) of sorghum (1) Promoted by plants compost tea (PCT) (2) Promoted by cattle manure compost tea (CMCT); Columns: means, n = 4; Bars: standard error; In each species, columns of same color, inside each treatment, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were associated to letters only the data which statistical difference was significant; Means associated to asterisks differ statistically from the mean of distilled water (n = 4) according to Dunnett's test at 5% significance: *=PCT and **=CMCT; Very thin: 0–0.5 mm, thin: 0.5–2.5 mm, thick: 2.5–4.5 mm, very thick: > 4.5 mm; SigmaPlot 14.5 program was used to produce the graphics and the figure was edited by PhotoScope program

4 DISCUSSION

The characterization data of the composts shows that both composts produced are of considerable quality, since they have desirable macro and micronutrients and total organic carbon contents, adequate pH, and a good C:N ratio for organic fertilizers (Erhart and Hartl 2010; Hubbe *et al.* 2010; Martínez-Blanco *et al.* 2013; Doan *et al.* 2014, 2015; Ramdani *et al.* 2015; Azim *et al.* 2018). The total N is below the optimal values, which is generally 1 to 4% of the total dry weight of compost, indicating a nitrogen loss probably by excess of moisturizing (Erhart and Hartl 2010; Azim *et al.* 2018), what is evident considering the moisture values. Moisture values were above than recommended as ideal (max. 65%), what can limit the oxygen transport due to the filling of smaller pores by water (Erhart and Hartl 2010; Hubbe *et al.* 2010; Azim *et al.* 2018), although it does not affect this study since the composts were used only as raw materials to extract the compost teas. In addition, although total N contents were lower than optimal, the C:N ratios presented desirable values for these type of raw materials for composting (Hubbe *et al.* 2010; Ramdani *et al.* 2015; Azim *et al.* 2018).

The maturity of both composts is evidenced by duration of maturation process, that is, from 40th to 90th day, defined by windrows reaching ambient temperature after thermophilic phase (Hubbe *et al.* 2010; Ramdani *et al.* 2015; Azim *et al.* 2018) as well as the values for total organic carbon and C:N ratio – which can be corelated to humification (Erhart and Hartl 2010; Hubbe *et al.* 2010; Ramdani *et al.* 2015; Azim *et al.* 2018; Spaccini *et al.* 2019). Furthermore, the content of heavy metals was non-existent or negligible – depending on the metal – in both composts, being significantly below the established limits and demonstrating a very low risk of contamination (Erhart and Hartl 2010).

Regarding the presence of pathogenic organisms, the plants compost showed higher quality than the cattle manure compost, considering the first is a waste with low risk of biological contamination in relation to the second (Leal *et al.* 2013; Reyes-Torres *et al.* 2018) besides the thermophilic phase was sufficient to produce a pathogen-free compost, which was not verified for cattle manure composting since its average temperature of thermophilic phase and the duration were lower and insufficient to kill the pathogens (Haug 2018). Although, considering that pathogenic organisms capable of infect humans were not present, as well as pathogenic microorganisms, both composts can be considered safe for human health. In addition, Orden *et al.* (2021) emphasize that the preparation of compost tea may be an appropriate form to control some undesirable effects of compost application, and in this case, this is valid for the presence of bovine parasites in cattle manure compost. Besides, according to our results, CMCT can be considered more microbiologically active than PCT, due to its higher values for population densities of undemanding bacteria and fungi, fungi that grow in high concentration of carbohydrates, slow-growing fungi, and saprophytic fungi.

Regarding the physicochemical characterization of the two compost teas, and considering Orden *et al.* (2021) suggestion – that there is a need for works on the solubility of nutrients present in compost and the concentrations of nutrients in compost tea produced –, the obtention method used in this study (stock solution of 1 compost : 1 distilled water [v:v], brewing for 24h with aeration) were capable of extract the compounds and proportionally enhance the values for macro and micronutrients in relation to the solid composts, besides it can be diluted if necessary. The values for macro and/or micronutrient content in compost teas are variable in the literature and its effects in crops promote increase in yield, biomass, length, and other parameters (Gutiérrez-Miceli *et al.* 2008; Pant *et al.* 2012; Taha *et al.* 2016; Otero *et al.* 2019; Fouda and Niel 2021; Orden *et al.* 2021), demonstrating the fertilizer potential of our compost teas. In addition, considering the nutrients reference values for liquid organic fertilizers in Brazil's legislation (Brasil 2020), our two compost teas can be considered as good liquid organic fertilizers. Furthermore, the dissolved oxygen levels are within the expected for compost teas after brewing for 24h with aeration, reflecting the microbial activity in the two compost teas (Pant *et al.* 2009; Shrestha *et al.* 2011).

In relation to enhancement of nutrients when compared to solid composts, Adejumo (2015) elucidates: organic amendments – including the compost teas – has been reported to be a major source of iron-humate complex, which has been used to correct Fe-deficiency in crop. This binding capacity of these complexes is probably responsible for the enhancement of Fe and Mn in relation to the solid composts, since both nutrients can present themselves as bivalent ions (Fe^{2+} and Mn^{2+}) and bind to humic substances – which is commonly associated with mature composts that are rich in organic matter –, forming complexes that was extracted from the solid composts to the teas. In the same way, enhanced values were also observed in both compost teas for Pb content, possibly due to heavy metal immobilizing potential of substances present in the solid composts, since mature composts have the tendency to form strong complexes with heavy metals (Lin and Su 2010; Lwin *et al.* 2018; Piccolo *et al.* 2019; Sayara *et al.* 2020). When the compost teas were extracted, Pb (Pb^{2+} as ion) was possibly complexed and consequently extracted with these substances.

The values obtained for compost teas pH were ideal for germination and root development – that is, proximal from neutrality – and corroborated with previous studies, although the electrical conductivity is considerably higher in relation to other studies, indicating a high concentration of soluble nutrients (Hargreaves *et al.* 2009; Pant *et al.* 2009, 2012; Taha *et al.* 2016; Otero *et al.* 2019; Samet *et al.* 2019; Fouda and Niel 2021; Orden *et al.* 2021). Such values are worrisome if they negatively affect germination since they make the environment hypertonic. In this context, when considered the effects of the tested compost teas on germination, both treatments reduced maize's germination especially in higher concentrations, although did not interfere with sorghum's germination, demonstrating that even with high electrical conductivity values the compost teas can still be beneficial, besides the dilution to lower concentrations can alleviate or annul the possible negative effect. In addition, our data demonstrate that cattle manure compost tea (CMCT) is more beneficial for germination when compared to plants compost tea (PCT), for both maize and sorghum, in normal conditions as well as under water deficit stress induced by mannitol.

It is noteworthy that it is difficult to impair the germination of highly improved species such as maize and sorghum, which have high seed vigor (Camargo and Vaughan 1973; Ghassemi-Golezani and Dalil 2014). Thus, germination is high, even under stress conditions such as water deficit. Under water deficit stress induced by mannitol, the concentrations CMCT did not interfere with maize's nor sorghum's germination, although PCT treatment reduced sorghum's germination percentage at 24h and Germination Speed Index (GSI) as the concentrations increased, probably enhancing mannitol effect in association with the high electrical conductivity of the compost tea. It demonstrates, as cited before, the advantage of CMCT using, despite its high electrical conductivity values.

Considering the effects of the compost teas on maize, the compost teas presented positive effects in lower or intermediate concentrations, with both treatments increasing shoot length, PCT enhancing root length and root surface area, and CMCT enhancing fresh biomass and APX activity. When considered the root diameter classes, some concentrations of PCT increased the length, surface area and volume of thin roots, while CMCT decreased them. Besides, also is noted a negative effect as the concentration increases depending on the treatment for some parameters, which demonstrates the necessity of dilute the compost teas for enhance maize growth. For sorghum, in general, the teas increased fresh biomass, total root length, root surface area, numbers of tips and forks, and root fineness, besides increased the length, surface area and volume of very thin roots, and volume of thin roots. The values obtained for compost teas effects under maize and sorghum sometimes even surpassed the values obtained for Hoagland's solution, and considering Hoagland's solution is a nutritive solution recognized worldwide, the teas can be considered as more than fertilizers, demonstrating their biostimulant potential.

Plant biostimulants are substances – other than nutrients, pesticides or soil additives – and microorganisms that promote increased growth and development when applied to plants or the rhizosphere, regardless of nutrient content. Biostimulants are so called because they stimulate natural processes, increasing or benefiting the absorption and efficiency

of nutrient use, crop quality, stress tolerance and improvement of tissue mineral composition (Bulgari *et al.* 2014; Calvo *et al.* 2014; du Jardin 2015). In addition, when compared the treatments, CMCT was more beneficial to root system of both species, since increased total root length and root surface area of maize, besides increased the length, surface area and volume of very thin and/or thin roots in relation to PCT, for both species.

The root system is responsible for providing vital nutrients and water, absorbing them from the soil, in addition to anchoring and stabilizing the plants, allowing them to grow (Taiz *et al.* 2017). According to Balemi and Negisho (2012), among the factors that influence the uptake efficiency, can be included modification of root architecture and thinner roots. The architecture of the root system is the spatial configuration – that is, the geometric arrangement – of the roots in the soil, which must have the ability to adapt since the roots are subjected to heterogeneous and often changing soil conditions (Balemi and Negisho 2012; Taiz *et al.* 2017). The root diameter is associated with the function: thinner roots are responsible for absorption of water and nutrients, while thicker roots are responsible for anchoring plants on the soil (Fitter 2002); so, the root diameter and root fineness parameters provide information about the function exerted by the roots. Moreover, as higher are the root length and the root surface area, as greater is the capacity of water and nutrient supply – that is, the foraging ability (Hodge *et al.* 2009). In this context, for maize, PCT in lower concentrations, and for sorghum, both compost teas in intermediate concentrations, improved water and nutrients absorption capacity, what also justifies the increases in fresh biomass, which is correlated to water content and plant growth.

Our compost teas also improved the architecture of sorghum roots. Fitter (2002) considers that root volume is the simplest geometrical correlate of root weight and can be correlated to sclerification/lignification. Other parameters, as the numbers of tips and forks in the roots, can be correlated to root architecture and can imply a greater foraging ability, playing an important role in root morphology considering their potential to enhance penetration through soil layers (Hodge *et al.* 2009; Soumya *et al.* 2021).

These effects are commonly related to biostimulant substances (Bulgari *et al.* 2014; Calvo *et al.* 2014; Canellas *et al.* 2015; du Jardin 2015), proving that our two compost teas treatments are biostimulants. Our results corroborate with González-Hernández *et al.* (2022) that investigated the effect of a green waste-based compost tea in tomato root development and verified that primary root length and lateral roots number were increased by the compost tea tested, indicating the capacity of improve root morphology and architecture, although our results could not be correlated with phytohormones regulation since their presence were not detected in our study.

Other studies also founded increase in biomass, root length and/or improvement in root morphology/architecture as effects of compost teas, humic substances or rich-carbon compounds and associate these effects with the physical-chemical and/or microbiological composition (Pant *et al.* 2012; Canellas *et al.* 2015; Taha *et al.* 2016; Otero *et al.* 2019; Fouda and Niel 2021; Chang *et al.* 2021), and in some cases, with phytohormones content (Pant *et al.* 2012; Otero *et al.* 2019). Eudoxie and Martin (2019) elucidate that the biostimulants effects of compost teas are associated to its composition: macro and micronutrient content, organic matter, humic substances, phytohormones, beneficial microorganisms, besides synergistic effect between two or more cited components.

So, the biostimulant effects observed in our results can be explained by the nutrient supply of the compost teas and by microbial activity, but not by phytohormones action, since they were not detected.

In this study, was used mannitol in -2.5 MPa osmotic pressure as water deficit inducer. Mannitol caused stress in maize and sorghum by reducing the parameters fresh biomass, shoot length and APX activity, besides increased lipid peroxidation. The compost teas, highlighting CMCT that clearly presented better effects, alleviate the stress of mannitol, sometimes even surpassing distilled water. The 1:5 concentration of CMCT decreased maize's number of anomalous seedlings even lower than distilled water, and intermediate concentrations enhanced the shoot length, root length, root surface area and CAT activity, although increased lipid peroxidation. The PCT treatment just increased maize's shoot

length at 1:10 concentration, even above distilled water, while the intermediate concentrations enhanced APX activity and decreased lipid peroxidation. Considering sorghum, both treatments – highlighting intermediate concentrations – reduced the number of anomalous seedlings, average root diameter and lipid peroxidation, sometimes reducing below distilled water. The intermediate concentrations of CMCT treatment also increased sorghum's fresh biomass, shoot length, all root morphology parameters analyzed and APX activity, sometimes even surpassing distilled water. On the other hand, the PCT treatment just increased shoot length, number of tips, number of forks and APX activity in the lowest concentration, although did not present a positive effect on root morphology parameters except average root diameter and root fineness.

When compared the treatments, CMCT must be highlighted since was more beneficial to both maize and sorghum than PCT, since CMCT was responsible for reducing the number of anomalous seedlings and increasing several root morphology parameters, especially those associated with the classes of diameter very thin and thin. It evidences, one more time, the biostimulant action of both compost teas, although the treatments apparently present different mechanisms of action to promote tolerance to water deficit. The better effects of CMCT are probably correlated to its microbiological composition, since presented higher microbial population density in most of the culture media analyzed.

As the period of the experiments did not exceed four days and considering the effects on maize and sorghum, the root morphology parameters, mainly those correlated to the root diameter that are indicative of thinner roots are desirable, since in the initial growth it is very important to absorb water and nutrients to ensure plant growth, and thinner roots are related to foraging capacity (Hodge *et al.* 2009). In this context, by enhancing root morphology and root architecture parameters in the presence of mannitol, the compost teas demonstrated being good alternatives to tolerate water deficit, that is, good biostimulants – since it was possible to verify a mitigation (attenuation) or nullification (neutralization) of mannitol effects –, ensuring maize and sorghum growth despite its presence (Calvo *et al.* 2014; du Jardin 2015).

In general, the intermediate concentrations of both treatments with the compost teas were capable of mitigate or nullify the damages, and some concentrations even stimulated some parameters beyond the stress alleviation. For both species, in general, among the concentrations tested, the lowest concentration (1:10) of PCT presented greater biostimulant effects, while for CMCT the same is verified for the intermediate concentrations (1:5 and 1:2.5). It demonstrates that is advantageous to use compost teas in diluted concentrations for greater biostimulant effect.

Studies that test compost teas as tolerance-inducers to water deficit may contribute to the understanding of our results. In the literature, in general, the application of compost teas under drought stress can alleviate the harmful effects besides enhance growth, yield, and quality of crops, and these effects are correlated to compost tea's capacity of increasing media moisture storage, enhance nutrient absorption, reestablish the ionic equilibrium, provide suitable nutrition, contribute to several physiological processes, protect against oxidative damage, produce or change the concentration of plant hormones, and act like plant growth-regulators (Pibars *et al.* 2018; Hussein *et al.* 2019; Osman *et al.* 2022; Omara *et al.* 2022); that is, the effects are correlated to the biostimulant action of the compost teas.

In this context, we correlate the tolerant effect to water deficit promoted by our compost teas, to the chemical and microbiological composition of the teas, since the teas presented a biostimulant action (du Jardin 2015; Eudoxie and Martin 2019). Noting that our compost teas can be considered as liquid organic fertilizers according to macro and micronutrients contents, Tripathi *et al.* (2014) highlight that macronutrients promote various beneficial activities in plant metabolism, including protect plants from abiotic and biotic stresses, among which can be cited water deficit. In addition, in general, PCT presented higher values for the antioxidant enzymes activities than CMCT, besides CMCT presented higher values for lipid peroxidation. It demonstrates that PCT increases the activity of antioxidant enzymes (Osman *et al.* 2022) as a defense mechanism against water deficit, while the tolerant effect in CMCT treatment is more related to the

biostimulant effect due chemical and microbiological composition (Gutiérrez-Miceli *et al.* 2008; Eudoxie and Martin 2019; Verrillo *et al.* 2021), which inclusive was more effective when considered the general results in both maize and sorghum growth and root system.

Pant *et al.* (2009) associate the lower non-enzymatic antioxidant activity in pak choi with the higher plant growth promoted by the treatment used, corroborating with our results for CMCT enzymatic antioxidant activity. Osman *et al.* (2022) verified that compost tea mitigated oxidative damage by boosting the enzymatic activity in sugar beet, which was verified for PCT. In this context, it becomes clear that the stress-tolerance mechanisms of our compost teas are associated with chemical and microbiological composition of PCT and CMCT, which becomes them as biostimulants.

The innovative character of this research is clear, since there are few studies in the literature that correlate the effects of compost teas as tolerance-inducers to water deficit stress, besides there are few studies with compost teas in maize and no studies in sorghum.

The two treatments of compost teas tested, plants compost tea (PCT) and cattle manure compost tea (CMCT), can be considered as good liquid organic fertilizers according to their macro and micronutrients contents, besides can be considered as biostimulants according to their effects on maize and sorghum initial growth and root morphology/architecture due to their chemical and microbiological composition, but not to phytohormones content. Under water deficit stress induced by mannitol, the stress-tolerance biostimulant effects are associated with the chemical and microbiological composition for both compost teas treatments, and to antioxidant enzymes activity for PCT.

Comparing the treatments, CMCT is clearly more beneficial than PCT. The best concentration for PCT was the lowest, 1:10 (volume of compost : volume of distilled water), while for CMCT the better effects were promoted by intermediate concentrations (1:5 and 1:2.5).

Furthermore, this study encourages future studies that seek to verify the compost teas action beyond the initial growth, in other developmental stages of maize and/or sorghum.

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STATEMENTS & DECLARATIONS

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

Not applicable

CODE AVAILABILITY

Not applicable

AUTHOR'S CONTRIBUTIONS

Kamilla Pacheco Govêa: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Visualization, Writing – Original Draft. **Yamka Sousa França:** Investigation. **Leticia Aparecida Bressanin:** Methodology, Investigation. **Kamila Rezende Dázio de Souza:** Validation, Investigation, Supervision. **Adriano Bortolotti da Silva:** Resources, Supervision. **Geraldo Alves da Silva:** Resources. **Paulo César Magalhães:** Resources, Supervision. **Thiago Corrêa de Souza:** Conceptualization, Methodology, Validation, Supervision, Resources, Writing – Review & Editing, Project administration, Funding acquisition.

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SUPPLEMENTARY FILE

INFLUENCE OF TWO COMPOST TEAS ON INITIAL GROWTH AND TOLERANCE TO WATER DEFICIT IN MAIZE AND SORGHUM

Journal of Plant Growth Regulation

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FIGURES

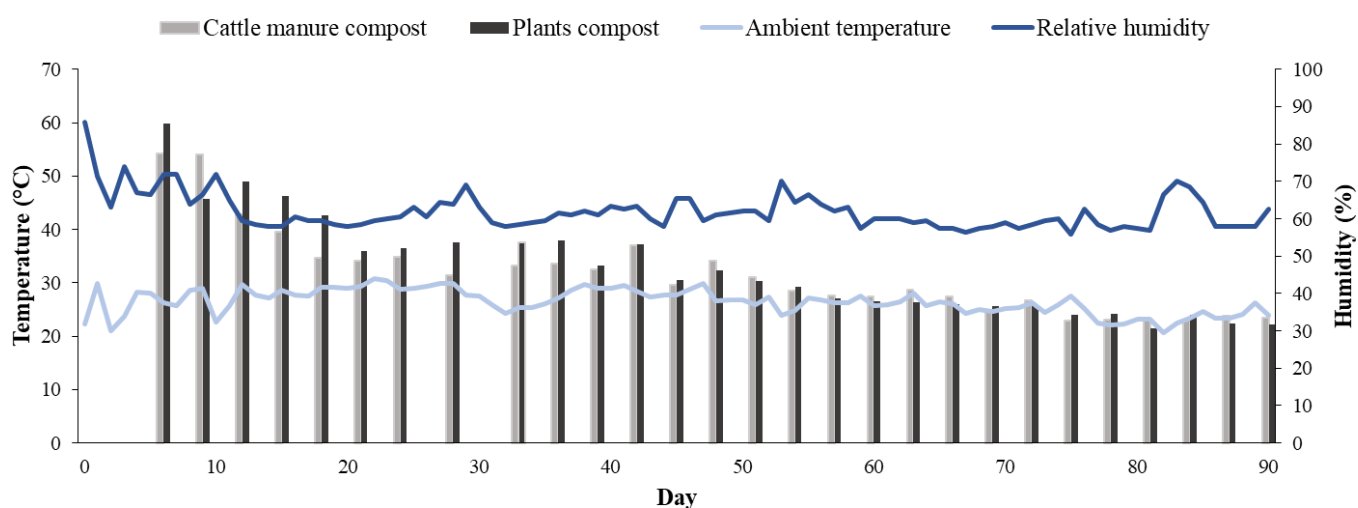


Fig. 1 Means of windrows temperature of cattle manure compost and plants compost and daily means of temperature (°C) and relative humidity (%), over 90 days; Office Excel was used to produce the graphic



Fig. 2 Windrows set up to obtain the composts: on the left, cattle manure compost windrow; on the right, plants compost windrow (a) On mounting day (b) Ninety days after mounting; The figure was edited by PhotoScape program

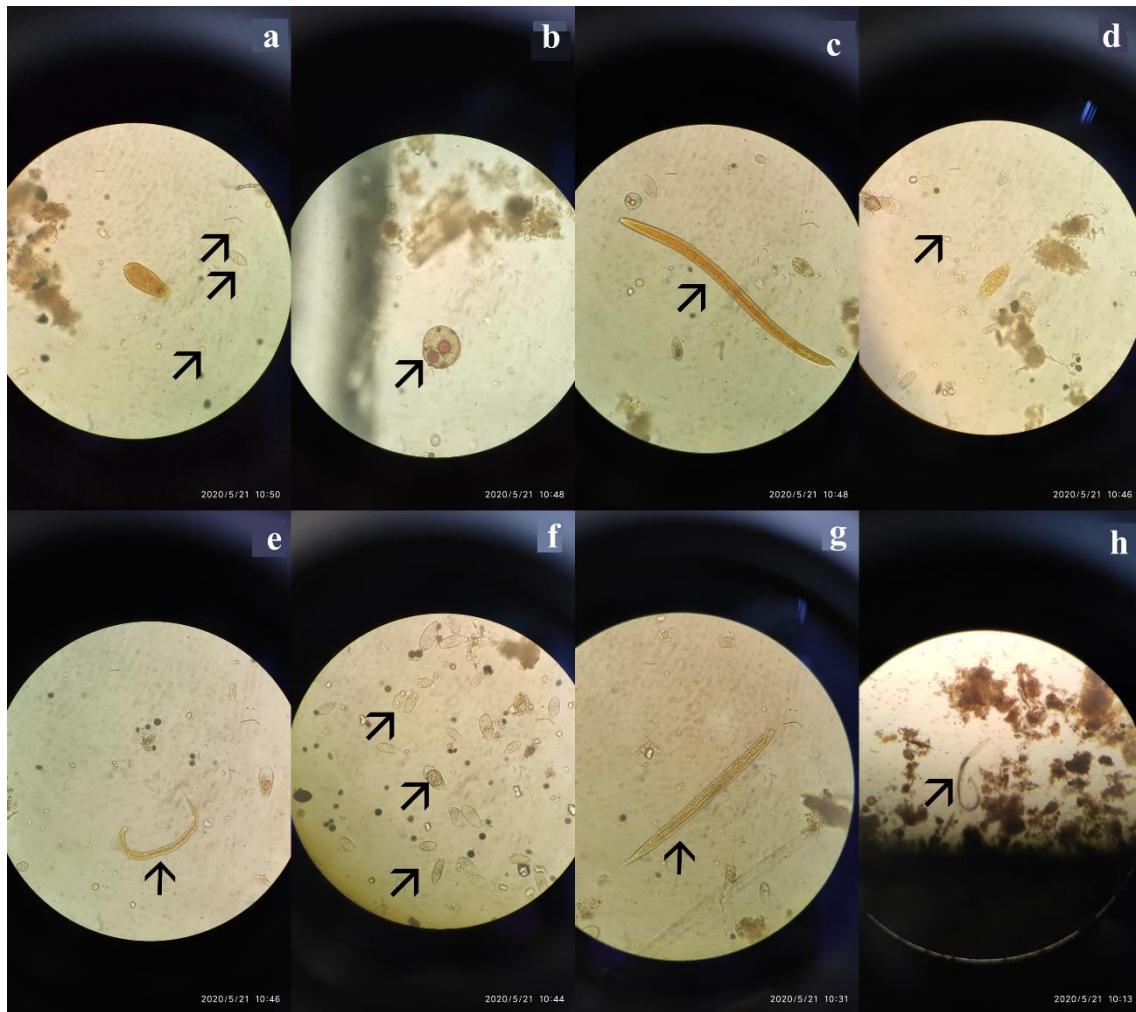


Fig. 3 Pathogenic organisms found in cattle manure compost (a) Eggs of *Haemonchus* spp, *Cooperia* spp, *Oesophagostomum* spp (b) Coccidial oocysts (c) Larva of *Strongyloides* spp (d) Eggs of *Cooperia* spp (e) Larva of *Oesophagostomum* spp (f) Strongyliform eggs (g) Larva of *Trichostrongylus* spp (h) Larva of *Oesophagostomum* spp; The figure was edited by PhotoScape program

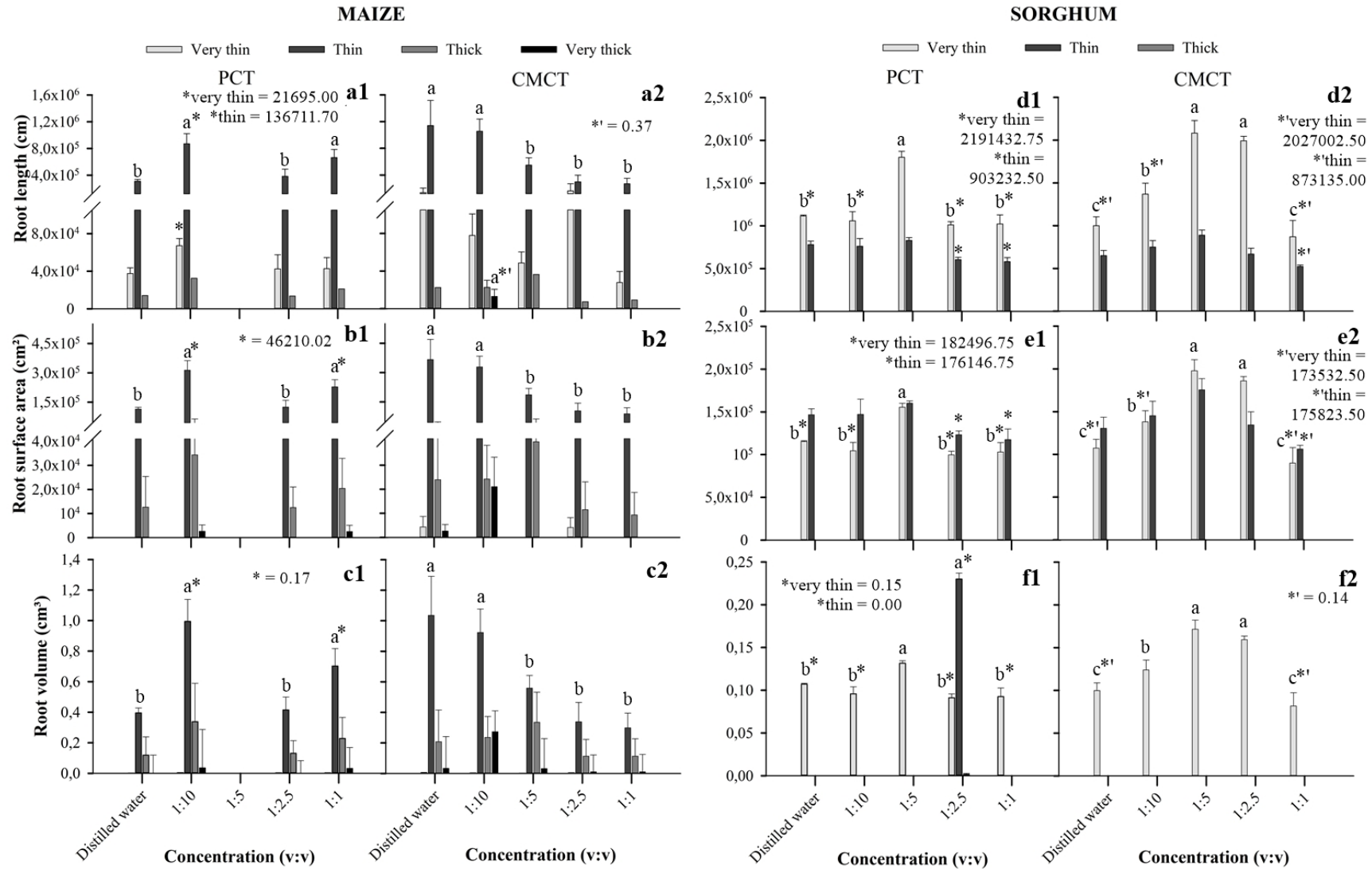


Fig. 4 Root morphology parameters of maize and sorghum under influence of compost teas, according to root diameter classes (a) Root length (cm) of maize (b) Root surface area (cm²) of maize (c) Root volume (cm³) of maize (d) Root length (cm) of sorghum (e) Root surface area (cm²) of sorghum (f) Root volume (cm³) of sorghum (1) Promoted by plants compost tea (PCT) (2) Promoted by cattle manure compost tea (CMCT); Columns: means, n = 4; Bars: standard error; In each species, columns of same color, inside each treatment, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were associated to letters only the data which statistical difference was significant; Means associated to asterisks differ statistically from the mean of Hoagland solution (n = 4) according to Dunnett's test at 5% significance: *=PCT and *'=CMCT; Very thin: 0–0.5 mm, thin: 0.5–2.5 mm, thick: 2.5–4.5 mm, very thick: > 4.5 mm; SigmaPlot 14.5 program was used to produce the graphics and the figure was edited by PhotoScape program

TABLES

Table 1. Physicochemical characterization of plants compost and cattle manure compost.

Parameters	Plants compost	Cattle manure compost	Unit	Reference values
Total N	0.50	0.40	%	-
Total P ₂ O ₅	0.30	0.30	%	-
P ₂ O ₅ ammonium citrate +H ₂ O	0.30	0.20	%	-
K ₂ O (water soluble)	0.70	0.50	%	-
Total Ca	0.44	0.34	%	-
Total Mg	0.22	0.15	%	-
Total S	0.12	0.07	%	-
Total B	< 0.010	N/D	%	-
Total Cu	< 0.010	< 0.010	%	-
Total Fe	0.03	0.10	%	-
Total Mn	< 0.010	< 0.010	%	-
Total Zn	< 0.010	< 0.010	%	-
Moisture	77.00	76.80	%	-
pH	6.90	8.20	-	-
Total organic carbon	36.10	30.40	%	-
C:N ratio	16.60	17.80	-	-
As	< 0.05	N/D	mg.kg ⁻¹	max 20.00
Cd	< 0.10	< 0.10	mg.kg ⁻¹	max 3.00
Hg	N/D	N/D	mg.kg ⁻¹	max 1.00
Ni	N/D	N/D	mg.kg ⁻¹	max 70.00
Pb	0.38	0.25	mg.kg ⁻¹	max 150.00
Se	N/D	N/D	mg.kg ⁻¹	max 90.00
Hexavalent Cr	N/D	N/D	mg.kg ⁻¹	max 2.00

N/D = Not detected

Table 2. Microbial population density and presence of pathogenic microorganisms in plants compost, cattle manure compost, plants compost tea (PCT) and cattle manure compost tea (CMCT) \pm standard error.

Culture medium	Application	Sample	Microbial population density	Unit
Levine EMB Agar	Cultivation of gram-negative pathogenic enterobacteria	Plants compost	Absent	CFU.g ⁻¹
		Cattle manure compost	Absent	CFU.g ⁻¹
		PCT	Absent	CFU.mL ⁻¹
		CMCT	Absent	CFU.mL ⁻¹
Nutrient Agar	Cultivation of undemanding bacteria and fungi	Plants compost	$1.04 \times 10^9 \pm 9.02 \times 10^7$	CFU.g ⁻¹
		Cattle manure compost	$1.48 \times 10^9 \pm 6.17 \times 10^7$	CFU.g ⁻¹
		PCT	$1.93 \times 10^8 \pm 2.73 \times 10^7$	CFU.mL ⁻¹
		CMCT	$6.63 \times 10^8 \pm 3.76 \times 10^7$	CFU.mL ⁻¹
Malt Extract Agar Base	Cultivation of fungi that grow in high concentration of carbohydrates	Plants compost	$7.60 \times 10^4 \pm 2.10 \times 10^4$	CFU.g ⁻¹
		Cattle manure compost	$1.48 \times 10^5 \pm 8.95 \times 10^3$	CFU.g ⁻¹
		PCT	$7.80 \times 10^4 \pm 3.51 \times 10^3$	CFU.mL ⁻¹
		CMCT	$2.60 \times 10^5 \pm 3.06 \times 10^4$	CFU.mL ⁻¹
Potato Dextrose Agar	Cultivation of fungi in general	Plants compost	$1.74 \times 10^5 \pm 3.44 \times 10^4$	CFU.g ⁻¹
		Cattle manure compost	$1.71 \times 10^5 \pm 1.56 \times 10^4$	CFU.g ⁻¹
		PCT	$1.60 \times 10^5 \pm 3.87 \times 10^4$	CFU.mL ⁻¹
		CMCT	$1.18 \times 10^5 \pm 1.33 \times 10^4$	CFU.mL ⁻¹
Czapek-Dox Agar Base	Cultivation of fungi that use sodium nitrate as a nitrogen source	Plants compost	$1.14 \times 10^5 \pm 1.39 \times 10^4$	CFU.g ⁻¹
		Cattle manure compost	$6.93 \times 10^5 \pm 1.83 \times 10^5$	CFU.g ⁻¹
		PCT	$4.87 \times 10^5 \pm 7.69 \times 10^4$	CFU.mL ⁻¹
		CMCT	$1.34 \times 10^5 \pm 4.14 \times 10^4$	CFU.mL ⁻¹
Dichloran Rose Bengal Chloramphenicol (DRBC) Agar Base	Cultivation of slow-growing fungi	Plants compost	$1.16 \times 10^5 \pm 3.74 \times 10^4$	CFU.g ⁻¹
		Cattle manure compost	$9.70 \times 10^4 \pm 3.22 \times 10^4$	CFU.g ⁻¹
		PCT	$< 30 \times 10^1$	CFU.mL ⁻¹
		CMCT	$6.33 \times 10^4 \pm 1.23 \times 10^4$	CFU.mL ⁻¹
Sabouraud Dextrose Agar	Cultivation of saprophytic and dermatophytic fungi, pathogenic and non-pathogenic	Plants compost	$5.60 \times 10^4 \pm 7.21 \times 10^3$	CFU.g ⁻¹
		Cattle manure compost	$3.37 \times 10^4 \pm 5.78 \times 10^3$	CFU.g ⁻¹
		PCT	$3.17 \times 10^4 \pm 1.86 \times 10^3$	CFU.mL ⁻¹
		CMCT	$5.83 \times 10^4 \pm 1.90 \times 10^4$	CFU.mL ⁻¹

Table 3. Results for *p*-value of Analysis of Variance (ANOVA) to verify difference between the treatments and the interaction treatments*concentrations for maize and sorghum under compost teas influence.

Parameters	MAIZE		SORGHUM	
	Treatments	Treatments*Concentrations	Treatments	Treatments*Concentrations
Germination percentage at 24h (%)	<i>p</i> = 0.0000	<i>p</i> = 0.0025	<i>p</i> = 0.2631	<i>p</i> = 0.2698
Germination percentage on the 4 th day (%)	<i>p</i> = 0.5962	<i>p</i> = 0.8662	<i>p</i> = 0.7717	<i>p</i> = 0.4570
Germination Speed Index (GSI)	<i>p</i> = 0.0000	<i>p</i> = 0.0064	<i>p</i> = 0.3891	<i>p</i> = 0.4029
Fresh biomass (g)	<i>p</i> = 0.4294	<i>p</i> = 0.0031	<i>p</i> = 0.5126	<i>p</i> = 0.0263
Dry biomass (g)	<i>p</i> = 0.5344	<i>p</i> = 0.7559	<i>p</i> = 0.8800	<i>p</i> = 0.0635
Shoot length (mm)	<i>p</i> = 0.2172	<i>p</i> = 0.0000	<i>p</i> = 0.0095	<i>p</i> = 0.2976
Total root length (cm)	<i>p</i> = 0.0081	<i>p</i> = 0.0116	<i>p</i> = 0.0649	<i>p</i> = 0.0013
Root surface area (cm ²)	<i>p</i> = 0.0347	<i>p</i> = 0.0260	<i>p</i> = 0.0642	<i>p</i> = 0.0047
Average root diameter (mm)	<i>p</i> = 0.6213	<i>p</i> = 0.0681	<i>p</i> = 0.7160	<i>p</i> = 0.0926
Root volume (cm ³)	<i>p</i> = 0.3762	<i>p</i> = 0.3647	<i>p</i> = 0.1284	<i>p</i> = 0.0612
Number of root tips	<i>p</i> = 0.1157	<i>p</i> = 0.5192	<i>p</i> = 0.3035	<i>p</i> = 0.0038
Number of root forks	<i>p</i> = 0.1426	<i>p</i> = 0.1700	<i>p</i> = 0.1224	<i>p</i> = 0.0004
Root fineness (cm/cm ³)	<i>p</i> = 0.2144	<i>p</i> = 0.4919	<i>p</i> = 0.6547	<i>p</i> = 0.0351
Length of very thin roots (cm)	<i>p</i> = 0.0451	<i>p</i> = 0.4804	<i>p</i> = 0.0108	<i>p</i> = 0.0002
Length of thin roots (cm)	<i>p</i> = 0.0100	<i>p</i> = 0.0035	<i>p</i> = 0.5929	<i>p</i> = 0.5516
Length of thick roots (cm)	<i>p</i> = 0.3440	<i>p</i> = 0.4425	<i>p</i> = 0.1665	<i>p</i> = 0.1043
Length of very thick roots (cm)	<i>p</i> = 0.0960	<i>p</i> = 0.0267	Non-existent data	Non-existent data
Surface area of very thin roots (cm ²)	<i>p</i> = 0.1730	<i>p</i> = 0.5735	<i>p</i> = 0.0014	<i>p</i> = 0.0003
Surface area of thin roots (cm ²)	<i>p</i> = 0.0126	<i>p</i> = 0.0012	<i>p</i> = 0.9453	<i>p</i> = 0.7340
Surface area of thick roots (cm ²)	<i>p</i> = 0.2664	<i>p</i> = 0.4913	<i>p</i> = 0.1667	<i>p</i> = 0.1045
Surface area of very thick roots (cm ²)	<i>p</i> = 0.0847	<i>p</i> = 0.1240	Non-existent data	Non-existent data
Volume of very thin roots (cm ³)	<i>p</i> = 0.2046	<i>p</i> = 0.4931	<i>p</i> = 0.0019	<i>p</i> = 0.0005
Volume of thin roots (cm ³)	<i>p</i> = 0.0298	<i>p</i> = 0.0007	<i>p</i> = 0.0000	<i>p</i> = 0.0000
Volume of thick roots (cm ³)	<i>p</i> = 0.3348	<i>p</i> = 0.5089	<i>p</i> = 0.1687	<i>p</i> = 0.1075
Volume of very thick roots (cm ³)	<i>p</i> = 0.0526	<i>p</i> = 0.1902	Non-existent data	Non-existent data
SOD (U min ⁻¹ g ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0002	<i>p</i> = 0.8238	<i>p</i> = 0.0000
APX (μM AsA min ⁻¹ g ⁻¹)	<i>p</i> = 0.0407	<i>p</i> = 0.0002	<i>p</i> = 0.2081	<i>p</i> = 0.0015
CAT (μM H ₂ O ₂ min ⁻¹ g ⁻¹)	<i>p</i> = 0.0004	<i>p</i> = 0.3811	<i>p</i> = 0.0005	<i>p</i> = 0.5218
Lipid peroxidation (ηmol MDA mg ⁻¹)	<i>p</i> = 0.4060	<i>p</i> = 0.0277	<i>p</i> = 0.2931	<i>p</i> = 0.4837

Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm. Were considered as significant the results for *p*-value < 0.05.

Table 4. Results for *p*-value of Analysis of Variance (ANOVA) to verify difference between the treatments and the interaction treatments*concentrations for maize and sorghum under compost teas associated to mannitol (-2.5 MPa) influence.

Parameters	MAIZE		SORGHUM	
	Treatments	Treatment*Concentrations	Treatments	Treatment*Concentrations
Germination percentage at 24h (%)	<i>p</i> = 0.0000	<i>p</i> = 0.0762	<i>p</i> = 0.0455	<i>p</i> = 0.0029
Germination percentage on the 4 th day(%)	<i>p</i> = 0.8264	<i>p</i> = 0.9945	<i>p</i> = 0.0717	<i>p</i> = 0.5663
Germination Speed Index (GSI)	<i>p</i> = 0.0000	<i>p</i> = 0.3082	<i>p</i> = 0.0372	<i>p</i> = 0.0214
Number of anomalous seedlings	<i>p</i> = 0.0149	<i>p</i> = 0.0007	<i>p</i> = 0.1649	<i>p</i> = 0.0098
Fresh biomass (g)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000
Dry biomass (g)	<i>p</i> = 0.2407	<i>p</i> = 0.2167	<i>p</i> = 0.5121	<i>p</i> = 0.1019
Shoot length (mm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000
Total root length (cm)	<i>p</i> = 0.0076	<i>p</i> = 0.0079	<i>p</i> = 0.0032	<i>p</i> = 0.0002
Root surface area (cm ²)	<i>p</i> = 0.0047	<i>p</i> = 0.0038	<i>p</i> = 0.0006	<i>p</i> = 0.0001
Average root diameter (mm)	<i>p</i> = 0.9746	<i>p</i> = 0.0065	<i>p</i> = 0.1606	<i>p</i> = 0.0031
Root volume (cm ³)	<i>p</i> = 0.0324	<i>p</i> = 0.1772	<i>p</i> = 0.0001	<i>p</i> = 0.0003
Number of root tips	<i>p</i> = 0.4349	<i>p</i> = 0.2076	<i>p</i> = 0.1979	<i>p</i> = 0.0002
Number of root forks	<i>p</i> = 0.1271	<i>p</i> = 0.5175	<i>p</i> = 0.0810	<i>p</i> = 0.0115
Root fineness (cm/cm ³)	<i>p</i> = 0.0861	<i>p</i> = 0.1232	<i>p</i> = 0.4075	<i>p</i> = 0.0076
Length of very thin roots (cm)	<i>p</i> = 0.3664	<i>p</i> = 0.2983	<i>p</i> = 0.0594	<i>p</i> = 0.0044
Length of thin roots (cm)	<i>p</i> = 0.0042	<i>p</i> = 0.0002	<i>p</i> = 0.0028	<i>p</i> = 0.0008
Length of thick roots (cm)	<i>p</i> = 0.0418	<i>p</i> = 0.2328	Non-existent data	Non-existent data
Length of very thick roots (cm)	<i>p</i> = 0.3234	<i>p</i> = 0.4390	Non-existent data	Non-existent data
Surface area of very thin roots (cm ²)	<i>p</i> = 0.7506	<i>p</i> = 0.3089	<i>p</i> = 0.0882	<i>p</i> = 0.0064
Surface area of thin roots (cm ²)	<i>p</i> = 0.0024	<i>p</i> = 0.0002	<i>p</i> = 0.0013	<i>p</i> = 0.0009
Surface area of thick roots (cm ²)	<i>p</i> = 0.0379	<i>p</i> = 0.2093	Non-existent data	Non-existent data
Surface area of very thick roots (cm ²)	<i>p</i> = 0.0536	<i>p</i> = 0.0959	Non-existent data	Non-existent data
Volume of very thin roots (cm ³)	<i>p</i> = 0.2953	<i>p</i> = 0.3543	<i>p</i> = 0.1240	<i>p</i> = 0.0080
Volume of thin roots (cm ³)	<i>p</i> = 0.0044	<i>p</i> = 0.0002	Non-existent data	Non-existent data
Volume of thick roots (cm ³)	<i>p</i> = 0.0184	<i>p</i> = 0.2582	Non-existent data	Non-existent data
Volume of very thick roots (cm ³)	<i>p</i> = 0.0341	<i>p</i> = 0.0595	Non-existent data	Non-existent data
SOD (U min ⁻¹ g ⁻¹)	<i>p</i> = 0.3890	<i>p</i> = 0.9357	<i>p</i> = 0.3527	<i>p</i> = 0.0527
APX (μM AsA min ⁻¹ g ⁻¹)	<i>p</i> = 0.0557	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000
CAT (μM H ₂ O ₂ min ⁻¹ g ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0001	<i>p</i> = 0.2184	<i>p</i> = 0.0301
Lipid peroxidation (ηmol MDA mg ⁻¹)	<i>p</i> = 0.0001	<i>p</i> = 0.0000	<i>p</i> = 0.0253	<i>p</i> = 0.0000

Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm. Were considered as significant the results for *p*-value < 0.05.

**5 ARTIGO III: EFFECT OF TWO COMPOST TEAS AS TOLERANCE-INDUCERS
TO LEAD AND ALUMINUM IN MAIZE AND SORGHUM**

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EFFECT OF TWO COMPOST TEAS AS TOLERANCE-INDUCERS TO LEAD AND ALUMINUM IN MAIZE AND SORGHUM

ABSTRACT

Compost teas have been reported as tolerance-inducers to biotic and abiotic stresses in plants, among which are few studies with heavy metals stress. Our hypothesis is that compost teas can mitigate the harmful effects of heavy metals in maize and sorghum. The objective of this work was to verify the effects of two compost teas, plants compost tea (PCT) and cattle manure compost tea (CMCT), as tolerance-inducers to lead (Pb) and aluminum (Al) in maize and sorghum. We produced and chemically analyzed the two compost teas, applying them to maize and sorghum exposed to Pb or Al, in order to evaluate their effects on germination, initial growth, root morphology/architecture, antioxidant enzyme activity and lipid peroxidation. The two treatments mitigated or nullified Pb and Al damages in several parameters analyzed, besides stimulated and improved initial growth and root morphology/architecture, demonstrating the biostimulant action. The effects observed are related to compost teas chemical and microbiological composition, in addition to enhancement of enzymatic antioxidant metabolism of maize.

Keywords: Biostimulant; Root morphology; Antioxidant enzymes; Heavy metals; *Zea mays*; *Sorghum bicolor*.

1 INTRODUCTION

Compost teas are derivatives of composts, obtained by mixing compost and water for a predetermined period, carrying useful and beneficial microorganisms as well as essential nutrients and other compost's soluble components, that are capable of confer resistance and stimulate plants' growth. Their advantage is related to the possibility of enhancing the reach and the benefits of composts (Eudoxie and Martin, 2019).

The scientific literature indicates that compost teas' application improves plants' growth and health, their nutritional quality, the productivity, besides can protect them from biotic and abiotic stresses (Amer *et al.*, 2020; Amos, 2017; Eudoxie and Martin, 2019; Fouda and Niel, 2021; Otero *et al.*, 2019). However, it should be noted that studies with compost teas and abiotic stresses are still scarce, being mostly related to saline stress (Adejumo, 2015; Amer, 2016; Amer *et al.*, 2021, 2020; Bayoumy *et al.*, 2019; Heba *et al.*, 2014; Li *et al.*, 2021).

Among abiotic stresses, can be mentioned heavy metals stress, such as lead (Pb) and aluminum (Al). The stress caused by the presence of heavy metals in the soil has notable adverse effects on the growth and productivity of agricultural crops, varying according to the species, the metal, the concentration, the chemical form, and the physicochemical properties of the soil, triggering from biochemical responses to a decrease in crop yield (Gill, 2014):

Among heavy metals, lead (Pb) is one of the main soil contaminants, resulting from human activities such as mining and smelting as well as natural climatic processes, which accumulates in roots, petioles and leaves (Ghori *et al.*, 2019). Various symptoms of toxicity to Pb in plants are reported in the literature, such as chlorosis, atrophies or reductions in growth, reduction in root length, change in membrane permeability, hormonal changes, inhibition of several enzymes' activity, reduction in water content, decreased capacity for mineral nutrition, inhibition of germination, damage to photosynthesis and cell respiration, and oxidative stress (Ali and Nas, 2018; Ghori *et al.*, 2019; Mitra *et al.*, 2020).

Aluminum (Al) is another heavy metal contaminating soil that is present in soils naturally and becomes available due to some natural events – such as spring snow melt, storms, etc. – and human activities – such as mining and aluminum-rich industrial and municipal discharges, among others (Rahman and Upadhyaya, 2021). Although, it has a particularity: it is a non-toxic element in places where the soil does not have acidity, but it causes toxicity in places with acidic soil – with pH between 4.5 and 5.5 (Chowra *et al.*, 2017; Rahman and Upadhyaya, 2021).

Al toxic effects commonly reported include reduced absorption of water and nutrients, inhibition of intercellular transport by plasmodesmata, reactive oxygen species (ROS) production, reduced photosynthetic rate, oxidative stress and damage to membranes, inhibition of root growth

and diameter, root discoloration, inhibition of shoot growth, leaf chlorosis and necrosis, impact on protein metabolism and/or enzymatic activity and damage to genetic material (Gupta *et al.*, 2013; Rahman and Upadhyaya, 2021). In Brazil, the Cerrado soil is known as acidic and, consequently, cultivated plants are susceptible to Al toxicity, representing a barrier to agriculture (Haridasan, 2008; Lopes and Guimarães Guilherme, 2016).

It is noteworthy that the scientific literature still does not present many studies that correlate the use of compost teas as tolerance inducers to heavy metals stress (Adejumo, 2015; Eftekhar and Fallah, 2018; Li *et al.*, 2021), which have been causing damage to agriculture around the world for a long time (Adejumo, 2015; Haridasan, 2008). It becomes clear the importance of studies that demonstrate compost teas' effects as tolerance inducers to heavy metals stress, besides the possibility of reducing organic residues and valuing compost products, contributing to circular economy (Bakan *et al.*, 2022).

In this context, maize (*Zea mays*) and sorghum (*Sorghum bicolor*) are crop species cultivated around the entire globe, used both for human and animal food, of considerable economic importance, on which there is no studies with compost teas effects as tolerance inducers to heavy metals stress. Our hypothesis is that compost teas can improve the response to the presence of lead and aluminum in maize and sorghum initial growth.

Therefore, the objective of this work was to verify the effects of two compost teas as tolerance-inducers to lead and aluminum in maize and sorghum.

2 MATERIALS AND METHODS

2.1 Compost teas

Were tested as treatments two different aerated compost teas: plants compost tea (PCT) and cattle manure compost tea (CMCT). The compost teas' physicochemical and microbiological compositions are shown in Table 1.

Table 1. Physicochemical and microbiological composition of plants compost tea (PCT) and cattle manure compost tea (CMCT).

Physicochemical composition	PCT	CMCT	Unit
pH	6.29	7.35	-
Dissolved oxygen	5.29	4.26	mg.L ⁻¹
N	29.74	25.12	g.kg ⁻¹
P	12.15	11.96	g.kg ⁻¹
K	61.69	71.14	g.kg ⁻¹
Ca	15.37	14.01	g.kg ⁻¹

Mg	9.83	9.1	g.kg ⁻¹
S	3.56	3.59	g.kg ⁻¹
B	0.0064	0.0034	g.kg ⁻¹
Cu	0.0218	0.0191	g.kg ⁻¹
Fe	0.6302	1.088	g.kg ⁻¹
Mn	0.233	0.1967	g.kg ⁻¹
Zn	0.0804	0.0832	g.kg ⁻¹
Microbiological composition	PCT	CMCT	Unit
Gram-negative pathogenic enterobacteria	Absent	Absent	CFU.mL ⁻¹
Undemanding bacteria and fungi	1.93 x 10 ⁸ ± 2.73 x 10 ⁷	6.63 x 10 ⁸ ± 3.76 x 10 ⁷	CFU.mL ⁻¹
Fungi that grow in high concentration of carbohydrates	7.80 x 10 ⁴ ± 3.51 x 10 ³	2.60 x 10 ⁵ ± 3.06 x 10 ⁴	CFU.mL ⁻¹
Fungi in general	1.60 x 10 ⁵ ± 3.87 x 10 ⁴	1.18 x 10 ⁵ ± 1.33 x 10 ⁴	CFU.mL ⁻¹
Fungi that use sodium nitrate as a nitrogen source	4.87 x 10 ⁵ ± 7.69 x 10 ⁴	1.34 x 10 ⁵ ± 4.14 x 10 ⁴	CFU.mL ⁻¹
Slow-growing fungi	< 30 x 10 ¹	6.33 x 10 ⁴ ± 1.23 x 10 ⁴	CFU.mL ⁻¹
Saprophytic fungi	3.17 x 10 ⁴ ± 1.86 x 10 ³	5.83 x 10 ⁴ ± 1.90 x 10 ⁴	CFU.mL ⁻¹

Macro and micronutrient contents are expressed as g.kg⁻¹ of lyophilized samples. Microbiological compositions are expressed as microbial population density ± standard error. Source: adapted from Govêa *et al.* (2023).

2.2 Germination and initial growth analysis

For the germination and initial growth analysis of maize (*Zea mays* – Pioneer® P30F53 hybrid), two growth chamber experiments were carried out, each one with a stressing solution: (1) Pb: lead nitrate (1 mM) and (2) Al: aluminum nitrate (1 mM). For sorghum (*Sorghum bicolor* – BRS 332), also two growth chamber experiments were carried out, but with a difference: the Al stressing solution was used in 2 mM concentration. The concentrations of heavy metals were defined in pilot experiments as stressful for maize and sorghum as they altered their initial growth.

For each experiment, were used the stressing solution added to four concentrations of compost tea: 1:10, 1:5, 1:2.5 and 1:1 (volume of compost : volume of distilled water), in addition to three controls: distilled water, stressing solution, and 100% Hoagland solution + stressing solution. Four repetitions were used to each concentration.

The seeds were placed to germinate in rolls with three sheets of Germitest® paper, two of which were used as a base and one to close the rolls, with each roll representing a repetition. The three sheets were weighed to determine the volume (mL) to be used for imbibition with the concentrations used, in the proportion of 2.5 mL x paper weight. After, the rolls were soaked with the concentrations, containing 50 seeds each and placed in plastic bags for autoclave. These were kept for four days in a B.O.D. germination chamber (Ethik Technology 411FPD, Brazil), with 30 °C

temperature and 12 hours photoperiod. The period of four days was determined in pilot experiments that elucidated as the necessary time to maize and sorghum growth without causing damage due to the mechanical shock caused by the autoclave bags in which the rolls were packed.

This experiment was repeated in order to collect samples for the analyzes of root morphology, antioxidant enzymes activity and lipid peroxidation.

For germination and initial growth analysis, after four days, the following parameters were evaluated: germination percentage at 24 hours and on the 4th day (%), Germination Speed Index (GSI), number of anomalous seedlings, fresh biomass (g), dry biomass (g) and shoot length (mm).

GSI was obtained as proposed by Chiapusio *et al.* (1997), as follows:

$$GSI = \frac{N_1}{1} + \dots + \frac{(N_n - N_{n-1})}{n}$$

where N_1 , N_n , and N_{n-1} correspond to the number of germinated seeds in the first, n , $n-1$ evaluations, respectively, and n is the evaluation number. Four evaluations were performed, every 24 hours.

Were considered as anomalous seedlings the visually defective seedlings, as twisted and/or blackened roots or shoots. Fresh and dry biomasses were measured using a precision balance with four decimal places (Shimadzu Marte AY220, Japan), and dry biomass were obtained after drying in an oven with forced air circulation at 40 °C (Ethik Technology 410/3ND, Brazil). For shoot length, the 10 visibly larger seedlings were selected and measured using a digital caliper (Digimes 100.170, Brazil).

2.3 Root morphology analysis

After repeating the experiment described in item 2.2, on the fourth day 10 seedlings were randomly collected from each repetition for root morphology analysis. The roots were fixed in 70% alcohol for further analysis in WinRhizo Pro 2007a (Regent Instruments, Canada). The parameters evaluated were total root length (cm), root surface area (cm²), average root diameter (mm), root volume (cm³), number of root tips, number of root forks, and root fineness (cm/cm³). Root fineness was measured according to Souza *et al.* (2012). In addition, also were evaluated the parameters root length, root surface area and root volume according to root diameter classes: very thin (0 – 0.5 mm), thin (0.5 – 2.5 mm), thick (2.5 – 4.5 mm) and very thick (>4.5 mm). Roots classified as very thick was not present in sorghum's data so was not analyzed for this species; besides, roots classified as thick was not present in sorghum's data of A1 experiment, so was not analyzed either.

An observation is necessary here: due to the pandemic, some concentrations could not be analyzed, due to the long storage time between the fixation in 70% alcohol and the analysis in

WinRhizo. For 1:10 concentration + Pb of PCT treatment, and for Hoagland + Pb control of CMCT treatment, in maize, all the four repetitions could not be analyzed in WinRhizo. Therefore, such data are not shown for any of the root morphology parameters in maize, nor were they considered in the statistical analysis.

2.4 Antioxidant enzymes activity and lipid peroxidation analyzes

For the analyzes of antioxidant enzymes activity and lipid peroxidation, after the fourth day of the experiment, the remaining fresh biomass – which was not collected for root morphology analysis – was collected and stored in liquid N₂ for further extraction.

For the extraction of antioxidant enzymes, 200 mg of seedlings were macerated in liquid N₂ with 50% PVPP and homogenized in 1.5 mL of extraction buffer containing: 375 µL of 400 mM potassium phosphate (pH 7.8), 15 µL of 10 mM EDTA and 75 µL of 200 mM ascorbic acid. The homogenates were centrifuged at 13,000 rpm for 10 minutes at 4°C and the supernatants were collected for enzymatic analyzes of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), quantified by spectrophotometry in ELISA reader (Biochrom Anthos Zenyth 200 RT, England). The final reaction volume for reading the enzymes was 2 mL, in a visible plate. All readings were performed in triplicate.

The quantification of SOD activity was performed according to the method proposed by Giannopolitis and Ries (1977), at 560nm, and a unit of SOD activity is defined as the amount of enzyme that inhibits the photoreduction of NBT by 50%. To quantify the CAT activity, the method of Havir and McHale (1987) was used, at 240 nm, every 15 seconds for 3 minutes, and a unit of CAT activity is defined as the amount of enzyme that catalyzes the decomposition of 1 µmol.min⁻¹ of H₂O₂. APX activity was quantified according to the method of Nakano and Asada (1981), at 290 nm, every 15 seconds for 3 minutes, and an APX activity unit is defined as the amount of enzyme that oxidizes 1 µmol.min⁻¹ of ascorbate.

For extraction of lipid peroxidation, 200 mg of seedlings were macerated in liquid N₂ with PVPP and homogenized in 1.5 mL of 0.1% trichloroacetic acid (TCA). The homogenates were centrifuged at 12,000 rpm for 15 minutes at 4°C, collecting the supernatants. Lipid peroxidation was determined by quantifying thiobarbituric acid reactive species, as described by Buege and Aust (1978), at 535 and 600 nm, quantified by spectrophotometry in an ELISA reader (Biochrom Anthos Zenyth 200 RT, England). The final reaction volume was 2 mL, in a visible plate. All readings were performed in triplicate.

2.5 Experimental design and statistical analysis

For statistical analysis, a completely randomized design (CRD) was used, with 2 treatments x 7 concentrations and 4 repetitions, for each species. The data obtained were submitted to analysis of variance (ANOVA), in order to verify difference between treatments and interaction between treatments and concentrations. The means were compared by the Scott-Knott test at 5% significance.

3 RESULTS

3.1 Maize and sorghum under lead (Pb) stress and compost teas influence

Under stress caused by lead (Pb), there was no statistical difference ($p>0.05$) between treatments nor interaction between treatments and concentrations for any of the germinative parameters, that is, the compost teas did not influence the germination percentage at 24h, on the 4th day or Germination Speed Index (GSI) in the presence of 1 mM of Pb, for both maize and sorghum (Supplementary File, Table 1).

Regarding the initial growth parameters, the cattle manure compost tea (CMCT), in the presence of Pb, was responsible for reducing the number of anomalous seedlings in relation to plants compost tea (PCT), while PCT promoted greater shoot length in relation to CMCT, for both maize and sorghum (Table 2). The fresh and dry biomasses showed no statistical difference ($p>0.05$) between treatments for any species (Supplementary File, Table 1).

For root morphology parameters, in maize, PCT was more beneficial than CMCT, once promoted higher values for all root morphology parameters analyzed, except for root fineness, compared to CMCT (Table 2). On the other hand, in sorghum, CMCT was more beneficial than PCT, since promoted higher total length, surface area and volume in sorghum roots than PCT, besides increased the length, surface area and volume in very thin roots, and enhanced values for surface area and root volume in thin roots compared PCT (Table 2). The further root morphology parameters (average root diameter, number of tips, number of forks, root fineness, length of thin roots and all thick roots parameters) showed no statistical difference ($p>0.05$) between the two treatments, for sorghum (Supplementary File, Table 1).

Considering antioxidant enzymes activity and lipid peroxidation, PCT increased ascorbate peroxidase (APX) activity and decreased lipid peroxidation in maize when compared to CMCT, while CMCT presented higher values for superoxide dismutase (SOD) and catalase (CAT) activities in relation to PCT (Table 2). In sorghum, PCT promoted higher activity of SOD and APX enzymes, besides decreased lipid peroxidation compared to CMCT (Table 2). The CAT activity presented no statistical difference ($p>0.05$) between treatments, for sorghum (Supplementary File, Table 1).

Table 2. Comparison between means of plants compost tea (PCT) and cattle manure compost tea (CMCT) + 1mM of lead (Pb) treatments for initial growth parameters, root morphology parameters, antioxidant enzymes activity and lipid peroxidation of maize and sorghum \pm standard error; n = 4.

Parameter	MAIZE				SORGHUM			
	PCT		CMCT		PCT		CMCT	
Number of anomalous seedlings	3.11 \pm 0.33	a	1.79 \pm 0.27	b	9.61 \pm 2.42	a	6.89 \pm 2.66	b
Shoot length (mm)	36.20 \pm 1.65	a	34.97 \pm 1.17	b	69.68 \pm 2.36	a	67.35 \pm 1.84	b
Total root length (cm)	1.61 $\times 10^6 \pm 1.60 \times 10^5$	a	9.95 $\times 10^5 \pm 9.87 \times 10^4$	b	1.64 $\times 10^6 \pm 1.26 \times 10^5$	b	1.83 $\times 10^6 \pm 1.68 \times 10^5$	a
Root surface area (cm ²)	6.43 $\times 10^5 \pm 5.71 \times 10^4$	a	3.95 $\times 10^5 \pm 3.94 \times 10^4$	b	2.51 $\times 10^5 \pm 1.46 \times 10^4$	b	2.80 $\times 10^5 \pm 2.10 \times 10^4$	a
Average root diameter (mm)	1.32 $\times 10^4 \pm 2.65 \times 10^2$	a	1.19 $\times 10^4 \pm 7.11 \times 10^2$	b				
Root volume (cm ³)	1960.93 ± 204.62	a	999.30 ± 181.66	b	0.3105 ± 0.0127	b	0.3481 ± 0.0212	a
Number of root tips	264.42 ± 16.19	a	211.18 ± 17.97	b				
Number of root forks	293.42 ± 33.18	a	177.36 ± 27.73	b				
Root fineness (cm/cm ³)	8.13 $\times 10^4 \pm 4.45 \times 10^4$	b	3.39 $\times 10^5 \pm 1.01 \times 10^5$	a				
Length of very thin roots (cm)	1.17 $\times 10^5 \pm 8.63 \times 10^3$	a	9.18 $\times 10^4 \pm 8.98 \times 10^3$	b	9.26 $\times 10^5 \pm 9.62 \times 10^4$	b	1.06 $\times 10^6 \pm 1.25 \times 10^5$	a
Length of thin roots (cm)	1.42 $\times 10^6 \pm 1.47 \times 10^5$	a	8.26 $\times 10^5 \pm 9.53 \times 10^4$	b				
Length of thick roots (cm)	5.28 $\times 10^4 \pm 6.60 \times 10^3$	a	3.39 $\times 10^4 \pm 4.51 \times 10^3$	b				
Length of very thick roots (cm)	1.03 $\times 10^4 \pm 2.68 \times 10^3$	a	2.01 $\times 10^3 \pm 1.06 \times 10^3$	b	N/D		N/D	
Surface area of very thin roots (cm ²)	3.38 $\times 10^3 \pm 1.05 \times 10^3$	a	1.10 $\times 10^3 \pm 6.48 \times 10^2$	b	8.20 $\times 10^4 \pm 7.76 \times 10^3$	b	9.57 $\times 10^4 \pm 1.05 \times 10^4$	a
Surface area of thin roots (cm ²)	4.90 $\times 10^5 \pm 4.60 \times 10^4$	a	2.91 $\times 10^5 \pm 3.25 \times 10^4$	b	1.41 $\times 10^5 \pm 6.47 \times 10^3$	b	1.56 $\times 10^5 \pm 1.07 \times 10^4$	a
Surface area of thick roots (cm ²)	5.93 $\times 10^4 \pm 6.99 \times 10^3$	a	3.64 $\times 10^4 \pm 5.07 \times 10^3$	b				
Surface area of very thick roots (cm ²)	1.72 $\times 10^4 \pm 4.11 \times 10^3$	a	3.72 $\times 10^3 \pm 1.67 \times 10^3$	b	N/D		N/D	
Volume of very thin roots (cm ³)	0.0057 ± 0.0005	a	0.0045 ± 0.0005	b	0.0695 ± 0.0336	b	0.0817 ± 0.0090	a
Volume of thin roots (cm ³)	908.17 ± 534.78	a	0.9187 ± 0.0981	b	0.0000 ± 0.0000	b	0.0473 ± 0.0223	a
Volume of thick roots (cm ³)	0.5689 ± 0.0528	a	0.3666 ± 0.0417	b				
Volume of very thick roots (cm ³)	0.2385 ± 0.0486	a	0.0641 ± 0.0200	b	N/D		N/D	
SOD (U min ⁻¹ g ⁻¹)	29.18 ± 0.60	b	32.57 ± 0.58	a	31.09 ± 1.27	a	24.80 ± 0.74	b
CAT (μ M H ₂ O ₂ min ⁻¹ g ⁻¹)	6.89 ± 0.34	b	9.66 ± 0.45	a				
APX (μ M ASA min ⁻¹ g ⁻¹)	78.52 ± 2.84	a	64.14 ± 1.27	b	103.28 ± 1.85	a	91.84 ± 2.01	b
Lipid peroxidation (η mol MDA mg ⁻¹)	38.39 ± 4.75	b	66.62 ± 2.54	a	57.88 ± 2.10	b	73.36 ± 2.58	a

Means followed by different letters, in the same row, inside each species, differ statistically by the Scott-Knott test at 5% significance. Were represented only the data which statistical difference was significant. Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm. N/D: Non-existent data.

Considering interaction between treatments and concentrations, both compost teas mitigated (that is, attenuated, but not nullified the toxic effect until it was equal to distilled water control) or nullified (that is, annulled the effect to the point of equaling to distilled water control) the negative effect of Pb in maize and sorghum (Figs. 1 and 2, respectively). In some cases, the teas even stimulated parameters (that is, enhanced, increased, promoted values statistically higher than distilled water control).

In maize initial growth parameters, 1:2.5 concentration of PCT increased fresh biomass (Fig. 1a) and shoot length (Fig. 1b) in relation to distilled water and Pb controls, while 1:1 concentration decreased both parameters. The CMCT treatment increased fresh biomass (Fig. 1a) in all

concentrations, except 1:5, in relation to all controls. All concentration of CMCT also nullified Pb toxic effect under maize's shoot length, besides 1:10, 1:2.5 and 1:1 concentrations stimulated shoot length, highlighting 1:2.5 concentration that even surpassed Hoagland solution control. The number of anomalous seedlings and dry biomass showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations for maize (Supplementary File, Table 1).

Regarding maize root morphology, 1:2.5 concentration and most of times also 1:5 concentration of PCT treatment enhanced total root length (Fig. 1c), root surface area (Fig. 1d), root volume (Fig. 1f), and the numbers of tips (Fig. 1g) and forks (Fig. 1h) in relation to distilled water and Pb controls, sometimes even surpassing Hoagland solution control. On the other hand, the 1:1 concentration of PCT decreased the same cited parameters in relation to Pb control. Average root diameter (Fig. 1e) was decreased by 1:5 concentration of PCT in relation to all controls and other concentrations, while the negative effect of Pb on root fineness (Fig. 1i) could not be mitigated by none of the PCT concentrations tested, neither by Hoagland solution control, presenting values proximal to zero while distilled water control was superior to 400 000 cm/cm³.

The CMCT concentrations, in general, were not as beneficial as PCT concentrations for root morphology parameters of maize exposed to Pb. The only CMCT concentration that deserves highlight is 1:2.5, which promoted higher values than Pb control for total root length (Fig. 1c) and number of root tips (Fig. 1g). None of CMCT concentrations were capable to mitigate the harmful effect of Pb on maize's average root diameter (Fig. 1e) or root fineness (Fig. 1i), besides presented no positive effect on root surface area (Fig. 1d), root volume (Fig. 1f) nor on number of root forks (Fig. 1h).

Considering antioxidant enzymes activity and lipid peroxidation in maize, the higher concentrations (1:2.5 and 1:1) of PCT decreased SOD activity (Fig. 1j) in relation to the other concentrations and all controls. Although, the lower concentrations (1:10 and 1:5) of PCT enhanced APX activity (Fig. 1k) and drastically decreased lipid peroxidation (Fig. 1l) when compared to the other concentrations and all controls, besides the other concentrations (1:2.5 and 1:1) were also capable of nullify the Pb harmful effect by decreasing lipid peroxidation (Fig. 1l).

For CMCT treatment, SOD activity (Fig. 1j) was decreased by 1:10 concentration as well as Pb and Hoagland solution controls. The APX activity (Fig. 1k) was increased by the lowest (1:10) concentration of CMCT, although was decreased by the highest (1:1) concentration, compared to all other concentrations and controls. In addition, all CMCT concentrations nullified the toxic effect of Pb in maize by decreasing lipid peroxidation, highlighting 1:10, 1:5 and 1:2.5 concentrations which reduced it even below to distilled water and Hoagland solution controls. There was no statistical difference ($p>0.05$) for interaction between treatments and concentrations for CAT activity in maize (Supplementary File, Table 1).

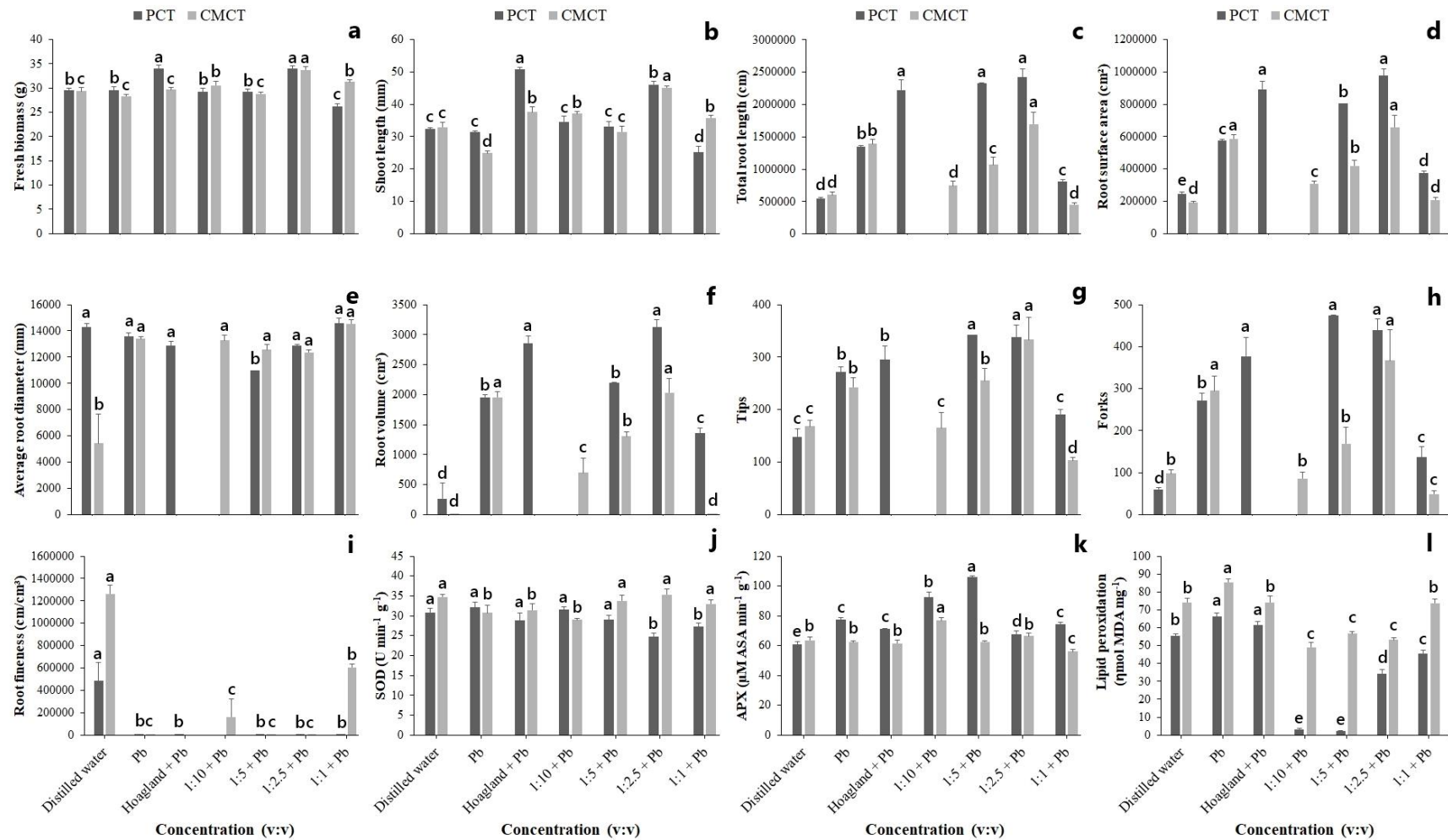


Fig. 1 Initial growth, root morphology and biochemical parameters of maize exposed to 1 mM of lead (Pb) associated with compost teas (a) Fresh biomass (g) (b) Shoot length (mm) (c) Total root length (cm) (d) Root surface area (cm²) (e) Average root diameter (mm) (f) Root volume (cm³) (g) Number of root tips (h) Number of root forks (i) Root fineness (cm/cm³) (j) Superoxide dismutase (SOD) activity (U min⁻¹ g⁻¹) (k) Ascorbate peroxidase (APX) activity (µM ASA min⁻¹ g⁻¹) (l) Lipid peroxidation (nmol MDA mg⁻¹); Columns: means, n = 4; Bars: standard error; Columns of same color, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScape program

For sorghum, both compost teas mitigated or nullified the negative effect in fresh biomass and in shoot length of sorghum compared to Pb control. In addition, the higher concentrations (1:2.5 and 1:1) enhanced both parameters (Figs. 2a and 2c), including with superior values than Hoagland solution control. In relation to dry biomass, some compost teas concentrations (1:5, 1:2.5 and 1:1) had the effect of decreasing it, which was also observed for Hoagland solution control (Figure 2b). The number of anomalous seedlings showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations (Supplementary File, Table 1).

For sorghum root morphology parameters, PCT mitigated or nullified Pb effect in lowest (1:10) and highest (1:1) concentrations for the parameters total root length (Fig. 2d), root surface area (Fig. 2e), average root diameter (Fig. 2f) and root fineness (Fig. 2j). In addition and more important, the same parameters were enhanced by the intermediate (1:5 and 1:2.5) concentrations, most often surpassing Hoagland solution control. The numbers of tips (Fig. 2h) and forks (Fig. 2i) were also enhanced by 1:5 and 1:2.5 concentrations of PCT, and the Pb effect under root volume (Fig. 2g) were nullified by all concentrations except the highest (1:1). For CMCT, this treatment reduced or nullified Pb effect in lower (1:10 and 1:5) concentrations for the parameters total root length (Fig. 2d), root surface area (Fig. 2e) and root volume (Fig.2g), besides stimulated the same parameters in higher concentrations (1:2.5 and 1:1). Average root diameter (Fig. 2f) was decreased while the numbers of tips (Fig. 2h) and forks (Fig. 2i) and root fineness (Fig. 2j) were enhanced by all concentrations of CMCT except the lowest (1:10), highlighting the higher ones (1:2.5 and 1:1), which most of times overcame Hoagland solution control.

Considering antioxidant enzymes activity and lipid peroxidation in sorghum, all concentrations of PCT enhanced SOD activity in relation to all controls and for CMCT this increase occurred only in 1:5 concentration (Fig. 2l). All concentrations of PCT increased APX activity in relation to Pb control, highlighting the lowest (1:10) and the highest (1:1) that presented higher values than distilled water and Hoagland solution controls; although none of CMCT concentrations differed statistically from Pb control (Fig. 2l). Lipid peroxidation was decreased by 1:2.5 concentration and increased by 1:1 concentration of CMCT in relation to all controls, while for PCT all concentrations except the highest (1:1) increased lipid peroxidation in relation to Pb control (Fig. 2m). CAT activity showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations (Supplementary File, Table 1).

The interaction between treatments and concentrations for root diameter classes demonstrated that, in maize, the 1:2.5 concentration of PCT stimulated the length (Fig. 3a1), surface area (Fig. 3b1) and volume (Fig. 3c1) of all root diameter classes, as well as Hoagland solution control. Stimulus is also verified in 1:5 concentration of PCT for length (Fig. 3a1), surface area (Fig. 3b1) and volume (Fig. 3c1) of very thin roots, and for length (Fig. 3a1) and surface area (Fig. 3b1) of thin roots.

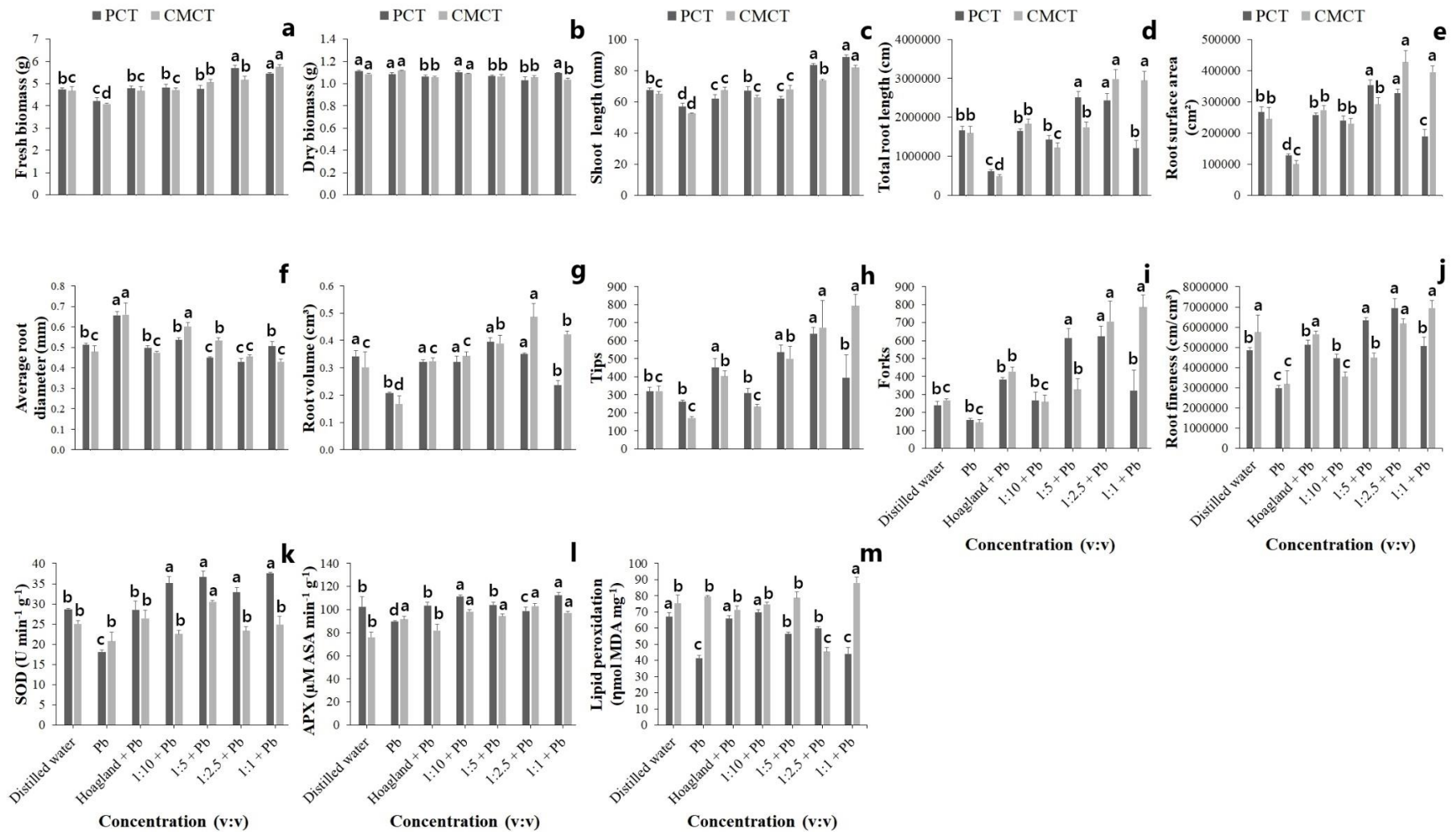


Fig. 2 Initial growth, root morphology and biochemical parameters of sorghum under lead (Pb) stress (1mM) and compost teas influence (a) Fresh biomass (g) (b) Dry biomass (g) (c) Shoot length (mm) (d) Total root length (cm) (e) Root surface area (cm²) (f) Average root diameter (mm) (g) Root volume (cm³) (h) Number of root tips (i) Number of root forks (j) Root fineness (cm/cm³) (k) Superoxide dismutase (SOD) activity (U min⁻¹ g⁻¹) (l) Ascorbate peroxidase (APX) activity (μM ASA min⁻¹ g⁻¹) (m) Lipid peroxidation (ηmol MDA mg⁻¹); Columns: means, n = 4; Bars: standard error; Columns of same color, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScape program

. The CMCT treatment, on the other hand, only increased the length (Fig. 3a2) of very thin roots and the volume (Fig. 3c2) of thick roots at 1:2.5 concentration. The other concentrations of CMCT were not capable of promote positive effects in maize roots, nor mitigate the Pb effects when applicable. In addition, under CMCT influence, very thick roots of maize presented no statistical difference ($p>0.05$) for root length or root surface area, and the same is verified for volume of thin roots. In sorghum, PCT was responsible for nullify Pb effect at 1:10 and 1:1 concentrations for length (Fig. 3d1) and mitigate Pb effect at the same concentrations for surface area (Fig. 3e1) and volume (Fig. 3f1) of very thin roots. Besides, all the parameters were stimulated by intermediate concentrations (1:5 and 1:2.5) of PCT in very thin roots. Considering thin roots, 1:1 concentration of PCT mitigated Pb effect under length besides nullified it in the other concentrations (Fig. 3d1), and for surface area (Fig. 3e1), all concentrations except 1:1 nullified Pb effect.'

Regarding CMCT, in very thin roots of sorghum, the concentration 1:10 mitigated Pb effect under root length (Fig. 3d2), surface area (Fig. 3e2) and volume (Fig. 3f2), while 1:5 concentration nullified Pb effect and the higher concentrations (1:2.5 and 1:1) stimulated all these parameters. For thin roots, 1:10 concentration nullified Pb effect under length (Fig. 3d2) and surface area (Fig. 3e2) while the other concentrations enhanced the same parameters. In addition, 1:1 concentration of CMCT also enhanced root volume in thin roots (Fig. 3f2). Thick roots presented no statistical difference ($p>0.05$) for any of the parameters in sorghum (Supplementary File, Table 1).

3.2 Maize and sorghum under aluminum (Al) stress and compost teas influence

In aluminum (Al) presence, in general, cattle manure compost tea (CMCT) was more beneficial than plants compost tea (PCT), for both maize and sorghum (Table 3).

Considering maize exposed to 1 mM of Al, CMCT promoted greater germination percentage at 24h and Germination Speed Index (GSI) compared to PCT (Table 3). There was no statistical difference ($p>0.05$) between treatments for germination percentage on the 4th day for maize (Supplementary File, Table 2). Besides, for sorghum, in the presence of 2 mM of aluminum (Al), there was no statistical difference ($p>0.05$) between treatments for any of the germinative parameters (Supplementary File, Table 2).

Regarding the initial growth parameters, PCT was responsible for reducing the number of anomalous seedlings in relation to CMCT, in both species, besides slightly increased maize's dry biomass. On the other hand, CMCT promoted greater fresh biomass and shoot length in maize compared to PCT (Table 3). For sorghum, the parameters fresh biomass, dry biomass, and shoot length showed no statistical difference ($p>0.05$) between treatments (Supplementary File, Table 2).

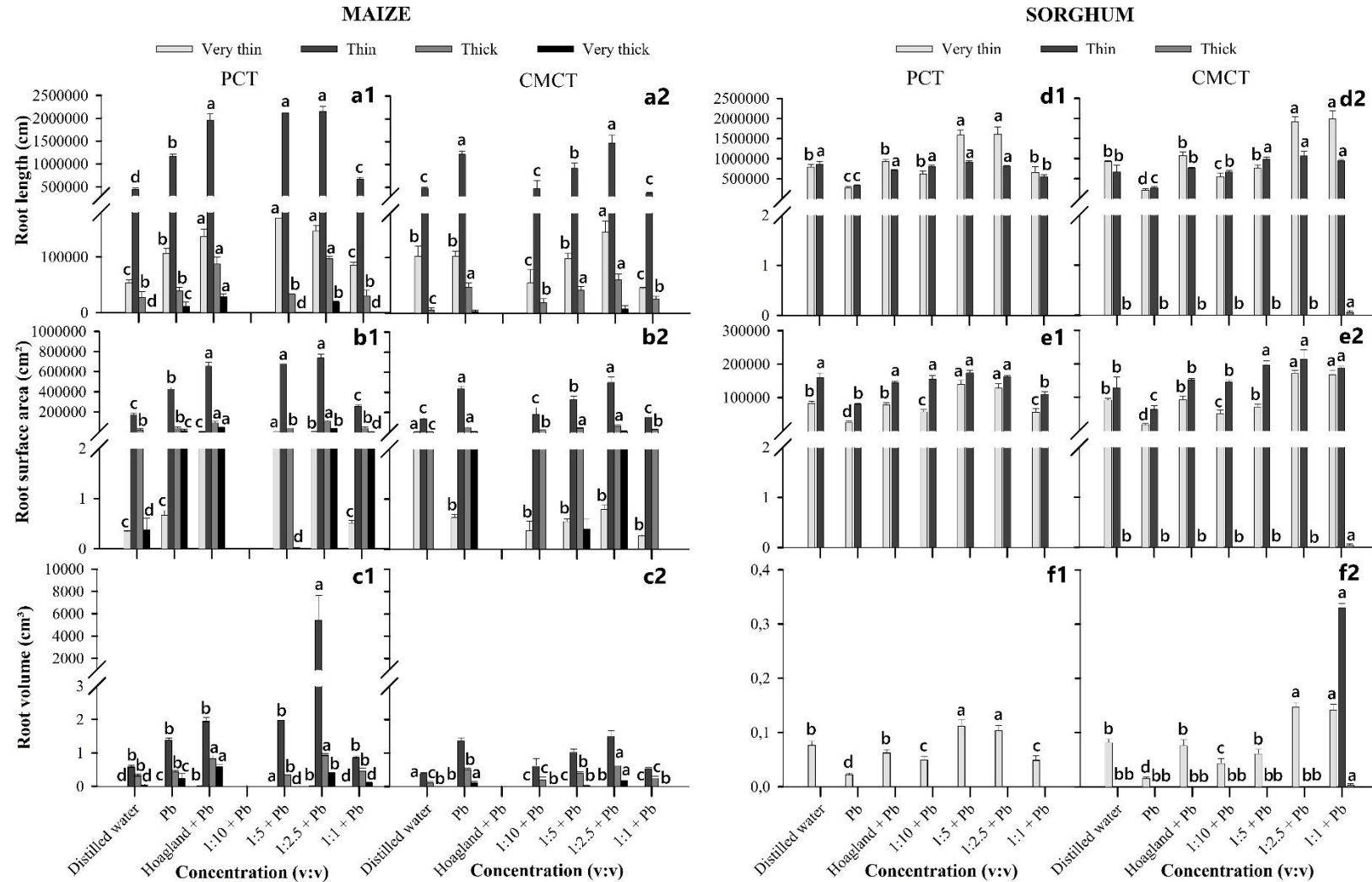


Fig. 3 Root morphology parameters of maize and sorghum under lead (Pb) stress (1mM) and compost teas influence, according to root diameter classes (a) Root length (cm) of maize (b) Root surface area (cm²) of maize (c) Root volume (cm³) of maize (d) Root length (cm) of sorghum (e) Root surface area (cm²) of sorghum (f) Root volume (cm³) of sorghum (1) Promoted by plants compost tea (PCT) (2) Promoted by cattle manure compost tea (CMCT); Columns: means, n = 4; Bars: standard error; In each species, columns of same color, inside each treatment, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were associated to letters only the data which statistical difference was significant; Very thin: 0–0.5 mm, thin: 0.5–2.5 mm, thick: 2.5–4.5 mm, very thick: > 4.5 mm; SigmaPlot 14.5 program was used to produce the graphics and the figure was edited by PhotoScape program

Table 3. Comparison between means of the treatments plants compost tea (PCT) and cattle manure compost tea (CMCT) + 1mM of aluminum (Al) for maize or 2mM for sorghum, for germination, initial growth and root morphology parameters, antioxidant enzymes activity and lipid peroxidation \pm standard error; n = 4.

Parameter	MAIZE				SORGHUM			
	PCT		CMCT		PCT		CMCT	
Germination percentage at 24h (%)	57.71 \pm 2.07	b	86.07 \pm 0.88	a				
Germination Speed Index (GSI)	37.57 \pm 0.53	b	44.66 \pm 0.36	a				
Number of anomalous seedlings	3.89 \pm 0.41	b	6.61 \pm 0.86	a	4.64 \pm 0.84	b	8.61 \pm 1.87	a
Fresh biomass (g)	30.92 \pm 0.55	b	32.07 \pm 0.35	a				
Dry biomass (g)	11.37 \pm 0.05	a	11.21 \pm 0.07	b				
Shoot length (mm)	37.94 \pm 1.25	b	44.35 \pm 1.47	a				
Total root length (cm)	6.43 x 10 ⁵ \pm 5.71 x 10 ⁴	b	1.61 x 10 ⁶ \pm 1.60 x 10 ⁵	a	1.93 x 10 ⁶ \pm 1.44 x 10 ⁵	b	2.17 x 10 ⁶ \pm 1.71 x 10 ⁵	a
Root surface area (cm ²)	2.09 x 10 ⁵ \pm 1.37 x 10 ⁴	b	5.16 x 10 ⁵ \pm 5.23 x 10 ⁴	a	2.71 x 10 ⁵ \pm 1.54 x 10 ⁴	b	3.09 x 10 ⁵ \pm 1.99 x 10 ⁴	a
Average root diameter (mm)	7.75 x 10 ³ \pm 1.29 x 10 ³	b	1.15 x 10 ⁴ \pm 8.09 x 10 ²	a				
Root volume (cm ³)	305.81 \pm 98.37	b	1482.88 \pm 193.80	a	0.3071 \pm 0.0127	b	0.3554 \pm 0.0181	a
Number of root tips	202.61 \pm 23.24	b	299.20 \pm 29.83	a				
Number of root forks	106.32 \pm 12.89	b	283.05 \pm 40.06	a	403.14 \pm 38.90	b	479.71 \pm 46.81	a
Root fineness (cm/cm ³)	1.05 x 10 ⁶ \pm 1.69 x 10 ⁵	a	2.78 x 10 ⁵ \pm 9.54 x 10 ⁴	b				
Length of thin roots (cm)	4.35 x 10 ⁵ \pm 1.59 x 10 ⁴	b	1.13 x 10 ⁶ \pm 1.25 x 10 ⁵	a	6.66 x 10 ⁵ \pm 1.26 x 10 ⁵	b	7.99 x 10 ⁵ \pm 1.51 x 10 ⁵	a
Length of thick roots (cm)	1.84 x 10 ⁴ \pm 4.95 x 10 ³	b	5.48 x 10 ⁴ \pm 6.97 x 10 ³	a	N/D		N/D	
Length of very thick roots (cm)	0.0981 \pm 0.0358	b	8.50 x 10 ³ \pm 2.14 x 10 ³	a	N/D		N/D	
Surface area of thin roots (cm ²)	1.48 x 10 ⁵ \pm 8.07 x 10 ³	b	3.80 x 10 ⁵ \pm 4.08 x 10 ⁴	a	1.28 x 10 ⁵ \pm 2.42 x 10 ⁴	b	1.57 x 10 ⁵ \pm 2.96 x 10 ⁴	a
Surface area of thick roots (cm ²)	1.94 x 10 ⁴ \pm 5.66 x 10 ³	b	5.81 x 10 ⁴ \pm 7.86 x 10 ³	a	N/D		N/D	
Surface area of very thick roots (cm ²)	438.35 \pm 438.25	b	1.50 x 10 ⁴ \pm 3.26 x 10 ³	a	N/D		N/D	
Volume of very thin roots (cm ³)	0.0070 \pm 0.0012	a	0.0055 \pm 0.0004	b				
Volume of thick roots (cm ³)	0.2177 \pm 0.0463	b	0.5538 \pm 0.0644	a	N/D		N/D	
Volume of very thick roots (cm ³)	0.0173 \pm 0.0062	b	0.2006 \pm 0.0401	a	N/D		N/D	
SOD (U min ⁻¹ g ⁻¹)					9.02 \pm 0.52	b	12.24 \pm 0.41	a
CAT (μ M H ₂ O ₂ min ⁻¹ g ⁻¹)	3.07 \pm 0.22	b	3.37 \pm 0.18	a	5.08 \pm 0.19	a	3.92 \pm 0.14	b
APX (μ M ASA min ⁻¹ g ⁻¹)	28.51 \pm 0.63	b	42.73 \pm 1.08	a	73.17 \pm 1.59	a	64.29 \pm 1.67	b
Lipid peroxidation (η mol MDA mg ⁻¹)	31.71 \pm 2.04	a	26.65 \pm 2.54	b	40.69 \pm 2.56	b	42.86 \pm 1.02	a

Means followed by different letters, in the same row, inside each species, differ statistically by the Scott-Knott test at 5% significance. Were represented only the data which statistical difference was significant. Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm. N/D: Non-existent data.

For root morphology parameters, in maize, CMCT increased all the parameters except root fineness and volume of very thin roots in relation to PCT. Considering sorghum, CMCT increased total root length, root surface area, root volume, number of forks, and length and surface area of thin roots when compared to PCT (Table 3). Treatments did not influence ($p > 0.05$) length of very thin roots, nor volume of thin roots, in maize. Besides, in sorghum, the treatments did not differ ($p > 0.05$) for average root diameter, number of tips, root fineness, length and surface area of very thin roots, and volume of any diameter class (Supplementary File, Table 2). In addition, sorghum did not present thick roots in Al experiment, so the data are not shown.

Considering antioxidant enzymes activity and lipid peroxidation, in maize, CMCT presented higher catalase (CAT) and ascorbate peroxidase (APX) activities, besides reduced lipid peroxidation, when compared to PCT. In sorghum, PCT was the responsible for enhancing the same enzymes activities and reducing lipid peroxidation in relation to CMCT, while CMCT promoted higher superoxide dismutase (SOD) activity than PCT (Table 3). SOD activity showed no statistical difference ($p>0.05$) between treatments for maize (Supplementary File, Table 2).

Regarding interaction between treatments and concentrations, for maize, 1:10 concentration of PCT increased the germination percentage at 24h while 1:1 concentration reduced it, in relation to the other concentrations and all controls (Fig. 4a). The concentrations of CMCT did not influence ($p>0.05$) germination percentage at 24h, and the further germinative parameters (germination percentage on the 4th day and GSI) showed no interaction between treatments and concentrations for maize (Supplementary File, Table 2).

For maize's initial growth parameters, all concentrations of CMCT were capable of mitigate the negative effect of Al by reducing the number of anomalous seedlings, highlighting 1:10, 1:2.5 and 1:1 concentrations that even nullified it (Fig. 4b). This parameter was not influenced ($p>0.05$) by the PCT concentrations. Fresh biomass was enhanced by all concentrations of PCT, except the lowest (1:10), and the intermediate concentrations (1:5 and 1:2.5) stimulated shoot length (Figs. 4c and 4d, respectively). For CMCT, the highest (1:1) concentration enhanced fresh biomass (Fig. 4c) and was the only capable of mitigate the negative effect of Al in shoot length (Fig. 4d). There was no interaction between treatments and concentrations for dry biomass (Supplementary File, Table 2).

Considering root morphology parameters of maize, the lowest (1:10) concentration of PCT was the only capable of nullify the increase promoted by Al in average root diameter (Fig. 4g), as well as the decrease in root fineness (Fig. 4k), besides increased the number of tips (Fig. 4i). None of the PCT concentrations positively influenced the root volume (Fig. 4h), and the other root morphology parameters (total root length, root surface area and number of forks) were not influenced ($p>0.05$) by PCT concentrations. For CMCT, on the other hand, the intermediate (1:5 and 1:2.5) concentrations stimulated the total root length (Fig. 4e), root surface area (Fig. 4f), root volume (Fig. 4h), and the numbers of tips (Fig. 4i) and forks (Fig. 4j). Stimulus was also promoted by 1:10 concentration of CMCT in the total root length (Fig. 4e) and number of forks (Fig. 4j), and all the stimuli responses cited surpassed the Hoagland solution control. The highest (1:1) concentration of CMCT was responsible for reduce maize's root surface area (Fig. 4f), average root diameter (Fig. 4g) and root volume (Fig. 4h), although was the only that increased root fineness (Fig. 4k), as well as Hoagland solution control.

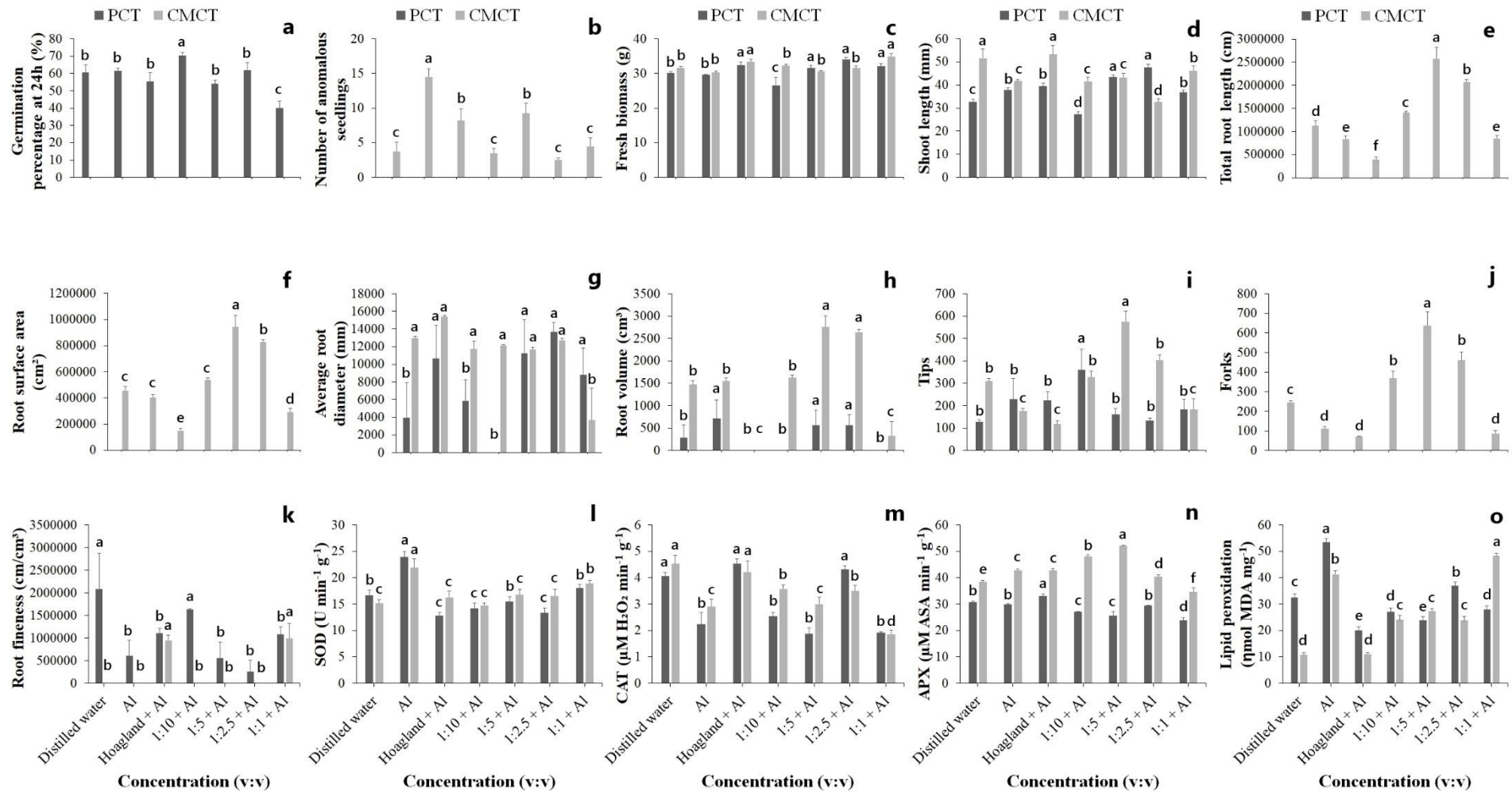


Fig. 4 Germination, initial growth, root morphology and biochemical parameters of maize under aluminum (Al) stress (1mM) and compost teas influence (a) Germination percentage at 24h (%) (b) Number of anomalous seedlings (c) Fresh biomass (g) (d) Shoot length (mm) (e) Total root length (cm) (f) Root surface area (cm²) (g) Average root diameter (mm) (h) Root volume (cm³) (i) Number of root tips (j) Number of root forks (k) Root fineness (cm/cm³) (l) Superoxide dismutase (SOD) activity (U min⁻¹ g⁻¹) (m) Catalase (CAT) activity (μM H₂O₂ min⁻¹ g⁻¹) (n) Ascorbate peroxidase (APX) activity (μM ASA min⁻¹ g⁻¹) (o) Lipid peroxidation (ηmol MDA mg⁻¹); Columns: means, n = 4; Bars: standard error; Columns of same color, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScope program

For antioxidant enzymes activity and lipid peroxidation, Al caused oxidative stress by increasing lipid peroxidation in maize, and all concentrations of PCT and CMCT treatments, except 1:1 of CMCT, mitigated or even nullified this effect (Fig. 4o). SOD activity was increased by Al, and all concentrations of the compost teas were capable of reduce it (Fig. 4l). CAT activity was decreased by Al, and the 1:2.5 concentration of PCT, as well as the 1:5 and 1:1 concentrations of CMCT nullified this effect, while the other concentrations (1:10 and 1:2.5) of CMCT mitigated it (Fig. 4m). All concentrations of PCT, except 1:2.5, reduced APX activity; while for CMCT it was increased by Al, being this effect mitigated or nullified by 1:2.5 and 1:1 concentrations, respectively (Fig. 4n).

For sorghum, CMCT treatment was responsible for nullify the effect of Al in germination percentage at 24h (Fig. 5a), while PCT did not influence ($p>0.05$) it. The other germinative parameters (germination percentage on 4th day and GSI) showed no interaction between treatments and concentrations for sorghum (Supplementary File, Table 2).

Considering the initial growth parameters, lower concentrations (1:10 and 1:5) of PCT mitigated Al effect of increasing the number of anomalous seedlings as well as Hoagland control, besides the higher concentrations (1:2.5 and 1:1) nullified Al effect; while all concentrations of CMCT nullified the effect of Al in sorghum's number of anomalous seedlings (Fig. 5b). The other initial growth parameters (fresh and dry biomasses, and shoot length) showed no statistical difference ($p>0.05$) for interaction between treatments and concentrations in sorghum (Supplementary File, Table 2).

For root morphology parameters, all concentrations of PCT were capable of nullify the Al effect in sorghum for the parameters total root length (Fig. 5c) and root surface area (Fig. 5d), besides intermediate concentrations (1:5 and 1:25) stimulated these same parameters, even surpassing Hoagland solution control. All concentrations of PCT also promoted a stimulus in the numbers of tips (Fig. 5f) and forks (Fig. 5g), and root fineness (Fig. 5h), highlighting the intermediate concentrations (1:5 and 1:2.5) which most of times overpassed Hoagland solution control. Average root diameter (Fig. 5e) was decreased by all concentrations of PCT.

Regarding CMCT, all concentrations at least nullified Al effect for the parameters total root length (Fig. 5c), root surface area (Fig. 5d), average root diameter (Fig. 5e), number of tips (Fig. 5f), and root fineness (Fig. 5h). In addition, the same parameters were stimulated, most of times even surpassing Hoagland solution control, highlighting the higher concentrations – mostly 1:2.5, but also 1:1, for number of tips and root fineness (Figs. 5f and 5h, respectively). Besides, the number of forks (Fig. 5g) was stimulated by all concentrations of CMCT, highlighting 1:5, 1:2.5 and 1:1 concentrations which presented higher values than Hoagland solution control. Root volume presented no interaction ($p>0.05$) between treatments and concentrations for sorghum (Supplementary File, Table 2).

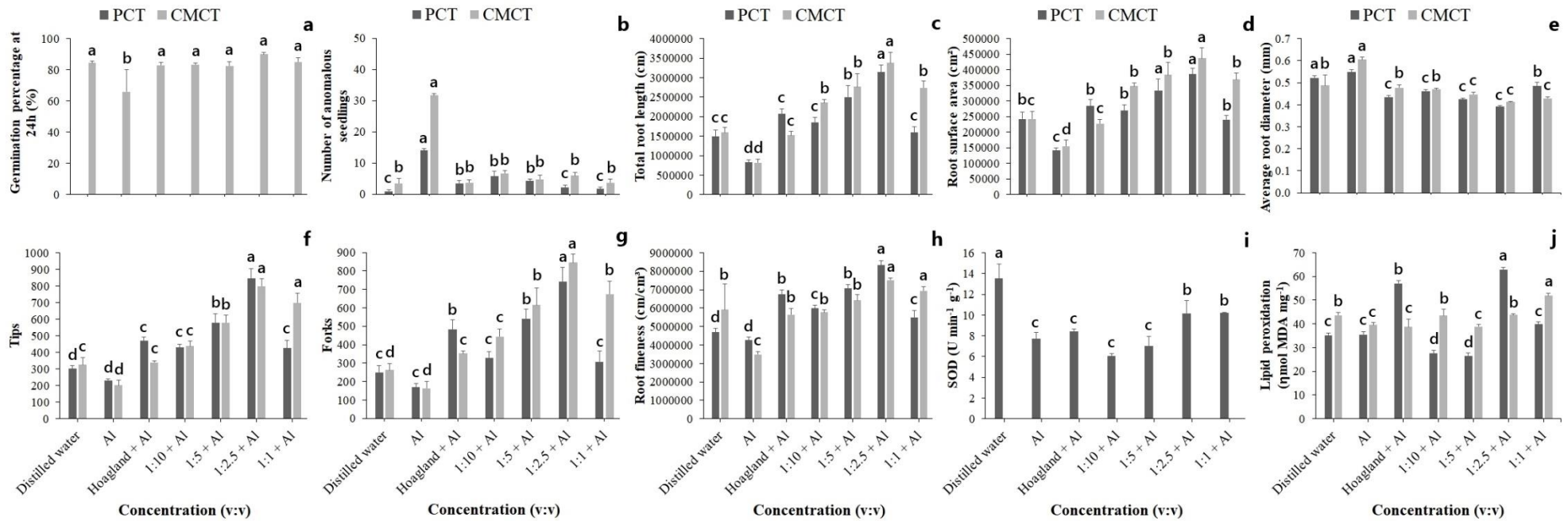


Fig. 5 Germination, initial growth, root morphology and biochemical parameters of sorghum under aluminum (Al) stress (2 mM) and compost teas influence (a) Germination percentage at 24h (%) (b) Number of anomalous seedlings (c) Total root length (cm) (d) Root surface area (cm²) (e) Average root diameter (mm) (f) Number of root tips (g) Number of root forks (h) Root fineness (cm/cm³) (i) Superoxide dismutase (SOD) activity (U min⁻¹ g⁻¹) (j) Lipid peroxidation (ηmol MDA mg⁻¹); Columns: means, n = 4; Bars: standard error; Columns of same color, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were represented only the parameters which statistical difference was significant; Office Excel program was used to produce the graphics and the figure was edited by PhotoScape program

For antioxidant enzymes activity and lipid peroxidation, the higher (1:2.5 and 1:1) concentrations of PCT mitigated Al effect of decreasing SOD activity (Fig. 5i), while CMCT did not influence ($p>0.05$) SOD activity. Lipid peroxidation in sorghum was decreased by lower concentrations (1:10 and 1:5) and increased by 1:2.5 concentration of PCT, besides the highest (1:1) concentration of CMCT increased lipid peroxidation (Fig. 5j). The further enzymes activities (CAT and APX) did not present statistical difference ($p>0.05$) for interaction between treatments and concentrations in sorghum (Supplementary File, Table 2).

Considering the root diameter classes for interaction between treatments and concentrations, for maize, the lowest (1:10) concentration of PCT stimulated the length (Fig. 6a1), surface area (Fig. 6b1) and volume (Fig. 6c1) of very thin roots in relation to the other concentrations and all controls. In addition, 1:10 and 1:1 concentrations of PCT were responsible for nullify Al effect of increasing the same parameters in thick roots (Figs. 6a1, 6b1 and 6c1). The very thick roots of maize were not influenced ($p>0.05$) by PCT concentrations.

On the other hand, the concentrations 1:10, 1:5 and 1:2.5 of CMCT stimulated the lengths of very thin and thin roots of maize, highlighting 1:5; while 1:10 and 1:1 concentrations decreased the length of thick roots, and 1:2.5 increased it. In addition, 1:5 concentration of CMCT stimulated the length of very thick roots in maize (Fig. 6a2). Al stress decreased the surface area of very thin roots in maize, and the only concentration of CMCT capable of nullify this effect was 1:5. The surface area of thin roots were stimulated by 1:10, 1:5 and 1:2.5 concentrations of CMCT, highlighting 1:5; while 1:10 and 1:1 concentrations decreased the surface area of thick roots, and 1:2.5 increased it. Besides, surface area and volume of very thick roots was increased by the intermediate (1:5 and 1:2.5) concentrations of CMCT (Figs. 6b2 and 6c2, respectively). Finally, all concentrations of CMCT, except 1:2.5, was capable of nullify Al effect of increasing the volume of thick roots in maize. CMCT concentrations did not influence ($p>0.05$) the volume of very thin roots, besides the volume of thin roots presented no interaction between treatments and concentrations for maize (Supplementary File, Table 2).

For sorghum, PCT not only nullified Al effect under very thin roots for all parameters analyzed, but also stimulated all the parameters in intermediate concentrations (1:5 and 1:2.5), and this enhance is even greater in 1:2.5 concentration, which overpassed Hoagland solution control (Figs. 6d1, 6e1 and 6f1). The decreasing promoted by Al in length of thin roots was also nullified by all PCT concentrations (Fig. 6d1). The CMCT treatment had the same effect in very thin roots, stimulating the three parameters in all concentrations, highlighting 1:5, 1:2.5 and 1:1, which also overpassed Hoagland control (Figs. 6d2, 6e2 and 6f2), besides all concentrations stimulated the length of thin roots (Fig. 6d2). The surface area of thin roots presented no statistical difference ($p>0.05$) for

interaction between treatments and concentrations for sorghum. In addition, for root volume, sorghum did not present data for thin roots, so the results are not shown (Supplementary File, Table 2).

The results demonstrate that lead (Pb) caused stress in maize by reducing shoot length and root fineness, and by increasing average root diameter, length, surface area and volume of very thick roots, and lipid peroxidation, demonstrating the occurrence of oxidative stress. In sorghum, it is possible to verify the negative effects of Pb due to decrease in fresh biomass, shoot length, total root length, root surface area, root volume, root fineness, and length, surface area and volume of very thin and thin roots; but also due to increase in average root diameter, length, surface area and volume of thick roots, although with no oxidative stress.

The presence of aluminum (Al) caused stress in maize by increasing the number of anomalous seedlings, average root diameter, length, surface area and volume of thick roots, SOD activity and lipid peroxidation, demonstrating oxidative damage; in addition to decreasing shoot length, total root length, numbers of tips and forks, and CAT activity. In sorghum, Al stress is verified by triggering reduction on germination percentage at 24h, total root length, root surface area, number of root tips, root fineness, length and surface area of very thin and thin roots, volume of very thin roots, and SOD activity, besides increasing number of anomalous seedlings and average root diameter, with no oxidative damage.

Considering the effects of compost teas in maize and sorghum initial growth and root morphology, in general, both compost teas were capable of mitigate or nullify the stress caused by the heavy metals, in addition to promote stimulus in several parameters, in most cases even surpassing Hoagland's solution effects. In addition, both treatments with compost teas also increased the numbers of tips and/or forks when submitted to Pb or Al, in both maize and sorghum, positively influencing the root architecture.

When compared the treatments, considering Pb stress, PCT was more beneficial to maize than CMCT, although CMCT was more beneficial to sorghum than PCT. And considering Al stress, CMCT was more beneficial to both species than PCT. In general, the greater stress-tolerance and stimulation effects are related to intermediate (1:5 and 1:2.5) concentrations, with few exceptions.

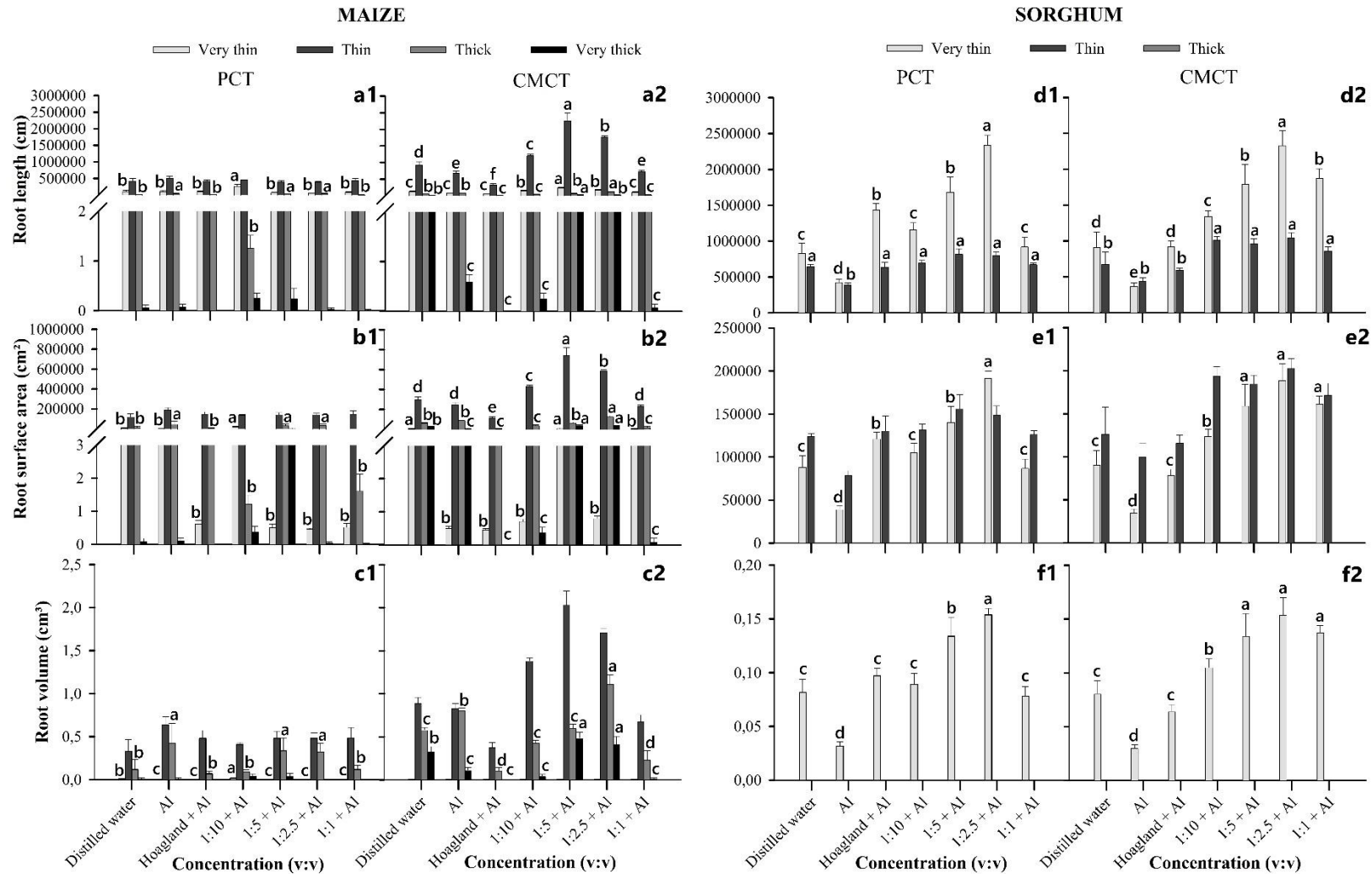


Fig. 6 Root morphology parameters of maize and sorghum under aluminum (Al) stress (1 mM and 2 mM, respectively) and compost teas influence, according to root diameter classes (a) Root length (cm) of maize (b) Root surface area (cm²) of maize (c) Root volume (cm³) of maize (d) Root length (cm) of sorghum (e) Root surface area (cm²) of sorghum (f) Root volume (cm³) of sorghum (1) Promoted by plants compost tea (PCT) (2) Promoted by cattle manure compost tea (CMCT); Columns: means, n = 4; Bars: standard error; In each species, columns of same color, inside each treatment, followed by different letters, differ statistically by the Scott-Knott test at 5% significance; Were associated to letters only the data which statistical difference was significant; Very thin: 0–0.5 mm, thin: 0.5–2.5 mm, thick: 2.5–4.5 mm, very thick: > 4.5 mm; SigmaPlot 14.5 program was used to produce the graphics and the figure was edited by PhotoScape program

4 DISCUSSION

The germination of highly improved species such as maize and sorghum, which present high seed vigor (Camargo and Vaughan, 1973; Ghassemi-Golezani and Dalil, 2014), is difficult to impair, making germination always high, even under heavy metals stress. This explains the absence or low interference of the metals on both species' germination process.

The observed effects, especially those that promoted stimulus beyond Hoagland solution, demonstrate the compost teas biostimulant action, considering that: 1) Hoagland solution is a nutritive solution recognized worldwide; 2) plant biostimulants are substances and microorganisms capable of enhance growth and development, regardless of nutrient content, by stimulating natural processes; and 3) plant biostimulants can also improve stress tolerance (Bulgari *et al.*, 2014; Calvo *et al.*, 2014; du Jardin, 2015).

The root system provides nutrients and water to plants, besides anchor and support their growth and development (Taiz *et al.*, 2017). Among the factors that influence the uptake efficiency of water and nutrients, is highlighted the modification of root architecture – that is, the spatial configuration – by investment in thinner roots (Balemi and Negisho, 2012). It is possible associate the root diameter with its function, once thinner roots are related to absorption of water and nutrients, while thicker roots are related to anchoring plants growth (Fitter, 2002). The numbers of root tips and forks can also be corelated to root architecture, as they increase the potential of roots penetration through soil layers (Soumya *et al.*, 2021). In this context, both compost teas improved water and nutrients absorption capacity in the presence of the heavy metals, by mitigating or nullifying their toxic effects on root morphology/architecture parameters, and/or also by stimulating some of these same parameters despite Pb or Al presence, demonstrating they are good stress-toleration alternatives to the heavy metals. It also explains the increases in fresh biomass, which is correlated to water content and plant growth.

It proves that our two compost teas treatments are biostimulants, since these positive effects are commonly associated to biostimulant substances (Bulgari *et al.*, 2014; du Jardin, 2015). The biostimulants effects of compost teas are related to their chemical and microbiological composition, that is, macro and micronutrient content, organic matter, humic substances, phytohormones, beneficial microorganisms, in addition to synergistic effect between two or more cited components (Eudoxie and Martin, 2019). In this context, the biostimulants effects observed for the compost teas tested in this research can be explained by the nutrient supply and microbial activity.

In the scientific literature, studies that test compost teas as toleration inducers to heavy metals are very scarce. Li *et al.* (2021) studied dissolved organic matter extracted at different times from pig compost and its copper binding capacity and suggest that shorter extraction times (6 and 12 h) enhance

Cu binding, but do not test the effect in plants. Eftekhar and Fallah (2018) studied the effect of compost tea and potassium humate on sunflower resistance to Pb toxicity and demonstrated that compost tea alleviated the Pb toxic effect under carotenoids and proline content and electrolyte leakage. Furthermore, Eudoxie and Martin (2019) demonstrated that even with heavy metals as components of several types of compost teas treatments, the positive effect in crops is still highlighted.

Considering this information, the exact mechanisms of action of compost teas as tolerance inducers to heavy metals are still not determined. However, they can be correlated to the mechanisms by which humic substances – in which the compost teas are commonly rich – can induce tolerance to heavy metals stress: 1) by complexing toxic forms of heavy metals binding its ions to carboxylic and phenolic hydroxyl groups of humic substances chemical structure, turning the ions unavailable to uptake; and 2) by activating and/or strengthening endogenous plant defense systems, as various enzymatic and non-enzymatic antioxidants (Canellas *et al.*, 2015).

Regarding the antioxidant enzymes activities and lipid peroxidation, in general, the antioxidant enzymes were responsible for reduce oxidative stress in maize, highlighting ascorbate peroxidase (APX), while in sorghum, there was no oxidative stress. It demonstrates that the compost teas increased the activity of antioxidant enzymes (Osman *et al.*, 2022) as a defense mechanism against Pb and Al stress in maize, while the stress-tolerant effect in sorghum is due the chemical and microbiological composition (Eudoxie and Martin, 2019; Gutiérrez-Miceli *et al.*, 2008; Verrillo *et al.*, 2021). Osman *et al.* (2022) elucidated that compost tea mitigates the oxidative damage promoted by salt stress by boosting the enzymatic antioxidant metabolism. Verrillo *et al.* (2021), on the other hand, elucidated that compost teas present antioxidant action due to humic substances content since these substances present capacity of either donate or accept electrons.

Finally, the stress-tolerant mechanisms of action of our compost teas, for both maize and sorghum, are associated to the chemical and microbiological compositions of the teas, and in case of maize, also by antioxidant enzymes activity (Eudoxie and Martin, 2019; Osman *et al.*, 2022; Verrillo *et al.*, 2021).

5 CONCLUSION

The innovation of this research is verified by the fact that are few studies in the literature that correlate the effects of compost teas as tolerance-inducers to stresses caused by the presence of heavy metals.

The two treatments of compost teas tested, plants compost tea (PCT) and cattle manure compost tea (CMCT), presented biostimulant action since they enhanced maize and sorghum stress-

toleration to lead (Pb) and aluminum (Al). The biostimulant effect is due to their chemical and microbiological composition, which mitigated or nullified Pb and Al toxic effects, and sometimes even stimulated initial growth and root morphology/architecture of both species. In addition, for maize, compost teas also increased APX activity to alleviate oxidative damage.

In maize, PCT was more effective to Pb-stress-toleration, while for Al-stress-toleration, CMCT was more effective. In sorghum, CMCT treatment was more effective in the presence of both heavy metals. In general, the intermediate concentrations (1:5 and 1:2.5) of the teas presented the greater biostimulant effects.

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SUPPLEMENTARY FILE

EFFECT OF TWO COMPOST TEAS AS TOLERANCE-INDUCERS TO LEAD AND ALUMINUM
IN MAIZE AND SORGHUM

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TABLES

Table 1. Results for *p*-value of Analysis of Variance (ANOVA) to verify difference between the treatments and the interaction treatments*concentrations for maize and sorghum under lead (Pb) stress (1 mM).

Parameters	MAIZE		SORGHUM	
	Treatments	Treatment*Concentrations	Treatments	Treatment*Concentrations
Germination percentage at 24h (%)	<i>p</i> = 0.9754	<i>p</i> = 0.2806	<i>p</i> = 0.7722	<i>p</i> = 0.6627
Germination percentage on the 4 th day (%)	<i>p</i> = 0.3722	<i>p</i> = 0.4469	<i>p</i> = 0.9592	<i>p</i> = 0.3235
Germination Speed Index (GSI)	<i>p</i> = 0.7844	<i>p</i> = 0.5789	<i>p</i> = 0.9115	<i>p</i> = 0.5006
Number of anomalous seedlings	<i>p</i> = 0.0015	<i>p</i> = 0.0314	<i>p</i> = 0.0012	<i>p</i> = 0.3416
Fresh biomass (g)	<i>p</i> = 0.9110	<i>p</i> = 0.0000	<i>p</i> = 0.4486	<i>p</i> = 0.0270
Dry biomass (g)	<i>p</i> = 0.4645	<i>p</i> = 0.7504	<i>p</i> = 0.3274	<i>p</i> = 0.0249
Shoot length (mm)	<i>p</i> = 0.0285	<i>p</i> = 0.0000	<i>p</i> = 0.0133	<i>p</i> = 0.0001
Total root length (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0190	<i>p</i> = 0.0000
Root surface area (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0073	<i>p</i> = 0.0000
Average root diameter (mm)	<i>p</i> = 0.0038	<i>p</i> = 0.0000	<i>p</i> = 0.5924	<i>p</i> = 0.0080
Root volume (cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0061	<i>p</i> = 0.0001
Number of root tips	<i>p</i> = 0.0001	<i>p</i> = 0.0000	<i>p</i> = 0.3657	<i>p</i> = 0.0030
Number of root forks	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.1948	<i>p</i> = 0.0000
Root fineness (cm/cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.9890	<i>p</i> = 0.0004
Length of very thin roots (cm)	<i>p</i> = 0.0014	<i>p</i> = 0.0000	<i>p</i> = 0.0245	<i>p</i> = 0.0000
Length of thin roots (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.1198	<i>p</i> = 0.0002
Length of thick roots (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0713	<i>p</i> = 0.0080
Length of very thick roots (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	Non-existent data	Non-existent data
Surface area of very thin roots (cm ²)	<i>p</i> = 0.0030	<i>p</i> = 0.0000	<i>p</i> = 0.0048	<i>p</i> = 0.0000
Surface area of thin roots (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0422	<i>p</i> = 0.0015
Surface area of thick roots (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0709	<i>p</i> = 0.0079
Surface area of very thick roots (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	Non-existent data	Non-existent data
Volume of very thin roots (cm ³)	<i>p</i> = 0.0071	<i>p</i> = 0.0000	<i>p</i> = 0.0028	<i>p</i> = 0.0000
Volume of thin roots (cm ³)	<i>p</i> = 0.0188	<i>p</i> = 0.0002	<i>p</i> = 0.0000	<i>p</i> = 0.0000
Volume of thick roots (cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0729	<i>p</i> = 0.0086
Volume of very thick roots (cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	Non-existent data	Non-existent data
SOD (U min ⁻¹ g ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0001	<i>p</i> = 0.0000	<i>p</i> = 0.0000
APX (μM AsA min ⁻¹ g ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000
CAT (μM H ₂ O ₂ min ⁻¹ g ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0533	<i>p</i> = 0.7667	<i>p</i> = 0.2494
Lipid peroxidation (ηmol MDA mg ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0000

Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm. Were considered as significant the results for *p*-value < 0.05.

Table 2. Results for *p*-value of Analysis of Variance (ANOVA) to verify difference between the treatments and the interaction treatments*concentrations for maize and sorghum under aluminum (Al) stress (1 mM and 2 mM, respectively).

Parameters	MAIZE		SORGHUM	
	Treatments	Treatment*Concentrations	Treatments	Treatment*Concentrations
Germination percentage at 24h (%)	<i>p</i> = 0.0000	<i>p</i> = 0.0029	<i>p</i> = 0.9274	<i>p</i> = 0.0210
Germination percentage on the 4 th day (%)	<i>p</i> = 0.8302	<i>p</i> = 0.2046	<i>p</i> = 0.2216	<i>p</i> = 0.4444
Germination Speed Index (GSI)	<i>p</i> = 0.0000	<i>p</i> = 0.0591	<i>p</i> = 0.7217	<i>p</i> = 0.0605
Number of anomalous seedlings	<i>p</i> = 0.0000	<i>p</i> = 0.0001	<i>p</i> = 0.0000	<i>p</i> = 0.0000
Fresh biomass (g)	<i>p</i> = 0.0110	<i>p</i> = 0.0007	<i>p</i> = 0.0833	<i>p</i> = 0.0737
Dry biomass (g)	<i>p</i> = 0.0467	<i>p</i> = 0.3388	<i>p</i> = 0.7574	<i>p</i> = 0.5663
Shoot length (mm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.5361	<i>p</i> = 0.0590
Total root length (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0156	<i>p</i> = 0.0022
Root surface area (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0035	<i>p</i> = 0.0088
Average root diameter (mm)	<i>p</i> = 0.0051	<i>p</i> = 0.0108	<i>p</i> = 0.3228	<i>p</i> = 0.0139
Root volume (cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0015	<i>p</i> = 0.0678
Number of root tips	<i>p</i> = 0.0003	<i>p</i> = 0.0000	<i>p</i> = 0.4784	<i>p</i> = 0.0004
Number of root forks	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0065	<i>p</i> = 0.0011
Root fineness (cm/cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0039	<i>p</i> = 0.5907	<i>p</i> = 0.0252
Length of very thin roots (cm)	<i>p</i> = 0.2446	<i>p</i> = 0.0000	<i>p</i> = 0.1800	<i>p</i> = 0.0016
Length of thin roots (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0006	<i>p</i> = 0.1178
Length of thick roots (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0125	Non-existent data	Non-existent data
Length of very thick roots (cm)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	Non-existent data	Non-existent data
Surface area of very thin roots (cm ²)	<i>p</i> = 0.1552	<i>p</i> = 0.0000	<i>p</i> = 0.1823	<i>p</i> = 0.0044
Surface area of thin roots (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0004	<i>p</i> = 0.0771
Surface area of thick roots (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0068	Non-existent data	Non-existent data
Surface area of very thick roots (cm ²)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	Non-existent data	Non-existent data
Volume of very thin roots (cm ³)	<i>p</i> = 0.0433	<i>p</i> = 0.0000	<i>p</i> = 0.1490	<i>p</i> = 0.0067
Volume of thin roots (cm ³)	<i>p</i> = 0.3226	<i>p</i> = 0.4387	Non-existent data	Non-existent data
Volume of thick roots (cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0070	Non-existent data	Non-existent data
Volume of very thick roots (cm ³)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	Non-existent data	Non-existent data
SOD (U min ⁻¹ g ⁻¹)	<i>p</i> = 0.1059	<i>p</i> = 0.0459	<i>p</i> = 0.0000	<i>p</i> = 0.0213
APX (μM AsA min ⁻¹ g ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0003	<i>p</i> = 0.5638
CAT (μM H ₂ O ₂ min ⁻¹ g ⁻¹)	<i>p</i> = 0.0290	<i>p</i> = 0.0026	<i>p</i> = 0.0000	<i>p</i> = 0.0417
Lipid peroxidation (ηmol MDA mg ⁻¹)	<i>p</i> = 0.0000	<i>p</i> = 0.0000	<i>p</i> = 0.0205	<i>p</i> = 0.0000

Very thin: 0–0.5 mm; thin: 0.5–2.5 mm; thick: 2.5–4.5 mm; very thick: > 4.5 mm. Were considered as significant the results for *p*-value < 0.05.

6 CONSIDERAÇÕES FINAIS GERAIS

Os chás de composto e de vermicomposto podem ser aplicados nas plantas como indutores de tolerância aos estresses abióticos, pois são capazes de mitigar os estresses e promover o crescimento e desenvolvimento das plantas apesar das condições estressantes. Os mecanismos de tolerância promovidos pelos chás aos estresses abióticos estão associados ao aumento do metabolismo antioxidante enzimático e não enzimático, à contribuição para a homeostase do K^+ e Na^+ , e à melhora do metabolismo fotossintético, consequentemente aumentando o crescimento e, em última análise, aumentando o rendimento das espécies cultivadas. Quando considerados os estresses abióticos individualmente, foi possível verificar que os chás foram benéficos tanto para o déficit hídrico quanto para o estresse salino.

Os dois tratamentos de chás de composto testados, *plants compost tea* (PCT) e *cattle manure compost tea* (CMCT), podem ser considerados bioestimulantes de acordo com seus efeitos sobre o crescimento inicial e morfologia/arquitetura radicular do milho e do sorgo, devido à sua composição química e microbiológica. Sob estresse por déficit hídrico, Pb ou Al, os efeitos bioestimulantes de tolerância ao estresse estão associados à composição química e microbiológica para ambos os tratamentos de chás de composto, e algumas vezes à atividade de enzimas antioxidantes.

O chá de composto que merece destaque em relação aos seus efeitos benéficos é o CMCT, principalmente quando é aplicado nas concentrações 1:5 ou 1:2.5 (v:v – volume de composto : volume de água destilada).

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